Investment patterns and kinship cues in a cooperatively breeding bird

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Contents

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Statement of intellectual contribution

As well as the assistance mentioned above, the research chapters (2-6) presented in this thesis have benefited from collaboration with a number of colleagues. To reflect this, they are presented in the style of scientific papers, with these collaborators listed as coauthors. As well as supervision from Ben Hatchwell and (for fieldwork) James Briskie, contributions made by the coauthors are detailed here. Other work is my own.

Chapter 2. I carried out the repeatability analysis with Isabel Winney, with whom I took the decision of what method to use for calculating R and implemented this method. Stephanie Preston provided provisioning data collected between 2008 and 2011, which were used in the turn-taking analysis. James Savage gave guidance on the turn-taking analysis and provided R code for implementing the runs test. All coauthors made comments that improved the manuscript.

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General summary

In cooperatively breeding species, ‘helpers’ provide care for other individuals’ offspring. Research into cooperative breeding, which initially asked the deceptively simple question ‘why?’, has continued to provide insights in behavioural ecology thanks to the opportunities for adaptation and coevolution that are generated in these unusual societies. I explore some of these potential adaptations in detail, mainly through studying a population of riflemen *Acanthisitta chloris*, which are passerine birds endemic to New Zealand. Previous work showed that riflemen are kin-based, facultative cooperative breeders. Most help is provided by adult birds, who have dispersed from their natal territory, but commute short distances to provision at the nests of relatives. Help is associated with enhanced recruitment of related offspring, and thus considered likely to confer indirect fitness benefits. These conclusions are substantiated by my results.

Provisioning by helpers is a special case of parental investment, and in Chapter 2 I characterise investment by rifleman carers. I find that sealed-bid and conditional cooperation models are inappropriate to describe investment in riflemen, and discuss possible reasons for this. I also demonstrate the validity of provisioning rate as a measure of food delivery in riflemen. In the following two chapters I test the hypothesis that helping drives adaptive sex allocation in cooperative breeders, first using data from riflemen, and then across 26 bird species. Surprisingly, the hypothesis is not supported in either case. In chapters 5 and 6 I consider how riflemen recognise their relatives in order to direct help to them. I identify candidate vocal and chemical kinship cues and test the responses of provisioning riflemen to olfactory manipulations and call playback.

My findings have implications for measuring parental investment in birds; show interesting discrepancies with evolutionary theory, and illustrate opportunities and challenges in sensory ecology. These themes are discussed in the final chapter.

INTRODUCTION
TO THE THESIS

1.

General introduction

This thesis describes research carried out into the evolution of parental investment and kin recognition in birds, mainly using the cooperatively breeding riflemen *Acanthisitta chloris* as a study species. In this chapter, I first introduce relevant concepts from the literature on parental investment, cooperative breeding and kin recognition. I then outline the scope of the thesis in more detail, and finally describe general methods that underpin the results presented in the following chapters.

Parental investment

For a gene to be favoured under natural selection, it must enhance the probability that copies of itself are present in its population in future generations (Dawkins, 1976). For this reason, most organisms devote some energetic investment to promoting the evolutionary fitness of offspring. Most commonly, this involves reproduction, sometimes followed by parental care (Smiseth et al., 2012). A parent's investment in its offspring can be formalised as the amount of resources it allocates to enhancing that offspring's survival and reproductive success, to the detriment of the potential allocation of those resources to other offspring (Trivers, 1972). This fundamental life-history tradeoff is widespread in nature, and arguably the most important of its kind, because for most organisms producing descendants that go on to reproduce themselves is the only long-term source of fitness.

The tradeoff is simplest where parental investment is limited to the production of gametes. In a given reproductive bout, the optimal investment is then a function of its effect on the probability and value of successful reproduction, traded off against the likelihood of being able to reproduce again. The situation is complicated when one parent provides post-fertilisation care for the offspring, because of parent-offspring conflict: each offspring is selected to demand more resources from the parent, than that parent is selected to provide (Trivers, 1974). Where there is more than one carer, it is complicated further, because there is not only conflict over *how much* investment each offspring should receive, but also over *who* provides it. This suite of potential coevolutionary interactions has made parental care a popular and productive topic in both theoretical and empirical behavioural ecology, and new insights continue to improve our understanding of this key life-history trait (Royle et al., 2012).

Offspring investment with multiple carers

In a number of animals, including most birds (Cockburn, 2006), offspring receive care from both of their parents, which adds another layer of potential conflict to the evolution of investment (Parker, 1979). In these species, raising offspring together is a cooperative venture, with positive outcomes for the fitness of both the mother and father. However, the alliance is uneasy, because it occurs between two unrelated individuals who would each fare better if the other worked harder. In terms of Trivers's (1972) tradeoff, high investment by an individual's partner maximises resource allocation to its current offspring without compromising its ability to invest in future offspring. The conflict that arises from this assumption has formed the basis of our understanding of how parental investment evolves with multiple carers.

