



MONASH University

Benefits of group living and helping behaviour in the cooperatively breeding purple-crowned fairy-wren, *Malurus coronatus*

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Abstract

Cooperative breeding involves individuals foregoing independent reproduction, and instead assisting others. As such, it has become a model system for understanding the evolution of altruism in general. Two types of benefits may lead individuals to stay in a social group as non-breeding subordinate and to engage in cooperative behaviour: benefits from group living itself, and benefits associated with helping raise offspring of the dominant breeding pair. My PhD project investigates both types of benefits in the facultatively cooperative breeding purple-crowned fairy-wren, *Malurus coronatus*. Specifically, my thesis investigates two important aspects of cooperative breeding that are rarely considered: (i) how the composition of social groups affects potential benefits of group living, and consequently helping behaviour; and (ii) how and why helpers may contribute to predator defence - which can enhance reproductive success as well as survival of group members - in addition to offspring provisioning, which is more commonly studied. In my first chapter, I studied patterns of social interactions within groups and show that subordinate fairy-wrens form close social bonds specifically with relatives and prospective mates – group members associated with kin-selected and potential mating benefits – while they form antagonistic relationships with reproductive competitors. This indicates that social group composition affects benefits of group living. In Chapter 2, I conducted experimental model presentations of predators of varying levels of threat at nests. I show that predator defence is complex and shaped by risk and multiple benefits. Importantly, defence by breeders and helpers is shaped by different benefits: breeders defend the brood more, presumably because they benefit more from brood survival, whereas helpers defend more against predators of adults in agreement with the important benefits they receive from social group members. Next, I tested whether individuals specialise in offspring feeding or nest defence (Chapter 3). This is an important question to test, as the presence of task specialisation could have important implications for our understanding of the evolutionary maintenance of cooperative behaviour in birds. I show that individuals in this species do not specialise, but there is considerable variation in helpers' contribution to cooperative tasks. Therefore, in Chapter 4, I test what benefits of group living and helping at the nest explain predator defence by helpers. I find that helpers invest more in nest defence when they have greater opportunities for breeding position inheritance, and engage in adult group member defence mainly to protect potential mates and relatives. Lastly, I show that variation in offspring feeding effort by helpers is not explained by hypotheses commonly proposed for the evolution of helping behaviour, but instead, by indirect and direct

benefits associated with enhanced survival of breeders they are assisting (Chapter 5). Again, group composition affects helping behaviour, with helpers working hardest when this enhances the fitness of a relative and a potential mate. Together, my thesis highlights the importance of considering social group composition as well as all costly forms of helping, in order to understand the origin and maintenance of complex cooperative social systems.

Publications during enrolment

- Teunissen, N., Kingma, S.A., Hall, M.L., Hidalgo Aranzamendi, N., Komdeur, J. & Peters, A. (2018). More than kin: subordinates foster strong bonds with relatives and potential mates in a social bird. *Behavioral Ecology*, **29**, 1316-1324. doi:10.1093/beheco/ary120
- McQueen, A., Naimo, A.C., Teunissen, N., Magrath, R.D., Delhey, K. & Peters, A. (2017). Bright birds are cautious: seasonally conspicuous plumage prompts risk avoidance by male superb fairy-wrens. *Proceedings of the Royal Society B: Biological Sciences*, **284**, 20170446. doi:10.1098/rspb.2017.0446
- Fan, M., Teunissen, N., Hall, M. L., Hidalgo Aranzamendi, N., Kingma, S. A., Roast, M., Delhey, K., & Peters, A. (2018). From ornament to armament or loss of function? Breeding plumage acquisition in a genetically monogamous bird. *Journal of Animal Ecology*, **87**, 1274-1285.
- Eastwood, J., Hall, M. L., Teunissen, N., Kingma, S. A., Hidalgo Aranzamendi, N., Fan, M., Roast, M., Verhulst, S., & Peters, A. (2019). Early life telomere length predicts lifespan and lifetime reproductive success in a wild bird. *Molecular Ecology*, **28**, 1127-1137. doi: 10.1111/mec.15002
- Roast, M., Aulsebrook, A., Fan, M., Hidalgo Aranzamendi, N., Teunissen, N., & Peters, A. (2019). Short-term climate variation drives baseline innate immune function and stress in a tropical bird: a reactive scope perspective. *Physiological and Biochemical Zoology*, **92**, 140-151. doi:10.1086/702310

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes one original paper published in a peer-reviewed journal and one submitted publication. The core theme of the thesis is behavioural and evolutionary ecology. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the School of Biological Sciences under the supervision of A/Prof Anne Peters, Dr. Michelle Hall (University of Melbourne), Dr. Kaspar Delhey, and Dr. Rohan Clarke.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

This thesis also includes 3 chapters which have not been submitted for publication. In the case of chapters 1 - 5 my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status	Nature and % of student contribution	Co-author name(s) Nature and % of Co-author's contribution	Co-author(s), Monash student Y/N
1	More than kin: subordinates foster strong social bonds with relatives and potential mates in a social bird	Published	70%. Concept, design, data collection, data analysis, and writing of manuscript	1) A. Peters: concept, design, data analysis, and edits to manuscript, 10% 2) S. A. Kingma: concept, design, and edits to manuscript, 7.5% 3) N. Hidalgo Aranzamendi: data collection and edits to manuscript, 5% 4) M. L. Hall: data collection and edits to manuscript, 5% 5) J. Komdeur: concept and edits to manuscript, 2.5%	No No Yes No No

2	Predator defense is shaped by risk, brood value and social group benefits in a cooperative breeder	Submitted (awaiting decision)	85%. Concept, design, data collection, data analysis, and writing of manuscript	1) A. Peters: concept, design, data analysis, and edits to manuscript, 10% 2) S. A. Kingma: concept, design, data analysis, and edits to manuscript, 5%	No No
3	Nest defence and offspring provisioning in a cooperative breeder: no evidence for task specialisation	Not yet submitted	85%. Concept, design, data collection, data analysis, and writing of manuscript	1) A. Peters: concept, design, data analysis, and edits to manuscript, 10% 2) S. A. Kingma: concept, design, data analysis, and edits to manuscript, 5%	No No
4	Predator defence and cooperative breeding: helpers defend to raise future helpers and to gain benefits of group living	Not yet submitted	80%. Concept, design, data collection, data analysis, and writing of manuscript	1) A. Peters: concept, design, data analysis, and edits to manuscript, 10% 2) S. A. Kingma: concept, design, data analysis, and edits to manuscript, 5% 3) M. Fan: data collection and edits to manuscript, 2.5% 4) M. Roast: data collection and edits to manuscript, 2.5%	No No Yes Yes
5	Helpers provision offspring of potential mates and relatives in a cooperative fairy-wren	Not yet submitted	80%. Concept, design, data collection, data analysis, and writing of manuscript	1) A. Peters: concept, design, data analysis, and edits to manuscript, 10% 2) M. Fan: data collection and edits to manuscript, 2.5% 3) M. Roast: data collection and edits to manuscript, 2.5% 4) N. Hidalgo Aranzamendi: data collection and edits to manuscript, 2.5% 5) S. A. Kingma: concept, design, data analysis, and edits to manuscript, 2.5%	No Yes Yes No No

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis. Tables and figures have not been renumbered and are therefore numbered chronologically within each chapter. Structure and formatting of chapters are in line with the requirements of journals to which manuscripts have been or will be submitted.

Niki Teunissen

Date: 9 October 2019

I hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I

have consulted with the responsible author to agree on the respective contributions of the authors.

Anne Peters

Date: 9 October 2019

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General introduction

Background

In cooperatively breeding species, individuals form social groups, and subordinate individuals forego their own reproduction and instead help the dominant breeding pair to raise their offspring (Emlen 1982; Brown 1987; Cockburn 1998). Cooperative breeding thereby challenges the Darwinian view that individuals should be selfish and only invest in offspring that are their own (Darwin 1859). Despite this, cooperative behaviour is found in a variety of vertebrate taxa, including mammals (e.g. mongooses), birds (e.g. corvids, fairy-wrens, and a range of other passerines) and fish (e.g. cichlids) (Taborsky and Limberger 1981; Emlen 1982; Jennions and Macdonald 1994; Cockburn 2006; Koenig and Dickinson 2016). Since help with reproduction is costly, resulting in lower survival, body condition, and/or future reproduction (Heinsohn and Cockburn 1994; Heinsohn and Legge 1999; Russell et al. 2003; van de Crommenacker et al. 2011), cooperative breeding has become a model system to study the evolution of altruism more broadly (Cockburn 1998; Bergmüller et al. 2007; Koenig and Dickinson 2016). The evolution of cooperative breeding in birds in particular has received considerable research attention (e.g. Cockburn 1998; Hatchwell 2009; Kingma 2017). Cooperative breeding occurs in 9% of bird species (Cockburn 2006), and is even obligate in some systems, where successful reproduction is impossible without cooperative brood care (e.g. white-winged choughs, *Corcorax melanorhamphos*; Heinsohn 1992; greater anis, *Crotophaga major*; Riehl 2011). However in most cooperative birds helping is facultative, making them a good candidate to study the proximate and ultimate drivers that promote cooperative behaviour (Hatchwell 2009).

Ultimately, cooperative breeding is assumed to be driven by ecological constraints on independent breeding (Emlen 1982; Cornwallis et al. 2017), whereby individuals stay on their natal territories without breeding independently because of a lack of outside opportunities, e.g. due to a shortage of available territories, nesting sites, or breeding partners (Pruett-Jones and Lewis 1990; Walters et al. 1992; Komdeur and Ekman 2010; Raihani et al. 2010). Even in the absence of strict habitat saturation, variation in territory quality can affect the formation of cooperative social groups. A good example of this is provided by the cooperative breeding system of the Seychelles warbler, *Acrocephalus sechellensis*. Here, offspring born on high-quality territories that stay at home as a subordinate and breed there later in life obtain greater lifetime inclusive fitness compared to ones that choose to leave home at an earlier age to breed in a low-quality territory instead (Komdeur 1992). Elegant experiments in this and other species, such as

the superb fairy-wren, *Malurus cyaneus*, demonstrate that the removal of a breeder in a group results in non-breeding subordinates rapidly taking over the vacant position (Pruett-Jones and Lewis 1990; Komdeur 1992), and indicate that individuals are often constrained from obtaining a breeding position by a shortage of (high-quality) breeding territories. Indeed, globally, cooperative breeding is particularly common in environments with high uncertainty and high climatic variability (i.e. greater ecological constraints), and when habitats are saturated, conditions that occur frequently in Australia (Komdeur and Ekman 2010; Jetz and Rubenstein 2011; Cornwallis et al. 2017). Additionally, cooperative breeding is particularly common among Australian birds (Russell 1989; Arnold and Owens 1998; Feeney et al. 2013). Therefore, studying this behaviour is not only important to understand its evolution, but also to predict how vulnerable species will cope in the changing Australian landscape and climate, particularly because cooperative breeders have low reproductive rates for population size (Arnold and Owens 1998).

Two stages of cooperative breeding: why stay, and why help?

Cooperative breeding involves two stages (Komdeur and Ekman 2010; Griesser et al. 2017). First, an individual has to make the decision to join and/or stay in a group without breeding independently (i.e. as a non-breeding subordinate*). Often subordinates in cooperative groups are offspring from previous broods that remained on their natal territory, therefore this first stage is generally assumed to involve ecological constraints that prevent individuals from dispersing and breeding independently, leading to delayed dispersal (Koenig et al. 1992; Hatchwell and Komdeur 2000; Ekman et al. 2004; Komdeur and Ekman 2010; Raihani et al. 2010). However, although the majority (55%) of cooperative birds breed in family groups, this is not always the case: in 30% of species, groups consist of a mix of relatives and non-relatives, and in 15% of species, groups primarily consist of non-relatives (Riehl 2013). Therefore, to understand the evolution of group formation in cooperative birds, it seems crucial to understand the formation of family units (Covas and Griesser 2007; Hatchwell 2009; Komdeur and Ekman 2010; Griesser et al. 2017), as well as drivers of group living in general that may promote the formation of mixed or non-kin units (Krause and Ruxton 2002). The second stage of cooperative breeding involves subordinate individuals then making the decision to help raise offspring in the group. This involves indirect

*Note that while subordinates can vary in the amount of help provided, and not all subordinates necessarily help with raising offspring in cooperative groups, throughout this thesis the term “helper” and “subordinate” are used interchangeably for the sake of simplicity.

and/or direct benefits associated with helping itself (Cockburn 1998; Bergmüller et al. 2007; Kingma 2017). Importantly, the costs and benefits of the decision to stay and the decision to help may be closely linked, but are not necessarily similar (Komdeur and Ekman 2010), providing the challenge for empirical studies to tease drivers of the two decisions apart. To gain a comprehensive understanding of the evolution of cooperative breeding it appears imperative for studies to simultaneously consider, and to tease apart, benefits of group living and benefits of helping raise offspring.

Offspring that delay dispersal and stay at home may receive various benefits of philopatry. This includes improved survival as a result of the natal territory providing a “safe haven” (Brown 1987; Kokko and Ekman 2002), or parents allowing offspring better access to resources or enhanced protection from predators (parental tolerance/nepotism; Ekman et al. 2000; Kraaijeveld and Dickinson 2001; Ekman et al. 2004; Griesser et al. 2006; Covas and Griesser 2007; Nelson-Flower and Ridley 2016; Nelson-Flower et al. 2018). More generally, group living is associated with various benefits to group members that may apply regardless of whether subordinates are retained offspring or not. For example, group members may experience improved survival through reduced predation rates in larger groups (Kokko et al. 2001; Krause and Ruxton 2002; Kingma et al. 2014). Additionally, subordinates may obtain reproductive benefits by eventually inheriting a breeding position, or by acquiring a mate within the group and budding off from the resident territory to establish a new breeding territory together (Koenig et al. 1992; Komdeur and Edelaar 2001; Kokko and Ekman 2002; Krause and Ruxton 2002; Ekman et al. 2004; Komdeur and Ekman 2010; Kingma 2017; Nelson-Flower et al. 2018). These survival and reproductive benefits associated with staying in a group as a subordinate may offset the costs of delaying independent reproduction and ultimately lead to a higher lifetime reproductive success (Covas and Griesser 2007; Komdeur and Ekman 2010). Benefits of group living are therefore in itself enough to drive the formation of social groups, even in the absence of subsequent cooperation (Kokko and Ekman 2002). Cooperative help therefore requires additional explanations.

As cooperative breeding often occurs in family groups (Riehl 2013), kin selection theory has been proposed as a likely explanation for the evolution of cooperative offspring care. This theory proposes that helpers gain indirect fitness benefits from helping closely related individuals (Hamilton 1964; Emlen 1995; Cockburn 1998; Bergmüller et al. 2007; Hatchwell 2009). Empirical and comparative studies have revealed that kin selection explains variation in helping behaviour within and across cooperatively breeding vertebrate species (e.g. Reyer 1984; Russell and

Hatchwell 2001; Griffin and West 2002; Baglione et al. 2003; Griffin and West 2003; Covas et al. 2006; Nam et al. 2010; Wright et al. 2010; Browning et al. 2012; Preston et al. 2013; Green et al. 2016; Dias et al. 2017; Barati et al. 2018). However, subordinates often help to raise unrelated offspring (e.g. Dunn et al. 1995; Magrath and Whittingham 1997; Legge 2000; Griffin and West 2003; Dickinson 2004; Hatchwell 2009; Wright et al. 2010; Riehl 2013; Barati et al. 2018), and meta-analyses have revealed that variation in relatedness to the brood alone explains as little as 10% of the variation in helping behaviour observed (Griffin and West 2003; Kingma 2017). It is therefore now clear that kin selection alone does not provide a general explanation for helping behaviour. Instead, direct benefits associated with helping explain a much greater proportion of variation in helping behaviour (up to 41%; Kingma 2017), and therefore seem to be more important drivers of helping.

Various direct benefits may be associated with helping raise offspring. Firstly, subordinates may benefit from staying in a group but need to provide help as a form of rent to avoid punishment or eviction by dominants (pay-to-stay hypothesis; Kokko et al. 2002; Bergmüller et al. 2007). Secondly, helpers may gain direct access to reproduction in the group, e.g. male helpers may receive access to extra-pair copulations with the breeding female or female helpers may lay eggs in the nest of the breeding pair (parentage acquisition hypothesis; Magrath and Whittingham 1997; Cockburn 1998; Dickinson 2004). Alternatively, helping may serve as a signal of quality and thereby helping raise offspring may increase subordinates' chances of finding a mate or obtaining a dominant position in the group (social prestige hypothesis; Zahavi 1995; Griffin and West 2002; Bergmüller et al. 2007). Lastly, the production of additional recruits itself may be beneficial to helpers. Enlarging group size may increase survival of group members in general due to e.g. safety-in-numbers or dilution effects (passive group augmentation). Additionally, the recruits produced may in turn offer helpers benefits through reciprocal interactions or by providing help with breeding themselves when the helper eventually obtains a breeding position in the group, thereby enhancing the helper's future reproductive success (active group augmentation) (Cockburn 1998; Kokko et al. 2001; Bergmüller et al. 2007; Kingma et al. 2014). These hypotheses have been investigated in a range of cooperative breeders, and different benefits seem to apply in different systems (e.g. Zahavi 1990; Mulder and Langmore 1993; Baglione et al. 2003; Nam et al. 2010; Dias et al. 2017). However, as proposed benefits are not mutually exclusive, it is important to consider the combined effect of multiple direct and indirect benefits and their interactions. This is however rarely done in studies on helping behaviour, possibly because it requires a range of detailed information on the study species,

including for example measures of relatedness, quantification of chances of breeding position inheritance prospects for individual helpers, and detailed parentage analyses (Kokko et al. 2001; Clutton-Brock 2002; Griffin and West 2002; Richardson et al. 2002; Dickinson 2004; Bergmüller et al. 2007; Kingma et al. 2011; Downing et al. 2018).

Social group composition and benefits of group living and helping

The type of group members that subordinates share a group with, i.e. the composition of the social group, will determine the type and relative magnitude of benefits of group living and helping that are relevant to individual helpers. Benefits of group living depend strongly on social group composition in groups that also include unrelated individuals (45% of cooperative bird species; Riehl 2013). Here, some individuals may be a relative, whereas others may be a potential mate (unrelated, opposite sex), or a reproductive competitor (unrelated, same sex). Hence, the relative sex and relatedness of group members affect the potential for and magnitude of group-living benefits. For example, nepotism is directed from parents to offspring only (Ekman et al. 2000; Ekman et al. 2004; Griesser et al. 2006; Nelson-Flower and Ridley 2016), and reproductive benefits resulting from acquiring a mate in the group are only possible when potential mates are present (Koenig et al. 1992; Komdeur and Edelaar 2001; Komdeur and Ekman 2010). Benefits and costs associated with certain group members may be escalated further through social interactions with group members. Social interactions are important in group-living animals for developing and maintaining relationships between individuals, such as pair-bonds and dominance hierarchies (Silk et al. 2006; Kutsukake 2009; Gill 2012). Aggressive interactions are costly in nature, associated with investment of time and energy and risk of injury (Rovero et al. 2000; Petit 2010), and negatively affect social relationships (Kutsukake and Clutton-Brock 2008). Affiliative interactions (e.g. allogrooming) on the other hand provide benefits such as reduced parasite load and reduced stress (Sanchez-Villagra et al. 1998; Aureli et al. 1999; McKechnie and Lovegrove 2001; Sapolsky 2005; Charpentier et al. 2012; Villa et al. 2016). Furthermore, affiliative interactions strengthen social bonds, which are additionally associated with e.g. improved reproductive success and earlier onset of reproduction (Silk et al. 2006; Silk et al. 2009; Seyfarth and Cheney 2012; Kenny et al. 2017; Riehl and Strong 2018). Importantly, social interactions tend to take place mostly between particular individuals: affiliation is more common among related and opposite-sex individuals while the reverse pattern holds for aggressive interactions (Seibert and Crowell-Davis 2001; Kutsukake and Clutton-Brock 2006; Silk et al. 2006; Dickinson et al. 2009; Kutsukake 2009; Mitchell et al. 2009; Charpentier et al. 2012). Our current understanding of social

interactions is based largely on studies on primates (e.g. Sanchez-Villagra et al. 1998; Shutt et al. 2007; Silk et al. 2009), but applying the knowledge acquired from these systems to cooperatively breeding vertebrates in general may reveal additional benefits of group living through social bonds formed between particular group members.

Potential benefits of help with breeding also vary with social group composition. For example, helpers can only obtain indirect kin-selected benefits when they are raising related offspring and/or enhancing the survival of related breeders through load-lightening (Hamilton 1964; Bergmüller et al. 2007; Meade et al. 2010; Johnstone 2011). Additionally, the potential for helping behaviour to increase social prestige assumes that potential mates are present to advertise to (Zahavi 1995; Bergmüller et al. 2007). Direct access to reproduction is generally also higher when helpers are unrelated to the opposite-sex breeder (Magrath and Whittingham 1997; Riehl 2017). Lastly, whether helpers might receive future help with breeding from the recruits they helped raise (as proposed by active group augmentation theory; Kokko et al. 2001; Kingma et al. 2014) depends on how likely they are to inherit a breeding position in the group in the future, and this depends on helpers' relatedness to the opposite-sex breeder as well as e.g. the presence of an older same-sex helper in the group (Kingma et al. 2011). Despite this clear effect of social group composition on benefits of group living and helping raise offspring, the social environment is rarely taken into account in studies on cooperative breeding. Since the composition of groups varies considerably between groups and species (Riehl 2013), taking this into consideration appears crucial to obtain a comprehensive understanding of the evolution of group living, and the evolutionary maintenance of cooperative behaviour.

Multiple forms of helping

Studies on cooperatively breeding birds in particular have generally quantified helping effort by measuring offspring feeding rates by helpers only, and the various hypotheses proposed for the evolution of helping behaviour are tested by relating variation in offspring provisioning by helpers to potential indirect and direct benefits of helping (Cockburn 1998; Bergmüller et al. 2007; Kingma 2017; Downing et al. 2018). However, helpers can contribute to raising offspring by participating in a variety of cooperative tasks, including nest building, defending young, territory maintenance, egg incubation, and nest cleaning (Taborsky 1984; Brown 1987; Clutton-Brock et al. 2003; Arnold et al. 2005; Bolopo et al. 2015). This means that commonly used measures of helping effort may not accurately reflect total helping effort by individuals, or costs of helping. This is particularly troubling if helpers may specialise in different forms of helping. For example, in noisy miners,

Manorina melanocephala, helpers specialise in offspring feeding and nest defence; some individuals help with nestling feeding only while others help with nest defence only, and individuals that contribute more to offspring feeding invest less in nest defence and vice versa (Arnold et al. 2005). Whether other facultatively cooperative birds may also specialise is unclear however. It is important to determine whether task specialisation may be a common feature of cooperative birds as this can have important implications for the accuracy of conventional measures of helping effort in birds, and thus for the conclusions drawn so far on the evolution of helping behaviour based on studies on offspring feeding by helpers.

Predation is a major cause of nest failure as well as adult mortality in birds (Ricklefs 1969; Lima and Dill 1990), therefore a particularly important way in which helpers can help is by defending offspring, and group members, from predators. They can do this by engaging in vigilance behaviour and actively defending against predators by mobbing the predator, attacking or distracting it, and/or by giving off frequent alarm calls (Montgomerie and Weatherhead 1988; McGowan and Woolfenden 1989; Arnold 2000; Caro 2005; Graw and Manser 2007). Indeed, cooperative predator defence increases nest success (Taborsky 1984; Boland 1998; Riehl 2011), as well as adult group member survival (McGowan and Woolfenden 1989; Garay 2009; Shen et al. 2017), and has been proposed as a key driver of group living (Caro 2005; Jungwirth et al. 2015; Groenewoud et al. 2016). Similarly, brood parasitism is common in cooperatively breeding birds (Langmore et al. 2011; Feeney et al. 2013), and cooperative defence against brood parasites also increases reproductive success and reduces the costs associated with providing parental care to heterospecific young (Rothstein 1990; Canestrari et al. 2009; Feeney et al. 2013). Thus, defence against predators and brood parasites is associated with high fitness benefits but also high costs, including risk of injury or mortality as well as time and energy expenditure (Montgomerie and Weatherhead 1988; Dugatkin and Godin 1992), and therefore may represent an equally or more costly and effective form of helping than offspring feeding. Since predator defence enhances survival of offspring as well as adult group members, studying predator defence in cooperative breeders should provide a unique opportunity to test how benefits of group living in general, and benefits of helping with reproduction, each drive cooperation. However, unlike offspring provisioning, we know very little about what benefits drive predator defence by helpers.

Thesis aims

In this thesis, I use the purple-crowned fairy-wren, *Malurus coronatus*, as a model species to investigate how benefits of group living, and benefits of helping raise offspring, drive cooperative

behaviour in a system with considerable variation in social group composition and high rates of nest predation. More specifically, I aim to determine (i) if and how the composition of social groups affects benefits of group living; (ii) whether helpers contribute to predator defence, if this affects their contribution to offspring feeding, and what consequences this has for the accuracy of measures of helping effort based on offspring feeding alone; and (iii) which benefits of group living and helping raise offspring drive investment in two important forms of helping - offspring provisioning and predator defence.

Study species: the purple-crowned fairy-wren

Purple-crowned fairy-wrens are small insectivorous passerines endemic to the monsoonal tropical region of northern Australia (Rowley and Russell 1993). I study the western subspecies, *M. coronatus coronatus*, which is listed as endangered under the Environment Protection and Biodiversity Conservation Act of Australia (2015). It is a riparian habitat specialist, restricted to patches of *Pandanus aquaticus* vegetation growing along edges of rivers and creeks (Rowley and Russell 1993). Territories are arranged in a linear fashion along watercourses, and territory boundaries and groups are stable year-round. Groups consist of a monogamous dominant breeding pair and often one or more subordinates (range 0 – 9) (Kingma et al. 2009; Kingma et al. 2010). The dominant breeding pair engages in duetting behaviour, providing a reliable cue for assigning dominance status to individuals (Hall and Peters 2008). Breeding can take place all year round but peaks during the monsoonal wet season (December – March) (Rowley and Russell 1993; Kingma et al. 2010) and is triggered by rainfall (Hidalgo Aranzamendi et al. 2019). Females usually lay 2 to 4 eggs, these are incubated for approximately 14 days and nestlings fledge another 13 days later (Kingma et al. 2010; personal observations). Occasionally, nests get parasitised by cuckoos (Langmore et al. 2011; personal observations). Purple-crowned fairy-wrens are facultative cooperative breeders; only the dominant female builds nests and incubates eggs, but all group members can participate in offspring feeding and predator defence (Kingma et al. 2011; personal observations).

The population of purple-crowned fairy-wrens that I study is located at Australian Wildlife Conservancy's Mornington Wildlife Sanctuary in northwest Australia (S17°31' E126°6'). This population comprises around 200-300 birds, all uniquely colour-banded, and is saturated; breeding vacancies only appear rarely (Fig. 1a). This population has been closely followed since 2005, therefore we have detailed information on each bird, including e.g. where it hatched, who its relatives are, where it dispersed to, and who it shares a group with. Research conducted on

this population over the years has established that while dispersal is female-biased, helpers of both sexes can stay as a subordinate and help feed offspring, and vary considerably in helping effort (Kingma et al. 2011). Additionally, group composition varies due to breeder turnover and subordinate dispersal, and many groups consist of a mix of relatives, potential mates (i.e. opposite-sex group members that are unrelated only, as the cost of inbreeding is high; Kingma et al. 2013), and reproductive competitors (i.e. unrelated same-sex group members) (Kingma et al. 2010; Kingma et al. 2011). Furthermore, nest success is low (only 22% of nests with eggs result in the production of at least one fledgling), and nest predation is the main source of nest failure (57% of nests) (Hidalgo Aranzamendi 2017). Therefore, cooperative offspring care and defence may have a considerable impact on reproductive success. This means that this population provides a good system to investigate how benefits of group living and of helping raise offspring may promote cooperative predator defence and offspring feeding, and particularly, how this relates to group composition (Fig. 1).

We know that various benefits of group living and of helping raise offspring apply in our study system. Firstly, subordinates are able to obtain a mate within their social group via two routes: they can sometimes pair up with an unrelated opposite-sex subordinate group member and split off from the resident territory to form their own breeding territory (Hidalgo Aranzamendi et al. 2016), or they may inherit a breeding position on the territory when the same-sex breeder dies or disperses (Kingma et al. 2011; Hidalgo Aranzamendi et al. 2016). Subordinates form stable queues for inheriting a breeding position this way: they never inherit a breeding position when an older same-sex subordinate is present, and the chances to become a breeder are greater for male compared to female helpers, and for helpers that are unrelated compared to related to the opposite-sex breeder (Kingma et al. 2011). Subordinates can also gain indirect kin-selected benefits from helping to raise related offspring (Kingma et al. 2011). Lastly, subordinates only rarely gain access to reproduction by gaining paternity or laying eggs, but are more likely to do so if they are unrelated to the opposite-sex breeder (Kingma et al. 2009; Kingma et al. 2011). This knowledge helps phrase hypotheses and design detailed tests of how cooperative behaviour relates to the full suite of benefits proposed to explain the evolution of cooperative breeding.

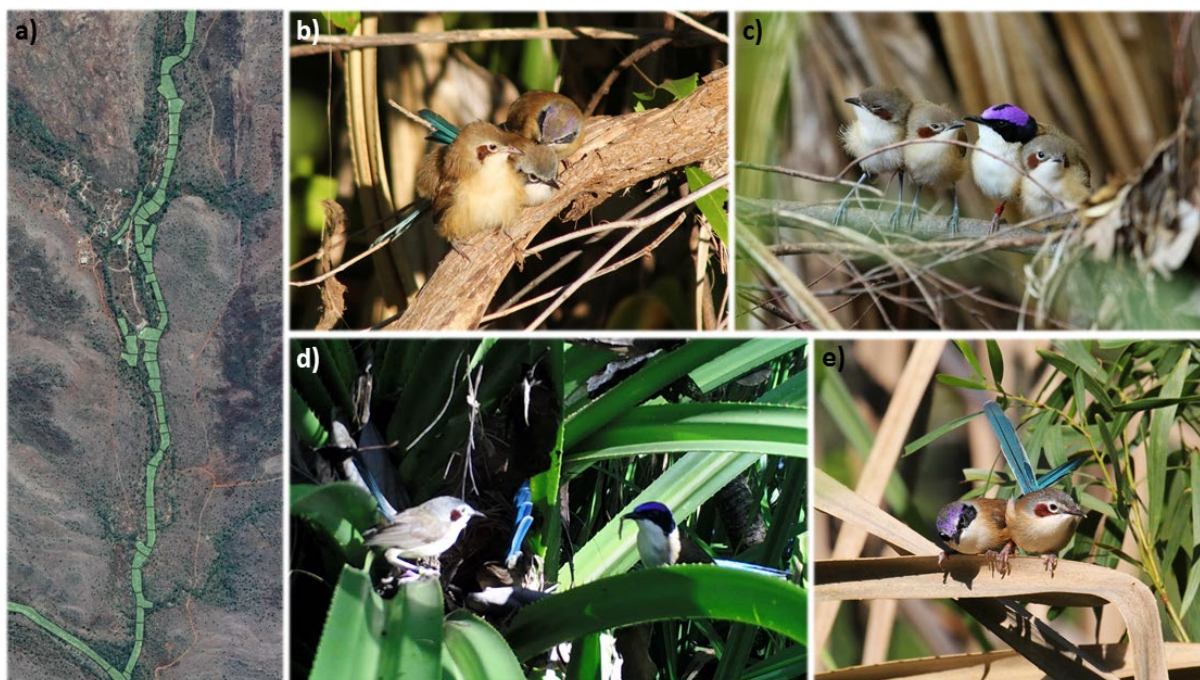


Figure 1. The purple-crowned fairy-wren provides a model system to study cooperative breeding and benefits of group living and helping at the nest. **a)** Arrangement of 50 fairy-wren groups that I followed closely for my study at AWC's Mornington Wildlife Sanctuary. Territories are aligned linearly along Annie creek, and all suitable habitat is occupied. **b)** Social group composition varies; depicted here are a juvenile (left), subordinate male (centre) and dominant male (right) engaging in allopreening. **c)** A dominant male resting in close proximity to his three offspring. Social interactions between group members such as the one depicted in (b) and (c) are common and establish social relationships within groups (Chapter 1). **d)** Group members cooperate in feeding offspring; here, a subordinate male is feeding at the nest while the dominant female and male are waiting their turn. **e)** Group members cooperate in defence against predators; nest predation is the main cause of nest failure. Image (a) was created using QGIS 3.4.8, image (d) was captured on a Bushnell Trophy Cam Aggressor Low Glow trail camera, and image (b), (c) and (e) were taken by Niki Teunissen/AWC.

Fieldwork

To address the aims of my thesis, I collected data over the course of three main field seasons: February – June 2016 (data collection for Chapters 1 and 5), December 2016 – May 2017 (data collection for Chapters 2 – 5), and December 2017 – May 2018 (data collection for Chapter 5). This encompasses three wet (breeding) seasons and (part of) one dry season. During each field season, I followed 50 fairy-wren groups along a single stretch of creek line closely (Fig. 1a), visiting each group at least once a week to record group size, social status of each group member, and to follow breeder females for any signs of breeding. Any nest found was checked regularly to determine laying date, clutch size, hatch date, and hatching and fledging success. Nestlings were

banded between 6 and 8 days of age. We determined relatedness for all birds used in this study by establishing for birds banded as nestlings or dependent fledglings who the social parents are, and by assigning paternity for birds banded as adults using 9 microsatellites (for details see Hidalgo Aranzamendi et al. 2016). Since extra-pair paternity is rare in this population (Kingma et al. 2009), social relatedness accurately reflects genetic relations. Wherever possible, two 1-hour nest watches were conducted on each nest to quantify offspring feeding rates by each group member. I also placed motion-triggered trail cameras (Bushnell Trophy Cam Aggressor Low Glow) at nests to additionally record feeding rates by individuals, and to record nest predation events. This allowed me to assess the type of predators that depredate fairy-wren nests and revealed that the most common nest predators are various species of varanid lizards (goannas; 61% of 31 predation events captured on camera), followed by avian predators (16%, including e.g. pheasant coucals, *Centropus phasianinus*, and blue-winged kookaburras, *Dacelo leachii*), snakes (10%), and centipedes (6%). It also revealed that social groups do indeed cooperate in predator defence and at times successfully deter predators from the nest. In addition, during the first field season (in 2016), I closely followed focal subordinates to collect data on the type and number of social interactions they engage in with group members. During the second field season (December 2016 – May 2017), I conducted experiments where I presented models of common predators and brood parasites at nests to record individual predator defence behaviour by individual fairy-wrens.

Thesis outline

My thesis investigates how benefits of group living and benefits of helping raise offspring drive helping behaviour in the purple-crowned fairy-wren, to gain a comprehensive understanding of how cooperative breeding is maintained in this species (Fig. 2). Importantly, I take into account the composition of social groups throughout, and how this affects benefits of group living and cooperation. I also study both tasks that helpers can assist with (offspring feeding and predator defence), to obtain an inclusive measure of helping effort and drivers of cooperation in this system.

In **Chapter 1**, I use intragroup patterns of social interactions to investigate whether group composition affects benefits of group living in this species. In group-living animals, social relationships are formed through repeated social interactions between individuals, where beneficial affiliative interactions strengthen social bonds and costly aggressive interactions weaken them (Silk et al. 2006; Kutsukake and Clutton-Brock 2008; Kutsukake 2009). Therefore, studying social interactions can provide insights into the cost and benefits of group living. I show

that the composition of social groups affects benefits of group living: subordinates form close social bonds with relatives and potential mates, but form antagonistic relationships with reproductive competitors.