Stable patterns of care require that neither parent would improve their fitness by changing their behaviour (Maynard Smith, 1977). An extreme example of a stable 'endpoint' of biparental care is sexual conflict driving the secondary evolution of uniparental care strategies, as appears to have happened in Eurasian penduline tits *Remiz pendulinus*, where after a clutch has been laid one parent deserts its partner, who then provides all of the care (Van Dijk et al., 2012). In most cases though the outcome is more subtle and biparental care is maintained, despite the apparent conflict over who provides it. Theoretical work has focused on explaining how this occurs. There are two mechanisms by which behavioural strategies might reach evolutionary stability: natural selection acting on fixed phenotypes ('sealed bids') on an evolutionary timescale, and behaviourally flexible individuals responding to one another in real time ('negotiation'). Initially, papers modelling both of these processes concluded that 'incomplete compensation' was a stable pattern of biparental care (Houston and Davies, 1985; McNamara et al., 1999). This describes a situation in which parents provide less care than is optimal for offspring; they increase their individual level of care in response to a reduction in their partner's effort, but will not compensate fully for this change. A meta-analysis showed that results of experiments on provisioning birds are generally consistent with this theory (Harrison et al., 2009). More recently, 'conditional cooperation' in which partners increase their investment in response to an *increase* by their partner (effectively, turn-taking), has also been demonstrated to be a stable pattern of care, and to result in greater fitness returns for offspring (Johnstone et al., 2014). Although this generates opposite predictions to incomplete compensation, it has been supported by at least four studies, including empirical data presented in the initial paper (Johnstone et al., 2014; Savage, 2014; Bebbington and Hatchwell, 2016; Koenig and Walters, 2016). This suggests that observed patterns in natural systems might result from the ecological feasibility of parents employing these respective strategies.

In cooperatively breeding species, reviewed below (see Cooperative Breeding in Birds), additional 'helpers' care for offspring as well as parents. I use 'carers' as an umbrella term for parents and helpers in this chapter and throughout the thesis. Combinations of strategies for carers in cooperative breeding systems are inevitably more diverse than those in biparental care, but the negotiation framework can be readily extended to cooperative groups (Johnstone, 2011); indeed, although turn-taking models have yet to be formally extended to cooperative breeders, two of the supporting studies cited above have come from such systems (Savage, 2014; Koenig and Walters, 2016). In cooperative breeders, relatedness between members of the group becomes crucial to predicting investment patterns (Johnstone, 2011; Savage et al., 2013). For example, helpers who are closely related to breeders might increase their indirect fitness if those breeders reduce their effort, known as load-lightening (Crick, 1992). This is generally observed in species where nestling starvation is rare (Hatchwell, 1999). Where breeders do not (fully) compensate for being helped, benefits of help accrue to offspring, and the indirect fitness gained by helpers then depends on their relatedness to the brood. There is evidence from species such as long-tailed tits *Aegithalos caudatus* that helpers adjust their investment according to their relatedness to the offspring they are helping (Nam et al., 2010).

Load-lightening is an example of how carers' fitness optima can align: relatedness reduces conflict over who provides care (though not completely, as in an outbred population each individual is at least twice as related to itself as to any other individual). Repeated reproductive bouts with the same partner (pair bonds) should also reduce this conflict: if two parents are likely to breed together again, the reproductive success of one depends on the fitness of the other, and exploitation becomes a relatively weaker strategy. A complete absence of sexual conflict is predicted only under 'true monogamy', where an individual will, with 100% certainty, only reproduce with one partner; this is considered 'to exist only as a theoretical reference point rather than a practical eventuality' (Lessells, 2006). Nevertheless, where there are long-term pair bonds partners have a considerable 'stake' in one another's fitness (Roberts, 2005). This has interesting implications for conflict-based models of the evolution of care.

Investment in relation to offspring sex

Fisher's (1930) models of optimal sex allocation formulated an elegant explanation for why sex ratios are so often equal in nature: because each offspring has a mother and father, each sex would immediately have a reproductive advantage if it became rarer than the other, and selection based on this advantage would return the sex ratio to parity. This remains a textbook example of negative frequency-dependent selection and refutation of group adaptation. Fisher (1930) went on to show that the expected sex ratio does not remain equal if one sex is more costly to produce than the other; at an equal sex ratio breeders would benefit from producing the cheaper sex, until the benefit of rarity for the other balanced the cost of its production. Therefore, the 'investment ratio' would remain equal, but the equilibrium sex ratio would be biased towards the cheaper sex. Cost differences are likely in size-dimorphic species, where the larger sex is expected to be more costly to produce. Using size dimorphism as an indication of cost differences shows that bird species generally support the theory (Benito and González-Solís, 2007). A more subtle source of cost difference comes in cooperative breeders where helpers are descendent kin, and more likely to be of one sex; here offspring of this more helpful sex should be effectively cheaper, because by helping they 'repay' some of the cost of rearing (Emlen et al., 1986). There is some support for this theory from single-species studies of cooperative breeders (e.g. Malcolm and Marten, 1982; Clarke et al., 2002).

While Fisher (1930) considered sex ratios at the population level, Trivers and Willard (1973) showed that equal sex ratios are not necessarily expected at the individual level. For example, in polygynous species, both sexes have equal reproductive success on average, but its variance is greater for males: some males monopolise reproduction and achieve high fitness, while others fail to reproduce and achieve none. This makes males a riskier investment than females. Therefore if a female can produce a high-quality offspring, she would do better to make it a male, and if not, she should produce a female. There are striking examples of this prediction being confirmed, for example in red deer *Cervus elaphus* (Clutton-Brock et al., 1984), blue tits *Cyanistes caeruleus* (Sheldon et al., 1999) and lance-tailed manakins *Chiroxiphia lanceolata* (Sardell and DuVal, 2014); on the other hand, a number of negative results have led to the suggestion that facultative sex allocation may be constrained or costly in taxa such as birds and mammals with chromosomal sex determination (West and Sheldon, 2002; but see Komdeur et al., 2002). An equivalent prediction for facultative sex ratio adjustment in cooperative breeders is that breeders who already have helpers will benefit less from producing the more helpful sex; this has been supported by meta-analyses (West and Sheldon, 2002; Griffin et al., 2005), but similarly, more recent negative studies have cast doubt on its generality (e.g. Cockburn and Double, 2008; Kingma et al., 2011).

Summary

Investment in offspring is arguably the most important trait in animal life-histories. Where this investment extends to providing care, complex behavioural patterns are expected to emerge from variation in levels of conflict, relatedness and interdependence in carer-carer and carer-offspring relationships. Investment may be allocated unequally among offspring, for example between males and females under certain conditions. The case of cooperative breeding generates distinct predictions regarding investment patterns, which are often directly testable in natural systems.

Cooperative breeding in birds

Cooperative breeding systems are characterised by helpers: non-parental carers who help to raise dependent offspring. Although such apparently altruistic behaviour was once considered an evolutionary paradox, the concept of inclusive fitness (Hamilton, 1964) has provided the adaptive framework through which it has been understood since the 1960s. Subsequently, the paradox has become a simple question, with an elusive answer: what explains the enormous variation in cooperation that exists in nature (e.g. Wilson, 1975; Emlen, 1997; Koenig and Dickinson, 2016)?

Cooperative breeding is diverse in taxonomically and in complexity, but has probably been studied most extensively in birds (Koenig and Dickinson, 2016). Birds possess certain traits that make them practical for studying evolution in the wild: they are easily colour-ringed; mostly diurnal and readily observed; have blood with nucleated red cells that can be genotyped from small samples; live at high enough densities that sufficient data can be collected on manageable study sites, and have the popular appeal to attract generations of fieldworkers to following their behaviour. Their cooperative breeding systems are also interesting scientifically because almost all are facultative, allowing meaningful comparisons to be made within species on the factors promoting cooperation (Hatchwell, 2009). Below, I review avian cooperative breeding ‘backwards’, by first looking at how helpers gain fitness, then the processes that make this their best option, and finally ultimate explanations for why some species have evolved cooperative breeding and others have not. In doing so I introduce the diversity of social systems covered by cooperative breeding, and discuss outstanding issues in this field of research.

Why helpers help

For helping to be evolutionarily stable, it must yield an inclusive fitness benefit that outweighs its cost (Hamilton, 1964). Understanding cooperative breeding is impossible without an explanation of why helpers help.

Immediate explanations for helping behaviour are reviewed in Table 1.1, with indication of their support. The non-adaptive explanation is something of a null hypothesis for the evolution of helping, which is difficult to test. It can however be ruled out with confidence in species where helpers have been shown to discriminate between recipients, such as long-tailed tits (Russell and Hatchwell, 2001). The likelihood that helping incurs a fitness cost (Heinsohn and Legge, 1999), also suggests that it is unlikely to be stable in the absence of any adaptive benefit.

Apart from parentage (see below), direct benefits of help have been rarely demonstrated, and only for future reproductive opportunities have they been confirmed in the absence of kinship. Although they should be more widely tested, and Cockburn (2004) and Riehl (2013) describe species which will be profitable to investigate, indirect benefits of helping kin are the most widely supported drivers of help in avian systems.

Indirect fitness benefits are supported by a number of studies relating help to enhanced breeding success of kin (e.g. Lennartz et al., 1987; Woxvold and Magrath, 2005; Preston et al., 2016), and correlations between relatedness and levels of care both within-species (Nam et al., 2010; Wright et al., 2010; Browning et al., 2012a) and between-species (Green et al., 2016). Through these studies cooperatively breeding birds have provided some of the strongest evidence available for the importance of kinship to the evolution of cooperation.

Table 1.1 Summary of explanations for why helpers raise non-descendent offspring in cooperatively breeding birds.