As this first chapter provided insight into why helpers might stay in a group, next, I focus on how and why they might cooperate. In **Chapter 2**, I investigate whether and how helpers contribute to predator and brood defence, and how this compares to defence behaviour by breeders. Since predator defence is inherently risky (Montgomerie and Weatherhead 1988), I test whether investment in defence reflects the risk of injury associated with the predator, and reproductive payoffs associated with survival of the brood. I also explicitly test whether benefits associated with survival of social group members may additionally determine investment in

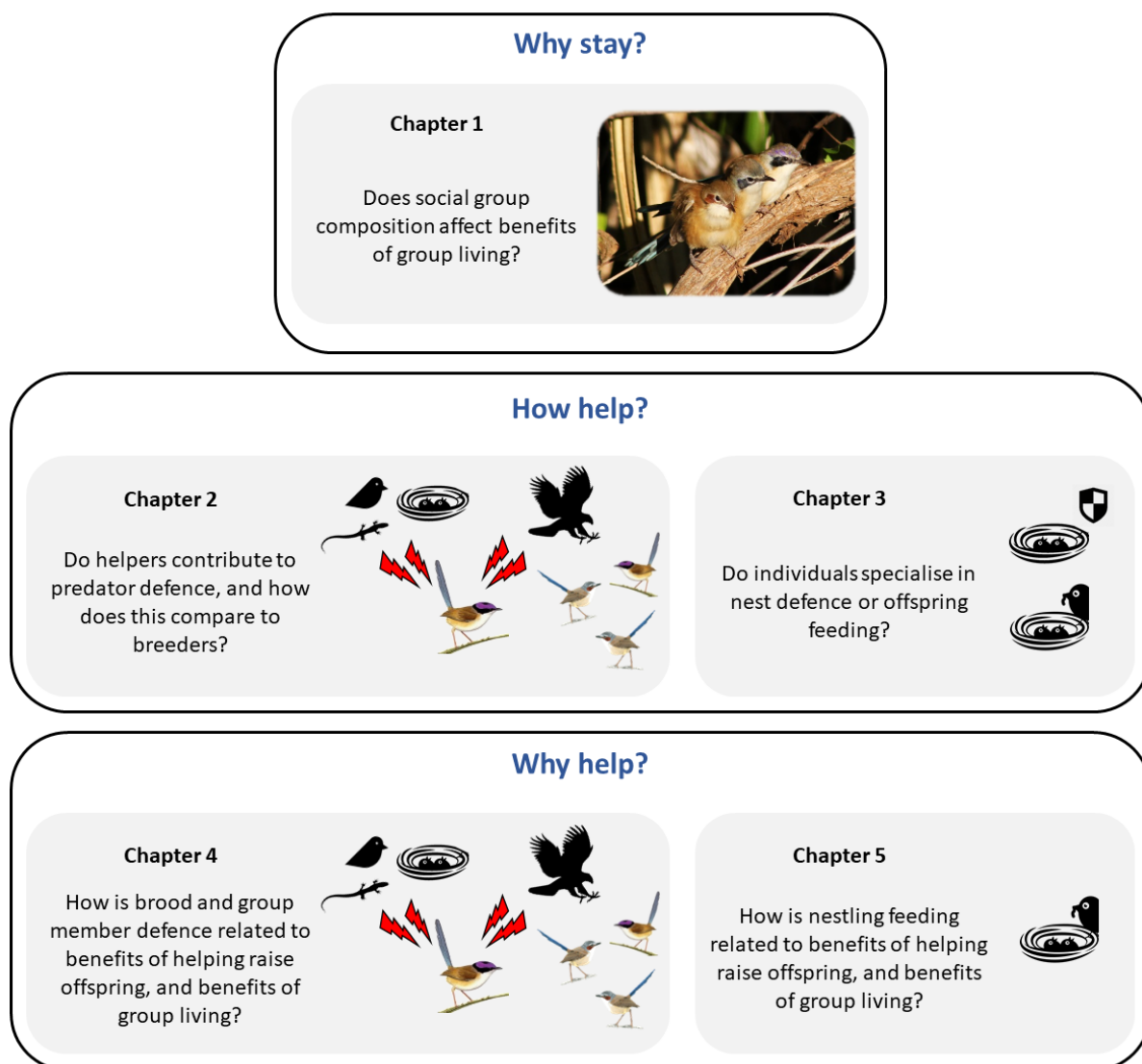


Figure 2. Thesis outline.

defence against predators that pose a threat to adult birds. This study shows that helper investment in nest defence can be substantial. Therefore, in **Chapter 3**, I investigate whether individuals specialise in cooperative tasks. This is an important question to address, since if task specialisation is common, this would have important consequences for the validity of conclusions drawn so far on the evolution of cooperative breeding based on measures on a single form of helping alone. I show that individuals do not specialise in the two forms of cooperative care observed in the purple-crowned fairy-wren - offspring feeding and nest defence - but helpers do vary considerably and consistently in their overall contribution to alloparental care.

Therefore, in Chapters 4 and 5 I study what benefits of group living and benefits of helping at the nest explain investment in predator defence and offspring feeding by helpers. First, in **Chapter 4**, I test what benefits drive predator defence by helpers. Since predator defence can enhance nest success as well as group member survival, studying defence behaviour allows to tease apart benefits of group living and benefits of helping raise offspring; nest defence should reflect benefits of helping raise offspring, while adult group member defence reflects benefits of group living. In **Chapter 5**, I use a similar approach to test what benefits drive help with offspring provisioning. Specifically, I test whether offspring feeding effort is explained by adaptive benefits commonly proposed for the evolution of helping behaviour, or by the type of breeders they are assisting and thereby enhancing the survival of (through load-lightening; Kingma et al. 2010). My findings from Chapters 4 and 5 highlight how benefits of group living and of helping raise offspring are influenced by the social environment and together drive cooperation.

Thesis organisation

This thesis is presented as a ‘thesis including published works’, consisting of a general introduction, five data chapters, and a general discussion. Chapter 1 is published in the journal *Behavioral Ecology*. Chapter 2 has also been submitted to the journal *Behavioral Ecology*, and is currently under review (Manuscript number BEHECO-2019-0419). Chapters 3-5 are to be revised prior to submission to peer-reviewed journals. I was responsible for project design, fieldwork, data analyses, and writing of each chapter, however, to reflect the collaborative nature of the research, the first-person plural is used throughout each data chapter.

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Chapter 1

More than kin: subordinates foster strong bonds with relatives and potential mates in a social bird

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Original Article

More than kin: subordinates foster strong bonds with relatives and potential mates in a social bird

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Social interactions shape relationships between individuals in complex societies. Affiliative interactions are associated with benefits and strengthen social bonds, while aggressive interactions are costly and negatively affect social bonds. Individuals may attempt to reduce aggressive encounters through submissive displays directed at higher-ranking individuals. Thus, fine-scale patterns of affiliative, aggressive, and submissive interactions may reflect costly and beneficial social relationships within groups, providing insight into the benefits of group living and the mechanisms of conflict resolution. So far, however, most studies have looked at social interactions and benefits of group living in isolation. We investigated how the strength of social bonds (affiliative vs. aggressive interactions) and submissive displays varied with kin-selected and potential mating benefits, and with reproductive conflict in the cooperatively breeding purple-crowned fairy-wren, *Malurus coronatus*. Our results revealed that subordinates formed equally strong social bonds with kin and potential mates (unrelated opposite-sex individuals) while they formed antagonistic relationships with reproductive competitors that offered no kin-selected or mating benefits (unrelated same-sex individuals). Submissive displays were directed exclusively at same-sex breeders, regardless of relatedness. Affiliation and submission were associated with reduced foraging time when food was limited, indicating a cost to maintaining positive relationships. Together, our results suggest that the strength of social bonds is determined by (potential) benefits obtained from group members, while submission likely serves to reduce conflict. Our findings highlight the importance of time-costly social interactions for maintaining relationships with group members, providing insight into how social groups of individuals with (partly) divergent interests can remain stable.

Key words: affiliation, cooperative breeding, group living, social interactions, submission, aggression.

INTRODUCTION

In complex societies, like those of humans and other group-living animals, individuals establish and maintain relationships, such as pair-bonds, alliances, and dominance hierarchies, by repeated social interactions (Hinde 1976; Silk et al. 2006; Kutsukake 2009; Gill 2012). Such social interactions, and the resulting social relationships, can be beneficial or costly in nature. Affiliative interactions (e.g., allogrooming, resting in contact) can provide benefits to the individuals involved, such as hygienic or thermoregulatory benefits (Hart et al. 1992; Sanchez-Villagra et al. 1998; McKechnie

and Lovegrove 2001; Radford and Du Plessis 2006; Villa et al. 2016) and reduction of stress, for both the recipient and the actor (Aureli et al. 1999; Detillion et al. 2004; Sapolsky 2005; Lewis et al. 2007; Shutt et al. 2007; Radford 2008; Sapolsky 2011; Ueno et al. 2015). Aggressive interactions on the other hand are associated with immediate costs to both parties involved, through time and energy expenditure, risk of injury, and elevated stress (Rovero et al. 2000; Petit 2010). Together, these interactions largely determine the nature of social relationships between group members. Affiliative interactions can strengthen social bonds between particular group members (Silk et al. 2006; Silk et al. 2009; Massen et al. 2010; Petit 2010; Gill 2012; Kenny et al. 2017). Conversely, although aggression can be a tool for negotiation, and save time and energy in the long-term by establishing and maintaining dominance hierarchies

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(De Waal 2000), aggressive interactions can have a long-lasting negative effect on social relationships, especially in societies where reconciliation after aggression is uncommon (De Waal 2000; Kutsukake and Clutton-Brock 2008). In addition, individuals may strategically use a third type of interaction, namely submissive displays, in an attempt to minimize negative relationships with group members; submission conveys the nonagonistic intentions of one individual to another, and may reduce the probability—and cost—of subsequently receiving aggression from others (Deag and Scott 1999; Flack and De Waal 2007; Petit 2010). Thereby, submissive displays may contribute to the stability of groups containing competitors for vital resources like food and reproduction.

Strong social bonds themselves are ultimately often associated with fitness benefits such as improved offspring survival and earlier onset of reproduction (Silk et al. 2009; Massen et al. 2010; Charpentier et al. 2012; Seyfarth and Cheney 2012). Therefore, fine-scale patterns of social interactions may reveal costs or benefits of social relationships. Both costly and beneficial social interactions are not random but instead take place mostly between particular individuals, to protect resources or to secure benefits. For example, generally, affiliative interactions are more common and aggressive interactions less common between related compared to unrelated individuals (e.g., Sanchez-Villagra et al. 1998; Kutsukake and Clutton-Brock 2006b; Silk et al. 2006; Dickinson et al. 2009; Chiarati et al. 2011; Charpentier et al. 2012; Napper et al. 2013; Viblanc et al. 2016), and between individuals of the opposite sex compared to individuals of the same sex (Seibert and Crowell-Davis 2001; Dickinson et al. 2009; Kutsukake 2009; Mitchell et al. 2009; Dey and Quinn 2014, but see Chiarati et al. 2011).

In complex avian societies in particular (e.g., cooperative breeders), groups may consist of a mix of kin and nonkin of either sex (regularly so in 30% of species; Riehl 2013). In such species, potential benefits of group living include kin-selected benefits (e.g., indirect fitness benefits from helping relatives; Hamilton 1964; Koenig & Dickinson 2016), benefits from parental nepotism (e.g., access to food, increased survival; Ekman et al. 2000; Kraaijeveld and Dickinson 2001; Kokko and Ekman 2002; Ekman et al. 2004; Griesser et al. 2006; Kingma et al. 2016), and reproductive benefits such as inheriting a breeding position or finding a mate within the group to establish a new breeding territory (Koenig et al. 1992;

Emlen 1994; Ekman et al. 2004; Kingma 2017). Since strong social bonds can improve the fitness of individuals involved (Silk et al. 2009; Charpentier et al. 2012), bonds with kin and potential mates are expected to provide the greatest potential benefits, whereas antagonistic and submissive behaviors are predicted to mainly occur between nonkin that may additionally be competitors for reproduction.

Here, we test whether social interactions match these predictions in the purple-crowned fairy-wren, *Malurus coronatus* (Table 1). In this cooperatively breeding passerine, many groups are a mix of related and unrelated group members of both sexes (Kingma et al. 2010; Kingma et al. 2011). Subordinates may benefit from being in a group with relatives if they increase their inclusive fitness by helping to raise related offspring and improving survival of parents, or through parental nepotism (Kingma et al. 2010; Kingma et al. 2011). In addition, unrelated opposite-sex group members represent potential current or future mates, whereas competition between same-sex individuals over breeding opportunities is high. Consequently, we predict that social bonds are strongest between related opposite-sex individuals (kin-selected benefits and benefits of parental nepotism) and unrelated opposite-sex group members (mating benefits), followed by related same-sex group members (that provide benefits of kin selection and parental nepotism but may also be in competition over reproduction), and weakest among unrelated same-sex individuals (reproductive competition; Table 1). We further predict that submissive displays will be targeted most at same-sex breeders (i.e., dominants; dominance is established by the fact that only dominant breeding pairs sing duets: Hall and Peters 2008, 2009). Since aggression is expected to be more likely between unrelated compared to related same-sex group members (no kin-selected benefits or nepotism to offset reproductive conflict), we expect rates of submission to be higher between these to minimize aggression received. We tested these predictions using behavioral observations of social interactions (affiliation, aggression, submission) of subordinate individuals with their group members. Time budgets were also quantified to investigate the impact of social interaction on time available for foraging. Together, these results enhance our understanding of the role social interactions may play in establishing relationships between particular group members and potential costs and benefits associated with this, which may

Table 1

Predictions of social bond strength (relative occurrence of affiliation and aggression) and submission between group members, and whether these were supported in the purple-crowned fairy-wren (pcfw)

Type of group member	Kin-selection/ nepotism benefit? ^a	Mating benefit? ^b	Reproductive conflict?	Predictions	Sample size (# dyads)	Predictions supported in pcfw?
Related, same-sex	Yes	No	Yes	1. Medium strength social bond 2. Intermediate level of submission directed at higher-ranked individuals	52 26	Partially ^c Yes ^d
Related, opposite-sex	Yes	No	No	1. Strong social bond 2. No or low level of submission directed at higher-ranked individuals	37 15	Yes Yes
Unrelated, same-sex	No	No	Yes	1. Weak/absent social bond 2. Highest level of submission directed at higher-ranked individuals	20 12	Yes Yes ^d
Unrelated, opposite-sex	No	Yes	No	1. Strong social bond 2. No or low level of submission directed at higher-ranked individuals	35 23	Yes Yes

Predictions are based on the balance of benefits and costs of group living according to relatedness and sex of individuals they may interact with, assuming that reproductive conflict may (partially) negate benefits.

^aKingma et al. 2010; Kingma et al. 2011; ^bKingma et al. 2011; Kingma et al. 2013; ^csimilar affiliation index as for related and unrelated opposite-sex group members; ^dbut note that the predicted difference in submission towards related and unrelated same-sex dominants is not significant in the analyses (see Figure 3).

ultimately provide insight into how social groups and relationships remain stable.

METHODS

Study site and species

Purple-crowned fairy-wrens are small insectivorous birds endemic to northern Australia, where they inhabit riparian vegetation (Rowley and Russell 1993; Skroblin and Legge 2010). Territories are aligned linearly along the rivers, and boundaries and groups are stable year-round and across years (Hall and Peters 2008; Kingma et al. 2011). Breeding can take place year-round but peaks in the monsoonal wet season (December to March) (Hall and Peters 2009; Kingma et al. 2012). A strength of our study system is that only the dominant breeding pair engages in duets (Hall and Peters 2008, 2009), providing a reliable cue to assign breeder (dominant) or subordinate status to each individual independent of submissive or aggressive interactions. Subordinate individuals of both sexes can help the breeding pair raise offspring (Kingma et al. 2010; Kingma et al. 2011). Due to breeder turnover and immigration into the group, many groups are composed of related and unrelated group members of both sexes (57% of groups with subordinates for the current study period; Kingma et al. 2010; Kingma et al. 2011). Subordinate individuals benefit from sharing a group with unrelated opposite-sex individuals (potential mates); although subordinate individuals never reproduce independently and rarely gain parentage (Kingma et al. 2009), they sometimes sire offspring if they are unrelated (7% of broods), but never if they are related, to the opposite-sex breeder (Kingma et al. 2011). Increased sperm production by such subordinates compared to related subordinates suggests that they are potential competitors for the breeding male (Kingma et al. 2012). In addition, subordinates that are unrelated to the opposite-sex breeder have higher chances of inheriting the breeding position when the same sex breeder dies or disappears (Kingma et al. 2011), and approximately 16% of subordinates gain their first breeding position this way (Hidalgo Aranzamendi et al. 2016). Subordinates may also pair with an unrelated subordinate group member of the opposite sex and bud off a new territory from their original territory (approximately 6% of subordinates gain their first breeding position this way; Hidalgo Aranzamendi et al. 2016). The benefits of acquiring a territory and an unrelated mate are substantial, as breeding vacancies are rare and the cost of inbreeding is high (Kingma et al. 2013).

We studied a color-banded population of approximately 250 purple-crowned fairy-wrens along Annie Creek and the Adcock River at Australian Wildlife Conservancy's Mornington Wildlife Sanctuary in northwest Australia (S17°31' E126°6') that has been monitored since 2005. Groups were observed year-round and all offspring banded as nestlings until 2010. From 2011, the population was monitored in 2 visits per year (May/June and November) and all unbanded birds (new offspring and immigrants from outside the core population) were banded (for details see Hidalgo Aranzamendi et al. 2016). From 2016, the population was additionally monitored during the main breeding season (February to April), using the same methods as in 2005–2010.

Behavioral observations

To study the frequency of social interactions between group members, we conducted behavioral observations where one observer (NT) followed individually color-banded subordinates ("focal

individual," $n = 25$ males and 15 females, average age 1.9 years, range: 0.6–5.0) from 23 groups (all consisting of a breeding pair with 1–4 subordinates, and 0–4 fledglings). Each focal individual was followed and observed until it had been in sight for approximately one hour (mean $\pm$ SE = 60 ± 1 min; total time followed including time out of sight = 115 ± 5 min). One or 2 focal observations were conducted per individual (mean = 1.2); if birds were observed twice, observations took place in different seasons. Focal observations took place during the dry season (May to June 2014 and 2016; $n = 23$ and $n = 12$ focal observations, respectively), when almost all birds had completed breeding, and during the wet season of 2016, when most groups were breeding (February to April; $n = 14$ focal observations); focal observations were not conducted if a nest with nestlings was present. All observations took place during the morning (5:50–11:00 am), when bird activity is highest, during calm, dry weather. The observer was blind to the relatedness of individuals in the group, but not to the sex of adults (which are sexually dichromatic). The individual to be observed was determined before the start of observations, to avoid any bias towards individuals that may be easier to follow. At the time we conducted behavioral observations, many groups had unbanded dependent fledglings (i.e., <3 months old) that were subsequently captured for banding and blood sampling. Genetic relationships of all individuals were confirmed by genotyping (for details see Hidalgo Aranzamendi et al. 2016) and were identical to social relatedness; individuals classified as "related" in our study were first-degree relatives (full sibling, parent-offspring; the 55 dyads considered unrelated included 5 half-siblings from different broods).

Focal animal continuous sampling (social interactions)

All interactions that took place between the focal individual and each of the other group members (i.e., a dyad, $n = 144$ dyads excluding fledgling group members) were recorded onto a voice activated sound recorder. This included aggressive interactions (chasing, physical attacks), submissive displays (characterized by bill-gaping and shivering of the wings), and affiliative interactions, which included allopreening and contact-sit (i.e., sitting closely side by side, (almost) touching, often while self-preening; see Figure 1) (Boucherie et al. 2016). Allopreening by definition involves contact-sit. Both these behaviors are considered good indicators of the strength of social bonds in other species (Cords 1997; Silk et al. 2006; Silk et al. 2009; Massen et al. 2010; Boucherie et al. 2016; Kenny et al. 2017). Affiliative and aggressive interactions were recorded during all observations, while submissive displays were recorded in 2016 only. The proportion of time birds were seen (i.e. total time seen/total time followed including out of sight) did not affect whether affiliative interactions (generalized linear mixed model with Territory ID and Bird ID as random effects; $z = 0.79$, $P = 0.43$), aggressive interactions ($z = 0.05$, $P = 0.96$), or submissive displays ($z = 0.29$, $P = 0.77$) were observed during an observation.

Focal animal instantaneous sampling (time budget)

Throughout each observation, the time budget of the focal subordinate was obtained by recording its behavior every 30 s while it was in sight. Behaviors included: foraging, resting, self-preening, flying, singing, and interacting with group members (aggressive, affiliative, submissive behavior).

Statistical analyses

All analyses were performed in R, version 3.3.2 (R Core Team 2017). For each dyad (i.e., each combination of focal subordinate

**Figure 1**

Affiliative interactions involve (a) contact-sit, where 2 birds sit in close proximity, and (b) allopreening. Photos by Niki Teunissen/Australian Wildlife Conservancy.

and each of its group members), we determined relatedness (unrelated/first-order relative) and sex (same/opposite) of the 2 individuals, and whether any affiliative interactions, aggressive interactions, and submissive displays were observed between them. Presence/absence of social interactions was used rather than frequencies since all types of social interactions were relatively uncommon (affiliative interactions: mean = 0.61 ± 0.12 per dyad per focal observation; aggressive interactions: mean = 0.05 ± 0.03 per dyad per focal observation; submissive displays: mean = 0.39 ± 0.16 per dyad per focal observation). For each focal subordinate the data consisted of $x-1$ dyads for groups of size x ; in all analyses we corrected for this replication across focal individuals by including its identity (Bird ID) nested within Territory ID as a random term in the models. As some groups contained unbanded (and thus not individually recognizable) fledglings at the time of observation, all potential interactions with fledgling group members ($n = 21$ individuals) were not included in analyses. We assessed seasonal differences by including one factor (“season”) with 3 levels (dry 2014, dry 2016, wet 2016).

We used a composite index of affiliation (relative affiliative vs. aggressive behavior) as an indicator of social bond strength. The relative frequency of affiliative versus aggressive interactions between 2 individuals is generally assumed to be a good measure of the strength of their social bond, since repeated affiliative interactions result in strong social bonds, whereas aggressive interactions negatively affect social bonds (Silk et al. 2006; Lewis et al. 2007; Kutsukake and Clutton-Brock 2008; Silk et al. 2009; Massen et al. 2010). Moreover, due to the rarity of these interactions in our data, combining interactions rather than analyzing them in isolation allowed for more robust statistical testing (see also Silk et al. 2006, 2009 for similar rationale). We computed the affiliation index by combining the occurrence of affiliative and aggressive interactions within dyads, giving each type of interaction equal weighting but opposite effects; a positive value of 1 was assigned if one or more affiliative interactions (allopreening or contact-sit) were observed between 2 individuals, and a negative value of 1 if one or more aggressive interactions were observed, giving a value of -1 , 0 , or 1 for each dyad. To test the hypothesis that the strength of social bonds is predicted by the relative balance of nepotism, kin-selected and mating benefits, and reproductive conflict, we included relative sex of both interacting individuals (same, opposite), relatedness of both interacting individuals (unrelated, related), and their interaction. We used a cumulative link mixed model (CLMM) with the ranked ($1 > 0 > -1$) composite index of affiliation per dyad as the dependent

variable using the package “ordinal” (Christensen 2015). The explanatory variables further included sex of the focal subordinate (male, female), status of the interacting individual (subordinate, breeder), group size (since there may be a saturation effect in larger groups) and season (dry 2014, dry 2016, wet 2016). Qualitatively similar results were obtained from separate statistical models for the occurrence of allopreening, contact-sit, and aggressive interactions, respectively (see Supplementary Material I). Although the interacting effect of relative sex and relatedness on affiliation index may differ for males and females, or vary with subordinates’ relatedness to the opposite-sex breeder (since subordinate sex and relatedness to breeders may for example influence the chance of inheriting a breeding position, or the extent of competition; Kingma et al. 2011, 2012), our sample size did not allow for the inclusion of such 3-way interactions in our analyses.

The occurrence of submissive displays during observations was only recorded for the wet and dry season of 2016 ($n = 76$ dyads) and analyzed separately. Since no submissive displays were directed at subordinate or opposite-sex group members (i.e., complete separation of the data; Figure 3), violating the assumption of homogeneity of variance, we fitted a generalized linear mixed model (GLMM) using Markov chain Monte Carlo (MCMC) techniques from the MCMCglmm package (Hadfield 2010, 2014), to analyze the effect of season (dry and wet 2016 only), status, sex and relatedness on the occurrence of submissive displays. This model included the presence/absence of submissive displays as a categorical response variable with the logit link. Relative sex (same/opposite) and relatedness (related/unrelated), as well as the interaction between them, and the main effects of sex of the focal bird, status of the second bird and season were included as fixed variables. Following the recommendations of Hadfield (2014), the residual variance was fixed at 1, and a Gelman prior with a scale of $\sigma^2 + \pi^2/3$ was used for the fixed effects, to deal with the issue of complete separation (Gelman et al. 2008). For the random factors, a parameter-expanded prior with a Cauchy distribution ($V = 1$, $\nu = 1$, $\alpha.\mu = 0$, and $\alpha.V = 25^2$) was used, as this prior puts less density on values close to zero. The chains were run for 11,000,000 iterations, with a thinning interval of 10,000, and a burn-in interval of 1,000,000, resulting in a sample size of 1000. Visual inspection of the time series and posterior density plots of the parameters confirmed convergence of the model, and the autocorrelation plots of the fixed and random effects showed no sign of autocorrelation. We present posterior mean, 95% credible interval (CI) and P -values. A similar model was used to test for a correlation between submissive displays and

aggressive interactions within dyads, with the presence/absence of submissive displays as response variable and the presence/absence of aggressive interactions as fixed variable. All other model parameters were the same.

We investigated whether there was a trade-off between birds' time allocation to foraging and interacting with group members by testing if engaging in affiliative or aggressive interactions or submissive displays was related to foraging time. In addition, we tested whether this trade-off might vary between the seasons because food availability is much higher during the wet season (Hidalgo Aranzamendi 2017) and this may reduce time budget constraints. We ran generalized linear mixed models with the proportion of time spent foraging as binomial response variable (using the c-bind function). The presence/absence of affiliative interactions, the start time of the observation (to control for diurnal variation in time allocation), season (dry 2014, dry 2016, wet 2016), and the 2-way interaction between season and the other 2 variables, were included as fixed effects. We constructed identical models with the presence/absence of aggressive interactions and the presence/absence of submissive displays as independent variables to test for their effect on time allocated to foraging.

RESULTS

The affiliation index did not differ depending on whether focal subordinates were interacting with subordinate or breeder (dominant) group members (no effect of status; CLMM: $\beta \pm \text{SE} = 0.20 \pm 0.48$, $z = 0.42$, $P = 0.68$), nor did it differ with group size ($\beta \pm \text{SE} = -0.19 \pm 0.28$, $z = -0.67$, $P = 0.50$), or between male and female subordinates ($\beta \pm \text{SE} = 0.54 \pm 0.51$, $z = 1.04$, $P = 0.30$). The effect of relative sex (same/opposite) on the affiliation index depended on relatedness (unrelated/related) of the dyad (interaction term: $\beta \pm \text{SE} = 3.11 \pm 1.33$, $z = 2.35$, $P = 0.02$; Figure 2). Subordinates interacting with related group members had a positive mean affiliation index regardless of the sex of the group member (mean affiliation index = 0.29 for same-sex dyads; 0.27 for opposite-sex dyads; Figure 2). In contrast, when subordinates were unrelated to a group member, interactions were more affiliative in opposite-sex dyads (mean affiliation index = 0.26), and more aggressive in dyads of the same sex (mean affiliation index = -0.10; Figure 2). The frequency of affiliative relative to aggressive interactions differed between seasons, with a higher mean affiliation index during the resource-rich wet season (2016) than the dry season of 2014 ($\beta \pm \text{SE} = 1.62 \pm 0.53$, $z = 3.07$, $P < 0.01$). Mean affiliation index did not differ significantly between the dry season of 2014 (mean $\pm \text{SE} = 0.68 \pm 0.46$) and 2016 (mean $\pm \text{SE} = 1.75 \pm 0.56$), nor between the dry season of 2016 and the wet season of 2016 (mean $\pm \text{SE} = 2.30 \pm 0.55$).

Subordinates always directed their submissive displays at breeders (never to other subordinates), with submissive displays recorded for 21% of subordinate-breeder dyads (MCMCglmm, effect of status: posterior mean = -5.02, 95% CI = -9.51 to -0.71, $P < 0.01$, $N = 76$ dyads). Furthermore, submissive displays were directed only at same-sex breeders (effect of relative sex: posterior mean = 6.80, 95% CI = 2.83–11.78, $P < 0.01$; Figure 3). Though submissive displays were recorded more often for unrelated (50% of 12 dyads) compared to related same-sex group members (19% of 25 dyads; Figure 3), we did not detect a significant interaction between relative sex and relatedness (posterior mean = -0.12, 95% CI = -5.74–5.09, $P = 0.96$) or a main effect of relatedness (posterior mean = -1.85, 95% CI = -7.07–2.93, $P = 0.49$). The

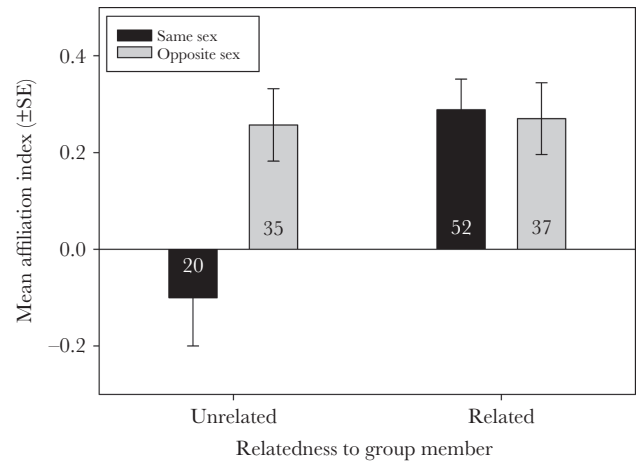


Figure 2

Variation in dyadic affiliation indices according to relative sex and relatedness of focal subordinates to other group members. Numbers represent total number of dyads in each category.

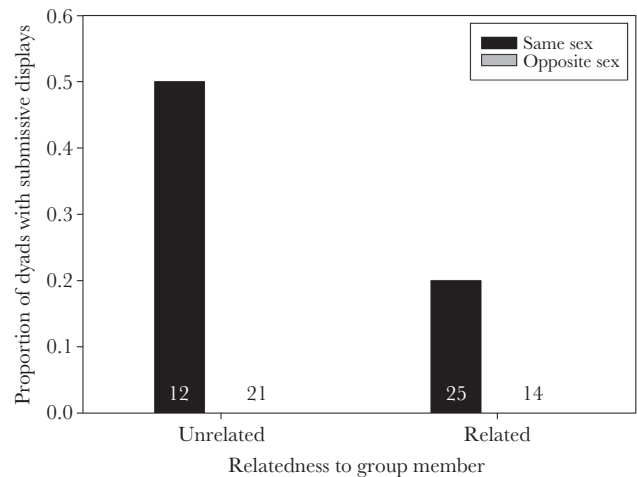


Figure 3

Proportion of dyads for which submissive displays by the focal subordinate were observed during a focal observation according to relative sex and relatedness to group members. Absent bars for opposite-sex dyads reflect the absence of any submissive displays for these. Numbers represent total number of dyads in each category.

occurrence of submissive displays did not differ between male and female subordinates (posterior mean = 1.77, 95% CI = -1.47–5.34, $P = 0.25$), nor with group size (posterior mean = -1.19, 95% CI = -3.30–1.06, $P = 0.28$), or between seasons (posterior mean = 0.63, 95% CI = -1.72–3.09, $P = 0.62$). The occurrence of submissive displays was positively correlated to aggression in dyads (posterior mean = 12.35, 95% CI = 2.43–24.57, $P < 0.01$); for every dyad in which aggression occurred, submissive behavior was also observed—focal subordinates were always submissive while the other member of the dyad (always a breeder) was aggressive—whereas for dyads where no aggression occurred, submissive behavior by the focal bird was observed in 11% of dyads. Most (82%) of submissive displays involved subordinates submitting spontaneously to an approaching breeder, with the remaining 18% (5/28) recorded in reaction to breeder aggression (i.e., <1 min post-aggression). Aggression followed spontaneous submission in only

9% (2/23) of cases, whereas 71% (5 of 7) of aggressive interactions were directed at nonsubmitting individuals. Within dyads, if subordinates submitted to the breeder immediately (<1 min) after aggression, aggression reoccurred in the same focal observation in only 1 of 4 cases, whereas aggression reoccurred in 2 of 3 cases when the subordinate did not submit to the dominant postaggression.

In the dry seasons birds spent a far greater proportion of time foraging overall ($89.8 \pm 1.1\%$) compared to the wet season ($71.3 \pm 2.7\%$) (Tukey's post hoc test: $\beta \pm \text{SE} = 1.36 \pm 0.14$, $z = -9.59$, $P < 0.01$ and $\beta \pm \text{SE} = 1.17 \pm 0.12$, $z = -9.91$, $P < 0.01$ for dry 2014 and dry 2016, respectively, foraging time between dry 2014 and dry 2016 did not differ: $\beta \pm \text{SE} = 0.19 \pm 0.16$, $z = -1.23$, $P = 0.43$). Engaging in affiliative interactions was associated with less time spent foraging, depending on the season (GLMM, interaction term: $\chi^2 = 12.34$, $\text{df} = 2$, $P < 0.01$): during both dry seasons, individuals spent less time foraging if they engaged in at least one affiliative interaction during an observation, while during the wet season, individuals spent the same (relatively lower) amount of time foraging regardless of whether they engaged in affiliative interactions (Figure 4a). A similar effect on foraging time was found for the interaction between season and submissive displays ($\chi^2 = 7.90$, $\text{df} = 1$, $P < 0.01$; Figure 4b), but the occurrence of aggressive interactions was unrelated to time spent foraging ($\chi^2 = 0.36$, $\text{df} = 1$, $P = 0.55$), regardless of season (interaction term: $\chi^2 = 3.08$, $\text{df} = 2$, $P = 0.21$). For full details on time budget analyses, see [Supplementary Material II](#).

DISCUSSION

We predicted that fine-scale patterns of affiliative and aggressive interactions between group members would reflect direct (current or future mating opportunities, benefits of parental nepotism) and indirect (kin-selected) benefits and reproductive conflict associated with being part of a social group (Table 1). In support of this prediction, we report a positive affiliation index between subordinate purple-crowned fairy-wrens and their related and opposite-sex unrelated group members, but a negative affiliation index between unrelated same-sex group members, indicating more aggression

and less affiliation between the latter. Furthermore, subordinates directed submissive displays at same-sex dominant breeders only, in line with predictions that this behavior serves to reduce reproductive conflict. Engaging in submissive or affiliative interactions was associated with decreases in time spent foraging during the dry season (when food availability is low) but not the wet season (when less time is spent foraging). Below, we discuss the implications of these findings.

Social bonds and benefits

Our findings support the prediction that subordinates form stronger social bonds with group members that may provide benefits, be they kin-selected or potential mating opportunities. Strong social bonds with kin can result in kin-selected fitness benefits through improved survival or reproduction of kin, as well as benefits from nepotism (Ekman et al. 1994, 2000; Kokko and Ekman 2002; Silk et al. 2009; Charpentier et al. 2012). Strong social bonds with potential mates can improve the likelihood of a future breeding partner being alive and/or in good condition once an opportunity arises to take up a breeding position or to establish together as a breeding pair (see e.g., Kingma et al. 2014), or can facilitate access to the breeding opportunity in case of competition with same-sex individuals from within and outside the group. Our results also affirm the importance of kin-based benefits in cooperative breeders—social bonds with relatives were equally strong between opposite and same-sex group members (Figure 2), suggesting that kin-affiliation and kin-selected benefits are not weakened by potential for reproductive conflict. Unrelated same-sex group members on the other hand offer no potential for kin-selected or mating benefits and, in line with our predictions (Table 1), we found a lack of social bonding between these; in fact, relationships were more aggressive (a mean affiliation index of <0; Figure 1), reflecting reproductive competition between these individuals.