Explanation for help	**Description and support**	**Type of fitness**	**Example species**	**Reference**
Non-adaptive	Help is a by-product of strong selection on parental care, which acts on simple rules such as 'provision nearby young'. Not empirically supported (but difficult to test).	Neutral	?Pukeko *Porphyrio melanotus*	Craig and Jamieson (1990)
Paternity sharing	Multiple males copulate with a female and help her to raise the brood; those that are not fathers to the offspring they care for may be considered helpers. Widespread, but often not considered true cooperative breeding (Koenig et al., 2016a).	Direct	Dunnock *Prunella modularis*	Burke et al. (1989)
Future reproductive opportunities	Helpers pair with recipient breeders after providing help. Recorded in a few species. Suggestion that helpers pair with recipient *offspring* after helping in another species not supported by molecular data (Preston et al., 2013a).	Direct	Moustached warbler *Acrocephalus melanopogon*	Fessl et al. (1996)
Raising related offspring	Helpers gain indirect fitness benefits by improving the productivity or recruitment of dependent kin. Widespread.	Indirect	Rifleman *Acanthisitta chloris*	Preston et al. (2016)
Load-lightening	Helpers improve the reproductive success of related breeders by allowing them to reduce their investment. Widespread.	Indirect	Long-tailed tit *Aegithalos caudatus*	Hatchwell and Russell (1996)
Avoiding punishment	Breeders behave aggressively towards subordinates that do not help, and so helpers avoid that cost. Supported in one species; rarely tested.	Direct	Superb fairy-wren *Malurus cyaneus*	Mulder and Langmore (1993)
Developing parental skills	Helpers learn skills that improve their parenting. Supported for *subordinates* in Seychelles warblers; now known that some were breeders (Komdeur et al., 2016).	Direct	?Seychelles warbler *Acrocephalus sechellensis*	Komdeur (1996)
Social prestige	Helping is a show of individual quality analogous to a sexual handicap that confers social advantages (e.g. perception as a desirable mate). Not empirically supported.	Direct	?Arabian babbler *Turdoides squamiceps*	Zahavi (1995)
Future help	Recipients of help reciprocate in kind, ameliorating its overall cost for the helper.	Direct	None	Clutton-Brock (2009)

The shared parentage that characterises ‘cooperatively polygamous’ systems is the other common driver of care for non-descendent offspring. Whether or not this constitutes help is a topic of some disagreement. Some authors separate these breeding systems from (other) cooperative breeders, defining the latter as requiring non-breeding helpers, while ostensible ‘helpers’ in the former should be referred to as cobreeders (Hartley and Davies, 1994; Boomsma, 2013; Koenig et al., 2016a). This reflects a fundamental difference in the type of fitness they attain through cooperation. Others have criticised this approach as drawing an unnecessary dichotomy within what might be better described as a continuum (Cockburn, 1998; Riehl, 2013). There are convincing arguments on each side of this debate. On one hand, the fitness effects of cobreeders provisioning someone else’s offspring have more in common with those for partially cuckolded males in biparental systems with extra-pair paternity (EPP), than non-breeding helpers raising kin as described above. On the other hand, to exclude cooperatively polygamous systems removes much of the diversity of avian cooperation. More critically, the two strategies are not always separable: considering a male’s decision to care for a brood in which he shares paternity with his brother, the marginal benefits may only exceed the costs because of the combination of his paternity share *and* relatedness to the non-offspring in the brood, where neither would have been a sufficient basis on its own. For this reason, although it is not critical to the remainder of this thesis, I favour an inclusive definition of cooperative breeding. This also solves the problem of classifying ‘hopeful reproductives’, where future breeding opportunities or social prestige may be drivers of helping.

Factors creating the conditions for helping

Though helping may be favoured in the immediate instance by the mechanisms considered above, it is still likely to yield a poorer fitness return than successful independent reproduction (Hatchwell and Komdeur, 2000). Explaining how helping becomes an individual’s best option is therefore a second critical process in understanding cooperative breeding.

The classic model of a cooperatively breeding bird has young delaying dispersal and helping their parents to raise more offspring on the home territory (Emlen, 1982). Delayed dispersal or the analogous retention of offspring in a mobile group is a feature of the majority of well-studied species, and explaining it has featured prominently in researchers' priorities (Koenig and Dickinson, 2016). A number of hypotheses, which are not fully distinct from one another, have been important here. Emlen's (1982) ecological constraints hypothesis emphasised the role of a limiting resource, which if unavailable makes dispersing unprofitable for juveniles. This principle has been experimentally confirmed to apply to nesting cavities in red-cockaded woodpeckers *Picoides borealis* (Walters et al., 1992), and territories in Seychelles warblers *Acrocephalus sechellensis* (Komdeur, 1992). Stacey and Ligon (1987) 'challenged' this interpretation with one that was quite similar: that when variation in habitat quality is high, individuals might enhance their lifetime fitness by initially remaining on their parents' high-quality territory (the 'benefits of philopatry hypothesis'); this was supported by their study of acorn woodpeckers *Melanerpes formicivorus*. Covas and Griesser (2007) suggested that this decision might derive from species' life-history traits rather than habitat variation. This has been invoked in green woodhoopoes *Phoeniculus purpureus*, where females that delay dispersal have a higher lifetime reproductive success (Hawn et al., 2007), and a version coined the 'delayed extra-pair benefits hypothesis' is suggested in western bluebirds *Sialia mexicana*, where older males are more successful at gaining EPP, and delaying breeding could positively affect lifespan (Dickinson et al., 2016). The 'group augmentation hypothesis' predicts that being part of an optimally-sized group can drive delayed dispersal (Kokko et al., 2001), and if the benefits of group size are high enough, might even lead to the formation of novel groups in non-kin systems (Riehl, 2013). Explanations for delayed dispersal are many, though all of course generalise to comparing its fitness outcomes with those of outside options, and some can be couched in the terms of others. Once dispersal has been delayed, the failure to reproduce independently usually has a simpler explanation: a lack of available unrelated mates (Koenig and Haydock, 2004).

Delayed dispersal is not, however, a prerequisite for cooperative breeding. In species such as riflemen, most helpers have dispersed onto their own territory, but visit the nests of relatives to help (Preston et al., 2013a). In this case *limited* natal dispersal generates a population kin structure in which relatives are close to one another and ‘commuting’ between territories is practical (Preston, 2012). In long-tailed tits, offspring also disperse and almost all attempt to breed independently. Some individuals whose nests fail become helpers; the rate of nest predation is so high that helping is a common strategy (Hatchwell, 2016). Not only is nest predation a proximate driver of helping decisions, it is also likely to promote the indirect benefits associated with them: because it is such a major driver of mortality, usually of an entire brood, those nests that are not depredated are the source of kin clusters that help one another in future seasons (Beckerman et al., 2011). The social systems exemplified by these species have been termed ‘kin neighbourhoods’ to differentiate them from those exhibiting delayed dispersal (Dickinson and Hatchwell, 2004). Though limited dispersal is still important in generating kin structure in these neighbourhoods, benefits of philopatry arguments do not apply because offspring do not remain on their parents’ territory. The colonies of species such as bell miners *Manorina melanophrys* and sociable weavers *Philetairus socius* lie somewhere in between these two definitions: although helpers have not dispersed, the presence of unrelated conspecifics within the colony means they are usually not constrained from breeding independently by inbreeding avoidance (Van Dijk et al., 2015; Wright and McDonald, 2016).

Factors promoting cooperative breeding between non-kin in shared-parentage groups have been less exhaustively studied, though well-reviewed by Riehl (2013). Unsurprisingly, this occurs more often when cooperative breeding is obligate. In species such as white-winged trumpeters *Psophia leucoptera*, it appears that females ‘tolerate’ cooperative breeding because it is the only route to reproducing successfully (Sherman, 1995). This is suggested to occur when ecological conditions are unfavourable enough to make independent reproduction unprofitable or impossible (e.g. Shen et al., 2016). Although the group augmentation hypothesis introduced above has had limited success in explaining cooperation in kin groups (Kingma et al., 2014), it may be more appropriate in this context. An alternative explanation is more analogous to the life-history hypothesis: that cooperative breeding first evolves between kin because of indirect fitness benefits and selection then favours traits expressed in cooperative groups until a species becomes specialised or obligately cooperative; relatedness within the group is then eroded by conspecific brood parasitism (CBP), immigration or extra-group mating, forming essentially non-kin cooperative breeding groups constrained by their specialisation (Cockburn, 2013). This appears relevant in *Malurus* fairy-wrens, but less so in joint-nesting species such as Taiwan yuhinas *Yuhina brunneiceps* in which an ecological explanation provides a better fit (Shen et al., 2016).

Ultimate predictors of cooperative breeding

Due to the diversity of the above processes favouring helping behaviour, explaining the global distribution of cooperative breeding in birds is especially challenging. Nevertheless, some broad-scale patterns have emerged that explain some of the variation in avian cooperative behaviour. First, cooperatively breeding species are more likely to live in unpredictable environments (Jetz and Rubenstein, 2011). This is consistent with the recurrent arguments that focus on ecological constraints in suggested explanations for cooperative breeding. Second, some studies suggest cooperative breeding is associated with long lifespan (Arnold and Owens, 1998; Beauchamp, 2014). This may make helping a relatively better strategy because of the likelihood of survival to breeding, and may also drive habitat saturation through low breeder turnover, in turn causing the ecological constraints that can drive delayed dispersal. Third, the above studies show a strong phylogenetic signal to cooperative breeding, supporting the hypothesis that it may be retained in lineages once they diversify, and therefore that current ecological or biological traits may not explain why a species is cooperative (Edwards and Naeem, 1993). Finally, higher levels of sexual monogamy have been observed in cooperative than non-cooperative species (Cornwallis et al., 2010). Monogamy drives up the indirect benefits available to helpers rearing social siblings, and these results have supported the theory that complex sociality evolves through a kin-selected ‘monogamy window’ (Boomsma, 2009). However, it is worth noting complications to this relationship: extreme promiscuity also favours helping, because a male who is likely to be cuckolded when breeding may be better off raising maternal half-siblings (Kramer and Russell, 2015); also, similar traits are likely to favour both monogamy and cooperation, obscuring any causal relationship (Dillard and Westneat, 2016).

Although these relationships are statistically significant within the reported datasets, they still leave much variation unexplained and fall short of acting as a predictive framework for cooperative breeding. There are opportunities to add insight, such as incorporating more detail on species' demography and life-history (Hatchwell, 2009), but it is unsurprising that broad-scale patterns provide an incomplete picture, given the diversity of processes that favour cooperation.

Summary

Cooperative breeding in birds encompasses a considerable array of social systems, and there is particular debate over whether communally breeding species should be included. As a result, it has become difficult to make generalisations about their characteristics. Indirect fitness benefits are widely implicated in driving the evolution of help, and ecological processes such as predation and poor or variable environmental quality are commonly, though not always, associated with creating permissive conditions. Evolutionary history, and life-history traits such as long lifespans and short dispersal distances, may also predispose species to cooperate. Sexual monogamy can promote indirect fitness in kin-based cooperative breeders, but a high diversity of genetic mating systems suggests that this is not a panacea.