Submissive displays: avoiding aggression from dominants?

In agreement with their different nature, submissive behaviors seem to be driven by different motivations than affiliative-aggressive

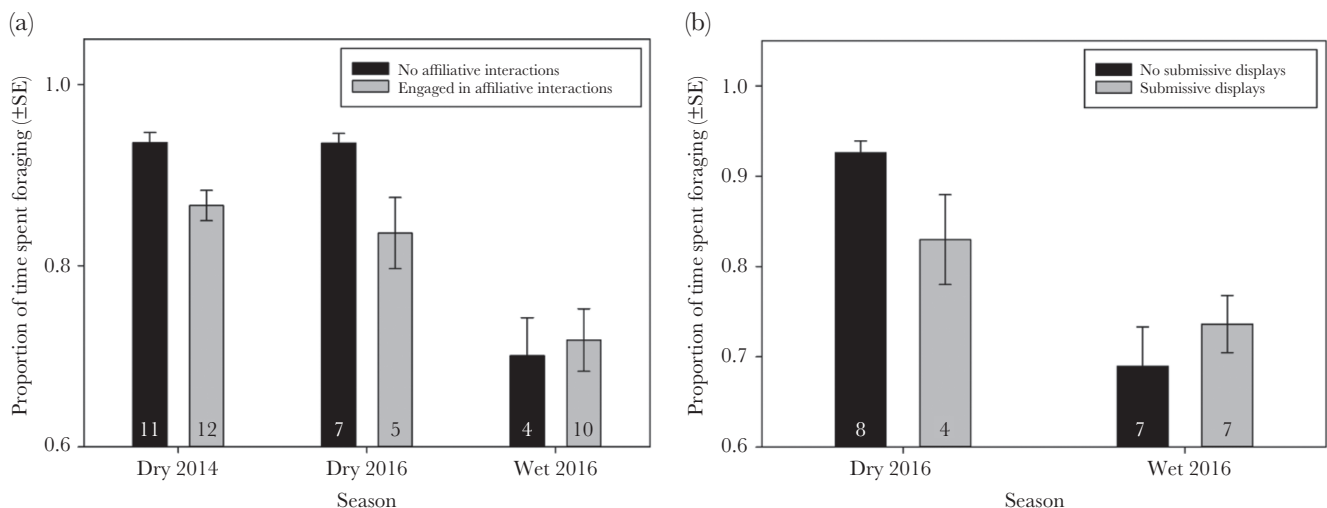


Figure 4

The mean proportion of time focal subordinates spent foraging in a given season (a) if they engaged in affiliative interactions during the focal observation compared to when they did not, and (b) if they performed submissive displays during the focal observation compared to when they did not. Note that submissive displays were not scored in 2014. Numbers represent total number of subordinates observed in each category.

behaviors. Submissive displays were only directed at same-sex breeders, suggesting a role in appeasement of higher-status individuals that are in reproductive conflict (Table 1). When breeders disappear, they are often replaced by a (related or unrelated) same-sex subordinate if there is one available (Kingma et al. 2011). This, in combination with immediate potential competition over fertilizations (males) and access to the nest (females), suggests a constant reproductive conflict between a breeder and same-sex subordinate. Subordinate group members on the other hand become competitors with each other only when a breeding vacancy appears, and competitive success in acquiring male vacancies is determined mainly by age or extent of breeding plumage (Kingma et al. 2011; Fan et al. 2018). This difference may explain why submissive behavior was never directed at other subordinates. In contrast to our predictions (Table 1), relatedness did not (significantly) affect submissive behavior, although the pattern suggests related subordinates might engage less in submissive behaviors (19% of related vs. 50% of unrelated dyads; Figure 3). Sample size may preclude us from concluding this firmly, however since subordinates can also replace their same-sex parent, submissive behavior to reduce reproductive conflict also applies for these same-sex relatives.

Submission may be a strategy to avoid or minimize aggression from dominant breeders in our study species. Breeders may use aggression strategically to suppress potential reproductive competitors (Kutsukake and Clutton-Brock 2006a; Nelson-Flower et al. 2013). Such targeted aggression can suppress reproductive hormone levels in the recipient, resulting in low or no parentage by subordinates (Young et al. 2006; Brouwer et al. 2009), or even eviction (Kutsukake and Clutton-Brock 2006a). Potential victims of aggression may adopt various strategies to avoid aggression from these group members. For example in meerkats, *Suricata suricatta*, female subordinates will unidirectionally groom the dominant female to avoid aggression (Kutsukake and Clutton-Brock 2008). Since allopreening was generally reciprocal in our study, *M. coronatus* does not seem to adopt a similar strategy. Instead, submissive displays likely function to reduce aggression. Submission was more likely in dyads where aggression was observed; all aggression recorded in our study, although it was quite rare, was directed from a same-sex breeder to a subordinate. Thus, submissive displays are directed only at group members that are reproductive competitors, and mainly at those most likely to be aggressive, as has been found in pukekos, *Porphyrio melanotus*; to our knowledge, the only other study on active submissive behavior in an avian cooperative breeder (Dey and Quinn 2014). The observations that subordinates generally submitted spontaneously to an approaching breeder, and that breeders rarely attacked a submitting subordinate, support that submission may serve to reduce the probability of aggression. This is in contrast to a previous study on meerkats, a species with high rates of aggression, where submission during agonistic situations was associated with an increased probability of reoccurrence of aggression (Kutsukake et al. 2008). This difference may reflect a different function of submission during an aggressive encounter: only submission in peaceful contexts, as seems to be the norm in our study, may function to decrease the probability of aggression and result in more positive relationships (Flack and De Waal 2007).

Although submissive displays come at a cost when resources are scarce (the dry season), high rates of aggression could incur much higher costs, including potential eviction (Kutsukake and Clutton-Brock 2006a; Kutsukake and Clutton-Brock 2008) which may reduce survival or condition (Ridley et al. 2008; Kingma et al. 2016, 2017). By signaling that they are not a threat, subordinates

may form relationships of a more positive nature by reducing aggression from breeders and continue to enjoy the benefits of living in a group. Ultimately, submissive displays may therefore allow social groups to remain stable despite potential conflict.

Implications: social group composition and benefits of group living

Our results indicate that social group composition should predict the frequency of affiliative and aggressive interactions of group members, and thereby generate individual-specific benefits of group living. Affiliative interactions and social bonds themselves are generally beneficial to the individuals involved (Detillion et al. 2004; Radford and Du Plessis 2006; Lewis et al. 2007; Silk et al. 2009; Sapolsky 2011; Charpentier et al. 2012; Fraser and Bugnyar 2012; Ueno et al. 2015; Villa et al. 2016), whereas aggressive interactions impose costs on individuals instead (Rovero et al. 2000; Lewis et al. 2007; Petit 2010). As a result, social group composition may determine the benefits that subordinates obtain from being part of a social group, and this may ultimately also influence subordinate dispersal. Subordinate purple-crowned fairy-wrens generally do not share groups with unrelated same-sex individuals as often as with unrelated opposite-sex or related individuals ($N = 20$ vs. $N = 35$ – 52 dyads for subordinates observed in current study, Table 1; $N = 39$ vs. $N = 75$ – 117 dyads for overall population in 2014–2016), which may be the result of subordinates dispersing more often when they share a group with unrelated same-sex individuals with whom they do not form positive social relationships (Figure 2). In turn, breeders could potentially also strategically use affiliative behavior to entice subordinates to remain in the group (Gill 2012). The higher affiliation index in the breeding season (wet season) compared to one of the 2 nonbreeding seasons suggests that this could be the case for purple-crowned fairy-wrens: affiliative behavior may function to encourage others to stay in the group and help with a breeding attempt. It should be noted though that while mean affiliation index was significantly higher in the wet season (2016) compared to the 2014 dry season, this difference was not significant for the 2016 dry season. Alternatively, birds may increase affiliative behavior during the wet season simply because they have more time available when food is more abundant and they spend less time foraging. It may be beneficial to use this time to invest more in social bonds: affiliating with group members is associated with a reduction in time spent foraging during the dry seasons. Future investigation into whether increases in affiliative behavior, especially during the breeding season, play a role in enticing group members to stay or increase their contributions to offspring care could further our understanding of how these behaviors evolved in the first place.

CONCLUSION

Our study highlights the important role that social interactions can play in complex social systems, and adds to the limited body of data available for avian systems (Seibert and Crowell-Davis 2001; Radford and Du Plessis 2006; Fraser and Bugnyar 2012; Gill 2012; Dey and Quinn 2014). We show that within-group patterns of affiliative, aggressive and submissive interactions in the purple-crowned fairy-wren coincide with kin-selected and mating benefits of group living as well as reproductive conflict. Moreover, these social interactions appear to affect birds' time allocation to essential maintenance behaviors such as foraging, reflecting a cost of interacting with group members in terms of

time expenditure. As these interactions may aid in subordinates staying and in the resolution of reproductive conflict, they may be crucial for social groups to remain stable. We encourage further studies on potential consequences of behavioral interactions for the occurrence of escalating conflict and subordinate dispersal. For instance, beneficial social bonds with group members may provide one incentive for offspring to delay dispersal and remain on their current territory. Thereby, our results may provide insight into not just social living in general, but also the proximate factors underlying the evolution of group living (e.g., Griesser et al. 2006; Covas and Griesser 2007; Komdeur and Ekman 2010; Kingma et al. 2016).

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Data accessibility: Analyses reported in this article can be reproduced using the data provided by Teunissen et al. (2018).

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Supplementary material for Chapter 1

Supplementary material I: Statistical analyses: contact-sit, allopreening, aggression

Running three separate statistical models for the occurrence of contact-sit, allopreening, and aggressive interactions, respectively (using an MCMCglmm rather than a CLMM for aggressive interactions due to rarity of events), yielded qualitatively similar results as our model for the affiliation index (model 1). Although non-significant due to the rarity of each interaction in isolation, the same patterns were observed according to sex and relatedness for each type of interaction. Combining the interactions into a single response variable (model 1) allowed for more robust statistical testing. Effect sizes for aggressive interactions are in the opposite direction relative to the other three response variables as it negatively affects social bonds, in contrast to the affiliation index, contact-sit, and allopreening, which positively affect social bonds.

	Model 1: affiliation index		Model 2: contact-sit		Model 3: allopreening		Model 4: aggression	
	Effect size ($\pm$ SE)	<i>P</i>	Effect size ($\pm$ SE)	<i>P</i>	Effect size ($\pm$ SE)	<i>P</i>	Effect size (95% CI)	<i>P</i>
Relatedness ^a	0.20 $\pm$ 0.65	0.76	0.03 $\pm$ 0.68	0.96	0.42 $\pm$ 0.79	0.60	-2.74 (-8.33, 2.36)	0.33
Sex ^b	-3.19 $\pm$ 1.22	<0.01	-1.77 $\pm$ 1.15	0.12	-0.67 $\pm$ 1.03	0.52	4.81 (0.97, 8.82)	<0.01
Status ^c	0.16 $\pm$ 0.47	0.73	0.05 $\pm$ 0.51	0.92	0.12 $\pm$ 0.59	0.84	-2.13 (-7.41, 2.25)	0.43
Season (Dry 2016) ^d	1.12 $\pm$ 0.56	0.046	1.32 $\pm$ 0.67	0.049	1.45 $\pm$ 0.81	0.08	-2.29 (-7.15, 2.61)	0.38
Season (Wet 2016) ^d	1.68 $\pm$ 0.54	<0.01	2.00 $\pm$ 0.62	<0.01	2.58 $\pm$ 0.73	<0.01	2.24 (-1.23, 5.42)	0.15
Relatedness x Sex ^e	3.13 $\pm$ 1.34	<0.01	1.68 $\pm$ 1.31	0.20	0.59 $\pm$ 1.27	0.64	-2.93 (-9.44, 2.27)	0.33

^a Effect sizes of related individuals relative to unrelated individuals

^b Effect sizes of same-sex individuals relative to opposite-sex individuals

^c Effect sizes of subordinates relative to dominant group members

^d Effect sizes relative to dry season of 2014

^e Effect sizes of related same-sex individuals relative to unrelated opposite-sex individual

Supplementary material II: Seasonal variation in time allocation

Seasonal variation in resource abundance (dry vs wet season, i.e. low vs high resource abundance) in a monsoonal climate was related to variation in a range of behaviors in our study species. In all seasons, subordinates spent most of their time foraging ($84.5 \pm 1.6\%$ of the observed time). In general, birds spent a greater proportion of time foraging in the dry seasons ($89.8 \pm 1.1\%$) compared to the wet season ($71.3 \pm 2.7\%$, Tukey's post hoc test: $z = -9.59$, $P < 0.01$ and $z = -9.91$, $P < 0.01$ for dry 2014 and dry 2016, respectively, foraging time between dry 2014 and dry 2016 did not differ: $z = -1.23$, $P = 0.43$). Decreased foraging time in the wet season was associated with increases in resting ($2.2 \pm 0.3\%$ vs $9.1 \pm 0.9\%$; $z = 8.95$, $P < 0.01$ and $z = 7.39$, $P < 0.01$ for dry 2014 and dry 2016, respectively) and self-preening ($5.0 \pm 0.8\%$ vs $13.5 \pm 2.3\%$; $z = 3.75$, $P < 0.01$ and $z = 2.03$, $P = 0.10$ for dry 2016 and dry 2014, respectively) during the wet compared to the dry season.

The relationship between affiliative interactions and time spent foraging differed between the seasons (GLMM, interaction term: $\chi^2 = 12.34$, $df = 2$, $P < 0.01$): during both dry seasons individuals spent less time foraging if they engaged in at least one affiliative interaction during an observation, while during the wet season individuals spent the same amount of time foraging regardless of whether they engaged in affiliative interactions (Fig. 4). A similar effect was found for the interaction between season and submissive displays (Fig. 5; GLMM: $\chi^2 = 7.90$, $df = 1$, $P < 0.01$), while aggressive interactions were not related to time spent foraging, neither in isolation (GLMM: $\chi^2 = 0.36$, $df = 1$, $P = 0.55$), nor in interaction with season (GLMM: $\chi^2 = 3.08$, $df = 2$, $P = 0.21$).

The amount of time subordinates spent self-preening was also predicted by season ($\chi^2 = 45.6$, $df = 2$, $P < 0.01$), with individuals spending more time self-preening during the wet season, significantly so compared to the dry season of 2016 (Tukey's post hoc test: $z = 3.75$, $P < 0.01$ and $z = 2.03$, $P = 0.10$ for dry 2016 and dry 2014, respectively). Time spent self-preening was also associated with engaging in affiliative interactions ($\chi^2 = 10.04$, $df = 1$, $P < 0.01$); those that affiliated with group members spent more time self-preening. There was no significant interacting effect of season and affiliative interactions on time spent preening ($\chi^2 = 3.86$, $df = 2$, $P = 0.15$), nor of season and aggressive interactions ($\chi^2 = 4.24$, $df = 2$, $P = 0.12$), season and submissive displays ($\chi^2 = 0.99$, $df = 1$, $P = 0.32$), nor a main effect of aggressive interactions ($\chi^2 = 0.10$, $df = 1$, $P = 0.75$) or submissive displays ($\chi^2 = 2.64$, $df = 1$, $P = 0.10$).

The proportion of time subordinates spent resting was predicted by an interacting effect of season and the presence of affiliative interactions ($\chi^2 = 8.90$, $df = 2$, $P = 0.01$). During both dry seasons, individuals that engaged in affiliative interactions spent slightly more time resting, while

during the wet season, individuals spent a much greater proportion of their time resting overall (Tukey's post hoc test: $z = 8.95$, $P < 0.01$ and $z = 7.39$, $P < 0.01$ for dry 2014 and dry 2016, respectively), and spent less time resting when they were observed to engage in affiliative interactions with group members. Aggressive interactions and submissive displays did not affect time spent resting, neither in isolation (GLMM: $\chi^2 = 0.55$, $df = 1$, $P = 0.46$ and $\chi^2 = 0.16$, $df = 1$, $P = 0.69$, respectively), nor in interaction with season (GLMM: $\chi^2 = 0.01$, $df = 2$, $P = 0.99$ and $\chi^2 = 0.94$, $df = 1$, $P = 0.33$, respectively).

Chapter 2

Predator defense is shaped by risk, brood value and social group benefits in a cooperative breeder

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Abstract

Predation is a major cause of mortality and nest failure in birds. Cooperative predator defense can enhance nest success and adult survival, but since it is inherently risky, dynamic risk assessment theory predicts that individuals modify defense behavior according to risk posed by the predator. Parental investment theory on the other hand predicts that reproductive payoffs (brood value) determine investment in nest defense. We propose that in cooperative breeders, fitness benefits deriving from survival of other group members may additionally influence defense behavior (social group benefits theory). We tested predictions of these theories in the cooperatively breeding purple-crowned fairy-wren, *Malurus coronatus*, where brood value is higher for breeders, but social group benefits more important for helpers. We recorded experimentally-induced individual defense behaviors in response to predator models presented near nests, representing differing levels of threat to nests and adults. As predicted, (i) individuals engaged in less risky defenses when encountering a more dangerous predator (dynamic risk assessment theory); (ii) individuals defended older broods more often, and breeders defended more than helpers (parental investment theory); and (iii) helpers were more likely to respond to a predator of adults (social group benefits theory). Our findings highlight that predator defense in cooperative breeders is complex, shaped by the combination of immediate risk and multiple benefits.

Keywords: altruism, anti-predator behavior, cooperation, helping, nest defense, predation

Introduction

Predation is a major cause of adult mortality and reproductive failure in birds (Ricklefs 1969; Lima & Dill 1990). Individuals may engage in active predator defense by attacking, mobbing, or distracting predators, and uttering frequent calls (Montgomerie and Weatherhead 1988; Arnold 2000; Caro 2005; Graw and Manser 2007). Parental investment theory predicts that the intensity of such nest defense increases with increasing fitness benefits associated with survival of the current brood (Trivers 1972; Montgomerie and Weatherhead 1988). Indeed, nest defense intensity has been found to increase with increasing brood value (e.g. larger, older, or higher quality brood; Olendorf and Robinson 2000; Rytönen 2002; Svagelj et al. 2012).

However, predator defense is costly as it involves increased risk of mortality and injury, lost foraging and mating opportunities, and energy expenditure (Montgomerie and Weatherhead 1988; Dugatkin and Godin 1992). Dynamic risk assessment theory therefore predicts that the intensity of nest defense will be lower when there are higher costs associated with defense, e.g. when the risk of mortality associated with the predator is higher, or expected future reproduction is higher, and the potential fitness lost is thus greater (Montgomerie and Weatherhead 1988; Dugatkin and Godin 1992). In support of the latter, nest defense intensity has been found to increase with age, and throughout the breeding season, as future breeding opportunities decline (Barash 1980; Regelman and Curio 1983; Thornhill 1989). However, various studies have reported an apparent increase in defense intensity for predators posing a greater risk (Edelaar and Wright 2006; Graw and Manser 2007; Griesser 2009; Colombelli-Negrel et al. 2010; Colombelli-Negrel et al. 2010; Dutour et al. 2016). But since these studies focused primarily on the vocal response by prey species, this does not necessarily reflect high risk-taking behavior. Instead, individuals may vary their defense strategy rather than intensity according to the type of predator, for example increasing their vocal response but keeping a greater distance from predators associated with greater risk (Swaigood et al. 1999; Strnad et al. 2012; Koboroff et al. 2013). This illustrates that when testing theories of predator defense, various types of defense need to be taken into consideration.

Cooperatively breeding species provide a particularly interesting system to study the complexities of predator defense and to examine predictions from parental investment and dynamic risk assessment theories simultaneously. Here, helpers can improve nest defense via increased vigilance or enhanced active predator defense through e.g. increased mobbing behavior (Boland 1998; McGowan and Woolfenden 1989; Hailman et al. 1994; Canestrari et al. 2009). Because breeders and helpers generally have different stakes in the breeding attempt, they

are predicted to engage differently in nest defense not only because of variation in risk posed by different predators but also variation in benefits associated with predator defense. Moreover, and importantly, in cooperative breeders individuals may not only defend the brood but also protect group members when a predator poses a threat to adults (e.g. Francis et al. 1989). Although this could potentially also be relevant in non-cooperative socially monogamous species (individuals protecting their partner), increased protection of conspecifics has been proposed as a key driver of group formation (Du Plessis et al. 1995; Caro 2005; Groenewoud et al. 2016). Protection of group members may therefore be an especially important reason for defense against predators in cooperative breeders (social group benefits theory).

Social group benefits vary within cooperative groups and may be more important for explaining predator defense behavior by helpers, for whom benefits associated with the social group may be greater than benefits directly associated with the brood. Depending on the social environment, individual helpers can obtain many different social benefits from group members; for example, through parental nepotism, breeding position inheritance, obtaining a mate, kin-selected benefits or group augmentation (Ekman et al. 2000; Kokko et al. 2001; Kingma 2017). While patterns of vigilance have been shown to vary at least to some extent with social benefits (presence of juveniles or kin) in several species (Griesser and Nystrand 2009; Santema and Clutton-Brock; Bednekoff 2015; but see Wright et al. 2001), social benefits have surprisingly not explicitly been considered when attempting to understand how variation in more risky, active, predator defense is shaped in cooperatively breeding species. In general, despite clear predictions, few studies have investigated helper contribution to active predator defense in cooperatively breeding species (Maklakov 2002; Arnold et al. 2005; van Asten et al. 2016), but doing so may also shed light on the adaptive benefits of group living and social structures in cooperative breeders.

Here, we test if combined predictions of the parental investment, dynamic risk assessment and social group benefits theories (see Table 1 for an overview) can explain investment in predator defense in a cooperative breeder. To do so we assess individual helper and breeder contribution to predator defense in response to three predator models, representing (i) a predator of nests only, (ii) a predator of nests and adults, (iii) a brood parasite (a large threat to the nest, but no threat to adults), and (iv) a non-threatening control. In our study species, the cooperatively breeding purple-crowned fairy-wren, *Malurus coronatus*, breeders are monogamous, helpers rarely gain paternity (Kingma et al. 2009) and vary in relatedness to the brood due to breeder turnover (Kingma et al. 2010; Kingma et al. 2011), so both male and female

breeders are on average more related to the brood than helpers ($r = 0.5$ for 98% of breeders compared to 62% of helpers; Kingma et al. 2011). Helpers can receive important social group benefits through group augmentation, parental nepotism, or future reproduction (i.e. potential future mates), and these benefits are greater for male helpers since they have greater chances of breeding position inheritance and are the more philopatric sex, thus staying on their natal territory for longer to reap those benefits (Kingma et al. 2010; Kingma et al. 2011; Margraf and Cockburn 2013; Teunissen et al. 2018). If individuals in this species indeed simultaneously balance risk of injury/mortality, fitness payoffs of the current brood surviving, and fitness payoffs of adult group members surviving, we predict that (1) individuals engage in less risk-taking when a predator poses greater risk of injury; (2) individuals respond more strongly with increasing brood value (i.e. brood age, size); (3) breeders defend more intensely overall; (4) male helpers defend

Table 1. Predictions for individual predator defense intensity based on the two main current theories for nest defense, and our proposed social group benefits theory for cooperative breeders. Predictions specific for purple-crowned fairy-wrens are given, and whether these were supported in this study.

Theory	Nest defense predicted to increase when:	Predictions in purple-crowned fairy-wrens	Supported?
Dynamic risk assessment	fitness costs are lower, e.g. lower risk of injury or mortality ^{a,b}	1. Less risk-taking when greater risk of injury	Yes (Fig. 1; Table 2)
Parental investment	brood survival yields greater fitness benefits (greater brood value) ^{a,c}	2. a. Defense increases with brood size	No ^d (Table 2)
		b. Defense increases with brood age (nest stage)	Yes ^e (Table 2)
		3. Breeders defend more than helpers (reflecting higher average relatedness to brood)	Yes (Fig. 1; Fig. 2; Table 2)
Social group benefits	greater fitness benefits are associated with group members' survival (e.g. through group augmentation, reciprocity, parental nepotism, obtaining a mate/breeding position)	4. Male helpers defend more than female helpers	Yes (Fig. 2)
		5. Helpers defend more when threat to adult group members	Yes (Fig. 1)

^a Montgomerie and Weatherhead 1988

^b Dugatkin and Godin 1992

^c Trivers 1972

^d Variation in brood size was limited (mean $\pm$ SD = 3 ± 0.8 , range = 2-5), potentially contributing to lack of detection of an effect

^e Likelihood of defense overall increased with brood age, while intensity of defense increased with brood age for helpers only

more intensely than female helpers; and (5) helpers defend relatively more strongly to predators of adults (Table 1).

Materials and methods

Study site and species

We studied a population of purple-crowned fairy-wrens that has been color-banded since 2005 at Australian Wildlife Conservancy's Mornington Wildlife Sanctuary in northwest Australia (S17°31' E126°6'). Purple-crowned fairy-wrens are riparian habitat specialists with stable year-round territories aligned linearly along watercourses (Rowley and Russell 1993). Groups consist of a socially monogamous dominant breeding pair that is often accompanied by one or more non-breeding subordinates (Kingma et al. 2010; Kingma et al. 2011). Only the dominant pair duets, providing a reliable cue to assign breeder status (Hall and Peters 2008), which our previous studies confirmed by parentage analyses (Kingma et al. 2009). Subordinates of both sexes can assist the breeding pair to provision offspring, and while they vary in the amount of help provided (Kingma et al. 2010; Kingma et al. 2011), here we use the term "helper" to refer to any subordinate.

Most breeding takes place during the monsoonal wet season (December – March) (Rowley and Russell 1993; Kingma et al. 2010). Only the breeder female builds the nest and incubates eggs. For the current experimental study, we followed 50 groups between December 2016 and May 2017, recording group size and social status of each group member during regular visits (at least once per week). Breeder females were followed closely for signs of breeding. All nests found were checked regularly to determine laying date, clutch size, hatch date, and number of nestlings. Clutch size ranges from 1 to 5 eggs (average $\pm$ SE = 3 ± 0.03), the incubation period lasts for around 14 days and the nestling period for 13 days (Kingma et al. 2010; personal observations). Only 22% of nests with eggs produce at least one fledgling, with nest predation being the main source of nest failure (57% of 685 nests) (Hidalgo Aranzamendi 2017; Hidalgo Aranzamendi et al. In press). Common nest predators at our study site include varanid lizards (goannas; 61% of 31 predation events captured on camera, unpublished data), avian predators (16%), and snakes (10%).

Experimental methods

We quantified individual contribution to predator defense, in response to models representing (1) a nest predator, (2) a predator of nests and adults, (3) a brood parasite and (4) a control model presenting no threat (see below). Experiments were conducted between 7 February – 24 April

2017, during the (early) incubation stage ($N = 29$ experiments, mean no. days since last egg laid at first trial $\pm$ SE = 3.3 ± 0.4 ; range = 1 to 11 days) and during the nestling stage ($N = 22$ experiments, mean no. days post-hatching at first trial $\pm$ SE = 6.3 ± 0.1 ; range = 5 to 7 days), adding up to a total of 51 experiments (i.e. 204 trials) at 37 nests, in 33 fairy-wren territories (group size ranged 2 – 7, mean $\pm$ SE = 3.3 ± 0.2). Responses were recorded for 37 helpers (24 males, 13 females) and 67 breeders (34 males, 33 females). At 14 nests, experiments were conducted twice – once during the egg stage and again during the nestling stage. Nestling banding always took place after the final trial had been concluded, to minimize carry-over effects of disturbance at the nest on anti-predator responses during the experiment.

For each experiment, we placed, in turn, four models near the nest (see electronic supplementary material, figure S1a; c):

- 1) Goanna; a plastic model of approximately 50cm in length (head to tail) representing a juvenile Merten's water monitor (*Varanus mertensi*) in standing posture. The model was painted based on photos of natural specimens, and we used reflectance spectra and psychophysical models of avian vision [38] to confirm that the painted model displayed natural colors (data not shown). Water monitors (*V. mertensi*, *V. mitchelli*) of this size are predators of both eggs and nestlings, but do not pose a threat to adult fairy-wrens.
- 2) Goshawk; a taxidermied brown goshawk (*Accipiter fasciatus*), mounted in a natural perched posture on a short stick. This species is a predator of both nests and adult fairy-wrens.
- 3) Cuckoo; a taxidermied shining bronze-cuckoo (*Chrysococcyx lucidus*), mounted in a natural perched posture on a short stick. Although this species does not occur at our study site, it is morphologically similar to the Horsfield's bronze-cuckoo (*C. basalis*), which occasionally parasitizes purple-crowned fairy-wren nests (Langmore et al. 2011), and mobbing responses of other fairy-wrens to both cuckoo species are equivalent (Payne et al. 1985). Bronze-cuckoos pose a threat to the nest at all stages; during the laying and early incubation stage they may parasitize fairy-wren nests, resulting in the fairy-wren eggs and/or nestlings being ejected from the nest by the cuckoo nestling, and during the nestling stage bronze-cuckoos can depredate the nest (Guppy et al. 2017). The reproductive costs associated with successful parasitism by a cuckoo are greater than those associated with nest predation alone, as it involves loss of the brood and extended costly parental care provided to cuckoo young (Rothstein 1990; Feeney et al. 2012). However, cuckoos do not depredate adults.
- 4) Control; a taxidermied peaceful dove (*Geopelia striata*), mounted in a natural perched posture on a short stick. Peaceful doves are a common species at our study site that are similar in size

to shining bronze-cuckoos (19-21cm vs 16-18cm; Pizzey and Knight 2012) and pose no threat to fairy-wren nests or adults.

Two exemplars of each model type were used and switched between trials to control for potential model effects. For each experiment, one exemplar of each model type was presented, with two presentations per day, over two days. Models were presented in a different order each time, in a balanced design of presentation sequences to account for any possible order effects. All trials took place between 5:30 am and 1:00 pm during calm, dry weather. During experiments, the stick supporting each taxidermied mount was fitted inside a 25x25x60cm cage constructed from mist net tightly strung around a wooden frame (see electronic supplementary material, figure S1b), to protect mounts from damage by fairy-wren attacks (although physical attacks were rare, the net did not seem to present a visual barrier since some individuals touched the net more than once; no birds got entangled). The cage was either suspended from a nearby branch, or in few cases placed on the ground, so the model was at nest height (range = 0.2 to 3.3m high). The cage was placed approximately 1m from the nest (mean $\pm$ SE = 1.2 ± 0.03 m). The goanna model was not placed in the cage, but instead, placed approximately 20cm from the nest on a branch or Pandanus leaf (mean $\pm$ SE = 21 ± 0.5 cm), facing the nest entrance, and attached to the vegetation with black wire (see electronic supplementary material, figure S1d). These different ways of presenting the models represent how avian and reptilian predators, respectively, naturally approach a fairy-wren nest, with their respective differences in mobility resulting in similar immediate risk to the nest (Montgomerie and Weatherhead 1988).

Prior to the first trial of the day, a camouflage hide was set up approximately 10m from the nest, providing a clear view of the nest and its surrounding area, and the empty cage was placed. Both were left for at least 30 min before the start of the first trial, to allow the birds to get accustomed. When none of the birds in the group were within visible distance of nest, the model was placed. The trial commenced as soon as at least one individual fairy-wren approached to within 2m of the model or started producing alarm calls, and continued for 10 min. One observer (N.T.) dictated the behavioral responses of each bird in the focal group on a voice recorder. For larger groups (≥ 5 individuals; $N = 7$ nests), a second observer was present to additionally record the behavioral responses of all group members; this confirmed that the first observer had not missed any of the birds' responses. At the end of the trial, the model was removed, and the next model was presented after an interval of at least 90 min.

Individuals were considered to respond to the model if they produced alarm calls, approached to < 2 m of the model but without going to the nest to incubate, feed or brood, and/or

stayed within 2-5m but intently focused on the model (often while exhibiting freezing behavior; see e.g. Cresswell et al. 2009; Cunningham and Magrath 2017). Thus, birds exhibiting normal nest attendance behavior or simply foraging near the nest by chance were never considered to respond to the model; approaches to the nest were only counted as a predator defense response if birds that were carrying food aborted their nest visit and directed their attention to the model or if they were directly approaching the predator itself rather than the nest. Predator defense was quantified as five measures of defense behavior; (1) likelihood of response, (2) nearest distance to the model, (3) time spent <2m and (4) <0.5m of the model (only including time spent near the model as part of predator defense behavior, not as part of normal nest attendance behavior; see criteria of response described above), and (5) number of alarm calls produced during each trial. Physical attacks only occurred in two trials and could therefore not be analyzed separately.

Statistical analyses

All analyses were performed in R 3.4.4 (R Core Team 2018). In 89% of 153 predator model trials, at least one individual in the group exhibited defense behavior in response to the model presented, whereas individuals responded to the control model in only 8% of trials. Control model presentations were therefore excluded from any further statistical analyses. Cases where the focal bird might not have been able to detect the model (i.e. where the bird was not seen at all during the trial and its group members did not give any alarm calls that may have alerted it to the presence of the model) were excluded from analyses (N = 71 of 498). In all trials, at least one individual in the group was able to detect the model. Only one group contained a juvenile (< 145 days old) at the time of the experiment; this bird was excluded from analyses since juveniles generally do not help (Kingma et al. 2010; Kingma et al. 2011).

We constructed five sets of statistical models to quantify how each of the five defense behaviors described above varied between breeders and helpers in response to predators of varying threat, and with brood value, to test predictions as outlined in Table 1. We included as explanatory variables: predator type (goanna, goshawk, cuckoo; prediction 1 in Table 1), brood size (prediction 2a), nest stage (eggs, nestlings; prediction 2b), status (breeder, helper; prediction 3), sex (male, female) and the interaction between status and sex (prediction 4), and the interaction between status and predator type (prediction 5). We also included the interaction between status and nest stage to test the possibility that helpers and breeders respond differently to changes in brood value, and the interaction between nest stage and predator type since we expect birds to respond more strongly to cuckoos during the early egg stage in particular, when

the risk for brood parasitism is highest. Additionally, we included trial number as covariate to control for order effects. Although individuals <1 year old provision nestlings less and may therefore be expected to also defend less against predators (Kingma et al. 2011), we did not include focal individual age in our models since nearly all (99%) breeders compared to 78% of helpers in our dataset were older than one year. All models included bird ID, nest ID, and exemplar (nested within predator type) as random effects. To analyze the likelihood of response, a generalized linear mixed model (GLMM) with binomial distribution was built using the packages 'lme4' (Bates et al. 2015) and 'lmerTest' (Kuznetsova et al. 2017). The nearest distance that the focal bird approached the model ((log+1)-transformed to meet assumptions of normality) was analyzed using a linear mixed model (LMM), including only birds that responded. Post-hoc tests were performed using the 'glht' function from the 'multcomp' package (Hothorn et al. 2008), controlling for any interaction effects. Since individuals often did not produce alarm calls (72% of trials), spent no time <2m (63%) or <0.5m of the model (92%), these response variables were analyzed with a Bayesian GLMM with a negative binomial distribution using the 'rstanarm' package (Goodrich et al. 2018). Priors were set to a normal distribution with mean = 0 and variance = 10, and variance = 100 for the intercept prior. Three chains were run, of 15,000 iterations, with a thinning interval of 20, and a warmup period of 5,000. Visual inspection of the trace, density and autocorrelation plots of the parameters using the 'rstan' package (Stan Development Team 2018) confirmed convergence of the model and showed no sign of autocorrelation. We present posterior means and 95% credible intervals (CI). Using GLMMs with binomial distribution instead (analyzing presence/absence of alarm calls, approach <2m and <0.5m) yielded qualitatively similar results for all three variables.

Results

Defense behavior and risk

Overall, individual fairy-wrens adjusted their defense response to the threat posed by the predator. First, birds were more likely to respond to goshawk models (70% of trials) compared to cuckoo models (56% of trials) (Tukey's HSD; $\beta \pm SE = 0.99 \pm 0.38$, $z = 2.62$, $P = 0.02$); with no difference in likelihood of response towards goanna (64% of trials) and cuckoo ($\beta \pm SE = 0.51 \pm 0.38$, $z = 1.35$, $P = 0.37$) or goanna and goshawk models ($\beta \pm SE = -0.48 \pm 0.38$, $z = -1.28$, $P = 0.41$) (Fig. 1a). Second, the physical response differed between predator types; birds approached both goanna and cuckoo models to a closer distance than goshawk models (Tukey's post hoc test: $\beta \pm SE = -0.32 \pm 0.06$, $z = -5.40$, $P < 0.01$, and $\beta \pm SE = -0.23 \pm 0.07$, $z = -3.49$, $P < 0.01$, respectively),

with no difference between goanna and cuckoo models ($\beta \pm SE = -0.09 \pm 0.07$, $z = -1.27$, $P = 0.41$; Fig. 1b). While there was no difference in the amount of time spent within 2m of models (Table 2; Fig. 1c), individuals never came within 0.5m of the goshawk, while they did get this close to goannas and cuckoos (Table 2). Physical attacks of the model were rare; one bird attacked a goanna model twice and another a cuckoo model twice; the goshawk was never physically attacked. The acoustic response (number of alarm calls produced) did not differ between predator types (Table 2; Fig. 1d). We found no significant interaction effect of predator type and nest stage for any of the response parameters (Table 2).

Defense behavior and social status

Breeders showed greater investment in nest defense than helpers: helpers were considerably less likely to respond (40% of trials compared to 59% for breeders; GLMM: $P < 0.01$; Table 2; Fig. 1a; Fig. 2a); did, on average, not approach as close to the predator model (LMM: $P < 0.01$; Table 2; Fig. 1b; Fig. 2b); spent less time within 2m of the model (Table 2; Fig. 1c; Fig. 2c) and within 0.5m of the model (Table 2); and produced fewer alarm calls (Table 2; Fig. 1d; Fig. 2d).

Among breeders, females generally showed stronger defense responses than males, while male helpers defended more strongly than female helpers (significant interaction effect of status and sex on likelihood of response, time spent <2m, and number of alarm calls; Table 2; Fig. 2). Generally, males produced fewer alarm calls compared to females, independent of social status (Table 2; Fig. 2d).

Breeders and helpers responded to the type of predator differently; helpers were more likely to respond to the goshawk compared to goanna and cuckoo models, while breeders were about equally likely to respond to all three types of predators (interaction term: $P = 0.01$; Table 2; Fig. 1a). Helpers also produced fewer alarm calls in response to goanna models in particular, while breeders produced equal numbers of alarm calls to all models (Table 2; Fig. 1d).

Individuals were more likely to respond to the predator during the nestling stage, when brood value is higher, compared to during the egg stage (Tukey's HSD; $\beta \pm SE = 0.62 \pm 0.29$, $z = 2.13$, $P = 0.03$). However, while the likelihood of response increased to a similar degree for breeders and helpers, they differed in how they adjusted the intensity of their response according to nest stage: during the nestling stage, helpers spent significantly more time <0.5m of the model than during the egg stage, and produced more alarm calls (Table 2), while breeders showed no difference in the intensity of response between nest stages. Neither breeders nor helpers adjusted their response with brood size (Table 2).

Table 2. The effect of a number of factors on five measures of predator defense behavior by individuals: (1) likelihood of response (N = 427 individual trials), (2) nearest distance to model (N = 270), (3) time spent <2m of the model (N = 426), (4) time spent <0.5m of the model (N = 427) and (5) number of alarm calls produced (N = 416). Effect size, standard error and *P*-values are given where frequentist statistical models were used and effect size and 95% confidence interval where Bayesian methods were used. For the main effects of variables that are also included in interaction terms, *P*-values presented were obtained through Tukey's HSD post-hoc analyses controlling for interaction effects. Significant terms are highlighted in bold.

	<i>Likelihood to respond</i>		<i>Log (Nearest distance to model)*</i>		<i>Time spent <2m</i>	<i>Time spent <0.5m</i>	<i>Number of alarm calls</i>
	Effect size ($\pm$ SE)	<i>P</i>	Effect size ($\pm$ SE)	<i>P</i>	Effect size (95% CI)	Effect size (95% CI)	Effect size (95% CI)
<i>Intercept</i>	1.02 $\pm$ 0.89	0.25	0.77 $\pm$ 0.15	<0.01	4.2 (2.5, 5.9)	0.4 (-4.3, 4.9)	3.0 (0.8, 5.3)
<i>Status</i> ^a	-2.68 $\pm$ 0.72	<0.01	0.19 $\pm$ 0.15	<0.01	-2.9 (-4.7, -1.1)	-6.5 (-12.5, -1.3)	-7.2 (-10.6, -4.0)
<i>Sex</i> ^b	-1.14 $\pm$ 0.39	0.52	0.17 $\pm$ 0.06	0.23	-0.5 (-1.3, 0.2)	-1.6 (-3.8, 0.7)	-1.2 (-2.1, -0.2)
<i>Brood size</i>	-0.03 $\pm$ 0.23	0.90	0.01 $\pm$ 0.04	0.73	0.0 (-0.5, 0.4)	0.0 (-1.2, 1.4)	0.1 (-0.5, 0.7)
<i>Predator type (goanna)</i> ^c	1.26 $\pm$ 0.52	0.37	-0.13 $\pm$ 0.08	0.41	1.0 (-0.2, 2.2)	2.2 (-0.4, 4.9)	-0.4 (-1.9, 1.0)
<i>Predator type (goshawk)</i> ^c	0.82 $\pm$ 0.50	0.02	0.20 $\pm$ 0.08	<0.01	0.3 (-1.0, 1.6)	-13.7 (-26.9, -5.4)	0.8 (-0.8, 2.5)
<i>Nest stage</i> ^d	0.50 $\pm$ 0.50	0.03	0.06 $\pm$ 0.09	0.69	0.7 (-0.8, 2.0)	-0.3 (-2.9, 2.8)	0.9 (-0.7, 2.6)
<i>Trial</i>	0.03 $\pm$ 0.11	0.82	-0.05 $\pm$ 0.02	0.01	0.2 (-0.1, 0.4)	0.7 (-0.1, 1.5)	0.3 (0.0, 0.7)
<i>Status (helper) x Sex (male)</i>	1.86 $\pm$ 0.68	<0.01	-0.18 $\pm$ 0.13	0.17	1.9 (0.2, 3.4)	3.6 (-1.2, 8.6)	3.7 (1.3, 6.2)
<i>Status (helper) x Predator type (goanna)</i>	-1.07 $\pm$ 0.65	0.01	0.24 $\pm$ 0.14	0.19	-0.1 (-2.2, 1.8)	-1.0 (-6.2, 4.7)	-2.7 (-5.7, 0.0)
<i>Status (helper) x Predator type (goshawk)</i>	0.67 $\pm$ 0.63	0.01	0.08 $\pm$ 0.13	0.19	0.1 (-1.9, 2.2)	-3.5 (-20.8, 11.8)	0.9 (-2.0, 3.9)
<i>Status (helper) x Nest stage (nestlings)</i>	0.74 $\pm$ 0.54	0.17	-0.05 $\pm$ 0.11	0.65	0.8 (-0.7, 2.5)	5.3 (0.5, 10.4)	4.3 (1.7, 7.2)
<i>Nest stage (nestlings) x Predator type (goanna)</i>	-0.43 $\pm$ 0.62	0.76	-0.15 $\pm$ 0.11	0.26	-0.3 (-2.1, 1.5)	1.2 (-3.3, 6.0)	-0.4 (-2.7, 2.1)
<i>Nest stage (nestlings) x Predator type (goshawk)</i>	-0.32 $\pm$ 0.60		-0.02 $\pm$ 0.11		-0.8 (-2.7, 1.0)	-4.9 (-20.6, 9.1)	-1.6 (-3.8, 0.6)

^a helper compared to breeder

^b male compared to female

^c compared to cuckoo model

^d nestling stage compared to egg stage

* only includes individuals that responded to the model

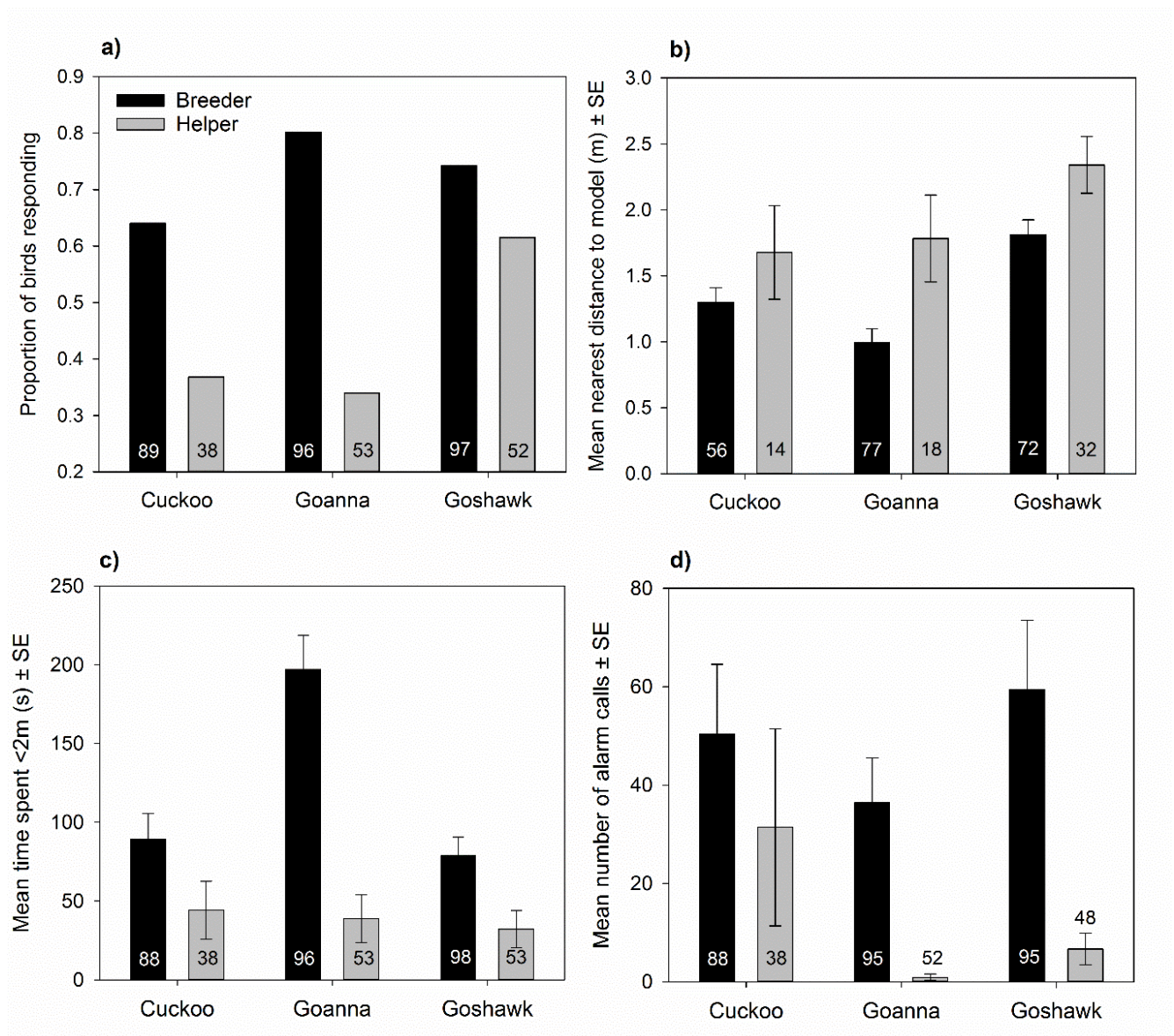


Figure 1. Responses by breeders (black bars) and helpers (grey bars) to each predator type, measured as (a) likelihood of response, (b) nearest distance to model, (c) time spent <2m of the model, and (d) number of alarm calls produced. Breeders defended more strongly than helpers overall, and responses differed with predator type (in interaction with social status for (a) and (d)). Values are based on raw data and results of statistical analyses are provided in Table 2. Numbers in bars indicate sample sizes. Sample sizes differ between predator types and response measures since only cases where birds were able to detect the model are included, and response measures could in a few instances not accurately be quantified. For nearest distance to the model (b), data are presented for individuals that responded only.

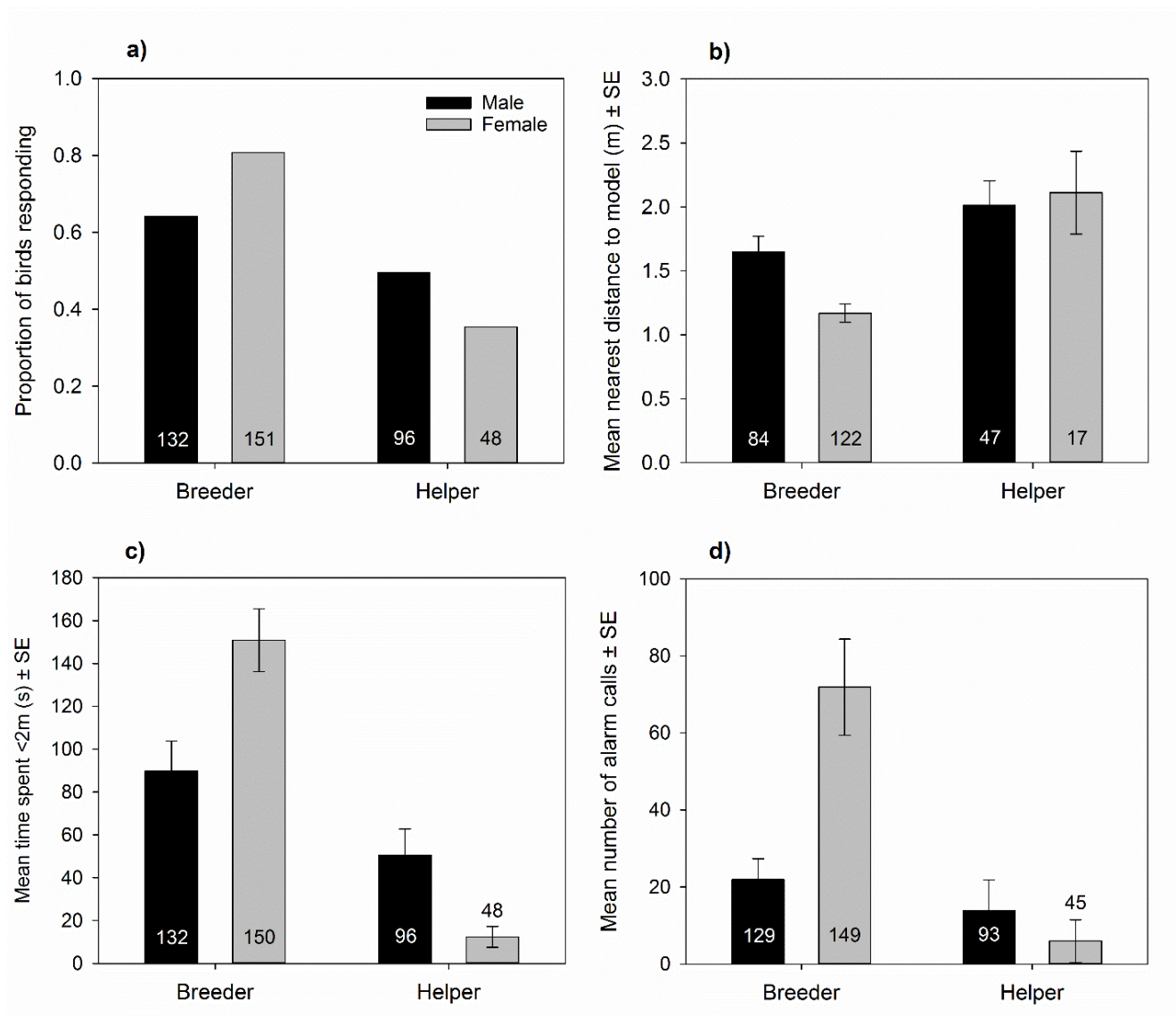


Figure 2. Responses by male (black bars) and female (grey bars) breeders and helpers to all predator types combined, measured as (a) likelihood of response, (b) nearest distance to model, (c) time spent <2m of the model, and (d) number of alarm calls produced. Breeders defended more strongly than helpers overall, and among breeders, females responded more strongly than males while among helpers, males responded more strongly than females (a, c, d). Values are based on raw data and results of statistical analyses are provided in Table 2. Numbers in bars indicate sample sizes. For nearest distance to the model (b), data are presented for individuals that responded only.

Discussion

We provide, as far as we are aware, the first simultaneous test of the dynamic risk assessment and parental investment theories for predator defense behavior in a cooperatively breeding bird species. Based on these theories, we predicted individuals to modify defense behavior with risk of injury and brood value, and predicted that breeders in this cooperative fairy-wren should defend more than helpers. Additionally, we proposed that in cooperative breeders, predator defense behavior should also be influenced by current and future social fitness benefits from adult group members surviving, and predicted that male helpers should therefore defend more than female helpers, and that helpers prioritize defense towards a predator of adults rather than the brood (see Table 1). Our results show that variation in defense behavior can be explained by each of these theories as we discuss below.

Dynamic risk assessment: Defense behavior and risk of injury

The key prediction of the dynamic risk assessment hypothesis, that individuals modulate their response according to level of threat and alter their behavior to minimize personal risk (Montgomerie and Weatherhead 1988; Dugatkin and Godin 1992), was clearly supported (Table 1). Both breeders and helpers changed their behavior according to the level of individual risk represented by the models, keeping greater distance from goshawk models - a predator that is associated with greater risk of injury for adults - compared to goanna and cuckoo models - predators that pose no risk to adults. The lack of response to control models in comparison indicates that goanna and cuckoo models were nonetheless perceived as a threat to the nest. Our findings suggest that fairy-wrens are able to distinguish between predator types and modify their defense behavior according to the degree of risk. Individual fairy-wrens in our study did not adjust the frequency of alarm calls according to the threat posed by the predator. This highlights the need to incorporate not only the commonly-studied acoustic response (as in e.g. Edelaar and Wright 2006; Graw and Manser 2007; Griesser 2009; Colombelli-Negrel et al. 2010; Colombelli-Negrel et al. 2010), but also the physical response, when testing predictions from risk assessment theory, as individuals may change defense strategy rather than intensity (Swaigood et al. 1999; Strnad et al. 2012; Koboroff et al. 2013).

Although goanna models were placed closer to the nest compared to goshawk and cuckoo models, and predator defense intensity has been found to decrease with predator distance from the nest in other species (Kleindorfer et al. 2005; Colombelli-Negrel et al. 2010), this is unlikely to have confounded our results. A goanna at 20cm from the nest is expected to represent a similar

level of threat to the nest as a goshawk or cuckoo at 1m from the nest, since avian predators have higher mobility (discussed in detail in Montgomerie and Weatherhead 1988). In addition, the closest approach and time spent within 0.5m of the model did not differ between cuckoos and goannas, despite their difference in distance from the nest, suggesting that differences in physical response to the goshawk indeed reflect a greater perceived threat to adults compared to a threat to the nest only for goannas and cuckoos.

Our expectation that the response to cuckoos should be stronger than to other nest predators, and particularly during the early egg stage (Feeney et al. 2013), was not supported. We expected this because cuckoos are associated with greater reproductive costs than other nest predators due to the risk of extended periods of care for the offspring of brood parasites (Rothstein 1990; Feeney et al. 2012). However, we found no interacting effect of predator type and nest stage on nest defense, and overall, fairy-wrens defended less often against cuckoos than against goshawks. Possibly, not all individuals in our study area were familiar with cuckoos as a result of relatively low brood parasitism rates at our study site (Langmore et al. 2012); brood parasitism of purple-crowned fairy-wren nests occurred in 4 of 9 years (2005-2010 and 2015-2017), with a mean $\pm$ SD of $4.4 \pm 4.5\%$ (maximum = 11%) of nests parasitized in years where at least some cuckoos hatched in fairy-wren nests. Since cuckoo recognition may require learning (Langmore et al. 2012; Feeney and Langmore 2013), unfamiliar individuals may respond less strongly, but we currently have insufficient data to test this idea.

Parental investment: Defense behavior and brood value

Parental investment theory predicts that individuals increase nest defense intensity with increasing brood value (relatedness, age, or size of the brood) (Trivers 1972; Montgomerie and Weatherhead 1988). These predictions were largely confirmed.

Although future analyses will possibly enable us to reveal a direct relationship between variation in relatedness and individual defense behavior, our observed greater investment in predator defense by breeders compared to helpers is in agreement with the prediction that relatedness affects defense (Table 1). Breeders on average have a higher genetic stake in the current brood compared to helpers: given near-monogamy in this species (Kingma et al. 2009), both breeders are full parents of the brood, while helpers are on average less related due to breeder turnover and dispersal (Kingma et al. 2010; Kingma, Hall and Peters 2011). Dynamic risk assessment theory may provide an alternative explanation, predicting that nest defense intensity increases when there are fewer opportunities for future reproduction. Breeders may invest more

in current relative to future reproduction, and therefore experience a lower cost of lost future reproduction and defend more intensely, compared to helpers. However to explore this possibility fully, we will first need to gain a better understanding of future prospects for reproduction for both breeders and helpers. It is worth noting that our finding that breeders defend more is unlikely to simply reflect breeders having higher probability to detect or be in the vicinity of the model because they attend the nest more often than helpers (Kingma et al. 2010; 2011): only individuals that were able to detect the model were included in analyses, and only birds that showed a response to the model were considered to engage in predator defense behavior.

Studies on non-cooperatively breeding species have generally reported increased defense of older and larger broods (e.g. Thornhill 1989; Lavery and Colgan 1991; Amat et al. 1996; Olendorf and Robinson 2000; Rytönen 2002; Svagelj et al. 2012). We found support for increased defense for older broods, with both breeders and helpers defending more often at later nest stages (Table 1). Contrary to parental investment theory however, defense behavior did not vary with brood size, possibly because variation in clutch size is relatively small (mean $\pm$ SD = 3 ± 0.8 for nests included in the current study) (Table 1). Moreover, previous studies on nest defense in cooperatively breeding birds have reported no effect of size or age of the brood (Arnold 2000) or a stronger response for older broods (van Asten et al. 2016), but these studies did not test for differences between helpers and breeders at different nest stages. While both breeders and helpers were more likely to defend in later nest stages, we found that the intensity of defense only increased for helpers and not for breeders. The latter might be a saturation effect, since breeder defense is always high, whereas helpers may be more sensitive to changes in brood value than breeders.

Social benefits: Social status and helper benefits

Breeders and helpers differed in how they modulated their response to predators of varying threat (interaction effect of social status and predator type), with helpers more likely to defend against a predator of adults than against nest predators (Table 1). This is in agreement with predictions based on social benefits: for helpers, adult group members' survival may be more important than the survival of the brood. Helpers may obtain significant benefits from adult group members through parental nepotism (i.e. facilitation of greater access to resources for mature offspring), reciprocal or mutualistic benefits of group augmentation (i.e. greater survival or future reproduction in larger groups due to e.g. reciprocal actions or safety in numbers), or the presence

of potential future mates in the group (i.e. unrelated opposite-sex group members). Hence, adult group members offer benefits that are larger and/or more immediate than young group members (Ekman et al. 2000; Kokko et al. 2001; Ekman et al. 2004; Kingma 2017; Teunissen et al. 2018), and their defence provides greater or more immediate payoffs.

Likewise, the observation that amongst helpers, males defend more intensely than females, can be explained by greater social benefits obtained from survival of the brood and adult group members (Table 1). In *M. coronatus*, male helpers are more likely to stay and have greater chances to inherit a breeding position in the group (Kingma et al. 2011) and therefore obtain greater immediate and future group augmentation benefits if the brood as well as adult group members survive (Kokko et al. 2001; Margraf and Cockburn 2013; Kingma 2017). Similarly, in cooperative meerkats, *Suricata suricatta*, female helpers – the philopatric sex – but not male helpers, increase vigilance behavior when pups are present (Santema and Clutton-Brock 2013), further supporting the notion that social benefits of predator defense may be closely linked to sex differences in philopatry. Possibly, nest defense by helpers may even serve to advertise quality to particular group members (Montgomerie and Weatherhead 1988; Zahavi 1995): for example in Arabian babblers, *Turdoides squamiceps*, subordinates mob predators of adults more than breeders, possibly to advertise their quality for the formation of dispersal coalitions (Maklakov 2002). Future research on *M. coronatus* will investigate if and how predator defense by helpers is aligned with individual benefits of helping and group living in this species, and is expected to enhance our understanding of predator defense in cooperative breeders and how helpers may balance contributions to individual and group success.

Conclusions

We show that individuals in a facultatively cooperative breeding fairy-wren modify their defense behavior in complex ways when detecting a predator and that this is aligned with relative risks as well as individual current and future benefits. Importantly, breeders and helpers seem to use different decision rules in predator defense. While breeders seem generally willing to defend the nest and group members - and they do so more intensely - helpers appear more responsive to changes in brood value and the social benefits associated with the survival of adult group members.

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Data accessibility

Analyses reported in this article can be reproduced using the data provided by Teunissen et al. (2019).

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Supplementary material for Chapter 2

Supplementary figure



Figure S1. (a) Taxidermied avian specimens used in experiments (left to right: control, cuckoo, goshawk), (b) which were presented in a cage constructed out of mist net; and (c) goanna specimens used in experiments, (d) attached to the vegetation near the nest during trials. Two exemplars of each model type were used.

Chapter 3

Nest defence and offspring provisioning in a cooperative bird: no evidence for task specialisation

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Abstract

In some obligate cooperative societies, successful reproduction requires individuals to specialise in different tasks. Such task specialisation is also predicted for facultative cooperative breeders when multiple costly activities are important for successful reproduction. However, in cooperative birds in particular, most research has focused on a single aspect of helping – offspring provisioning – although other costly activities are important too. If individuals specialise in certain tasks, such emphasis on provisioning might prevent a full understanding of the social structure of cooperative societies and how seemingly altruistic helping behaviour is evolutionarily stable. Here we tested whether individual purple-crowned fairy-wrens, *Malurus coronatus*, specialise in the two forms of costly offspring care important for reproductive success: nestling provisioning and nest defence (to predator models presented at nests). Helpers and breeders generally contributed to both tasks, and we found no evidence for individual task specialisation. Breeders consistently performed all tasks at a high level, whereas individual subordinates varied in overall contribution to both types of care. Thus, task specialisation may be absent in facultatively cooperative birds, even when activities are costly and important for reproduction, suggesting that in such species enhanced investment across multiple tasks may potentially be more important than enhanced efficiency of different tasks.

Keywords: cooperation, predator defence, task division, altruism, parental care, predation

Introduction

Cooperative offspring care, whereby individuals forego independent reproduction and instead assist others in raising their offspring, challenges the evolutionary premise that individuals should be selfish [1-4]. In some cooperative species, such as social insects or obligate cooperative breeders, successful reproduction requires individuals to cooperate in order to effectively defend and raise offspring in the group [3,5-9]. In eusocial insects, this is achieved through the formation of caste systems with strict division of labour, whereby individuals are highly specialised in performing certain tasks within the colony. This is associated with permanent morphological specialisation and results in high colony efficiency and productivity [3,6]. However, the formation of such distinct castes is limited to eusocial species (insects and eusocial mole-rats; [10]). A similar morphology-based social structure, with size-dependent division of different forms of labour, has been demonstrated in cooperative cichlids, where differences in body size determine the efficiency at certain tasks among individuals [11,12]. However, in most cooperatively breeding vertebrates, morphological differences between individuals are not as distinct, and the efficiency benefit of specialisation is predicted to be smaller. Consequently, it remains largely unexplored whether task specialisation occurs in such species.

In cooperative vertebrates, task specialisation is predicted to evolve when activities are costly, and/or important for fitness [13-15], and to manifest as consistent individual differences in the propensity to perform various tasks (as in e.g. social spiders [16-18]). Efficiency-based specialisation can result from various differences between classes of cooperating individuals (generally breeders and non-breeding subordinates or helpers [1,2]). For example, differences in behaviour [19], foraging efficiency and lactation demands [20], direct benefits of tasks [21], and condition-dependency of different tasks, can result in division of labour, with each social class contributing mainly to different activities. Within each social class, individuals also vary in relevant traits such as benefits associated with tasks, body condition, and age [1,2,5,22], likely affecting their efficiency at various tasks [11,14]. Nonetheless, whether individuals within social classes specialise in particular tasks, and how differences between social classes may affect propensity of individual breeders and helpers to specialise in tasks, is poorly understood.

If task specialisation were commonly undetected, this could affect our understanding of the social structure of cooperatively breeding species and the evolutionary maintenance of costly helping behaviour [1,2]. Despite helpers and breeders generally contributing to a range of cooperative tasks [2,5,13,21,23], studies on cooperative breeding in birds in particular have mostly focused on a single form of helping, usually offspring provisioning. Consequently, adaptive

explanations for helping are most often tested by studying whether variation in offspring provisioning rates is related to potential benefits of helping within species [2,5,22,24-26], and comparisons across species are generally made using offspring provisioning rates by helpers relative to breeders as measure of helping effort (e.g. [22,25]). This research focus on offspring provisioning is particularly troubling if task specialisation is common among breeders and/or helpers, in which case measures of one form of helping alone do not necessarily reflect total helping effort or costs of helping. Determining whether task specialisation occurs in cooperatively breeding vertebrates is therefore important for understanding the accuracy of conventional measures of helping effort and, ultimately, the adaptive benefits that lead to the formation of social groups.

Here, we test whether individuals specialise in costly tasks associated with (allo)parental care in the facultative cooperatively breeding purple-crowned fairy-wren, *Malurus coronatus*. In this species, breeders and helpers may participate in offspring provisioning, which is costly [27] and increases nest productivity [27], and defence against nest predators. Nest predation is common (57% of 685 nests; [28]), thus both behaviours have the potential for large fitness benefits in this species and are likely candidates for task specialisation. We quantified contribution to both forms of care by breeders and subordinates using observations of nestling provisioning and defence responses to predator models presented at nests, and tested for individual task specialisation by breeders and helpers separately. We predict a negative correlation between individual contribution to nest defence and nestling provisioning if task specialisation occurs, and a positive or no correlation if task specialisation is absent.

Methods

Study site and species

We studied a colour-banded population comprising 50 purple-crowned fairy-wren groups at Australian Wildlife Conservancy's Mornington Wildlife Sanctuary in northwest Australia (S17°31' E126°6'). Groups form stable year-round territories and consist of a breeding pair (status confirmed by duetting behaviour [29]), that are monogamous, and that can be accompanied by one or more non-breeding subordinates of both sexes (range 0-9), that rarely sire offspring [27,30-32]. Groups were followed closely throughout the wet season (December 2016 to May 2017), when most breeding takes place [27,32], recording individuals' social status and checking for signs of breeding. Nests were monitored for egg laying, hatching and fledging. Only the breeder female engages in nest building, incubation and brooding, but all group members may

participate in nestling feeding and nest defence. The incubation period lasts for 14 days and the nestling period for 13 days [27].

Nest defence

We quantified individual nest defence effort by presenting models of common threats at 22 nests on 21 fairy-wren territories (mean group size $\pm$ SE = 3.8 ± 0.3 , range = 2-7): a nest predator (plastic Merten's water monitor, *Varanus mertensi*), a predator of nests and adults (taxidermied brown goshawk, *Accipiter fasciatus*), a brood parasite (taxidermied shining bronze-cuckoo, *Chrysococcyx lucidus*), and a control (taxidermied peaceful dove, *Geopelia striata*). Goannas and avian predators are common nest predators at our study site (61% and 16% of 31 predation events captured on camera, respectively; unpublished data). While shining bronze-cuckoos do not occur at our study site, Horsfield's bronze-cuckoos, *Chrysococcyx basalis*, are morphologically similar and sometimes parasitise purple-crowned fairy-wren nests at our study site [33], and both species elicit similar mobbing responses from other fairy-wrens [34]. We used two exemplars of each model type and switched these between trials to control for model effects. The models were presented at the nest over two days, with two presentations per day, and at least 90 min in between trials, in a different order each time. We observed no sign of habituation; the likelihood for individuals to engage in predator defence did not vary with trial number (GLMM including Bird ID and Nest ID as random terms: $\beta \pm$ SE = 0.07 ± 0.11 , $z = 0.61$, $P = 0.54$). Trials were conducted during calm, dry weather, between 6:00 am and 12:30 pm, when nestlings were 4-7 days old (mean $\pm$ SE = 5.3 ± 0.1). Taxidermied models were mounted on a stick in a natural posture and presented in a 25x25x60cm cage, placed 1m from the nest, at nest height, while the goanna was attached to the vegetation with wire 20cm from the nest, representing how these predators naturally approach nests. The cage was constructed out of mist net tightly strung around a wooden frame, to protect the model from fairy-wren attacks while minimising the potential for altering the perceived risk or cost of predator defence for focal birds (Fig. 1). At least 30 min before the start of the first trial of the day, a camouflage hide was set up approximately 10m from the nest, as well as the empty cage, and left to allow the birds to get accustomed (as in [35, 36]). Additionally, for the first five experiments conducted, we observed the nest from a distance for ≥ 30 min after placing the empty cage and in all cases, birds did not react to the empty cage and resumed normal activities (no alarm calls, approached the nest and fed as normal). The model was placed at the start of the trial, when no birds in the focal group were within visible distance



Figure 1. (a) Goanna models were attached to the vegetation near the nest, and (b) taxidermied avian predators were presented in a cage constructed out of mist net. This reflects the natural behaviour of the predators, just before predation.

of the nest. The trial continued for 10 min from the moment the first fairy-wren approached to <2m of the model and/or produced alarm calls. Observing from the camouflaged hide, one observer (N.T.) recorded the behavioural response of each bird in the group, including the closest approach to the model, the number of alarm calls given, and the time spent within 2m of the model.

Responses were recorded for 42 breeders and 25 subordinates. During predator model trials, 64% of individuals engaged in predator defence compared to only 7% for control trials. Since only 43% of birds overall, and only 25% of subordinates, approached to within 2m of predator models in a given predator trial (i.e. goanna, goshawk, or cuckoo trials), and only 34% of birds (and 21% of subordinates) produced alarm calls, data was zero-inflated. Therefore, variation in nest defence effort among individuals was best reflected by an ordinal variable quantifying nest defence effort as the number of predator model trials (i.e. excluding control trials) for which the focal individual engaged in defence behaviour (0-3). Individuals were considered to engage in defence behaviour if they produced alarm calls, approached the model to <2m (without going to the nest to feed or brood), or stayed within 2-5m but intently focused on the model. This quantification thus provides a combined nest defence response to a variety of threats. While engaging in nest defence from a distance is arguably a less risky behaviour than approaching to a

close distance, it is still considered a costly activity, potentially attracting attention from predators to the individual, and entailing costs such as lost foraging and mating opportunities, and energy expenditure [7,37].

Nestling provisioning

For the same 22 nests, we quantified individual nestling provisioning effort for all group members with two 1-hour observations at each nest – one the day before the first and one the day after the last nest defence trial, with a few exceptions: one nest observation was conducted two days prior to the first trial, one on the day but prior to the first trial, and one nest could not be observed after the trials as it had been depredated. Nest observations were conducted during the morning (starting between 5:45 and 10:00 am), in calm, dry weather, from a camouflaged hide approximately 10m from the nest. The identity of each bird bringing food to the nestlings was recorded; individuals could be unambiguously identified through their unique combination of colour bands in 98% (497 of 509) of feeding visits.