Kin recognition

As researchers studying various cooperative breeding systems have concluded that indirect fitness benefits have played a key role in the evolution of helping, interest has grown in the mechanistic basis of how these benefits are realised (Komdeur and Hatchwell, 1999). Being able to discriminate between kin and non-kin can be crucial to allocating help adaptively, where potential helpers regularly encounter the latter. It can also be beneficial for non-cooperative species where there is strong selective pressure on avoiding inbreeding. Although kin recognition is a relatively new field (see Grafen, 1990), it has provided important context to understanding behavioural decisions.

Adaptive basis

The adaptive value of help influences the potential benefits of evolving a mechanism to recognise kin in cooperative breeders (Griffin and West, 2003). These benefits are also influenced by social structure. In species where cooperative groups are formed by parents retaining philopatric offspring, within-group relatedness is likely to be high, and helpers might gain indirect fitness benefits from helping any young born in the group. A comparative analysis has shown that these species are less likely to have helping patterns that suggest kin discrimination (Cornwallis et al., 2009). By contrast, the benefits of kin recognition are likely to be considerable in kin neighbourhoods, because individuals have dispersed from their natal territory meaning that close relatives, although likely to be nearby, are not reliably structured in space. Species living in these societies are more likely to be able to discriminate kin. For example, the long-tailed tit shows an overwhelming preference for helping close kin and generally will not help if there are none available (Russell and Hatchwell, 2001). Similar selective pressures operate in colonial species where often hundreds of conspecifics of variable relatedness live in close proximity. In the colonial bell miner, Wright et al. (2010) showed that helpers work harder when provisioning close relatives, indicating an ability to discriminate. In both species, individuals are able to distinguish kin from non-kin based on vocalisations (Hatchwell et al., 2001; McDonald and Wright, 2011), reflecting the norm in successful studies of kin recognition in avian cooperative breeders (Crane et al., 2015).

Beyond helping, kin recognition may be important for inbreeding avoidance, in species where mating with relatives is likely (under random mate choice), and carries a fitness cost through inbreeding depression. This is especially plausible in the kin-structured populations of cooperative breeders where interactions between relatives are frequent. However, kin structure is widespread even in non-cooperative species (Hatchwell, 2010). Here, substantial risks of inbreeding can select for mechanisms of kin recognition (or at least recognition of genetically dissimilar individuals) in the absence of helping benefits, for example in colonies of the black-legged kittiwake *Rissa tridactyla* (Mulard et al., 2009). Though the mechanism by which kittiwakes avoid inbreeding is unknown, chemical compounds present in preen wax have been demonstrated to contain some relatedness information and may act as an olfactory cue (Leclaire et al., 2014). In both cooperative and non-cooperative species, sex-biased dispersal has been suggested to act as a mechanism of passive inbreeding avoidance, which may weaken selection on kin recognition mechanisms (Koenig and Haydock, 2004). Recent models suggest that this is unlikely to be sufficient in all populations (Blyton et al., 2015). This supports the contention that kin recognition is adaptive in some non-cooperative species, and the results may add insight to our currently helping-centred view of its distribution among cooperative species.

Mechanism and development

As social structure should influence the likelihood of kin recognition evolving, it might also influence the mode in which kinship cues develop. One mode is for cues to be individually distinct; these can then be learned from association with nestmates, offspring and parents, with individuals later choosing these individuals to help, or avoiding them when finding a mate. However, this is only likely to be beneficial when these relationships reliably form between kin; individuals using these cues in systems with high levels of EPP and CBP will make frequent ‘mistakes’ in such decisions. On the other hand, cues might accurately encode information on genetic relatedness; in this case, they would be robust to the destabilising influence of EPP and CBP. Genetic cues also carry the advantage of enabling recognition between unfamiliar relatives, but the feasibility of their evolution has been questioned because of the conceptual difficulty of a trait accurately representing an individual genotype (Crozier, 1986; Grafen, 1990). Recent work demonstrating genetic kin recognition in house mice *Mus musculus* suggests that stable genetic cues can arise from diverse gene complexes and be detected through olfaction (Green et al., 2015). The dependence of the value of learned versus genetic kinship cues on social structure means that we can make predictions about the types of system in which they are likely to occur, although this may also be influenced by the historical constraint.

Among cooperatively breeding birds, all studies demonstrating kin recognition have done so using vocal cues, and in most cases evidence suggests that these are learned associatively (Crane et al., 2015; Riehl and Stern, 2015). The most complete study of learned vocal recognition comes from long-tailed tits (Sharp et al., 2005), in which calls are individual-specific, more similar between family members, and learned from familiar kin at the nest: cross-fostered nestlings had calls that were more similar to their foster families, and later helped at the nests of these social relatives rather than genetic kin. Similar processes appear to operate in other species, but even in long-tailed tits understanding of the recognition process is incomplete: helpers make fine-scale decisions such as working harder when provisioning closer relatives, which are not attributable to a simple ‘help my social family’ recognition rule (Hatchwell, 2016). Whether this is explained by the action of learned and genetic cues together, or by the diversification of learned cues in a way that reflects pedigree relatedness, is still an open question. An exception to the general trend of learned cues in avian cooperative breeders comes from bell miners, where the structure of a simple contact call is apparently innate, and differences in this structure reflect genetic relatedness (McDonald and Wright, 2011). This appears to underlie the type of adjustments whose mechanism is unknown in long-tailed tits, and may be particularly advantageous given the complex social organisation of the bell miner’s colonies.