For 12 of these nests (41 individuals), feeding rates were additionally quantified from videos recorded by motion-triggered trail cameras (Bushnell Trophy Cam Aggressor Low Glow) placed approximately 1-3m from the nest. There are random differences between nests in how many feeding events are recorded by cameras (possibly owing to differences in sensitivity between cameras, density and type of vegetation surrounding the nest, distance from camera to the nest, etc.), thereby not allowing us to compare total feeding rates by individuals, but we can instead compare individual proportion of feeds delivered to the nest (relative to the total number of feeds by the group) obtained from camera footage with feeding effort obtained from nest observations. The proportion of feeds delivered by the focal individual as determined from a 1-hour nest watch on a given day (based on a total number of feeds of mean $\pm$ SE = 10 ± 0.7 , range = 3-21) was highly correlated to the proportion of feeds determined from videos recorded throughout the entire day on the same day (mean total number of feeds $\pm$ SE = 72 ± 3.6 , range = 31-147) (linear regression: $R = 0.72$, $\beta \pm$ SE = 0.62 ± 0.07 , $t = 8.74$, $P < 0.01$). Similarly, when comparing the proportion of feeds by focal individuals as determined from our two nest watches (mean total number of feeds $\pm$ SE = 23 ± 1.3 , range = 8-40) to the overall proportion of feeds over the entire nestling period as determined from video footage (mean total number feeds $\pm$ SE = 393 ± 34 , range = 46-924), these are highly correlated ($R = 0.69$, $\beta \pm$ SE = 0.61 ± 0.09 , $t = 7.01$, $P < 0.01$). Furthermore, of 28 cases where the focal bird did not feed during a given nest watch, camera footage confirmed that the majority (75%; 21 of 28 cases) of these birds did not feed for that

entire day (based on a total number of feeds by the group of mean $\pm$ SE = 56 ± 5.4 , range = 12-115). Hence, this confirms that two hours of nest observations accurately reflect individual nestling feeding effort.

Statistical analyses

We fitted a generalised linear mixed model (GLMM) with nestling provisioning rate (number of feeds by focal bird over a single nest observation, i.e. two measures per focal bird) as response variable, using the 'lme4' [38] and 'lmerTest' [39] packages in R 3.4.4 [40]. Since focal birds did not feed in 25% of cases, a GLMM with negative binomial distribution was used. To test for covariation between individual nest defence and nestling provisioning effort for breeders and/or helpers, we included nest defence effort (0-3), social status (breeder, subordinate), and their interaction. Additionally, we included focal birds' sex (male, female) and the interaction sex*social status, since male and female breeders provision equally [27], but male subordinates provision more than females (related to their greater prospects for breeding position inheritance; [30]). Lastly, our model controlled for nestling age (range 4-9 days; as feeding rate during a single nest observation was used as response variable), brood size (range 1-4), and group size (range 2-7; no group contained juveniles (< 145 days old) at the time of the breeding attempt), as they potentially affect provisioning rates [30]. Although division of labour may also occur between individuals of different age classes [11,14,18], and subordinates <1 year old provision less than older subordinates in this species [30], we did not include focal individual age since all dominants and 84% of subordinates in our dataset were older than one year. Bird ID and Nest ID were included as random terms to account for replication across individuals and nests. A post-hoc Tukey's Honest Significant Difference test controlling for significant interaction effects was performed for the main effect of social status, using the 'glht' function from the 'multcomp' package [41]. Analysing relative instead of absolute provisioning rates (controlling for total feeds by the group), or analysing the presence/absence of response to each predator model separately, yielded qualitatively similar results (Supplementary material, Tables S1-2). If birds that provision more often are more likely to detect a predator, this could result in a correlation between individual nest defence and provisioning effort. However, most (81% of 67) birds were able to detect all three predator models (i.e. seen within visible distance of model and/or group members producing alarm calls), 18% were able to detect two predator models, and only one bird was able to detect one predator model only. Nonetheless, we additionally analysed nest defence effort as the proportion of predator trials in which an individual showed defence behaviour relative to the

total number of predator trials where the bird was able to detect the model, which yielded similar results (Supplementary material, Table S3), indicating that this is unlikely to cause a bias.

Results

The majority of breeders (40 of 42 individuals) contributed to both nestling provisioning and nest defence; two breeders provisioned but did not respond to the nest predator. Subordinates showed greater variation: while 44% (11 of 25) of subordinates contributed to both forms of care, 32% only contributed to nest defence, 24% did not provide any form of care, and none contributed to nestling provisioning only.

The relationship between nest defence effort and nestling provisioning effort varied with social status (interaction nest defence effort*status; Table 1); while nest defence and provisioning effort did not co-vary for breeders, subordinates who contributed more to nest defence generally also provisioned more (Fig. 2). Among breeders, males and females contributed equally to nestling provisioning (mean $\pm$ SE = 5.0 ± 0.5 and 5.1 ± 0.4 feeds/hr, respectively) whereas among subordinates, males provisioned more than females (mean $\pm$ SE = 1.5 ± 0.4 and 0.4 ± 0.3 feeds/hr, respectively) (interaction sex*status; Table 1). Breeders provisioned more frequently than subordinates overall (Tukey's HSD: $\beta \pm$ SE = 2.51 ± 0.37 , $z = 6.77$, $P < 0.01$; Fig. 2).

Table 1. The effect of various factors on nestling provisioning effort (feeds/hr) by focal individual purple-crowned fairy-wrens. Significant terms are highlighted in bold.

Parameter	β	SE	z value	P
Intercept	0.62	0.40	1.56	0.12
Nest defence effort	0.09	0.08	1.14	0.25
Social status ^a	-3.85	0.67	-5.76	<0.01
Sex ^b	-0.01	0.16	-0.07	0.94
Nestling age	0.08	0.03	2.54	0.01
Brood size	0.12	0.08	1.39	0.16
Group size	-0.03	0.05	-0.60	0.55
Nest defence effort x Status (subordinate)	0.82	0.22	3.80	<0.01
Sex ^b x Status ^a	1.02	0.51	1.98	0.047

^a subordinate compared to breeder

^b male compared to female

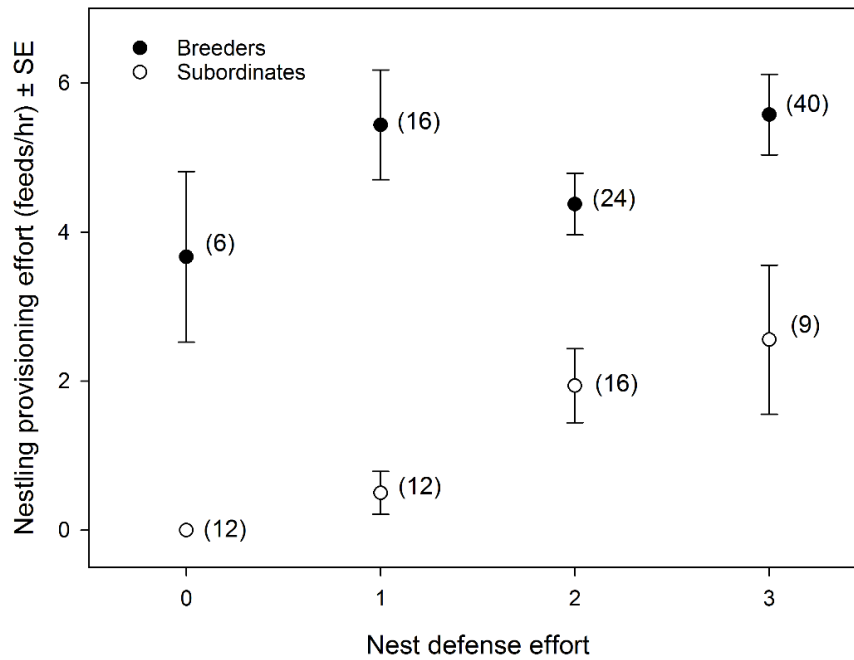


Figure 2. Individual nestling provisioning effort in relation to nest defence effort for breeder (black) and subordinate (white) purple-crowned fairy-wrens. Nestling provisioning and nest defence effort are unrelated among breeders but positively correlated among subordinates. Nest defence effort is quantified as the number of predator model presentation trials during which individuals engaged in defence behavior. Values are based on raw data. Numbers in brackets indicate the number of measures of individual nestling provisioning effort.

Discussion

Understanding task specialisation is important as it can potentially alter our view of the accuracy of conventional measures of helping effort, and how helping behaviour is evolutionarily stable. Our study revealed no negative correlation between individual nestling provisioning and nest defence effort in purple-crowned fairy-wrens. Both activities are costly and important for successful breeding, an important condition for task specialisation [15]: nestling provisioning reduces adult survival ([27]; see also [42]), and increases fledgling production [27]; predator defence is associated with risk of injury or mortality and costs such as lost foraging and mating opportunities and energy expenditure [7,37], and can increase nest success [35,43-45]. Thus, breeders and subordinates do not show specialisation for costly, important tasks in this facultative cooperative breeder. This is broadly in agreement with empirical studies on two cooperative mammals revealing no specialisation by breeders or helpers for a range of activities associated with breeding [46,47], or task specialisation by helpers according to body mass in their first year of life only [14]. In birds, as far as we are aware, only one previous study tested for task

specialisation in risky predator defence and offspring provisioning by helpers: in a socially highly complex obligate cooperative breeder, notorious for their vigorous coordinated mobbing behaviour (noisy miners, *Manorina melanocephala*), helpers often specialise in one of these two forms of helping behaviour [13]. Obligate cooperation is rare in birds however, and most species do not breed in large coalitions [48,49]. Therefore, our results of lack of task specialisation might be more broadly applicable to facultative cooperative breeders, and suggest that in such species enhanced investment across multiple tasks may be more important than enhanced efficiency of different tasks.

Breeders invested more in both tasks than subordinates in purple-crowned fairy-wrens: they almost always defended the brood and invested more in offspring provisioning compared to subordinates (Fig. 2). Most likely this reflects large and universal benefits obtained from the brood for breeders; the near-absence of extra-pair paternity in this species means that 98% of breeders are full parents [31], whereas subordinates are on average less related to the brood due to breeder turnover [30]. The positive correlation between provisioning and defence in subordinates indicates that subordinates vary in overall effort, with some consistently contributing more to all activities (as in meerkats [14,46,50] and banded mongooses [47], but not superb fairy-wrens, *Malurus cyaneus* [51]). Presumably, this reflects variation in individual benefits obtained from helping, such as territory inheritance and indirect fitness benefits, which can vary greatly between individuals in this species [30,52]. This explanation is further supported by our finding that nestling provisioning rates by male and female breeders are similar, whereas among subordinates, males provision more than females: male subordinates have greater prospects of breeding position inheritance [30]. Future analyses will aim to reveal whether and how individual variation in benefits obtained from helping may indeed explain differences in overall cooperativeness.

Our results showing lack of task specialisation are reassuring for our understanding of the evolution of cooperative breeding in birds, which currently is often based on studies on offspring provisioning alone. We encourage further research on the potential for task specialisation in other cooperative birds, and cooperatively breeding vertebrates in general, and also highlight that other costly, important tasks, for example those associated with territory defence, should be assessed. Moreover, whether task specialisation occurs may also depend on species-specific relative magnitude of benefits of each task, efficiency benefits of cooperation (which can be associated with differences in morphology, e.g. related to age [11,14]), and degree of within-group conflict [15], which can become clear once multiple studies have been conducted on different species.

Such research will aid in our understanding of individual variation in various forms of helping, and may indicate whether conclusions on the evolution of cooperative breeding drawn from studies on a single aspect of helping only are reliable.

Ethics. Research was approved by the Monash University Animal Ethics Committee (BSCI/2015/11), Western Australian Department of Environment and Conservation (U29/2015-2018; BB003658), the Australian Bird and Bat Banding Scheme (ABBBS, license number 2230 to A.P.), and Australian Wildlife Conservancy (AWC).

Data accessibility. The dataset supporting this article are available in the supplementary material.

Competing interests. We have no competing interests.

Authors' contributions. N.T. and A.P. conceived study, with contributions from S.A.K.; N.T. collected and analysed data; N.T. wrote manuscript, with input from A.P. and S.A.K.

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Supplementary material for Chapter 3

Supplementary tables

Table S1. Results for the analysis of relative nestling provisioning effort by focal individual purple-crowned fairy-wrens. The analysis was run using a generalised linear mixed model with the proportion of feeds by the focal subordinate relative to the total number of feeds by the group (using the c-bind function) as binomial response variable. Note that the results are similar to those for absolute provisioning effort (Table 1 in the main article). Significant terms are highlighted in bold.

Parameter	β	SE	z value	P
Intercept	-0.98	0.37	-2.66	0.01
Nest defence effort	0.17	0.07	2.43	0.02
Social status ^a	-3.28	0.61	-5.38	<0.01
Sex ^b	-0.03	0.13	-0.20	0.84
Nestling age	0.00	0.03	0.07	0.95
Brood size	0.02	0.08	0.26	0.80
Group size	-0.09	0.04	-2.17	0.03
Nest defence effort x Status (subordinate)	0.62	0.20	3.17	<0.01
Sex (male) x Status (subordinate)	0.85	0.48	1.77	0.08

^a subordinate compared to breeder

^b male compared to female

Table S2. Results for the analysis of nestling provisioning effort (feeds/hr) by individual purple-crowned fairy-wrens according to the nest defence response to each predator model separately: goanna (nest predator), goshawk (adult and nest predator), and cuckoo (brood parasite). A separate generalised linear mixed model was run for each predator model using presence versus absence of nest defence behaviour to the model. Note the generally similar effects of nest defence effort, sex, social status, and their interactions as for the analysis on nest defence response to all predator models combined (Table 1 in main article). Significant terms are highlighted in bold.

	<i>Goanna</i>				<i>Goshawk</i>				<i>Cuckoo</i>			
Parameter	β	SE	z value	P	β	SE	z value	P	β	SE	z value	P
Intercept	0.53	0.45	1.19	0.23	0.89	0.41	2.18	0.03	0.70	0.37	1.87	0.06
Nest defence effort ^a	0.32	0.22	1.43	0.15	-0.02	0.21	-0.09	0.93	0.24	0.18	1.36	0.17
Social status ^b	-2.58	0.53	-4.92	<0.01	-3.40	0.66	-5.12	<0.01	-3.22	0.58	-5.53	<0.01
Sex ^c	-0.03	0.17	-0.19	0.85	-0.01	0.18	-0.07	0.94	0.01	0.17	0.09	0.93
Nestling age	0.08	0.03	2.55	0.01	0.08	0.03	2.54	0.01	0.07	0.03	2.42	0.02
Brood size	0.13	0.10	1.22	0.22	0.09	0.09	0.92	0.36	0.16	0.09	1.78	0.08
Group size	-0.02	0.06	-0.40	0.69	-0.02	0.06	-0.41	0.68	-0.07	0.05	-1.37	0.17
Nest defence effort x Status (subordinate)	0.55	0.41	1.33	0.18	1.32	0.53	2.47	0.01	1.26	0.40	3.12	<0.01
Sex (male) x Status (subordinate)	1.04	0.52	2.00	0.045	1.04	0.55	1.90	0.06	1.29	0.52	2.47	0.01

^a presence compared to absence of nest defence behaviour

^b subordinate compared to breeder

^c male compared to female

Table S3. Results for the analysis of nestling provisioning effort (feeds/hr) by individual purple-crowned fairy-wrens according to nest defence effort controlling for the likelihood of detecting the predator model. A generalised linear mixed model was run with nest defence effort as the proportion of predator trials in which an individual showed defence behaviour relative to the total number of predator trials where the bird was able to detect the model (i.e. seen within visible distance of model and/or group members producing alarm calls). Note the similar effects of nest defence effort, sex, social status, and their interactions as for the analysis on nest defence effort uncorrected for model detection (Table 1 in main article), indicating that the positive correlation between individual nest defence and nestling provisioning effort is unlikely a result of birds that feed more being more likely to detect a predator. Significant terms are highlighted in bold.

Parameter	β	SE	z value	P
Intercept	0.62	0.42	1.49	0.14
Nest defence effort*	0.26	0.30	0.86	0.39
Social status ^a	-3.51	0.68	-5.16	<0.01
Sex ^b	-0.01	0.16	-0.06	0.95
Nestling age	0.08	0.03	2.58	<0.01
Brood size	0.09	0.09	1.00	0.32
Group size	-0.01	0.06	-0.21	0.83
Nest defence effort x Status (subordinate)	1.92	0.66	2.89	<0.01
Sex (male) x Status (subordinate)	0.85	0.53	1.61	0.11

* proportion of trials with nest defence behaviour, relative to total models detected

^a subordinate compared to breeder

^b male compared to female

Chapter 4

Predator defence and cooperative breeding: helpers defend to raise future helpers and to gain benefits of group living

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Abstract

In cooperative breeders, helpers may cooperate to obtain benefits of group living and of helping raise offspring. However, group-living benefits are often not explicitly considered, and most studies focus on offspring provisioning only, particularly for cooperative birds, which limits our understanding of the evolution of cooperative breeding. Predator defence in particular may not only enhance reproductive success but also survival of group members. Therefore, studying cooperative predator defence allows to tease apart benefits of helping raise offspring from group-living benefits. We use this approach to test what benefits drive nest defence and adult group member defence, respectively, by subordinate purple-crowned fairy-wrens, *Malurus coronatus*. We recorded individual defence behaviour to predator models presented near the nest, posing differing levels of threat to nests and adults. We show that nest defence effort did not increase when broods were more related (kin selection), when groups were smaller (passive group augmentation), or when potential future mates were observing defence effort (social prestige). Instead, subordinate investment in nest defence was greater when chances of territory inheritance were higher, suggesting that nest defence is driven by the benefits of raising future helpers (active group augmentation theory). Defence effort for adult group members depended on the type of individuals under direct threat, with subordinates mainly protecting kin and potential mates, individuals that offer multiple benefits of group living and mutualistic social bonds. Thus, predator defence by subordinates in this cooperative fairy-wren is driven by benefits of producing additional recruits that might help in the future *and* benefits of group living. Our

findings highlight the value of explicitly considering the social environment in analyses of helping behaviour, and how helping effort relates to the full suite of proposed benefits of cooperation, to gain a complete picture of the complexity of social systems.

Keywords: social context, territory inheritance, anti-predator behaviour, predation, sociality, alloparental care

Introduction

Cooperative breeding is a model system to study the evolutionary maintenance of cooperation in general [1-5]. In cooperatively breeding species, two types of benefits may drive individuals to stay in a social group as a non-breeding subordinate, and engage in social cooperation with group members: benefits from group living itself, and benefits associated with helping raise offspring of the dominant breeding pair [6-9]. Although the proximate and ultimate drivers of cooperative offspring care have received considerable attention (e.g. [10]), our understanding of benefits of cooperative breeding deriving from group living is still somewhat limited. While group-living benefits depend on the type of group members helpers share a group with (e.g. only relatives offer parental nepotism or kin-selected benefits, only potential mates offer current or future reproductive benefits; [9, 11-16]), social group composition is not often considered in studies on helping behaviour. Moreover, tests of specific hypotheses have focused mainly on testing whether offspring provisioning by helpers is related to potential benefits of helping with reproduction [2, 3, 7, 17]. As a result, the relative importance of group living benefits in addition to benefits of help with reproduction has generally not been explicitly assessed in the context of the evolutionary maintenance of cooperation. However, encompassing the entire suite of adaptive benefits driving cooperative breeding is important in order to develop a complete picture of the complexity of cooperative social systems.

Helpers can assist with various cooperative tasks [6, 7, 18, 19], and predator defence may be a particularly important form of cooperation and promote group living [20-23]. Predation is a major cause of adult mortality and the primary cause of nest failure in most cooperatively breeding birds [24-26]. Consequently, cooperative defence against predators can increase survival of group members [8, 22, 27, 28], and investment in their defence likely reflects benefits of group living. Additionally, cooperative nest defence increases reproductive success [18, 24, 29-31], possibly to a greater extent than the provision of food to offspring, and should be driven by benefits of helping raise offspring. Therefore, studying helper investment in predator defence would allow to explicitly take group-living benefits into account when assessing drivers of cooperative behaviour, and importantly, to tease those apart from benefits associated with helping raise offspring in the group.

To address this, here, we provide a systematic test of the relative importance of both types of benefits in a cooperative bird. To do so, we presented models representing a threat to nests, a threat to nests and adults, and a control, at nests of purple-crowned fairy-wrens (*Malurus coronatus*). This experimental approach combined with existing variation in the social

environment (groups often consist of a mix of related and unrelated individuals of both sexes; [32-34]), and thereby group-living benefits, allows us to test whether subordinates' investment in predator defence depends on group-living benefits associated with group members that are under direct threat of death or injury, and to tease this apart from benefits associated with defending the brood.

We systematically test the full suite of hypotheses proposed for the evolutionary maintenance of helping behaviour (see Table 1 for full overview of predictions). First, if subordinates engage in brood defence for indirect fitness benefits associated with raising closely related individuals (kin selection theory; [3-5, 7, 35]), they will defend more when they are more related to the brood. Second, subordinates may use brood defence to advertise quality and increase their chances of finding a mate in the group (social prestige hypothesis; [3, 36, 37]). Subordinates form stable queues for breeding position inheritance on the territory [33], ruling out the potential for defence to signal quality to opposite-sex breeders. However, since subordinates may sometimes pair with an unrelated opposite-sex subordinate within the group and bud off to form a new territory [38], investment in nest defence may serve as a signal of quality to subordinate potential mates. Third, subordinates may receive direct benefits from increasing group size. This can be passive, through a positive effect of additional recruits on helper survival, e.g. due to mutualistic benefits like safety-in-numbers or dilution effects (passive group augmentation). It can also be active benefits obtained from recruits such as reciprocal interactions or the provision of help by recruits when the helper later obtains the breeding position (active group augmentation) [3, 7, 17, 39]. Finally, in purple-crowned fairy-wrens, non-cooperative individuals are not punished or evicted [32, 33], indicating that help does not serve as payment of rent (pay-to-stay hypothesis; [3, 40]). Furthermore, helpers rarely sire offspring or lay eggs (1.8% of offspring; [32, 33, 41]), making direct access to reproduction an unlikely driver of helping behaviour in this species (parentage acquisition hypothesis; [7, 42, 43]), therefore these hypotheses were ruled out to explain the evolutionary maintenance of helping in this population of fairy-wrens.

Group composition affects benefits of group living in this population; subordinates form close social bonds with kin and potential mates (unrelated opposite-sex individuals) in their group [34], which may offer group living benefits through parental nepotism (e.g. enhanced access to food and predator protection; [11-13, 16, 44]), kin-selected benefits [4, 7, 35], and potential mate acquisition, either through breeding position inheritance or by pairing with an unrelated subordinate within the group to form a new territory (20% and 7% of subordinates in our study

population gain their first breeding position this way, respectively; [38]) [2, 9, 14-16]. We therefore predict subordinates to invest more in adult group member defence when kin and potential mates (dominant or subordinate) are under threat, if they engage in predator defence to protect those group members they share mutualistic social bonds with (as in crested macaques, *Macaca nigra*; [45]) (see Table 1). Alternatively, if group living benefits include reduced predation in larger groups (passive group augmentation) [13, 15, 17, 39], subordinates should invest more in predator defence when more group members are under threat, regardless of what type of group members they are protecting [46] (Table 1). Here, we use an integrative framework to simultaneously test the importance of the kin selection, social prestige and group augmentation hypotheses for subordinate investment in nest defence, and the mutualistic social bond and passive group augmentation hypotheses for investment in adult group member defence.

Table 1. Predictions for individual subordinates' likelihood to engage in nest defence and adult group member defence. Predictions are based on the main hypotheses for explaining helping behaviour, and benefits of group living, respectively. Predictions specific for purple-crowned fairy-wrens are given, and whether these were supported in this study.

Nest defence (all models; irrespective of whether or not group member present)		
Hypothesis	Predictions in PCFW:	Supported?
Kin selection	Defence increases with relatedness to the brood	No (Fig. 1; Table S1)
	Subordinates do not defend unrelated broods	No (Fig. 1; Table S1)
Social prestige	Defence increases when subordinate potential mate is present	No (Table S1)
Passive group augmentation	Defence decreases with group size ^a	No ^b (Table 3; Table S1)
Active group augmentation	Defence increases with probability of breeding position inheritance	Yes (Fig. 2; Table 3)
Adult group member defence (goshawk; when group member present)		
Hypothesis	Predictions in PCFW:	Supported?
Mutualistic social bond	Defence increases when kin or a potential mate are present	Yes (Fig. 3b; Fig. 4b; Table 4)
Passive group augmentation	Defence increases with greater number of group members present	No (Table S2)

^a Due to diminishing returns of additional group members [39].

^b Defence only decreased with group size if the probability of breeding position inheritance - a better predictor of nest defence - was not accounted for.

Methods

Study site and species

Purple-crowned fairy-wrens are riparian habitat specialists endemic to the wet-dry tropics of northern Australia [47, 48]. Territories are stable year-round, and groups consist of a socially monogamous breeding pair and often one or more subordinates [32, 33, 47]. Social status can reliably be assigned through duetting behaviour, which only the dominant breeding pair engages in [49]. Subordinates of both sexes can assist the breeding pair to raise offspring, and vary in relatedness to the brood due to breeder turnover and subordinate dispersal [33]. Breeding can take place year-round but most breeding occurs in the monsoonal wet season (December – March) [32, 47]. Only the breeding female builds nests and incubates eggs but all group members can help with nest defence and offspring provisioning.

We studied a colour-banded population of approximately 300 purple-crowned fairy-wrens, monitored since 2005 at Australian Wildlife Conservancy's Mornington Wildlife Sanctuary in northwest Australia (S17°31' E126°6'). From 2012 to 2018, the population was monitored in two visits per year (May/June and November), where group membership and social status was documented for each individual, and all unbanded birds (new offspring and immigrants) were banded. From 2016 to 2018, 50 groups in the population were additionally monitored during the main breeding season. Breeding activity was monitored during regular visits (at least once per week) by following breeder females. All nests found were checked regularly to determine laying date and number of nestlings. *M. coronatus* usually lay 3 or 4 eggs (range 1-5, mean $\pm$ SE = 3 ± 0.03), and the nestling period lasts for approximately 13 days ([32]; personal observations). Nest predation is the main source of nest failure for this population (57% of nests are depredated; [50]), and most commonly occurs by varanid lizards (goannas; 61% of 31 observed predation events), avian predators (16%), and snakes (10%) (unpublished data).

Model presentations

We quantified individual predator defence effort for 37 subordinates (25 males, 12 females) by presenting models of three common threats and a control at fairy-wren nests, representing:

- 1) A nest predator (goanna); a plastic model of approximately 50 cm in length from head to tail, painted to represent a juvenile Merten's water monitor (*Varanus mertensi*). Reflectance spectra and psychophysical models of avian vision [51] were used to confirm the model displayed natural colours.

- 2) A predator of nests and adults (goshawk); a taxidermied brown goshawk (*Accipiter fasciatus*).
- 3) A brood parasite (cuckoo); a taxidermied shining bronze-cuckoo (*Chrysococcyx lucidus*). While absent from our study site, this species is morphologically similar to the Horsfield's bronze-cuckoo (*C. basalis*), which occasionally parasitises purple-crowned fairy-wren nests at our study site [52], and defence responses to both species are similar for other fairy-wrens [53].
- 4) A control; a taxidermied peaceful dove (*Geopelia striata*), a common species at our study site presenting no threat to adult fairy-wrens or nests.

Taxidermied models were mounted in a natural posture on a short stick. For each experiment, all four model types were presented at the nest, one at a time, over the course of two days, with two presentations per day. Two exemplars of each model type were used and switched between trials. To control for any order effects, models were presented in a different order for each experiment, in a balanced design of model sequences. Experiments were conducted in February-April 2017, between 6:00 am and 12:30 pm during calm, dry weather. Experiments were conducted during the early incubation stage (mean no. days since last egg laid at first trial $\pm$ SE = 3.3 ± 0.5) at 19 nests, and during the nestling stage (mean no. days since hatching at first trial $\pm$ SE = 5.2 ± 0.2) at 12 nests; at 10 of these, experiments were conducted during both the incubation and the nestling stage.

Prior to the start of the first trial of the day, a camouflage hide was set up approximately 10m from the nest, and a cage constructed out of mistnet tightly strung around a 25 x 25 x 60 cm wooden frame placed 1m from the nest, at nest height. Both were left at least 30 min to allow the birds to get accustomed. At the start of the trial, when no fairy-wrens were within visible distance, the taxidermied mount was placed inside the cage (to protect it from damage by fairy-wren attacks), or the goanna model was placed 20cm from the nest facing the nest entrance and attached to the vegetation with black wire; representing how avian and reptilian predators, respectively, naturally approach nests, with their respective differences in mobility resulting in similar immediate risk to the nest [54]. The trial lasted for 10 min from the moment the first fairy-wren approached the model to within 2m or started producing alarm calls. From the camouflage hide, one observer (N.T.) recorded for each bird in the focal group its response to the model, including the closest approach to the model, the time spent within 2m of the model, and the number of alarm calls given off. The model was then removed and the next trial started after an interval of at least 90 min, to minimise carry-over effects.

Relatedness and breeding position inheritance

We defined relatedness of subordinates to the breeding pair, and thus to the brood, using social pedigree data. Birds probably infer relatedness through associative learning [55], and they are thus likely unaware of any discrepancies between social and genetic relatedness resulting from extra-pair mating, which is very rare (4.4% of offspring; [41]) in our population anyway. Individuals were classified as 'related' if they were first-order relatives only (full sibling, parent-offspring). Subordinates were classified as full siblings to the brood (predicted relatedness to the brood = 0.50; related to both breeders), half-siblings or aunts/uncles ($r = 0.25$; related to one breeder only), or unrelated to the brood and breeders ($r = 0$).

Previous research on this population established that the main determinants of breeding position inheritance prospects are subordinates' sex, relatedness to the opposite-sex breeder, and the presence of an older same-sex subordinate in the group [33]. Following a similar approach to [32], we quantified the proportion of subordinates that inherit the breeding position when a vacancy opens up on the territory (i.e. the same-sex breeder dies or disperses), for a recent study period (November 2012 – November 2018) according to the same parameters (subordinates' sex, relatedness to the opposite-sex breeder, and the presence of an older same-sex subordinate). The calculated proportions were used as the "probability of breeding position inheritance" for analyses of nest defence (for a detailed description of calculations and findings for breeding position inheritance, see Supplementary material I).

Statistical analyses

All analyses were performed in R 3.4.4 [56]. Individuals were considered to engage in predator defence if they produced alarm calls, approached the model to <2m but did not go to the nest to incubate, feed or brood, and/or approached to 2-5m and stayed intently focused on the model. Birds exhibiting normal nest attendance behaviour or simply foraging nearby were thus not considered to respond to the model; approaches to the nest were considered a predator defence response if birds aborted their nest visit (i.e. did not bring prey item to nest) instead directing their attention to the model. Only trials where the focal subordinate would have been able to detect the model (i.e. the bird was seen within visible distance of the model during the trial and/or group members produced alarm calls that may have alerted it to its presence) were included in analyses ($N = 163$ of 255 cases). Subordinates engaged in defence behaviour in 45% of predator model trials but only very rarely (5%) in control trials. Control model presentations were therefore excluded from analyses ($N = 20$ of 163 cases). One group contained one unbanded subordinate at

the time of the experiment; this individual was excluded from analyses since we could not assign parentage or probability of breeding position inheritance for this bird. All observed subordinates were old enough to participate in predator defence; the youngest observed subordinate was 146 days old and engaged in predator defence.

Since subordinates rarely approached <2m of the model (20% of trials) or produced alarm calls (13%), we tested our predictions (Table 1) by analysing the likelihood of focal subordinates to engage in any defence behaviour. First, we tested all hypotheses proposed for why subordinates engage in nest defence, namely to gain (i) kin-selected benefits, (ii) social prestige and/or (iii) passive or (iv) active group augmentation benefits (Table 1). We quantified (i) kin-selected benefits of helping at the nest as focal subordinates' relatedness to the brood (as categorical variable; $r = 0$, $r = 0.25$, or $r = 0.5$). (ii) Regarding social prestige, since breeding position inheritance queues are stable, nest defence would only serve to advertise quality to subordinate, rather than dominant, potential mates (i.e. unrelated opposite-sex subordinates). Therefore, we tested whether the likelihood to defend in a trial was greater when there was an unrelated opposite sex subordinate present nearby (i.e. alarming or <5m of the model). Furthermore, we quantified (iii) passive group augmentation benefits as group size (with smaller group size indicating a greater potential increase in benefits, due to diminishing returns of additional group members; [39]), and (iv) active group augmentation benefits as focal subordinates' probability to inherit a breeding position in the group (since only subordinates that eventually become breeders in the group will benefit from future help by new recruits). To test predictions of all four hypotheses, we constructed and compared (using AICc) seven GLMMs, including the presence/absence of predator defence behaviour as binomial response variable and as independent variable any possible combination of main effects of relatedness to the brood, the presence of a subordinate potential mate nearby, and probability of breeding position inheritance, both in isolation and in concert. Group size was included in all models as it not only reflects potential benefits of passive group augmentation, but is also necessary to control for potential load-lightening effects in larger groups [57-59], which may affect nest defence effort overall. Additional variables that are expected to affect nest defence effort were also included in all models: sex (male, female), age (first-year, older), brood size, nest stage (eggs, nestlings), predator type (goanna, goshawk, cuckoo), trial no. (1-4), and time of day (hr). Bird ID, nest ID, and exemplar (nested within predator type) were included as random effects to account for exemplar effects and replication across individuals and nests. Only the statistical model that best explains subordinates' likelihood to engage in nest defence is presented in the results section ($\Delta AICc \geq 2.1$

compared to all other models), but the full results of all seven models and corresponding AICc values are given in the supplementary material (Table S2).

Although group size is negatively correlated with the probability of breeding position inheritance (LM: $R = 0.55$, $t = -7.84$, $P < 0.01$), both variables were included in models since for models without inheritance (Table S2, model 1, 2 and 4), this yielded a better fit to the data compared to the same models excluding group size ($\Delta\text{AICc} = 4.3, 4.4$ and 5.7 , respectively), while excluding group size yielded a non-significantly better fit for models including inheritance (Table S2, model 3, 5, 6 and 7; $\Delta\text{AICc} = 0.9, 0.9, 0.4$ and 0.4 , respectively). When including one of the two variables only in the final ‘best’ model (Table 3; Table S2, model 3), the model including the probability of breeding position inheritance was better than the same model including group size only ($\text{AICc} = 176.3$ vs 179.9 , respectively), and excluding group size from the model increased the effect size and significance of the probability of breeding position inheritance ($\beta \pm \text{SE} = 1.99 \pm 0.61$, $P < 0.01$ vs $\beta \pm \text{SE} = 1.56 \pm 0.69$, $P = 0.02$).

Secondly, we tested whether subordinates engage in adult group member defence to protect individuals that provide mutualistic social bonds, i.e. kin and potential mates, or to maintain larger group size, i.e. protecting group members in general (Table 1). This can only be tested by taking into account which individuals were directly present at the predator model. Hence, we determined for each focal subordinate whether kin group members (i.e. first-order relatives) and potential mates were present near the model during each trial (i.e. approached to $<5\text{m}$ and stayed, focused on the model, or produced alarm calls, thus alerting the predator to their presence). Potential mates were defined as any unrelated opposite-sex subordinates, and unrelated opposite-sex dominants only if no older same-sex subordinates were present in the group (since the focal subordinate will generally not inherit a breeding position in this case; Table S1; [33]). Additionally, we determined the overall number of group members present near the model ($\text{mean} \pm \text{SE} = 2.1 \pm 0.1$, range = 0-5). Since group members are only under threat of death or injury during goshawk model presentations – the only predator of adult birds presented at nests – we tested predictions of our two hypotheses (Table 1) by testing for an interaction between predator type and the presence of kin, potential mates, or group members in general, respectively, at the model. Similar to the analyses for nest defence described above, we constructed and compared seven GLMMs, including any possible combination of the interaction effect of predator type and: the presence/absence of kin, number of group members, and presence/absence of potential mates at the model. All models included the same covariates and random effects as described previously. Again, only the statistical model that best explains

subordinates' likelihood to engage in group member defence is presented in the results section ($\Delta\text{AICc} = 1.3$ compared to the next-best model, $\Delta\text{AICc} \geq 3.9$ compared to all other models), but full results of all models are given in the supplementary material (Table S3).

Lastly, we constructed one final GLMM to confirm that our findings for increased defence when potential mates and kin are under threat (Table 3) truly reflect active protection of such group members and not overall increased investment in defence when such individuals are present in the group. This model was similar to the model that best explained subordinate investment in group member defence, including the interaction between predator type and the presence of kin, and potential mates at the model, respectively (Table 3; Table S3, model 5), except the presence of kin and potential mates at the model was replaced by the presence of kin and potential mates in the group (regardless of whether they were near the predator model) (Table S4).

Results

Nest defence

Subordinates engaged in predator defence in 45% (64 of 143) of predator model presentations. Of models testing the predictions of hypotheses explaining subordinate investment in nest defence, the model including the probability of breeding position inheritance only yielded the best fit to the data ($\Delta\text{AICc} \geq 2.1$; Table 2; Table S2); subordinates were more likely to engage in nest defence with increasing prospects for breeding position inheritance ($\beta \pm \text{SE} = 1.56 \pm 0.69$, $z = 2.25$, $P = 0.02$; Table 2; Fig. 2). In contrast, likelihood of nest defence did not vary with subordinates' relatedness to the brood ($P \geq 0.08$; Table S2; Fig. 1), nor did it increase when subordinate potential mates were present to advertise quality to ($P \geq 0.72$; Table S2). Likelihood of nest defence was only negatively correlated to group size when probability of breeding position inheritance was unaccounted for (Table S2). This reflects a negative correlation between group size and breeding position inheritance prospects, rather than a direct effect of group size on nest defence effort, as breeding position inheritance was a better predictor of nest defence ($\Delta\text{AICc} = 3.6$; see *Statistical analyses*).

Table 2. Results of the statistical model that best explains subordinates' likelihood to engage in nest defence (AICc = 177.2, compared to all other models $\Delta\text{AICc} \geq 2.1$), including benefits obtained through breeding position inheritance only. Significant terms are highlighted in bold.

	Effect size $\pm$ SE	<i>P</i>
<i>Intercept</i>	-4.37 $\pm$ 2.15	0.04
<i>Probability of breeding position inheritance</i>	1.56 $\pm$ 0.69	0.02
<i>Sex^a</i>	0.87 $\pm$ 0.45	0.055
<i>Subordinate age^b</i>	-0.10 $\pm$ 0.58	0.86
<i>Group size</i>	-0.24 $\pm$ 0.19	0.21
<i>Brood size</i>	-0.36 $\pm$ 0.36	0.32
<i>Nest stage^c</i>	1.21 $\pm$ 0.46	<0.01
<i>Predator type (goanna)^d</i>	0.35 $\pm$ 0.55	0.53
<i>Predator type (goshawk)^d</i>	1.72 $\pm$ 0.57	<0.01
<i>Trial no.</i>	0.42 $\pm$ 0.20	0.04
<i>Time of day</i>	0.32 $\pm$ 0.15	0.03

^a male relative to female

^b older relative to first-year

^c nestling relative to egg stage

^d relative to cuckoo model

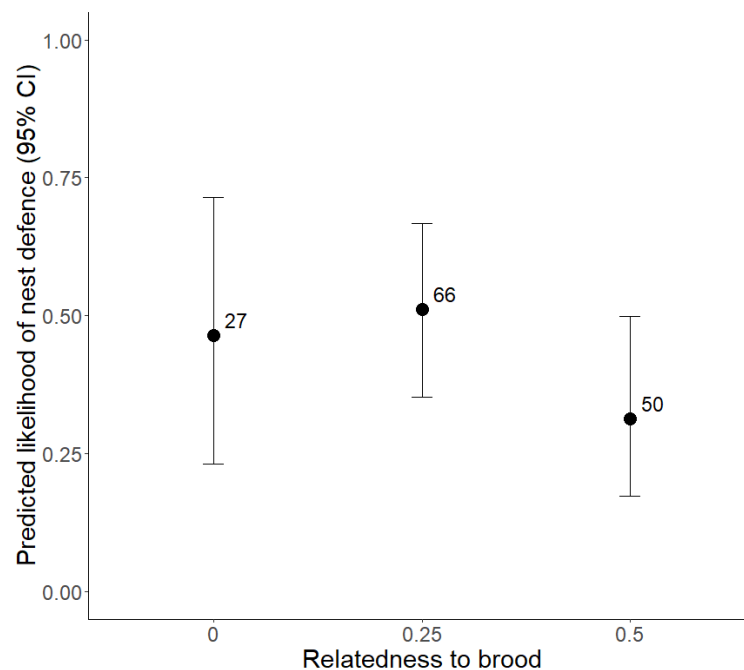


Figure 1. Investment in brood defence is unrelated to kin-selected benefits: subordinate purple-crowned fairy-wrens that are more closely related to the brood are not more likely to engage in nest defence. Shown is the effect of relatedness to the brood on the predicted likelihood of engaging in nest defence. Error bars indicate 95% confidence intervals, numbers next to predicted means indicate sample sizes (number of predator model presentations for which responses were recorded for subordinates of given relatedness to the brood).

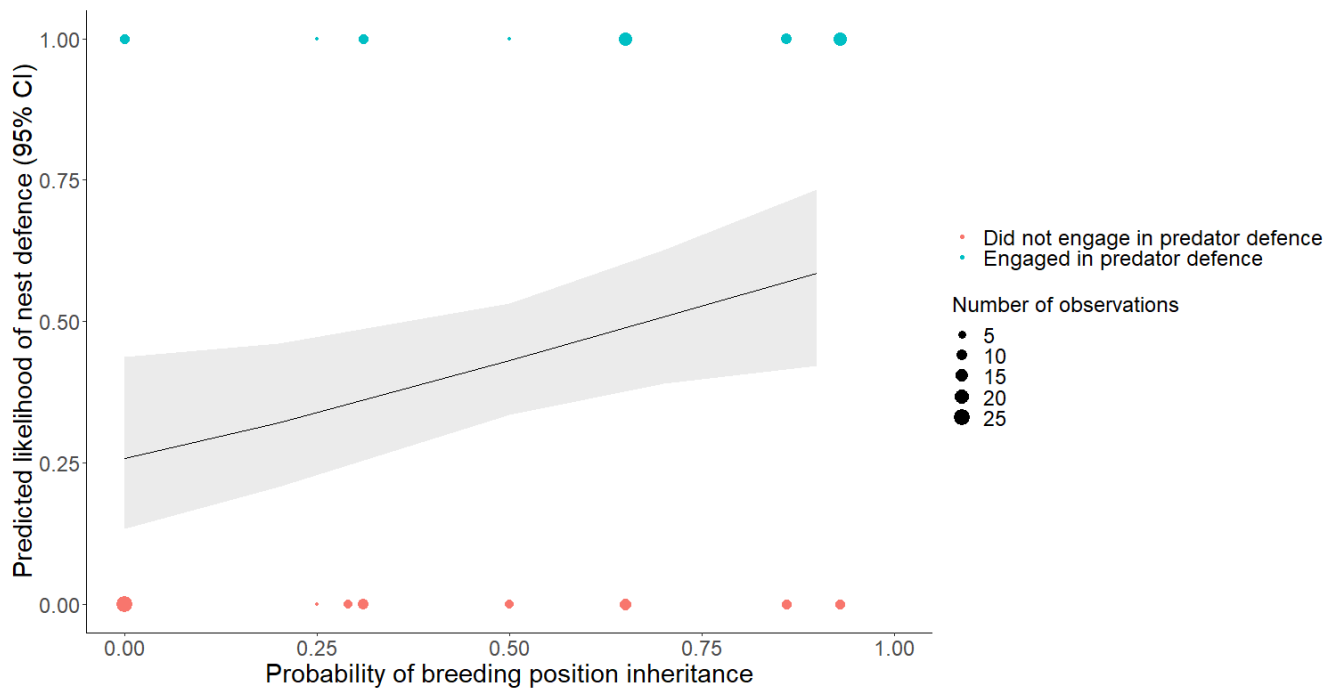


Figure 2. The likelihood of subordinate purple-crowned fairy-wrens to engage in predator defence increases with increasing probability to inherit a breeding position on the territory. The line represents predicted values, confidence bands indicate 95% confidence intervals. Dots depict the observations of subordinates that did and did not engage in predator defence, area of dots indicates the number of observations at each level of probability of inheritance.

Adult group member defence

The model including the presence of kin and the presence of (a) potential mate(s) near the predator best explained subordinate investment in adult group member defence. While it was only marginally better than the same model excluding the presence of kin ($\Delta\text{AICc} = 1.3$; Table S3), it was significantly better compared to all other models ($\Delta\text{AICc} \geq 3.9$; Table S3), and the presence of kin significantly affected subordinates' likelihood to engage in predator defence, in interaction with predator type; subordinates were about 1.5-2 times more likely to defend when kin was present at the goshawk model, compared to when they were not, and compared to goanna and cuckoo model presentations ($\beta \pm \text{SE} = 1.75 \pm 1.56$, $P = 0.01$; Table 3; Fig. 3b), indicating greater investment in defence when kin is under direct threat. Similarly, the presence of a potential mate at the model and predator type had interacting effects on the likelihood of predator defence; subordinates were thrice as likely to engage in defence when a potential mate was present at the goshawk model and thus under direct threat ($\beta \pm \text{SE} = 3.31 \pm 1.45$, $P < 0.01$; Table 3; Fig. 4b). Importantly, the presence of kin or potential mates in the social group, as opposed to those present at the predator model, did not affect the likelihood of predator defence, neither in

isolation ($\beta \pm SE = -0.42 \pm 1.59$, $P = 0.37$ and $\beta \pm SE = -0.40 \pm 0.91$, $P = 0.60$ for kin and potential mates, respectively; Table S4; Fig. 3a; 4a) nor in interaction with predator type (goshawk; $\beta \pm SE = 0.68 \pm 1.79$, $P = 0.22$ and $\beta \pm SE = -1.62 \pm 1.28$, $P = 0.43$ for kin and potential mates, respectively; Table S4; Fig. 3a; 4a), indicating that the observed increased investment in defence when kin or potential mates are present at the model reflects active protection of such group members. The number of group members present at the model in general, regardless of the types of group members, did not affect predator defence behaviour, neither in isolation ($P \geq 0.44$; Table S3), nor in interaction with predator type ($P \geq 0.12$; Table S3).

Table 3. Results of the statistical model that best explains subordinates' likelihood to engage in adult group member defence (AICc = 175.1), including the presence of kin and the presence of potential mates near the model in interaction with predator type. The model was marginally better than the model including the presence of potential mates only ($\Delta AICc = 1.3$), and significantly better than all other models ($\Delta AICc \geq 3.9$). Significant terms are highlighted in bold. P -values presented for main effects of terms that are also included in interaction effects were obtained from post-hoc Tukey's HSD tests controlling for interaction effects.

	Effect size ($\pm SE$)	P
<i>Intercept</i>	-3.02 ± 2.30	0.19
<i>Kin^a</i>	0.55 ± 0.95	0.48
<i>Kin (present) x Predator type (goanna)</i>	-2.09 ± 1.23	0.01
<i>Kin (present) x Predator type (goshawk)</i>	1.75 ± 1.56	
<i>Potential mate^a</i>	0.12 ± 0.86	0.11
<i>Potential mate (present) x Predator type (goanna)</i>	-0.97 ± 1.16	<0.01
<i>Potential mate (present) x Predator type (goshawk)</i>	3.31 ± 1.45	
<i>Sex^b</i>	0.54 ± 0.50	0.28
<i>Subordinate age^c</i>	-0.23 ± 0.62	0.71
<i>Group size</i>	-0.47 ± 0.19	0.01
<i>Brood size</i>	-0.23 ± 0.40	0.57
<i>Nest stage^d</i>	1.38 ± 0.52	<0.01
<i>Predator type (goanna)^e</i>	2.18 ± 1.35	0.57
<i>Predator type (goshawk)^e</i>	-1.15 ± 1.69	0.13
<i>Trial no.</i>	0.34 ± 0.21	0.11
<i>Time of day</i>	0.33 ± 0.17	0.052

^a present at the predator model relative to absent

^b male relative to female

^c older relative to first-year

^d nestling relative to egg stage

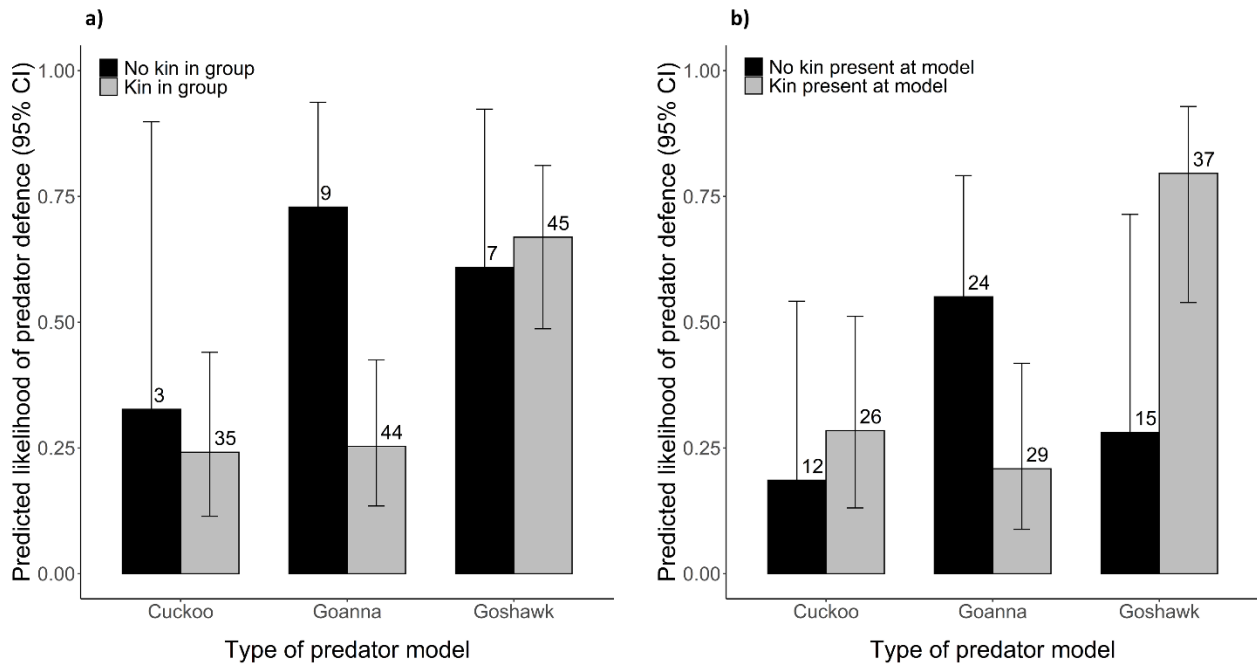


Figure 3. Subordinates defend more against predators of adults when kin are present. (a) The presence of kin in the social group does not affect the likelihood of subordinates to engage in predator defence, but (b) the presence of kin at the predator model and predator type have interacting effects on the likelihood of predator defence, with subordinates defending more often against goshawk models (a predator of adults) when kin is present. Predicted values are presented. Error bars indicate 95% confidence intervals, numbers above bars indicate sample sizes.

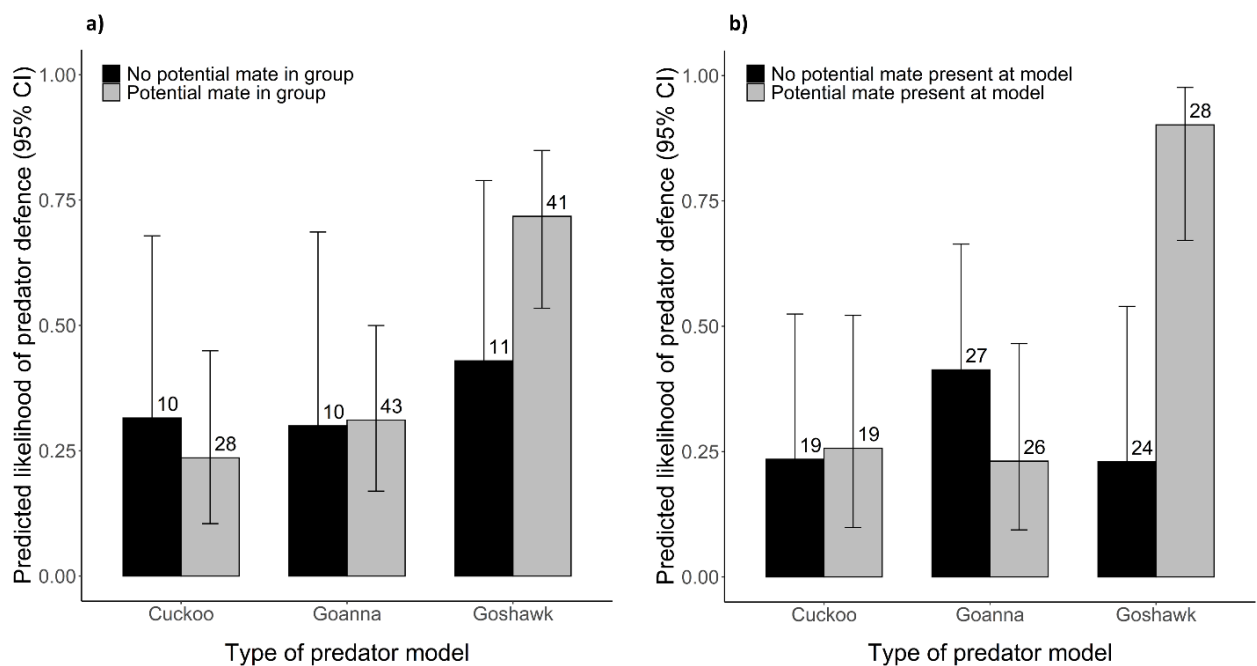


Figure 4. Subordinates defend more against predators of adults when a potential mate is present. (a) The presence of potential mates in the social group does not affect the likelihood of predator defence by subordinates, and had no interacting effect with predator type, but (b) the presence of potential mates at the predator model and predator type have interacting effects on the likelihood of subordinates to engage in predator defence, with subordinates defending more often against goshawks when a potential mate is present. Predicted values are presented. Error bars indicate 95% confidence intervals, numbers above bars indicate sample sizes.

Discussion

We provide a systematic test of the relative importance of indirect and direct benefits of helping at the nest as well as group living per se, in driving subordinate investment in predator defence in a cooperative breeder. We thereby take the full suite of proposed benefits of helping into consideration [8], for a form of help that is considered to be particularly important for reproductive success and adult survival and a key driver of group living in general [18, 20-23, 30], yet is rarely studied in this context. With our integrative approach, we show that nest defence by subordinates in *M. coronatus* is driven by direct benefits associated with territory inheritance, supporting active group augmentation theory, while group member defence is driven by benefits from protecting potential mates and kin group members specifically, supporting our proposed mutualistic social bond theory. Below, we discuss implications of our findings in turn.

Brood defence and benefits of helping raise offspring

Contrary to predictions from kin selection theory [3-5, 7, 35], subordinates did not invest more in nest defence when they shared a higher relatedness with the brood, and often helped defend unrelated broods. This is in contrast to empirical and comparative studies showing that kin selection explains variation in offspring provisioning effort by helpers within and across cooperatively breeding vertebrate species (e.g. [37, 58, 60-70]). It has become clear, however, that kin selection theory cannot provide a general explanation for helping behaviour: individuals are not always related to the offspring they help raise (e.g. [4, 31, 42, 43, 64, 66, 70-73]), and variation in relatedness only explains 10% of the observed variation in helping behaviour when no other, direct, benefits of helping are involved [2, 66].

Helping has been proposed to serve as a signal of quality (social prestige) to potential mates, although evidence is limited [36, 37, 74, 75]. Since predator defence is a high-risk activity, associated with significant costs such as energy expenditure, lost foraging opportunities, and risk of mortality and injury, it may serve as a reliable signal of quality, perhaps even more so than offspring provisioning [54, 76]. However, signalling quality does not seem to be an important driver of brood defence in our study species: subordinate purple-crowned fairy-wrens were no more likely to defend the nest when subordinate potential mates were present as potential targets of advertising quality. Possibly, predator defence may be more likely to serve to enhance social prestige in systems where subordinates do not form stable queues for breeding position inheritance, and they would thus also benefit from advertising their quality to dominant breeders,

or where individuals can disperse in coalitions, and subordinates can advertise to potential coalition members [77].

Although variation in nest defence effort by helpers was not explained by passive group augmentation benefits (direct effect of group size per se), it was strongly predicted by opportunity to inherit a breeding position in the resident territory (Fig. 2). While helping effort is predicted to increase with territory inheritance prospects by several hypotheses, active group augmentation theory provides the most likely explanation. Helpers are unlikely to increase defence effort in order to increase their chances of inheriting a breeding position, since inheritance queues are stable. And although helpers may be willing to pay more to stay when they have greater chances to eventually become a breeder in the group [3, 40], there is no evidence for punishment or eviction of non-helping subordinates in this species [33], a critical assumption of the pay-to-stay hypothesis. It has been hypothesised that sex differences in helping behaviour cannot easily be distinguished from inheritance effects [78] since the non-dispersing sex is more likely to inherit the resident territory and often helps more, at least with offspring provisioning [7, 70, 79]. This potential confound does not pose an issue for the interpretation of our findings however: males invested marginally more in nest defence than female helpers, but prospects of territory inheritance did not differ between the sexes (Supplementary material I). Instead, our results suggest that helpers are more willing to defend the nest when they are more likely to derive future benefits from recruits that may offer future help with reproduction or reciprocal interactions when the helper inherits the breeding position [3, 17, 39]. Our findings thereby support recent evidence from meta-analyses on the importance of direct benefits of breeding position inheritance in driving helping behaviour, even when controlling for sex differences in dispersal [2, 80].

Adult group member defence and benefits of group living

Increased protection from and defence against predators may enhance adult survival and promote the formation of social groups in group living species [20, 22, 81, 82]. However, we lack an understanding of what benefits of group living drive individual predator defence behaviour to protect adult group members. The number of group members present (i.e. at risk of getting injured or killed by the predator) did not affect defence behaviour by subordinate purple-crowned fairy-wrens, indicating that group member defence is not driven simply by benefits associated with living in larger groups [15, 17, 39, 46]. Possibly living in larger groups is not associated with significant benefits in this species, or there is an intermediate optimal group size. This is in

agreement with our observation that helpers in larger groups were less likely to engage in adult group member defence (regardless of number of group members under threat), suggesting that additional group members may indeed be associated with diminishing returns [39], or indicating load-lightening in larger groups [57]. However, likely this reflects increased defence effort by helpers with greater chances of breeding position inheritance, which is negatively correlated with group size but a better predictor of predator defence (see *Statistical analyses*).

The type of group members present at the dangerous predator presentation, and thus at risk, predicted defence effort: subordinates were more likely to engage in defence when kin or potential mates were near a goshawk. It could be argued that helpers may merely be alerting such group members to the presence of the predator, rather than actively defending them. Even if this is the case however, approaching the predator, even if at a distance, and alarm calling, is still a costly activity, associated with increased chance of being detected by the predator, and lost foraging and mating opportunities and energy expenditure [54, 76]. Engaging in defence to protect kin and potential mates may be hugely beneficial as these group members are associated with several benefits of group living: kin-selected benefits and benefits of parental nepotism for kin [11, 13, 16, 35], future reproductive benefits for potential mates [2, 14, 16, 38], and benefits from mutualistic social bonds for both [34]. The presence of potential mates in the group is expected to be particularly important, since breeding vacancies are rare and the cost of inbreeding high [83]; this may explain the greater likelihood of response when potential mates were under threat relative to kin. The few studies that have addressed predator defence in relation to the social environment suggest that predator defence may be greater in family groups [77, 84, 85]; but see [86], or when individuals share a close social bond [45]. Additional studies on the role of social group composition in driving predator defence could provide important insights into the function of predator defence and how it relates to benefits of group living.

Conclusion

We show that predator defence by subordinates in a cooperative fairy-wren is driven by benefits associated with helping raise offspring *and* benefits of group living. Our findings highlight the value of studying forms of helping other than the commonly studied offspring feeding for cooperative birds [2, 3, 7], and to take social group composition into account when studying helping behaviour. This approach allows to take the full suite of proposed benefits of cooperation into account [8], which is necessary for a comprehensive understanding of the complexity of cooperative systems.

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Supplementary material for Chapter 4

Supplementary material I: Probability of breeding position inheritance

Previous research on this population by Kingma et al. [1] established that the main determinants of breeding position inheritance prospects are subordinates' sex, relatedness to the opposite-sex breeder, and the presence of an older same-sex subordinate in the group. We used a similar approach to quantify the proportion of subordinates that inherited the breeding position when a vacancy opened up on the territory (i.e. the same-sex breeder died or dispersed), for all cases in a recent 6-year study period (November 2012 – November 2018).

Since most vacancies appeared in between field seasons, only subordinates that were present both before and after the changeover were included in calculations. Subordinates that dispersed away from the territory in between seasons were not included since we were unable to establish in these cases whether they were present on the territory when the vacancy appeared. While the youngest individual to have been observed taking up a breeder position over the entire study period was 92 days old, subordinates that were younger than this at the end of the field season prior to the vacancy were included in calculations ($N = 15$ of 83 subordinates; mean age $\pm$ SE = 61 ± 4 days), since a gap of ~4 months in between field seasons means they were more likely than not to be old enough to become a breeder at the time the vacancy appeared (and indeed did in 2 of 15 cases).

The calculated proportions (see Table S1) were used as the “probability of breeding position inheritance” for analyses of nest defence, except for a few cases where multiple same-sex subordinates were the oldest and of exactly the same age (from the same brood). Since male subordinates are about half as likely to inherit the breeding position when another male subordinate of the same age is present (0.30 compared to 0.65 when related to the opposite-sex breeder; based on 10 subordinates, 5 cases; Table S1), in one case where 3 male subordinates of the same age were present, the probability of breeding position inheritance was divided equally between them (since all unrelated to the opposite-sex breeder: $0.93/3 = 0.31$ for each). Since the probability of breeding position inheritance presented in Table S1 for females of the same age is based on only 2 cases, we used a similar approach for one case where 2 and one case where 3 females of the same age were present, dividing the presented probability of inheritance for the oldest female equally between them. Running the same analyses with an assigned probability of breeding position inheritance of 0 for these females instead yielded the same results. Our

datasets included no cases where a subordinate female was unrelated to the opposite-sex breeder and an older same-sex subordinate was present.

To statistically test whether the probability that a subordinate filled a breeding vacancy depended on its relatedness to the remaining opposite-sex breeder, its own sex, and the presence of older same-sex subordinates, we fitted a generalised linear mixed model (GLMM) with binomial distribution using the 'lme4' [2] and 'lmerTest' [3] packages. Sex, relatedness, and the presence of an older subordinate ('yes', 'no', 'same age') were included as independent variables, and territory ID was included as a random variable to control for repeated observations across territories. Post-hoc Tukey's Honest Significant Difference (HSD) tests were performed using the 'glht' function from the 'multcomp' package [4].

In 73% (40 of 55) of cases where the resident breeder disappeared, a subordinate in the group inherited the breeding position. Subordinates rarely inherited a vacant breeding position when older same-sex subordinates were present (1 of 21 cases). Subordinates were much more likely to inherit a breeding position when they were the oldest in the group compared to when older same-sex subordinates were present (Tukey's HSD: $\beta \pm SE = 4.28 \pm 1.14$, $z = 3.75$, $P < 0.01$), or those of the same age ($\beta \pm SE = 2.20 \pm 0.76$, $z = 2.90$, $P < 0.01$) (Table S1). Fewer subordinates inherited breeding positions when older compared to same-age same-sex subordinates were present (5% vs. 21%, respectively; Table S1), but this difference was not statistically significant ($\beta \pm SE = 2.08 \pm 1.26$, $z = 1.65$, $P = 0.22$). The probability of breeding position inheritance depended on subordinates' relatedness to the remaining opposite-sex breeder (Table S1): subordinates were more likely to fill a vacancy if they were unrelated to the opposite-sex breeder (GLMM: $\beta \pm SE = 1.65 \pm 0.70$, $z = 2.34$, $P = 0.02$). The probability of inheritance did not differ significantly between males and females (males relative to females: $\beta \pm SE = 0.82 \pm 0.65$, $z = 1.27$, $P = 0.20$; Table S1).

Table S1. The proportion of subordinates that inherited a vacant breeding position when the same-sex breeder disappeared, according to subordinate's sex, relatedness to the remaining opposite-sex breeder, and the presence of older same-sex subordinates in the group. In all cases where subordinates were the same age, they were the oldest in the group. The number of cases is shown in parentheses. There were no cases where male subordinates were of the same age and unrelated to the opposite-sex breeder.

Relatedness to opposite-sex breeder	Older subordinate present	Male	Female
Unrelated	Yes	0 (3)	1 (1)
	No	0.93 (15)	0.86 (7)
	Same age	-	0 (2)
Related	Yes	0 (15)	0 (2)
	No	0.65 (17)	0.50 (10)
	Same age	0.30 (10)	0 (2)

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Supplementary material II: Supplementary tables

Table S2. Results of the statistical models testing the hypotheses for subordinate's likelihood to engage in nest defence. Significant terms are highlighted in bold.

	1. Presence of subordinate potential mate AICc = 182.2		2. Relatedness to brood AICc = 179.3		3. Breeding position inheritance AICc = 177.2		4. Subordinate potential mate + Relatedness AICc = 181.8		5. Subordinate potential mate + Inheritance AICc = 179.6	
	Effect size (±SE)	P	Effect size (±SE)	P	Effect size (±SE)	P	Effect size (±SE)	P	Effect size (±SE)	P
Intercept	-2.80 ± 1.98	0.16	-2.27 ± 2.05	0.27	-4.37 ± 2.15	0.04	-2.22 ± 2.09	0.29	-4.43 ± 2.17	0.04
Presence of subordinate potential mate ^a	-0.18 ± 0.51	0.72					0.06 ± 0.53	0.91	-0.11 ± 0.52	0.84
Relatedness ($r = 0.25$) ^b			-0.02 ± 0.61	0.97			-0.02 ± 0.61	0.98		
Relatedness ($r = 0.5$) ^b			-1.10 ± 0.63	0.08			-1.11 ± 0.64	0.08		
Probability of breeding position inheritance					1.56 ± 0.69	0.02			1.55 ± 0.70	0.03
Sex ^c	0.73 ± 0.46	0.11	0.63 ± 0.49	0.20	0.87 ± 0.45	0.055	0.64 ± 0.49	0.20	0.85 ± 0.47	0.07
Subordinate age ^d	0.14 ± 0.56	0.81	-0.05 ± 0.60	0.93	-0.10 ± 0.58	0.86	-0.07 ± 0.61	0.91	-0.08 ± 0.60	0.90
Group size	-0.45 ± 0.17	<0.01	-0.44 ± 0.17	0.01	-0.24 ± 0.19	0.21	-0.45 ± 0.17	0.01	-0.24 ± 0.19	0.22
Brood size	-0.20 ± 0.34	0.56	-0.40 ± 0.36	0.26	-0.36 ± 0.36	0.32	-0.40 ± 0.36	0.26	-0.36 ± 0.36	0.31
Nest stage ^e	1.21 ± 0.46	<0.01	1.06 ± 0.46	0.02	1.21 ± 0.46	<0.01	1.04 ± 0.47	0.03	1.23 ± 0.47	<0.01
Predator type (goanna) ^f	0.34 ± 0.54	0.54	0.37 ± 0.54	0.50	0.35 ± 0.55	0.53	0.36 ± 0.55	0.51	0.36 ± 0.56	0.51
Predator type (goshawk) ^f	1.68 ± 0.57	<0.01	1.76 ± 0.58	<0.01	1.72 ± 0.57	<0.01	1.75 ± 0.59	<0.01	1.75 ± 0.59	<0.01
Trial no.	0.41 ± 0.20	0.04	0.38 ± 0.21	0.06	0.42 ± 0.20	0.04	0.38 ± 0.21	0.07	0.43 ± 0.21	0.04
Time of day	0.30 ± 0.14	0.04	0.38 ± 0.15	0.01	0.32 ± 0.15	0.03	0.38 ± 0.15	0.01	0.33 ± 0.15	0.03

^a present relative to absent

^b relative to unrelated subordinates ($r = 0$)

^c male relative to female

^d older relative to first-year

^e nestling relative to egg stage

^f relative to cuckoo model

	6. Relatedness + Inheritance <i>AICc</i> = 179.8		7. Subordinate potential mate + Relatedness + Inheritance <i>AICc</i> = 182.4	
	Effect size ($\pm$ SE)	<i>P</i>	Effect size ($\pm$ SE)	<i>P</i>
<i>Intercept</i>	-3.76 $\pm$ 2.34	0.11	-3.71 $\pm$ 2.39	0.12
<i>Presence of subordinate potential mate^a</i>			0.05 $\pm$ 0.53	0.92
<i>Relatedness (r = 0.25)^b</i>	0.19 $\pm$ 0.63	0.77	0.19 $\pm$ 0.63	0.77
<i>Relatedness (r = 0.5)^b</i>	-0.64 $\pm$ 0.72	0.37	-0.65 $\pm$ 0.73	0.37
<i>Probability of breeding position inheritance</i>	1.12 $\pm$ 0.79	0.16	1.12 $\pm$ 0.79	0.16
<i>Sex^c</i>	0.69 $\pm$ 0.49	0.16	0.70 $\pm$ 0.50	0.16
<i>Subordinate age^d</i>	-0.11 $\pm$ 0.61	0.85	-0.12 $\pm$ 0.62	0.84
<i>Group size</i>	-0.30 $\pm$ 0.20	0.14	-0.30 $\pm$ 0.20	0.14
<i>Brood size</i>	-0.44 $\pm$ 0.36	0.23	-0.44 $\pm$ 0.36	0.23
<i>Nest stage^e</i>	1.11 $\pm$ 0.46	0.02	1.10 $\pm$ 0.48	0.02
<i>Predator type (goanna)^f</i>	0.39 $\pm$ 0.55	0.49	0.38 $\pm$ 0.56	0.49
<i>Predator type (goshawk)^f</i>	1.78 $\pm$ 0.58	<0.01	1.77 $\pm$ 0.60	<0.01
<i>Trial no.</i>	0.39 $\pm$ 0.21	0.06	0.38 $\pm$ 0.21	0.07
<i>Time of day</i>	0.38 $\pm$ 0.15	0.01	0.38 $\pm$ 0.16	0.01

^a present relative to absent

^b relative to unrelated subordinates ($r = 0$)

^c male relative to female

^d older relative to first-year

^e nestling relative to egg stage

^f relative to cuckoo model

Table S3. Results of the statistical models testing the hypotheses for subordinate's likelihood to engage in adult group member defence. Significant terms are highlighted in bold. *P*-values presented for main effects of terms that are also included in interaction effects were obtained from post-hoc Tukey's HSD tests controlling for interaction effects.