Summary

Mechanisms of kin recognition are likely to evolve in species where there are opportunities to direct cooperative behaviours such as helping towards relatives, and/or there is a substantial risk of inbreeding, and neither are avoidable by following simple spatial rules. These criteria are more likely to be met in cooperative breeders, particularly those living in kin neighbourhoods or colonies. Whether cues are genetic or learned may be subject to selection; although genetic kinship cues have been considered conceptually problematic, there are good examples from birds and other taxa. Among cooperatively breeding birds, most demonstrated kinship cues are learned associatively, and all are vocalisations; however, olfactory cues have been rarely investigated, and evidence from non-cooperative species suggests they play a role in avian communication.

Scope of the thesis

Riflemen

The research presented in this thesis addresses the themes introduced above, primarily using the facultatively cooperative rifleman as a study species.

Riflemen are members of the ancient New Zealand wren family Acanthisittidae, which has historically inhabited an uncertain position within the passerine order Passeriformes (e.g. Sibley, 1970; Raikow, 1987; Sibley and Ahlquist, 1990). The family was traditionally omitted from the two major radiations of passerines (the oscines Passeri and suboscines Tyranni), on the basis of lacking the derived features characteristic of each, and molecular data have now confirmed its position as an outgroup to the rest of the order (Ericson et al., 2002; Jetz et al., 2012). New Zealand wrens thus constitute the third (albeit humble) radiation of passerine birds: the Acanthisitti. Eight species are known: four from subfossils, which probably became extinct before 1500; Lyall's wren *Traversia lyalli*, which became extinct in 1895; the bush wren *Xenicus longipes*, which became extinct in the late 20th century; riflemen, and their only surviving cousins the rock wren *X. gilviventris* (Gill et al., 2010; Worthy et al., 2010). All are or were poor flyers at best, with four of the extinct species, including Lyall's wren, known to have been flightless, and likely to have been easy prey for introduced mammalian predators (Gill, 2004; Tennyson and Martinson, 2006). There is no evidence for cooperative breeding elsewhere in the family, though Lyall's wren and the bush wren were poorly known while extant, leaving the rock wren as the only candidate species (Gill, 2004). Rock wrens are classified as globally vulnerable; despite once occurring throughout New Zealand they are now restricted to open areas in the South Island high country (BirdLife International, 2016). A study in Fiordland showed that both parents share nest-building, incubation and provisioning in this species (as they do in riflemen), but no extra helpers were observed at any nests (Michelsen-Heath, 1989).

Riflemen cooperate within kin neighbourhoods; there is a strong kin bias to helping in this species, and helpers improve the recruitment of offspring, suggesting a central role for indirect fitness benefits in driving the behaviour (Preston et al., 2013a, 2016). I review more detail of the ecology, life-history and cooperative breeding system of the species in Chapter 7, which incorporates research conducted for this thesis, so here I will simply outline salient points concerning their suitability as a study species. Firstly, relatedness between helpers and recipients is high, carrying implications for the evolution of investment strategies, and implying that helpers actively recognise kin (Preston et al., 2013a). Kin recognition is also expected because of the kin-neighbourhood structure of the population (see Kin Recognition), and a substantial risk of inbreeding (Preston, 2012). Secondly, riflemen deliver food to dependent nestlings at a high rate (Sherley, 1990a), making this a potentially powerful measure of offspring investment in the species. Finally, riflemen exhibit sexual size dimorphism (Sherley, 1993) and sex-biased helping (Preston et al., 2013a), generating strong, testable predictions about expected investment in the two sexes (see Parental Investment).

Thesis outline

In this thesis I investigate patterns of offspring investment, and potential kinship cues, in the rifleman cooperative breeding system. Below, I summarise the methods used in the fieldwork and genetic analysis that underpins the research presented in the following chapters. In Chapter 2, I describe patterns of provisioning by parents and helpers in this species. Chapter 3 examines the effect of offspring sex on investment, and in Chapter 4 I expand this investigation, using a comparative approach to assess cross-species support for the repayment hypothesis of sex allocation. In the next two chapters, I focus on potential kinship cues: Chapter 5 looks at the potential for chemical signals to communicate kinship, and Chapter 6 does likewise for vocalisations. Work towards this thesis is synthesised in the final two chapters, with Chapter 7 considering its implications for our understanding of rifleman cooperative breeding, and Chapter 8 the broader themes to which it provides insight.

General methods

Kowhai Bush

I carried out field observations and collected samples between September and January from 2012-2015, at Kowhai Bush, near to Kaikoura on New Zealand’s South Island (173° 37’ E, 42° 23’ S). The site is a temperate regrowth forest dominated by kanuka *Kunzea ericoides* on the floodplain of the Kowhai River, with a mean annual temperature of 12 C, and mean annual rainfall of 865 mm (Gill, 1980). The population of riflemen here has undergone a recent decline and numbered between 6 and 11 pairs during the study period. The total forest area comprises 240 ha, but riflemen are generally restricted to a 30 ha section, with generally sparser understorey, where a grid of 230 nestboxes has been provided. The vast majority of rifleman pairs use these nestboxes (just four natural nests were recorded in three seasons), which protect nests from the depredations of introduced mammals (Briskie et al., 2014).

Field methods

I collected basic field data and samples as follows. For procedures used in specific studies see the methods sections of the following chapters.