	1. Kin <i>AICc</i> = 182.3		2. Group members <i>AICc</i> = 179.0		3. Potential mate <i>AICc</i> = 176.4		4. Kin + Group members <i>AICc</i> = 183.6	
	Effect size ($\pm$ SE)	<i>P</i>	Effect size ($\pm$ SE)	<i>P</i>	Effect size ($\pm$ SE)	<i>P</i>	Effect size ($\pm$ SE)	<i>P</i>
<i>Intercept</i>	-3.50 $\pm$ 2.12	0.10	-2.19 $\pm$ 2.24	0.33	-1.87 $\pm$ 2.07	0.36	-2.60 $\pm$ 2.23	0.24
<i>Kin</i> ^a	0.41 $\pm$ 0.90	1.00					0.26 $\pm$ 1.16	0.79
<i>Kin (present) x Predator type (goanna)</i>	-1.69 $\pm$ 1.12	0.08					-1.54 $\pm$ 1.34	0.22
<i>Kin (present) x Predator type (goshawk)</i>	0.45 $\pm$ 1.18						0.29 $\pm$ 1.39	
<i>No. group members present</i>			0.27 $\pm$ 0.41	0.51			0.18 $\pm$ 0.49	0.71
<i>No. group members present x Predator type (goanna)</i>			-0.36 $\pm$ 0.57				0.03 $\pm$ 0.67	
<i>No. group members present x Predator type (goshawk)</i>				0.12				0.37
<i>Potential mate</i> ^a					-0.06 $\pm$ 0.84	0.11		
<i>Potential mate (present) x Predator type (goanna)</i>					-0.17 $\pm$ 1.06	0.02		
<i>Potential mate (present) x Predator type (goshawk)</i>					2.54 $\pm$ 1.16			
<i>Sex</i> ^b	0.75 $\pm$.46	0.10	0.84 $\pm$ 0.51	0.10	0.65 $\pm$ 0.48	0.17	0.89 $\pm$ 0.49	0.07
<i>Subordinate age</i> ^c	0.07 $\pm$ 0.57	0.90	-0.06 $\pm$ 0.60	0.92	-0.17 $\pm$ 0.60	0.78	-0.13 $\pm$ 0.62	0.83
<i>Group size</i>	-0.42 $\pm$ 0.17	0.01	-0.58 $\pm$ 0.19	<0.01	-0.51 $\pm$ 0.18	<0.01	-0.53 $\pm$ 0.18	<0.01
<i>Brood size</i>	-0.25 $\pm$ 0.36	0.49	-0.08 $\pm$ 0.36	0.81	-0.24 $\pm$ 0.38	0.52	-0.17 $\pm$.38	0.65
<i>Nest stage</i> ^d	1.14 $\pm$ 0.45	0.01	1.10 $\pm$ 0.48	0.02	1.42 $\pm$ 0.50	<0.01	1.09 $\pm$ 0.49	0.03
<i>Predator type (goanna)</i> ^e	1.32 $\pm$ 0.94	0.70	0.99 $\pm$ 1.34	0.75	0.46 $\pm$ 0.80	0.78	1.15 $\pm$ 1.35	0.89
<i>Predator type (goshawk)</i> ^e	1.38 $\pm$ 1.04	0.03	-0.08 $\pm$ 1.38	0.80	0.37 $\pm$ 0.81	0.01	-0.30 $\pm$ 1.52	0.83
<i>Trial no.</i>	0.42 $\pm$ 0.20	0.04	0.41 $\pm$ 0.21	0.048	0.38 $\pm$ 0.21	0.07	0.44 $\pm$ 0.21	0.04
<i>Time of day</i>	0.34 $\pm$ 0.16	0.03	0.20 $\pm$ 0.17	0.22	0.27 $\pm$ 0.15	0.07	0.24 $\pm$ 0.17	0.15

^a present at the predator model relative to absent

^b male relative to female

^c older relative to first-year

^d nestling relative to egg stage

^e relative to cuckoo model

	5. Kin + Potential mate		6. Group members + Potential mate		7. Kin + Group members + Potential mate	
	AICc = 175.1		AICc = 182.2		AICc = 182.0	
	Effect size ($\pm$ SE)	P	Effect size ($\pm$ SE)	P	Effect size ($\pm$ SE)	P
Intercept	-3.02 $\pm$ 2.30	0.19	-1.99 $\pm$ 2.23	0.37	-2.71 $\pm$ 2.44	0.27
Kin ^a	0.55 $\pm$ 0.95	0.48			0.14 $\pm$ 1.28	0.88
Kin (present) x Predator type (goanna)	-2.09 $\pm$ 1.23	0.01			-2.15 $\pm$ 1.56	0.02
Kin (present) x Predator type (goshawk)	1.75 $\pm$ 1.56				2.05 $\pm$ 1.84	
No. group members present			0.33 $\pm$ 0.42	0.44	0.30 $\pm$ 0.61	0.62
No. group members present x Predator type (goanna)			-0.41 $\pm$ 0.59		0.21 $\pm$ 0.77	
No. group members present x Predator type (goshawk)				0.58		0.92
Potential mate ^a	0.12 $\pm$ 0.86	0.11	-0.33 $\pm$ 0.90	0.39	-0.20 $\pm$ 1.05	0.40
Potential mate (present) x Predator type (goanna)	-0.97 $\pm$ 1.16	<0.01	0.09 $\pm$ 1.10		-1.05 $\pm$ 1.30	<0.01
Potential mate (present) x Predator type (goshawk)	3.31 $\pm$ 1.45		2.23 $\pm$ 1.27	0.12	3.36 $\pm$ 1.67	
Sex ^b	0.54 $\pm$ 0.50	0.28	0.67 $\pm$ 0.49	0.17	0.64 $\pm$ 0.56	0.25
Subordinate age ^c	-0.23 $\pm$ 0.62	0.71	-0.21 $\pm$ 0.61	0.73	-0.36 $\pm$ 0.66	0.58
Group size	-0.47 $\pm$ 0.19	0.01	-0.58 $\pm$ 0.20	<0.01	-0.53 $\pm$ 0.22	0.02
			1			
Brood size	-0.23 $\pm$ 0.40	0.57	-0.13 $\pm$ 0.39	0.74	-0.18 $\pm$ 0.43	0.68
Nest stage ^d	1.38 $\pm$ 0.52	<0.01	1.31 $\pm$ 0.51	0.01	1.33 $\pm$ 0.54	0.01
Predator type (goanna) ^e	2.18 $\pm$ 1.35	0.57	1.12 $\pm$ 1.39	0.66	1.84 $\pm$ 1.53	0.88
Predator type (goshawk) ^e	-1.15 $\pm$ 1.69	0.13	-0.00 $\pm$ 1.43	0.42	-1.35 $\pm$ 1.92	0.48
Trial no.	0.34 $\pm$ 0.21	0.11	0.37 $\pm$ 0.21	0.08	0.36 $\pm$ 0.22	0.10
Time of day	0.33 $\pm$ 0.17	0.052	0.22 $\pm$ 0.17	0.19	0.29 $\pm$ 0.18	0.11

^a present at the predator model relative to absent

^b male relative to female

^c older relative to first-year

^d nestling relative to egg stage

^e relative to cuckoo model

Table S4. Comparison of the statistical model that best explains subordinate's likelihood to engage in adult group member defence, and a similar model containing the mere presence of kin and potential mates in the group rather than their presence near the predator model. Significant terms are highlighted in bold. *P*-values presented for main effects of terms that are also included in interaction effects were obtained from post-hoc Tukey's HSD tests controlling for interaction effects.

	1. Kin + Potential mate in group <i>AICc</i> = 187.6		2. Kin + Potential mate at predator model <i>AICc</i> = 175.1	
	Effect size ($\pm$ SE)	<i>P</i>	Effect size ($\pm$ SE)	<i>P</i>
<i>Intercept</i>	-2.60 $\pm$ 2.50	0.30	-3.02 $\pm$ 2.30	0.19
<i>Kin</i> ^a	-0.42 $\pm$ 1.59	0.37	0.55 $\pm$ 0.95	0.48
<i>Kin (present) x Predator type (goanna)</i>	-1.65 $\pm$ 1.69	0.22	-2.09 $\pm$ 1.23	0.01
<i>Kin (present) x Predator type (goshawk)</i>	0.68 $\pm$ 1.79		1.75 $\pm$ 1.56	
<i>Potential mate</i> ^a	-0.40 $\pm$ 0.91	0.60	0.12 $\pm$ 0.86	0.11
<i>Potential mate (present) x Predator type (goanna)</i>	0.45 $\pm$ 1.27	0.43	-0.97 $\pm$ 1.16	<0.01
<i>Potential mate (present) x Predator type (goshawk)</i>	1.62 $\pm$ 1.28		3.31 $\pm$ 1.45	
<i>Sex</i> ^b	1.11 $\pm$ 0.53	0.04	0.54 $\pm$ 0.50	0.28
<i>Subordinate age</i> ^c	-0.26 $\pm$ 0.60	0.66	-0.23 $\pm$ 0.62	0.71
<i>Group size</i>	-0.39 $\pm$ 0.19	0.04	-0.47 $\pm$ 0.19	0.01
<i>Brood size</i>	-0.29 $\pm$ 0.38	0.45	-0.23 $\pm$ 0.40	0.57
<i>Nest stage</i> ^d	1.21 $\pm$ 0.47	0.01	1.38 $\pm$ 0.52	<0.01
<i>Predator type (goanna)</i> ^e	1.36 $\pm$ 1.99	0.70	2.18 $\pm$ 1.35	0.57
<i>Predator type (goshawk)</i> ^e	-0.10 $\pm$ 2.05	0.53	-1.15 $\pm$ 1.69	0.13
<i>Trial no.</i>	0.47 $\pm$ 0.21	0.03	0.34 $\pm$ 0.21	0.11
<i>Time of day</i>	0.33 $\pm$ 0.15	0.03	0.33 $\pm$ 0.17	0.052

^a present relative to absent/responding relative to not responding

^b male relative to female

^c older relative to first-year

^d nestling relative to egg stage

^e relative to cuckoo model

Chapter 5

Helpers provision offspring of potential mates and relatives in a cooperative fairy-wren

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Abstract

Cooperative breeding involves individuals foregoing independent reproduction and instead helping others raise their offspring. Multiple hypotheses have been proposed to explain the evolution of such helping behaviour. These predict that helpers receive indirect or direct benefits from helping, either via the production of additional recruits or via benefits associated directly with the act of helping itself. However, while there is considerable evidence for reduced parental care by assisted breeders (load-lightening), theoretical predictions, and consequently empirical tests, generally neglect to take into account how fitness effects on breeders may additionally affect alloparental care. In particular, social group composition, and thereby the benefits associated with enhanced survival of breeders, often varies in cooperative breeders. Therefore, the type of breeders helpers are assisting is expected to additionally affect investment in cooperative care by helpers. Here, we provide a systematic test of the benefits driving help with offspring provisioning in a cooperative bird; including direct and indirect benefits deriving from helping raise the brood (kin selection, social prestige, passive and active group augmentation theories), and fitness benefits associated with load lightening of breeders. In our study species, the purple-crowned fairy-wren (*Malurus coronatus*), offspring provisioning by helpers enhances survival of breeders. Moreover, group composition, and therefore, benefits of helping, vary. We show that commonly proposed hypotheses do not clearly explain variation in offspring provisioning effort by helpers. Instead, helper investment in offspring provisioning was explained by an interaction effect of indirect and direct benefits associated with enhanced survival of

breeders rather than offspring. We show that kin-selected benefits are a prerequisite for help with offspring feeding, but that helpers provide the most care when they are assisting a relative and a potential mate; breeders that are associated with important immediate and potential future benefits. Our findings highlight the importance of taking improved survival of breeders as well as social group composition into account when explaining helping behaviour, to achieve a comprehensive understanding of the evolution of helping behaviour in cooperative breeders.

Keywords: alloparental care, nestling feeding, altruism, cooperation, helping, social environment

Introduction

Cooperative breeding, where subordinate individuals forego their own reproduction and instead help raise the offspring of others, has become a model system to study the evolution of costly, altruistic behaviour (Emlen 1982; Brown 1987; Cockburn 1998; Bergmüller et al. 2007). To enhance our understanding of how such seemingly altruistic helping behaviour can evolve, a number of studies have tested if and how variation in helping effort – usually offspring provisioning rate – is related to proposed benefits of helping. Most research initially focussed on indirect benefits to helpers associated with the production of related offspring (kin selection) (Brown 1987; Griffin and West 2002; Baglione et al. 2003; Griffin and West 2003; Bergmüller et al. 2007). More recently, the importance of direct benefits to helpers has been recognised (Cockburn 1998; Kokko et al. 2001; Kokko et al. 2002; Bergmüller et al. 2007; Riehl 2013; Kingma et al. 2014); direct benefits can drive helping behaviour along with or instead of indirect benefits (Clutton-Brock 2002; Richardson et al. 2002; Ekman et al. 2004; Kingma et al. 2011; Riehl 2011), and explain a substantial amount of variation in helping effort in cooperatively breeding birds (Kingma 2017; Downing et al. 2018).

These studies of direct and indirect benefits of helping, and the theories they are testing, focus primarily on how helping may increase focal helper's fitness via the production of additional recruits or via immediate or future benefits directly to helpers (e.g. access to reproduction as a result of helping) (Hamilton 1964; Kokko et al. 2001; Kokko et al. 2002; Bergmüller et al. 2007). However, when breeders receive assistance with reproduction, they often lower their parental effort in response (load-lightening; Russell et al. 2008; Meade et al. 2010; Johnstone 2011; Bruintjes et al. 2013; Zottl et al. 2013; Brouwer et al. 2014; Dias et al. 2017; van Boheemen et al. 2019), and this can result in their improved survival and/or condition (Khan and Walters 2002; Cockburn et al. 2008; Kingma et al. 2010; Meade et al. 2010). Thus, the effect of helping on the dominant breeding pair should also be considered when aiming to understand the complexity of altruistic helping behaviour in cooperative breeders. More specifically, the type of breeders receiving help (e.g. sex, relatedness) often varies (Riehl 2013), affecting the benefits associated with enhanced survival of these breeders. For instance, related breeders are associated with indirect benefits (Hamilton 1964; Meade et al. 2010) and potentially benefits from parental nepotism (Ekman et al. 2000; Ekman et al. 2004; Griesser et al. 2006; Nelson-Flower and Ridley 2016), while unrelated opposite-sex breeders may offer current (Magrath and Whittingham 1997; Riehl 2017) or future mating benefits (Kingma et al. 2011). Therefore, complex analyses of helping behaviour are required, that include direct and indirect benefits deriving from the brood, as well

as benefits from positive fitness effects on breeders, taking into account the type of breeders helpers are assisting.

Here, we use such a comprehensive approach to test what fitness benefits drive helper investment in offspring feeding in a facultatively cooperative breeding bird, the purple-crowned fairy-wren, *Malurus coronatus*. Subordinates in this species vary in relatedness to the brood as well as to breeders due to breeder turnover and subordinate dispersal (Kingma et al. 2011; Teunissen et al. 2018). Breeders decrease their feeding rates when helpers contribute to nestling feeding, and this is associated with increased survival for breeders (Kingma et al. 2010). Subordinates benefit from sharing a group with breeders that are kin and/or potential mates (unrelated, opposite-sex): these offer benefits from mutualistic social bonds, as well as the potential for breeding position inheritance and kin-selected benefits (Kingma et al. 2011; Teunissen et al. 2018). Thus, we have a good understanding of the various benefits of group living and helping raise offspring that might apply in this system (see e.g. Kingma et al. 2011; Teunissen et al. 2018), therefore we can differentiate between benefits deriving from the brood, and from enhanced survival of breeders that may include relatives, potential mates and/or reproductive competitors.

We tested all hypotheses relating to indirect and direct benefits commonly proposed for helping that might apply in our system, and fitness benefits from helping different types of breeders. (1) Kin selection theory proposes that helping provides indirect fitness benefits by producing related recruits (Hamilton 1964; Emlen 1982; Cockburn 1998; Bergmüller et al. 2007; Hatchwell 2009). Alternatively, helpers may feed offspring for direct fitness benefits. If helping raise young increases group size, (2) subordinates may benefit from increased survival in larger groups (passive group augmentation theory; Kokko et al. 2001; Kingma et al. 2014), or (3) subordinates may benefit from recruits in the future because they offer reciprocal interactions or help with breeding when the helper obtains a breeding position in the resident territory (active group augmentation theory; Cockburn 1998; Kokko et al. 2001; Bergmüller et al. 2007; Kingma et al. 2014). Additionally, (4) social prestige theory proposes that helping serves as a signal of quality, and therefore individuals that help more increase their chances of obtaining a mate, either by inheriting a breeding position or pairing up with a subordinate group member (Zahavi 1995; Komdeur and Edelaar 2001; Griffin and West 2002; Bergmüller et al. 2007). Lastly, we propose that (5) helping can serve to increase the fitness of valuable breeders, i.e. kin and/or potential mates, through load-lightening effects (termed here ‘load lightening of valuable breeders’; see Table 1 for full overview of predictions). Using this integrative approach, we highlight how

neglecting to take group composition into account can lead to misinterpretation of results: we show that the main hypotheses proposed for the adaptive indirect and direct benefits of helping do not fully explain helping effort, but social group composition does.

Table 1. Support for predicted investment in offspring feeding by individual subordinate purple-crowned fairy-wrens according to 5 hypotheses proposed.

Hypothesis ^a	Predictions in <i>M. coronatus</i>	Supported?
1. Kin selection (Hamilton 1964)	Subordinates do not feed unrelated nestlings	Yes (Fig. 1; Table 2)
	Feeding rates increase with increasing relatedness to the brood	No (Fig. 1; Table 2)
2. Passive group augmentation (Kokko et al. 2001)	Feeding rates decrease with group size ^b	No (Table 2; Table S2)
3. Active group augmentation (Kokko et al. 2001)	Feeding rates increase with probability of breeding position inheritance ^c	No (Table 2; Table S2)
4. Social prestige (Zahavi 1995)	Feeding rates increase when a subordinate potential mate is present ^d	No (Table 2; Table S2)
5. Load lightening of valuable breeders	Feeding rates increase when one of the breeders is a potential future mate (unrelated, opposite sex) ^e	Yes, if the other breeder is a relative (Fig. 2; Table 3)
	Feeding rates increase when one or both breeders are relatives (independent of breeder sex) ^e	Yes (Fig. 2; Table 3)

^a Helping is unlikely to be driven by opportunities for direct access to reproduction (parentage acquisition hypothesis; Magrath and Whittingham 1997; Cockburn 1998; Ekman et al. 2004), or to serve as payment of rent (pay-to-stay hypothesis; Kokko et al. 2002; Bergmüller et al. 2007), as helpers rarely gain paternity or lay eggs (Kingma et al. 2009), and non-cooperative individuals do not seem to get punished or evicted (Kingma et al. 2010; Kingma et al. 2011), hence these hypotheses are not tested in this study.

^b Benefits of increasing group size are expected to be greater in small compared to large groups, due to diminishing returns of additional group members (Kingma et al. 2014).

^c Subordinates can only benefit from recruits helping with breeding in the future if they can inherit a breeding position in the territory, thus helping effort should be related to chances of breeding position inheritance (Kokko et al. 2001; Kingma et al. 2014).

^d Since queues for breeding position inheritance are stable (Table S1; Kingma et al. 2011), helping is unlikely to serve as a signal of quality to breeders, but may serve to advertise quality to subordinate potential mates with which to establish a new territory (as 7% of subordinates do; Aranzamendi et al. 2016).

^e Related breeders offer kin-selected benefits and, possibly, benefits of parental nepotism; potential mates offer potential mating benefits through breeding position inheritance; both relatives and potential mates offer benefits from mutualistic social bonds (Teunissen et al. 2018).

Methods

Study site and species

Purple-crowned fairy-wrens are endemic to the wet-dry tropics of northern Australia, where they inhabit riparian vegetation (Rowley and Russell 1993; Skroblin and Legge 2010). Groups and territories are stable year-round and consist of a monogamous breeding pair (which engage in duetting behaviour, providing a reliable cue to assign social status; Hall and Peters 2008) and often one or more subordinates (Rowley and Russell 1993; Kingma et al. 2010; Kingma et al. 2011). Extra-pair paternity is rare (4.4% of offspring), and subordinates rarely gain direct access to reproduction (1.8% of offspring) (Kingma et al. 2009; Kingma et al. 2011). Breeding vacancies are rare, and the cost of inbreeding is high (Kingma et al. 2013). Subordinates can inherit a breeding position in the group when the same-sex breeder disappears (20% of first breeding positions), or in rarer cases, pair with an unrelated opposite-sex subordinate in the group and bud off to form a new territory (7% of first breeding positions) (Hidalgo Aranzamendi et al. 2016). Subordinates of both sexes can help the breeding pair to raise offspring, and vary in offspring provisioning rates (Kingma et al. 2011). Most breeding takes place during the monsoonal wet season (December – March) (Rowley and Russell 1993; Kingma et al. 2010).

We studied a population of approximately 300 uniquely colour-banded individuals, monitored since 2005 at Australian Wildlife Conservancy's Mornington Wildlife Sanctuary in northwest Australia (S17°31' E126°6'). From 2012 to 2018, the population was monitored in two visits per year (May/June and November), where group membership and social status of each individual was documented, and all unbanded birds (new offspring and immigrants) were banded. For the current study, 50 fairy-wren groups were followed closely during the main breeding season from 2016 to 2018, recording individuals' social status and breeding activity. Nests were monitored for egg laying, hatching, and fledging. *M. coronatus* generally lay 3 or 4 eggs (range 1-5, mean $\pm$ SE = 3 ± 0.03), and nestlings fledge approximately 13 days after hatching (Kingma et al. 2011; personal observations).

Nestling feeding observations

We quantified individual nestling feeding rate for 71 subordinates in February – April 2016, March – April 2017, and December 2017 – April 2018 (year '2016', '2017' and '2018', respectively). We conducted 77 nestling feeding watches at 45 nests on 32 territories containing subordinate group members, resulting in a dataset of 145 individual observation hours. Two 1-hour feeding watches were conducted at each nest where possible (due to nest failure, some nests were only watched

once; mean $\pm$ SE = 1.7 ± 0.1 watches per nest), between 3 and 8 days after hatching (mean $\pm$ SE = 5.6 ± 0.2 days post-hatching). Feeding watches were conducted during the morning (starting between 5:25 and 10:25 am) during calm, dry weather, from a camouflaged hide approximately 10m from the nest. The identity of each bird bringing food to the nestlings was recorded. Group members could be unambiguously identified by their colour bands in 96% (937 of 974) of feeding visits. Only individuals older than 94 days, the youngest age of a bird that was seen feeding, were considered as focal subordinates. The youngest age of a subordinate that was unrelated to one or more of the breeders in our dataset was 82 days.

Potential benefits of helping

Since extra-pair paternity is rare (Kingma et al. 2009), social relatedness reflects genetic relatedness in this species. We therefore determined relatedness of subordinates to breeders and to the brood using social pedigree data, where only first-order relatives (full sibling, parent-offspring) were classified as 'related'. We quantified potential benefits of helping as proposed by each of the hypotheses outlined in Table 1 as follows:

- 1) Kin-selected benefits of raising the brood were quantified as focal subordinates' relatedness to the brood, classified as $r = 0$ (unrelated to both breeders), $r = 0.25$ (related to one breeder only), or $r = 0.50$ (related to both breeders).
- 2) Passive group augmentation benefits were reflected by group size (mean group size $\pm$ SE = 3.8 ± 0.2 , range = 3-7).
- 3) We quantified active group augmentation benefits by determining how likely subordinates were to inherit the breeding position on the territory when a vacancy opened up. For this we used data from a recent study period (2012-2018) since population density has increased by $\geq 60\%$, and number of helpers by $\geq 65\%$, resulting in saturation of the population, since previous quantifications of inheritance prospects (Kingma et al. 2011). We calculated the likelihood of inheritance based on focal subordinates' sex, relatedness to the opposite-sex breeder, and whether an older same-sex subordinate group member was present, similar to Kingma et al. (2011) (for a detailed description of how breeding position inheritance prospects were calculated, see Supplementary material I).
- 4) Since breeding position inheritance queues are stable (Table S1; Kingma et al. 2011), offspring feeding may only serve to advertise quality to subordinate and not to dominant group members. We therefore determined the potential for subordinates to obtain benefits from

social prestige by establishing for each focal subordinate, whether a subordinate potential mate (i.e. unrelated opposite-sex subordinate) was present in the group.

- 5) We quantified benefits of load lightening of breeders by determining whether focal subordinates were related to the same-sex and to the opposite-sex breeder, respectively, as relative sex and relatedness together determine whether breeders represent a relative, potential mate (unrelated, opposite sex), and/or a reproductive competitor (unrelated, same sex).

Statistical analyses

We used a model comparison approach to test predictions of hypotheses proposed to explain helping behaviour (Table 1). All analyses were performed in R 3.4.4 (R Core Team 2018). Since subordinates did not feed nestlings when they were unrelated to both dominants (i.e. complete separation of the data; Fig. 1), we analysed nestling feeding rates with Bayesian GLMMs with a negative binomial distribution using the 'rstanarm' package (Goodrich et al. 2018). We fitted models including the number of feeds at the nest by the focal subordinate during a 1-hour nest watch as response variable. As independent variable, we included any possible combination of the main effects of relatedness to the brood, probability of breeding position inheritance, and the presence of a subordinate potential mate, as well as the interaction between relatedness and probability of inheritance (previously found to affect feeding effort by helpers; Kingma et al. 2011), both in isolation and in concert (testing hypothesis 1, 3 and 4, respectively; Table 1). Models were compared using the widely applicable information criterion (WAIC). Only the statistical model that best explains feeding effort by subordinates ($\Delta\text{WAIC} = 0.2 - 4.7$ compared to other models) is presented in the results section, but the full results of all nine models and corresponding WAIC values are given in the supplementary material (Table S2).

Since relatedness to the brood as well as prospects for breeding position inheritance depend on subordinates' relatedness to breeders (Kingma et al. 2011), we ran a separate model including focal subordinate relatedness to the opposite-sex and to the same-sex breeder, and their interaction (Table 1, hypothesis 5). Group size (predicting benefits of passive group augmentation; Table 1, hypothesis 2) was included in all models since it may also be a confounding variable due to load lightening effects in larger groups (i.e. the more individuals feed offspring, the smaller the effect of help by the focal subordinate on offspring production; see Kingma et al. 2014). Although group size was negatively correlated with the probability of breeding position inheritance (LM: $R = 0.48$, $t = -6.64$, $P < 0.01$), excluding group size from models did not yield a

significantly better fit to the data for any of our models (excluding vs including group size; ΔWAIC = -0.8, 1.8, 0.0, -0.5, 0.9, 0.9, -2.5, -0.1, and -0.1 for models 1-9, respectively; Table S2), and did not change the effect of inheritance prospects on nestling feeding rates (CI remained overlapping with zero for all models).

Other potentially confounding variables known to explain variation in feeding rates were additionally included in all models: sex (male, female), age (first-year, older), brood size, brood age (days), and time of day (hr). Year (2016, 2017, 2018) was also included to account for variation across years. Bird ID and nest ID were included as random effects to account for replication across individuals and nests. For all models, priors were set to a normal distribution with mean = 0 and variance = 10, and variance = 100 for the intercept prior. Three chains were run of 15,000 iterations each, with a thinning interval of 20 and a warmup period of 5,000. Visual inspection of the trace, density, autocorrelation, and posterior predictive plots using the 'rstan' package (Stan Development Team 2018) confirmed convergence of the model and showed no sign of autocorrelation. We present posterior means and 95% credible intervals (CI) for all effects.

Results

Focal subordinates were observed feeding nestlings in 40% (58 of 145) of nestling feeding observations. Of models comparing benefits associated with kin selection, active group augmentation, and social prestige, the model including the main effect and the interaction effect of relatedness and the likelihood of breeding position inheritance best explained investment in nestling feeding by subordinates (Table 2; Table S2). This model indicates that relatedness to the brood affected nestling feeding rates by focal subordinates (Table 2): subordinates never fed unrelated nestlings, and surprisingly, fed nestlings more frequently when they were raising second-order relatives ($r = 0.25$) compared to first-order relatives ($r = 0.50$) (Fig. 1). Thereby our results support one of the two predictions for kin selection theory only (Table 1). This effect was consistent across models, and all models that did not include relatedness to the brood provided a significantly worse fit to the data than our top model ($\Delta\text{WAIC} \geq 3.0$; Table S2).

The top model further indicates that relatedness to the brood and breeding position inheritance prospects had no interacting effects on offspring feeding rates, nor did we detect a main effect of breeding position inheritance (Table 2). Additionally, helping effort did not vary with group size (Table 2), nor did it change when a subordinate potential mate was present to advertise to (Table S2), thereby not supporting predictions of active or passive group

augmentation, or social prestige theory (Table 1). These findings are consistent across models (Table S2), indicating the robustness of these findings.

Table 2. Results of the top statistical model that best explains nestling feeding effort by subordinates when taking traditional adaptive benefits of helping into account only, including the main effects of and interaction between relatedness to the brood and probability of breeding position inheritance. Significant terms are highlighted in bold.

	Effect size (95% CI)
<i>Intercept</i>	-4.5 (-16.4, 5.4)
<i>Relatedness (R) ($r = 0.25$)^a</i>	8.1 (0.3, 19.4)
<i>Relatedness (R) ($r = 0.5$)^a</i>	4.0 (-4.0, 14.9)
<i>Probability of breeding position inheritance (I)</i>	-12.7 (-39.8, 7.0)
<i>R ($r = 0.25$) $\times$ I^a</i>	10.7 (-9.3, 37.4)
<i>R ($r = 0.5$) $\times$ I^a</i>	13.3 (-6.6, 40.3)
<i>Group size</i>	-0.2 (-0.8, 0.4)
<i>Sex^b</i>	-0.1 (-1.3, 1.1)
<i>Age^c</i>	-0.4 (-2.0, 1.0)
<i>Brood size</i>	0.2 (-0.5, 0.8)
<i>Brood age</i>	0.3 (0.1, 0.5)
<i>Year^d (2017)</i>	-2.4 (-5.0, -0.2)
<i>Year^d (2018)</i>	-1.1 (-3.4, 1.1)
<i>Time of day</i>	-0.1 (-0.5, 0.2)

^a relative to subordinates unrelated to the brood ($r = 0$)

^b male relative to female

^c older relative to first-year

^d relative to 2016

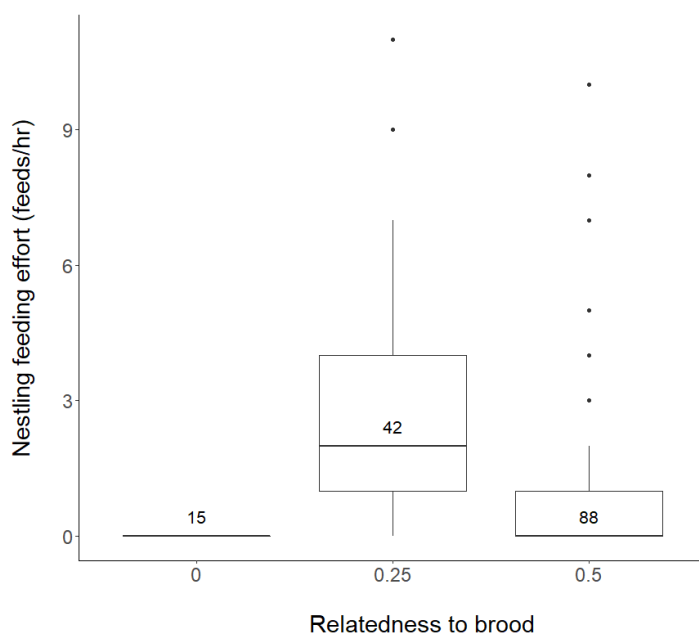


Figure 1. Individual nestling feeding rates by subordinate purple-crowned fairy-wrens vary with their relatedness to the brood. Subordinates that were unrelated to the brood ($r = 0$) never fed nestlings, and subordinates that were raising second-order relatives ($r = 0.25$) fed most often. Raw values are presented. Numbers in boxplots indicate sample sizes.

Although the top model including adaptive benefits of helping commonly proposed provided a slightly better fit to the data (WAIC = 374.9 compared to 377.2), the type of breeders subordinates are assisting (according to sex and relatedness) additionally explained variation in helping effort, and explains why subordinates fed second-order relatives more often (Table 3). Relatedness to breeders positively affected helping effort: feeding rates were higher when subordinates were related compared to unrelated to the same-sex breeder, and to the opposite-sex breeder (main effect of both; Table 3). Moreover, relatedness to the same-sex and to the opposite-sex breeder had interacting effects on subordinate feeding rates (Table 3). When subordinates were unrelated to both breeders, they did not feed nestlings at all. When they were related to both breeders they fed at similar rates compared to when they were related to the opposite-sex breeder but unrelated to the same-sex breeder (i.e. assisting a relative and a competitor). Interestingly, subordinates fed nestlings more than twice as often when they were related to the same-sex breeder and unrelated to the opposite-sex (i.e. assisting a relative and a potential mate) (Fig. 2), indicating that feeding rates are higher when breeders are relatives, and that feeding rates are higher when one of the breeders is a potential future mate only if the other breeder represents a relative, supporting load lightening of valuable breeders (Table 1).

Table 3. Results of the statistical model testing for the effect of subordinates' relatedness to the same- and opposite-sex breeder on nestling feeding rates. Significant terms are highlighted in bold.

	Effect size (95% CI)
<i>Intercept</i>	-6.0 (-14.5, 1.3)
<i>Relatedness to same-sex breeder^a</i>	7.4 (2.8, 14.4)
<i>Relatedness to opposite-sex breeder^a</i>	6.1 (1.2, 13.3)
<i>Relatedness to same-sex (related) x</i> <i>Relatedness to opposite-sex breeder (related)</i>	-8.4 (-15.8, -3.2)
<i>Group size</i>	-0.2 (-0.7, 0.4)
<i>Sex^b</i>	0.1 (-1.0, 1.3)
<i>Age^c</i>	-0.6 (-2.0, 0.7)
<i>Brood size</i>	0.2 (-0.5, 0.8)
<i>Brood age</i>	0.3 (0.1, 0.5)
<i>Year^d (2017)</i>	-2.0 (-4.3, 0.2)
<i>Year^d (2018)</i>	-1.1 (-3.5, 1.2)
<i>Time of day</i>	-0.1 (-0.5, 0.3)

^a related relative to unrelated

^b male relative to female

^c older relative to first-year

^d relative to 2016

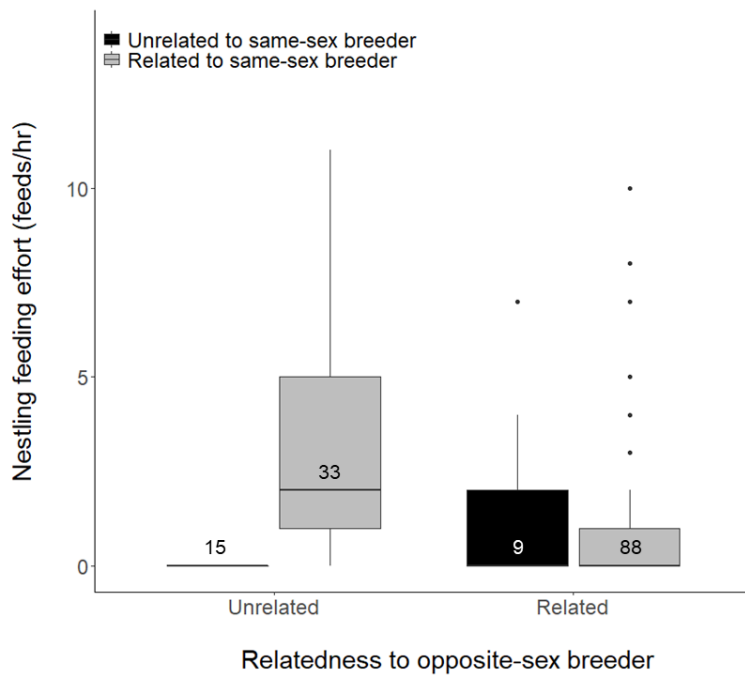


Figure 2. Subordinates assisting a related same-sex breeder and unrelated opposite-sex breeder fed offspring most frequently, as indicated by an interacting effect of focal subordinates' relatedness to the opposite-sex and to the same-sex breeder. Raw values are presented. Numbers in boxplots indicate sample sizes.

Discussion

We provide a systematic test of the importance of indirect and various direct benefits in driving helping behaviour in a cooperative breeder. We show that offspring provisioning by subordinates is not driven by direct fitness benefits commonly proposed, and is explained only to a limited extent by indirect benefits associated with the enhanced production of related offspring. Instead, our findings can only be fully explained by taking into account the type of breeders subordinates are assisting, and indicate that subordinates are most willing to assist with offspring feeding when this enhances survival of breeders that are associated with indirect kin-selected and potential mating benefits. We discuss our findings in detail below.

Three commonly proposed hypotheses to explain helping behaviour that focus on fitness benefits deriving directly from the brood being raised, did not clearly explain variation in offspring feeding by subordinates in our study. First, feeding rates did not vary with group size, indicating that investment in offspring feeding is not driven by passive benefits resulting from larger group size (passive group augmentation; Kokko et al. 2001; Kingma et al. 2014). Second, there was no effect of subordinates' probability to inherit a breeding position on the territory, suggesting that helping raise offspring does not serve to enhance the production of recruits that might in the future act as helpers themselves when the subordinate has potentially obtained a breeding position in the group (active group augmentation; Kokko et al. 2001; Bergmüller et al. 2007; Kingma et al. 2014). Lastly, feeding rates did not increase when a (subordinate) potential mate

was present, suggesting that helping does not serve as an advertisement signal either (Zahavi 1990; Zahavi 1995; Griffin and West 2002; Bergmüller et al. 2007).

Our study did find support for kin selection as an important factor in explaining helping, since no subordinate ever helped to raise unrelated broods (Hamilton 1964; Emlen 1982; Cockburn 1998; Bergmüller et al. 2007). Kin selection is often shown to explain helping behaviour within and between species (Griffin and West 2002; Griffin and West 2003; Nam et al. 2010; Green et al. 2016; Dias et al. 2017; Barati et al. 2018). Indeed, the fact that subordinate fairy-wrens in our study never fed nestlings when they were unrelated to both breeders, despite one of them being a potential mate, suggests that kin-selected benefits are a prerequisite for help with offspring feeding. However, kin selection generally explains only a small proportion of variation in helping effort among cooperative breeders, and is often not the only driver of helping (Richardson et al. 2002; Griffin and West 2003; Kingma et al. 2011; Kingma 2017; Downing et al. 2018). Similarly, in our study feeding rates did not increase when subordinates shared a higher degree of relatedness to the brood (in contrast to e.g. Baglione et al. 2003; Griffin and West 2003; Nam et al. 2010; Wright et al. 2010; Green et al. 2016; Dias et al. 2017; Barati et al. 2018), and the indirect fitness benefits of increased production of offspring, and enhanced survival of related breeders, were thus greater. Instead, subordinates fed most frequently when nestlings were half-siblings rather than full siblings, and therefore, kin selection theory alone cannot fully explain variation in helping effort in this species.

The fact that nestling feeding by subordinates also increases survival of the breeders they are assisting, as a result of load-lightening, is common in cooperative breeders (Khan and Walters 2002; Cockburn et al. 2008; Kingma et al. 2010; Meade et al. 2010) but it is rarely considered that this in itself may also constitute a benefit to helpers. By taking into account what sort of breeders helpers are assisting, we are able to show that helpers that are related to the same-sex breeder but not to the opposite-sex breeder, work the hardest. Unrelated breeders offer no kin-based or other benefits if they are of the same sex (and in fact, represent reproductive competitors) while unrelated breeders of the opposite sex represent potential mates associated with future reproductive benefits (Magrath and Whittingham 1997; Kingma et al. 2011; Riehl 2017; Nelson-Flower et al. 2018), and mutualistic social bonds (Teunissen et al. 2018). Indeed, breeding vacancies are rare and the cost of inbreeding is high (Kingma et al. 2013), suggesting that unrelated opposite-sex group members are valuable. Our findings highlight that offspring feeding by helpers is driven by both indirect kin-selected benefits from raising relatives and enhancing survival of related breeders, and by direct benefits associated with enhanced survival of potential

mates, in interaction. Our findings thereby support those from a range of studies indicating that kin selection alone cannot explain variation in helping behaviour, and direct benefits should be considered alongside indirect benefits when attempting to understand variation in helping behaviour in cooperative breeders (Richardson et al. 2002; Griffin and West 2003; Ekman et al. 2004; Covas et al. 2006; Wright et al. 2010; Kingma et al. 2011; Riehl 2011; Kingma 2017; Downing et al. 2018).

Our findings are partly in line with previous research on this species, which showed that offspring feeding by subordinates is driven by indirect benefits as well as active group augmentation benefits through breeding position inheritance, acting in interaction (Kingma et al. 2011). While we found an effect of indirect fitness benefits – albeit more so through survival of breeders than offspring – we did not detect higher feeding rates with increased chances of breeding position inheritance, but instead, when breeders included a potential mate. This suggests that it is survival of potential mates, rather than future help provided by additional recruits, that is currently driving helping behaviour in the population. Possibly, this reflects changed conditions at our field site over time: fairy-wren density has increased by $\geq 60\%$; from a mean $\pm$ SE of 10 ± 0.6 individuals km^{-1} in 2005-2009, the study period used by Kingma et al. (2011), to 17 ± 1.3 individuals km^{-1} at the time this study was conducted, in 2016-2018. Concurrently, the number of helpers in our study population has increased from a mean $\pm$ SE of 60 ± 5.6 in 2005-2009 to 113 ± 20 in 2016-2018, and the number of territories has increased from 48 ± 3.5 to 74 ± 0.6 , and seems to have reached stable levels, indicating saturation of the population (unpublished data). Thus, a shortage of vacant breeding positions appears to currently be the limiting factor for independent reproduction by subordinates, and likely results in individuals staying on the resident territory as a subordinate for longer. This may explain why benefits associated with survival of breeders now appear to be more important in driving offspring feeding by helpers than benefits associated with the production of additional offspring.

Conclusion

Commonly proposed adaptive hypotheses for the evolution of helping behaviour do not fully explain offspring provisioning effort by subordinate purple-crowned fairy-wrens. By taking into account the type of breeders subordinates are assisting, we show that indirect and direct fitness benefits associated with increased survival of valuable breeders drive helping behaviour. Load-lightening appears common among cooperative breeders (Khan and Walters 2002; Meade et al. 2010; Johnstone 2011; Zottl et al. 2013; van Boheemen et al. 2019), and social group composition

varies considerably between and within species (Riehl 2013). Therefore, it seems imperative to incorporate effects of helping on the fitness of breeders in studies on cooperative behaviour, as well as what sort of benefits these breeders might offer to helpers in return, to achieve a comprehensive understanding of the complexity of helping behaviour in cooperative breeders.

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Supplementary material for Chapter 5

Supplementary material I: Probability of breeding position inheritance

We determined how likely subordinates were to inherit the breeding position on the territory when a vacancy opened up (i.e. the same-sex breeder died or dispersed), following approaches by Kingma et al. (2011). We did this by determining, for all cases between November 2012 and November 2018 where a vacancy opened up, the likelihood of inheritance based on subordinates' sex, relatedness to the opposite-sex breeder, and whether an older same-sex subordinate group member was present; all factors known to affect breeding position inheritance prospects (Kingma et al. 2011). For cases where the vacancy appeared in between field seasons, only subordinates present on the territory before and after the vacancy were included. In line with earlier findings, the likelihood of breeding position inheritance was greater when subordinates were the oldest in the group (compared to when an older same-sex subordinate was present; GLMM including sex, relatedness, and presence of older subordinate: $\beta \pm SE = 4.28 \pm 1.14$, $z = 3.75$, $P < 0.01$, compared to same-age subordinates; $\beta \pm SE = 2.20 \pm 0.76$, $z = 2.90$, $P < 0.01$, no significant difference when older compared to same-age same-sex subordinates were present; $\beta \pm SE = 2.08 \pm 1.26$, $z = 1.65$, $P = 0.22$) (Table S1). Furthermore, subordinates were more likely to fill a vacancy if they were unrelated to the opposite-sex breeder (compared to related: $\beta \pm SE = 1.65 \pm 0.70$, $z = 2.34$, $P = 0.02$), while males were no more likely to inherit than females ($\beta \pm SE = 0.82 \pm 0.65$, $z = 1.27$, $P = 0.20$) (Table S1).

The obtained values (see Table S1) were used as the “probability of breeding position inheritance” for analyses, with the exception of cases where multiple same-sex subordinates were the oldest in the group (i.e. from the same brood). Our calculated values show that males are about half as likely to inherit the breeding position when another male of the same age is present and they are related to the opposite-sex breeder (0.30 vs 0.65; Table 2), based on 5 cases, whereas our dataset only contained 1 case where two unrelated females, 1 case where two related females, and no cases where multiple unrelated males of the same age were present, making these values unreliable. Therefore, in two cases where two females, one case where three females, and one case where three males of the same age were present, the probability of breeding position inheritance was divided equally between them. Our dataset included no cases where a subordinate female was not the oldest and unrelated to the opposite-sex breeder.

Table S1. The proportion of subordinates that inherited a vacant breeding position when the same-sex breeder disappeared, according to subordinate's sex, relatedness to the remaining opposite-sex breeder, and the presence of older same-sex subordinates in the group. Where subordinates were of the same age, they were the oldest in the group. Number of cases is shown in parentheses. There were no cases where male subordinates were of the same age and unrelated to the opposite-sex breeder.

Relatedness to opposite-sex breeder	Older subordinate present	Male	Female
Unrelated	Yes	0 (3)	1 (1)
	No	0.93 (15)	0.86 (7)
	Same age	-	0 (2)
Related	Yes	0 (15)	0 (2)
	No	0.65 (17)	0.50 (10)
	Same age	0.30 (10)	0 (2)

Reference

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Supplementary material II: Supplementary table

Table S2. Results of the statistical models testing the hypotheses for subordinates' helping effort at the nest. Significant terms are highlighted in bold.

	1. Relatedness to brood WAIC = 375.1	2. Breeding position inheritance WAIC = 377.9	3. Subordinate potential mate WAIC = 378.4	4. Relatedness + Inheritance WAIC = 378.3	5. Relatedness + Subordinate potential mate WAIC = 376.1	6. Inheritance + Subordinate potential mate WAIC = 378.0
	Effect size (95% CI)	Effect size (95% CI)	Effect size (95% CI)	Effect size (95% CI)	Effect size (95% CI)	Effect size (95% CI)
<i>Intercept</i>	-7.4 (-17.6, 0.3)	-1.8 (-7.5, 3.9)	-0.8 (-6.3, 4.5)	-6.5 (-17.1, 2.2)	-7.0 (-17.9, 0.9)	-1.1 (-6.8, 4.6)
<i>Relatedness (R) (r = 0.25)^a</i>	8.5 (2.8, 17.7)			8.7 (3.0, 18.6)	8.4 (2.7, 18.0)	
<i>Relatedness (R) (r = 0.5)^a</i>	6.4 (0.7, 15.7)			6.2 (0.4, 16.1)	6.4 (0.6, 16.2)	
<i>Probability of breeding position inheritance (I)</i>		0.7 (-1.1, 2.5)		-0.9 (-2.8, 1.0)		0.7 (-1.0, 2.5)
<i>R (r = 0.25) x I^a</i>						
<i>R (r = 0.5) x I^a</i>						
<i>Subordinate potential mate^b</i>			0.8 (-0.8, 2.4)		0.4 (-1.1, 1.8)	0.8 (-0.9, 2.4)
<i>Group size</i>	-0.1 (-0.6, 0.4)	0.1 (-0.6, 0.8)	-0.2 (-0.9, 0.5)	-0.2 (-0.8, 0.3)	-0.2 (-0.8, 0.4)	-0.1 (-0.9, 0.8)
<i>Sex^c</i>	0.1 (-1.1, 1.3)	0.8 (-0.4, 2.1)	0.9 (-0.3, 2.1)	0.0 (-1.1, 1.2)	0.2 (-0.9, 1.3)	0.9 (-0.3, 2.2)
<i>Age^d</i>	-0.5 (-2.0, 0.9)	-0.3 (-1.9, 1.2)	-0.2 (-1.7, 1.2)	-0.4 (-1.8, 1.1)	-0.6 (-2.2, 0.9)	-0.6 (-2.3, 1.2)
<i>Brood size</i>	0.2 (-0.4, 0.8)	0.0 (-0.8, 0.7)	0.0 (-0.8, 0.7)	0.2 (-0.5, 0.9)	0.2 (-0.6, 0.8)	0.0 (-0.8, 0.7)
<i>Brood age</i>	0.3 (0.1, 0.5)	0.3 (0.1, 0.5)	0.3 (0.1, 0.5)	0.3 (0.1, 0.5)	0.3 (0.1, 0.5)	0.3 (0.1, 0.5)
<i>Year^e (2017)</i>	-2.2 (-4.4, -0.1)	-2.2 (-5.1, 0.2)	-2.3 (-5.3, 0.3)	-2.3 (-4.8, 0.0)	-2.3 (-4.7, 0.1)	-2.5 (-5.4, 0.3)
<i>Year^e (2018)</i>	-1.2 (-3.4, 1.0)	-1.4 (-4.2, 1.2)	-1.4 (-4.4, 1.3)	-1.1 (-3.5, 1.1)	-1.3 (-3.7, 0.9)	-1.6 (-4.5, 1.3)
<i>Time of day</i>	-0.1 (-0.4, 0.3)	-0.1 (-0.5, 0.3)	-0.1 (-0.5, 0.3)	-0.1 (-0.5, 0.3)	-0.1 (-0.5, 0.3)	-0.1 (-0.5, 0.3)

^a relative to subordinates unrelated to the brood (r = 0)

^b present relative to absent

^c male relative to female

^d older relative to first-year

^e relative to 2016

	7. Relatedness + Inheritance + Subordinate potential mate WAIC = 377.5	8. Relatedness* Inheritance WAIC = 374.9	9. Relatedness* Inheritance + Subordinate potential mate WAIC = 375.0
	Effect size (95% CI)	Effect size (95% CI)	Effect size (95% CI)
<i>Intercept</i>	-6.3 (-17.2, 2.5)	-4.5 (-16.4, 5.4)	-4.2 (-16.0, 6.1)
<i>Relatedness (R) (r = 0.25)^a</i>	8.7 (3.0, 18.4)	8.1 (0.3, 19.4)	7.8 (0.1, 18.1)
<i>Relatedness (R) (r = 0.5)^a</i>	6.2 (0.3, 15.8)	4.0 (-4.0, 14.9)	3.8 (-4.1, 14.2)
<i>Probability of breeding position inheritance (I)</i>	-0.9 (-2.9, 1.1)	-12.7 (-39.8, 7.0)	-13.0 (-38.5, 6.1)
<i>R (r = 0.25) x I^a</i>		10.7 (-9.3, 37.4)	11.0 (-8.4, 36.5)
<i>R (r = 0.5) x I^a</i>		13.3 (-6.6, 40.3)	13.5 (-6.1, 38.7)
<i>Subordinate potential mate^b</i>	0.4 (-1.0, 1.7)		0.2 (-1.1, 1.7)
<i>Group size</i>	-0.3 (-0.9, 0.4)	-0.2 (-0.8, 0.4)	-0.2 (-0.9, 0.5)
<i>Sex^b</i>	0.1 (-1.2, 1.3)	-0.1 (-1.3, 1.1)	-0.1 (-1.2, 1.3)
<i>Age^c</i>	-0.5 (-2.1, 1.1)	-0.4 (-2.0, 1.0)	-0.5 (-2.2, 1.0)
<i>Brood size</i>	0.2 (-0.5, 0.9)	0.2 (-0.5, 0.8)	0.1 (-0.6, 0.8)
<i>Brood age</i>	0.3 (0.1, 0.5)	0.3 (0.1, 0.5)	0.3 (0.1, 0.5)
<i>Year^d (2017)</i>	-2.4 (-5.0, 0.0)	-2.4 (-5.0, -0.2)	-2.5 (-5.2, -0.1)
<i>Year^d (2018)</i>	-1.2 (-3.8, 1.1)	-1.1 (-3.4, 1.1)	-1.1 (-3.7, 1.3)
<i>Time of day</i>	-0.1 (-0.5, 0.3)	-0.1 (-0.5, 0.2)	-0.1 (-0.5, 0.2)

^a relative to unrelated subordinates (r = 0)

^b present relative to absent

^c male relative to female

^d older relative to first-year

^e relative to 2016

General discussion

Cooperative breeding provides a model system for understanding the evolution of cooperation in general (Cockburn 1998; Bergmüller et al. 2007; Koenig and Dickinson 2016). It involves the decision for an individual to stay in a group as a non-breeding subordinate, and subsequently, the decision to contribute to cooperative tasks (Komdeur and Ekman 2010). Therefore, understanding how cooperative breeding can evolve requires an understanding of the benefits of group living as well as benefits of helping, and how these relate to cooperative behaviour by individuals (Komdeur and Ekman 2010; Shen et al. 2017). In my PhD research, I explored how both types of benefits drive cooperative behaviour in the purple-crowned fairy-wren by studying the two forms of costly cooperation helpers may engage in, offspring feeding and predator defence. By relating cooperative behaviour to the composition of social groups, which varies greatly in this species, I was able to study the full suite of benefits proposed for the evolution of helping behaviour, and to tease apart to what extent helpers cooperate for benefits associated with group living relative to benefits associated with helping at the nest itself. My study revealed that investment in cooperative tasks by helpers reflects both types of benefits, and that social group composition plays a crucial role in determining individual behaviour. Additionally, my research showed that predator defence constitutes a potentially important form of cooperation to protect nests and group members, and that while investment in predator defence is positively related to investment in offspring provisioning, individual investment in the two forms of cooperation is not necessarily driven by the same adaptive benefits of helping. Below, I discuss the main findings of my thesis and their implications (see Fig. 1 for an overview of results for each chapter), and I provide suggestions for further research.

Group composition determines benefits of group living

In the purple-crowned fairy-wren, groups are often composed of a mix of relatives, potential mates, and reproductive competitors, and the composition of social groups influences potential costs and benefits of group living. Subordinates benefit from sharing a group with relatives as they can receive indirect fitness benefits through acts that enhance the fitness of related group members (Kingma et al. 2011). Although untested in this species, parents could further provide offspring with enhanced access to resources or protection from predators (Ekman et al. 2000; Griesser et al. 2006). Since breeding vacancies are uncommon, the presence of potential mates

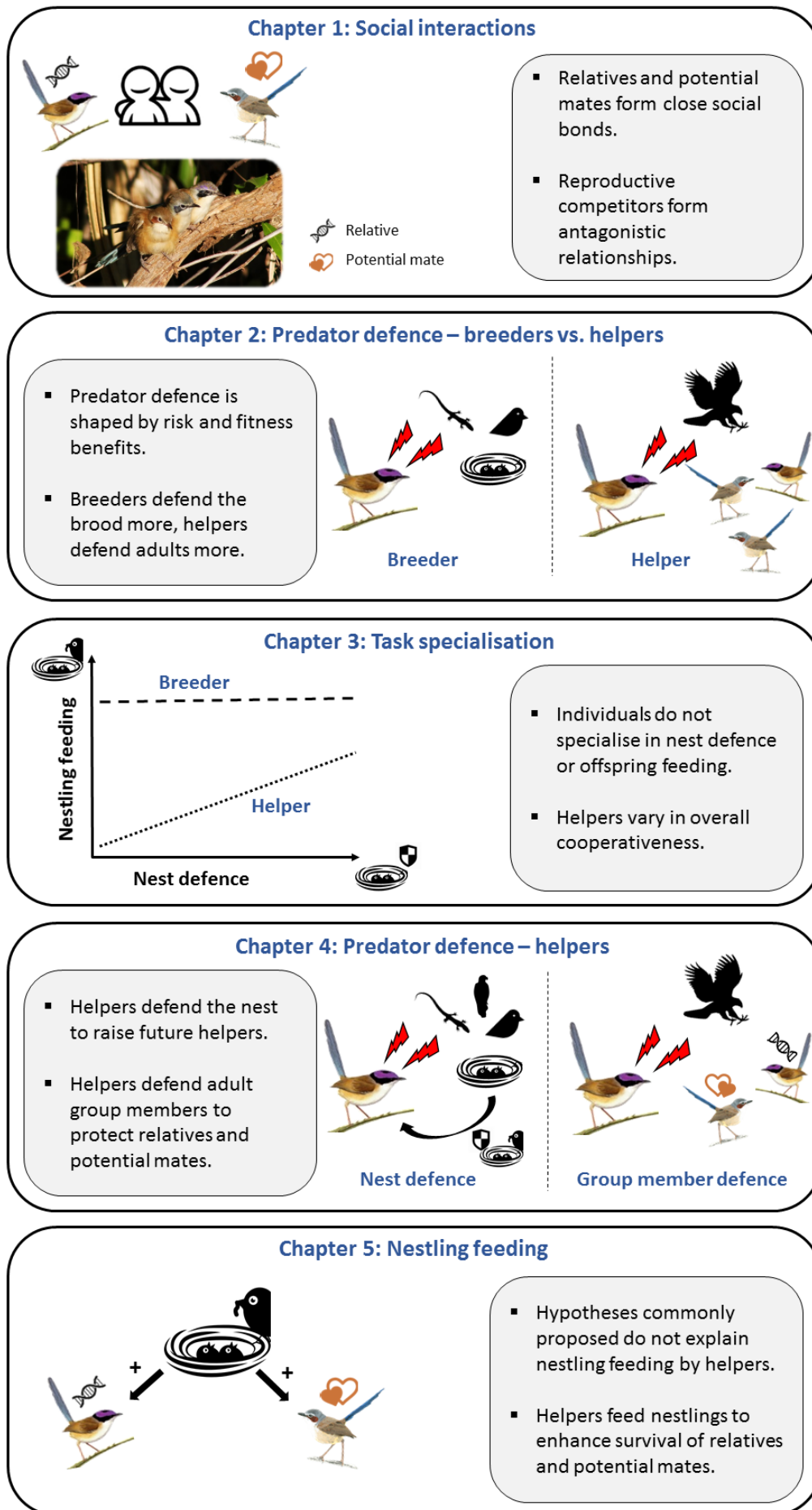


Figure 1. Outline of thesis chapters and key findings.

(unrelated opposite-sex individuals) in the group is highly beneficial. Subordinates can pair up with a subordinate in the group and bud off from the resident territory, or inherit the breeding position when the same-sex breeder in the group disappears (Hidalgo Aranzamendi et al. 2016; Fan et al. 2018); chances of inheritance are greater when subordinates are unrelated to the opposite-sex breeder (Kingma et al. 2011). Although extra-pair paternity is rare, subordinates at times also gain success via this route when they are unrelated to the opposite-sex breeder only (Kingma et al. 2009; Kingma et al. 2011). However competition over reproduction may be substantial; while queues for breeding position inheritance are stable (Kingma et al. 2011), subordinates can compete over potential subordinate mates within the group, and over vacant positions elsewhere (Fan et al. 2018).

Chapter 1 revealed an additional benefit of group living that depends on social group composition: subordinates affiliated most frequently and thus formed strong social bonds with potential mates and relatives specifically. These affiliative interactions and social bonds are known to be beneficial to individuals (Sanchez-Villagra et al. 1998; Aureli et al. 1999; Sapolsky 2005; Silk et al. 2009; Villa et al. 2016; Riehl and Strong 2018). Thus, affiliating with relatives and potential mates provides indirect benefits associated with enhanced survival or condition of relatives; increased chances of a potential mate still being alive and in good condition when a breeding vacancy opens up; and the formation of mutualistic social bonds, a benefit of group living not often considered for cooperative breeders. Further studies could investigate whether the strength of individual social bonds predicts the likelihood of potential future benefits (e.g. forming a breeding pair or receiving reciprocal actions) to come to fruition. Individuals could also use social interactions strategically to entice other group members to stay in the group or engage in subsequent cooperative behaviour (as in Radford 2011; Gill 2012). Further studies on the purple-crowned fairy-wren could test whether affiliative behaviour from dominant breeders to subordinates may entice them to help raise offspring in the group, and whether affiliative behaviour directed at specific group members affect their likelihood to disperse; if this is the case, affiliation with relatives and potential mates would further prolong the benefits associated with these group members.

Since social group composition determines how beneficial it is for an individual to stay, logically, I predict that social group composition affects dispersal behaviour. My study system is unique in that these fairy-wrens are highly restricted to edges of waterways with *Pandanus aquaticus* vegetation (Rowley and Russell 1993; Skroblin and Legge 2012), and respond strongly to playback of duet songs (Hall and Peters 2008). This combination of factors has allowed us to

conduct highly accurate yearly censuses from 2005 onwards along all waterways with suitable habitat in a 40km radius of the core study population to find birds that have dispersed out of the population. This allows us to distinguish dispersal from death for each individual with unusually high accuracy (see Koenig et al. 1996). Furthermore, we have detailed social information both before and after individuals disperse, which could be used to test whether subordinates are more likely to disperse when they share a social group with particular types of group members (e.g. more competitors and fewer relatives and potential mates). The fact that subordinate fairy-wrens in our population share groups with potential mates and relatives about twice as often compared to reproductive competitors (see Chapter 1, *Discussion*; Chapter 5, Fig. 2) suggests that they might be more likely to stay when sharing a group with beneficial group members compared to competitors (similar to male southern pied babblers, *Turdoides bicolor*; Nelson-Flower and Ridley 2016). Since aggression is rare (Chapter 1), it appears unlikely that competing subordinates are evicted from social groups, and a more likely explanation is provided by a greater probability of dispersing when the benefits of group living are smaller and the cost of reproductive competition greater (Nelson-Flower and Ridley 2016). This also indicates that dispersal from the natal territory to a new group with only unrelated individuals could be a highly costly activity, associated with costs of dispersal itself (Pasinelli et al. 2004), reduced benefits of group living, and increased costs of reproductive competition (which can be substantial; Nelson-Flower et al. 2013).

Group composition determines how much subordinates help

My thesis shows a clear and consistent effect of social group composition on cooperative behaviour. Helpers were more willing to cooperate in defence against a dangerous predator when doing so might prevent potential mates and relatives from getting hurt (Chapter 4), and cooperated more with offspring provisioning when this benefitted breeders that were relatives and/or a potential mate (Chapter 5). Moreover, these benefits associated with valuable group members were a more important driver of cooperation than benefits associated with helping raise the brood: nestling feeding was not explained by benefits associated with raising the brood (Chapter 5), and helpers were twice as willing to protect group members from predators compared to the brood, despite greater risk involved with defence against predators of adults (Chapter 2). My PhD research highlights the value of using an integrative approach to simultaneously test the full suite of proposed benefits of group living and of helping raise offspring that might be driving cooperative behaviour. Had I only considered benefits to the brood and directly to helpers, and not explicitly taken into account how cooperation by helpers benefits

other group members, I would have concluded that (1) help with predator defence occurs but is much lower than contribution by breeders, and against predictions by dynamic risk assessment theory (Montgomerie and Weatherhead 1988; Dugatkin and Godin 1992), increases with risk of injury (Chapter 2); (2) help with predator defence is only driven by active group augmentation benefits (Chapter 4); and (3) help with offspring feeding is not clearly explained by any of the proposed benefits of helping at the nest (Chapter 5). Thus, taking the full suite of benefits into account allows us to gain a more comprehensive picture of the complexity of this social system. Thereby, my research shows that social group composition determines benefits of group living and investment in cooperative behaviour. Social group composition is therefore important in both stages of cooperative breeding: the decision to stay in a group, and the decision to help (Komdeur and Ekman 2010; Shen et al. 2017).

Considering group composition may be particularly important for understanding the evolution of non-kin cooperation: 45% of cooperatively breeding birds breed in groups that are not composed primarily of kin (Riehl 2013), and the type of individuals present in the group may therefore be more generally important in such cooperative systems. A few studies have shown that investment in cooperative care by subordinates is related to group composition: in white-browed scrubwrens, *Sericornis frontalis*, helpers provide more care when they are unrelated to the opposite-sex breeder (Magrath and Whittingham 1997); in red-winged fairy-wrens, *Malurus elegans*, the sex of helpers present in the group affects provisioning effort by all other group members (Brouwer et al. 2014); and in Siberian jays, *Perisoreus infaustus* (Griesser 2009), and Arabian babblers, *Turdoides squamiceps* (Maklakov 2002), helpers contribute more to active predator defence when kin are present. In Arabian babblers, even the composition of the brood affects provisioning effort, with helpers preferentially feeding offspring that are of the opposite sex, presumably to avoid reproductive competition with same-sex offspring in the future (Ridley and Huyvaert 2007). Thus, the identity or type of recipients of cooperative behaviour constitutes an important consideration when activities are costly and associated with significant fitness benefits for the recipients.

A better understanding of how social group composition results in the level of care provided by individual group members could be obtained from studies on negotiation over (allo)parental care in cooperative breeders. It is well established that whenever multiple individuals are involved in providing care to offspring, conflict is expected to arise over the relative investment of each carer (Trivers 1972). As a result, parental decisions are not made independently and carers 'negotiate' over their relative share of care (McNamara et al. 1999;

McNamara et al. 2002; McNamara et al. 2003; Johnstone and Hinde 2006). How individuals adjust nestling feeding rates in response to a change in level of care by the other parent has been tested empirically in a range of species exhibiting biparental care (reviewed in (Houston et al. 2005; Harrison et al. 2009), but in few cooperative breeders (Wright and Dingemanse 1999; McDonald et al. 2009; Zottl et al. 2013). Recently, theoretical models on negotiation over offspring care in biparental care systems have been extended to cooperatively breeding systems (Johnstone 2011; Savage et al. 2013). These models predict that group composition determines the outcome of negotiation over offspring care, with optimal levels of alloparental care effort influenced by e.g. whether subordinates are helping maternal or paternal kin, and degree of relatedness to the parent providing a greater share of care. Additionally, the degree by which breeders compensate for a change in helper effort depends on the degree of relatedness to the helper. Empirical tests of the predictions of these models may yield important insights into how conflict over (allo)parental care is resolved in cooperative breeders, and how the composition of social groups determines the level of care provided by each group member. Predictions could be tested experimentally by decreasing (e.g. by handicapping; as in Wright and Cuthill 1990; Griggio et al. 2005) or increasing (e.g. by using targeted playback of extra begging calls; as in Hinde and Kilner 2007; McDonald et al. 2009) the amount of care provided by one of the group members, and monitoring nestling feeding effort by all individuals in the group both before and after the manipulation, and relating the change in feeding effort by individuals to patterns of relatedness between group members.

Predator defence: enhancing reproductive success and group member survival

Defence against predators and brood parasites constitutes a particularly important form of cooperation, enhancing nest success and group member survival (Taborsky 1984; Boland 1998; Canestrari et al. 2009; Garay 2009; Riehl 2011; Feeney et al. 2013). Yet, how helpers contribute to predator defence is relatively unexplored in cooperative birds (but see Maklakov 2002; Arnold et al. 2005; van Asten et al. 2016). My PhD research shows that helpers can contribute substantially to predator defence to protect the brood and group members (Chapter 2; 3; 4). Active predator defence by helpers thus seems to constitute an important benefit of living in groups and breeding cooperatively in this species. Individuals can also reduce the risk of predation through other, arguably less costly, behaviours. While the purple-crowned fairy-wren does not have a sentinel system, sentinel behaviour in other cooperative birds can reduce predation of adult group members (McGowan and Woolfenden 1989; Hailman et al. 1994; Wright et al. 2001;

Ridley et al. 2010; McQueen et al. 2017). Additionally, synchronisation of feeding visits to the nest by group members can reduce nest predation rates (Raihani et al. 2010). Additional studies on the various forms of predator defence behaviour in cooperative breeders are needed to reveal the benefit of cooperative defence to reproductive success and adult survival. These would provide insight into whether and how predator defence promotes the formation of social groups and the evolution of cooperative breeding (see Feeney et al. 2013; Jungwirth et al. 2015; Groenewoud et al. 2016).

My research assumes that predator defence by purple-crowned fairy-wrens results in increased nest success and adult survival, as it does in other species (e.g. Taborsky 1984; Boland 1998; Canestrari et al. 2009; Riehl 2011; Feeney et al. 2013), and as my personal observations of successful nest defence by groups attest. While it was beyond the scope of my PhD to explicitly test this, future research could focus on relating the incidence of nest predation to group size and group composition. A negative relationship between nest predation and group size would indicate that the presence of more helpers enhances nest defence, either through an increase in vigilance or active mobbing behaviour (Francis et al. 1989; McGowan and Woolfenden 1989; Boland 1998; Riehl 2011; Feeney et al. 2013), thereby enhancing nest survival. Additionally, placing trail cameras at additional nests to record natural predation attempts would allow for a quantification of the likelihood of successful nest defence according to the number of individuals actively defending the nest. I was unable to capture footage of sufficient nest predation attempts for official analyses throughout my PhD, however the limited data I was able to collect seems to suggest that larger groups mob predators more often, and more individuals mobbing together are more successful at warding off predators. Fairy-wrens mobbed nest predators in 19 of 29 (66%) of predation events recorded on motion-triggered trail cameras during the day. Groups consisting of unassisted pairs ($N = 11$) mobbed the predator in 55% of cases, whereas pairs with at least one subordinate (range = 1-3 subordinates, number of mobbing individuals = 1-3, $N = 18$) mobbed the predator in 72% of predation attempts. When one individual mobbed the predator alone, or two individuals mobbed the predator together, this was successful in 1 of 7 cases, and 1 of 9 cases, respectively. When three individuals mobbed together however, the predator was successfully deterred in all 3 observed cases. It is worth noting that the 5 cases of successful predator defence were against four goannas (*Varanus mitchelli*, *V. mertensi*, and *V. scalaris*) and one common tree snake (*Dendrelaphis punctulatus*), indicating that cooperative defence can successfully deter the most common nest predators (together responsible for 76% of 29 recorded predation events).

Helpers feed offspring and defend against predators for different benefits

Despite a positive correlation between nest defence and nestling feeding effort overall for helpers, 32% of subordinates contributed to predator defence but not to offspring feeding, while none contributed to nestling feeding only (Chapter 3). This means that if I had used only offspring provisioning rates as a measure of helping effort, nearly a third of subordinates would have been erroneously classified as non-cooperative. Instead, only 24% of subordinates were classified as non-cooperative when taking into account both forms of helping, compared to 56% when only considering offspring provisioning. Possibly, subordinates are more likely to engage in predator defence than offspring feeding because individuals differ in their ability to perform specific tasks (Bruitjes and Taborsky 2011; Wright et al. 2014), or because individuals of different age classes are more likely to contribute to particular tasks due to different costs associated with tasks (Heinsohn and Cockburn 1994; Clutton-Brock et al. 2003; Bruitjes and Taborsky 2011; Settepani et al. 2013). I consider this unlikely since breeders did not show similar preference to invest in predator defence, and contribution to tasks did not vary with age (Chapter 3). However future studies relating helping effort to e.g. body condition may shed light on the costs associated with both forms of helping. I consider the most likely explanation that subordinates are more likely to engage in predator defence than nestling feeding because the two tasks incur different benefits. Although predator defence is associated with risk of injury (Montgomerie and Weatherhead 1988; Dugatkin and Godin 1992), investment in a single predator defence event may greatly increase the prospects of brood and group member survival whereas many repeated feeding events may be required to similarly increase chances of survival. In support of this argument, I showed that the benefits explaining both forms of helping are only partly the same (Chapter 4; 5). Predator defence and nestling feeding behaviour were both predicted by benefits deriving from the enhanced survival of adult group members that represent relatives and potential mates. However, predator defence to protect the brood was additionally predicted by breeding position inheritance prospects, whereas nestling feeding effort was unrelated to probability of breeding position inheritance or other benefits associated with raising the brood. My findings thereby highlight how studying multiple forms of helping in a single system can inform us on the specific drivers of cooperative behaviour, and how these may according to costs and benefits associated with particular tasks, resulting in complex cooperative behaviour.

Conclusions

Taken together, my PhD thesis has allowed us to gain insights into how and why helpers contribute to multiple tasks associated with cooperative breeding. The most surprising findings of my research are the overwhelming importance of adult group members as a motivation for subordinate investment in cooperative tasks, specifically those that provide immediate beneficial interactions and delayed benefits of potential reproduction and kin selection. Contrary to common belief, stemming from most studies focusing on offspring provisioning and benefits of helping directly to subordinates and via enhanced production of offspring, improving the survival of valuable group members appears to be the most important driver of predator defence and offspring provisioning in this species, and possibly more broadly. My approach also highlights the value of studying cooperative predator defence: not only are helpers more likely to contribute to this, it also allows us to explicitly study how the full suite of benefits proposed for the evolution of cooperative breeding is related to cooperative behaviour, and to tease apart effects of benefits of group living from benefits associated with helping raise offspring. Thereby, my research expands our common explanations for why subordinates choose to stay in a group, and why they help (Komdeur and Ekman 2010), and specifically, how non-kin cooperative breeding may be maintained (Riehl 2013). Evidently, helping behaviour is complex and reflects multiple benefits associated with group members and raising the brood.

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