I identified active nests before eggs hatched by weekly checking of all nestboxes on the study site for the presence of nests, and followed the progress of those containing nests by checking them daily. Each individual in the population was given a unique combination of two colour rings and a metal Department of Conservation AP ring for identification, either as a 15-day-old nestling, or as an adult or juvenile caught by mist-netting near to known nests.

I filmed nests using a digital camcorder every 3 days after hatching when possible, starting at day 3, where hatching is defined as day 0, and continuing until fledging (typically around day 24). Each recording started with a 15-minute acclimatisation period for which footage was discarded, with data then collected from the following hour. Recording start time varied between 0700 and 1700 NZST. Carers were not caught on the days their nests were filmed.

After nests were filmed on day 15, each nestling was temporarily removed from the nest to be weighed, measured, ringed, sexed, and have samples taken of blood for genetic analysis, and preen wax for chemical analysis. Riflemen can be sexed reliably in the hand at day 15, females being larger than males with differently coloured plumage. At least one nestling was left in each nest at all times so that adults did not return to an empty nest, which may stimulate abandonment. I collected blood samples using brachial venipuncture, and stored them in plastic screw-cap microfuge tubes along with c.1 mL pure ethanol. Blood samples were also collected from all adults and juveniles who were caught by mist-netting and had not been previously sampled.

Genotyping

Genotyping was carried out at the University of Sheffield's NERC Biomolecular Analysis Facility. I extracted DNA from blood and tissue using ammonium acetate precipitation (Nicholls et al., 2000), quantified it using fluorometry, and diluted samples to c.10-20 ng/µL. I genotyped samples at 17 loci using 13 primers designed by Preston et al. (2013b), as well as 3 general markers designed by Dawson et al. (2010) and the Z043B sex marker (Dawson et al., 2016), in a novel combination of 3 multiplexes (Table 1.2). Each sample was amplified at all 17 loci using PCR, in which 1 µL of sample was run with 1 µL of multiplex mix (within which all primers were at 0.2 µM) and 1 µL of PCR Master Mix (Qiagen, Hilden), with one PCR for each multiplex. I ran PCR product through an ABI 3730 96-well capillary sequencer and exported the results to the program GeneMapper (Applied Biosystems, Foster City), which I used to score alleles at each locus for each sample. Allele scores were standardised with Preston et al. (2013b). In total I genotyped 128 individuals from Kowhai Bush, and combined these with genotypes at the corresponding loci for 226 individuals genotyped by Preston et al. (2013b), to give a total dataset of 354 individuals. I obtained Queller and Goodnight's (1989) estimate of relatedness between each dyad using the program SpAGeDi (Hardy and Vekemens, 2002), and reconstructed pedigree relationships using the program Colony (Jones and Wang, 2010).

Ethical note

All research was carried out in accordance with New Zealand law and under approval from the University of Canterbury's Animal Ethics Committee. Behavioural experiments, ringing and the collection of blood samples were carried out under Department of Conservation permit NM-34956-FAU. Preen wax samples were collected under Department of Conservation permit NM-34075-FAU. Birds were handled only for ringing, measurements and the collection of blood and preen wax samples. All adults were released at the capture location within 45 minutes of initial capture, and all nestlings were carefully replaced in their nest.

Table 1.2 Markers used to genotype riflemen from Kowhai Bush (2008-2015), ordered into multiplexes with their annealing temperature provided in brackets. TG~ markers were developed by Dawson et al. (2010), the Z043B sex marker was developed by Dawson et al. (2016), and Ach~ markers were developed by Preston et al. (2013b). Observed heterozygosity was calculated in the program SpAGeDi (Hardy and Vekemans, 2002).

Marker name	**Dye**	**Individuals genotyped (/354)**	**No. alleles**	**Size range (base pairs)**	**Observed heterozygosity**
Multiplex 1 (56 C)					
TG01-147	HEX	336	2	278-280	0.50
TG04-004	HEX	336	2	164-166	0.49
TG13-009	HEX	332	4	189-199	0.53
Z043B (sex marker)	6FAM	321	2	262-272	0.50
Multiplex 2 (60 C)					
Ach006	HEX	346	5	225-245	0.77
Ach007	6FAM	345	9	232-268	0.86
Ach008	HEX	341	5	264-280	0.70
Ach010	6FAM	339	11	191-217	0.77
Ach012	HEX	340	5	329-356	0.77
Ach013	6FAM	339	7	147-175	0.78
Ach014	HEX	347	5	184-200	0.74
Ach020	HEX	344	3	153-163	0.53
Multiplex 3 (60 C)					
Ach001	6FAM	324	9	189-227	0.85
Ach011	6FAM	323	8	266-280	0.81
Ach019	HEX	323	5	174-184	0.72
Ach023	6FAM	324	12	328-357	0.87
Ach030	HEX	322	10	217-244	0.79

INVESTMENT
PATTERNS OF
PARENTS AND HELPERS

2.

Flexibility but no coordination of visits in provisioning riflemen

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James V. Briskie, Isabel S. Winney and James L. Savage

Parental care strategies occupy a continuum from fixed investments that are consistent across contexts, to flexible behaviour that largely depends on external social and environmental cues. Determining the flexibility of care behaviour is important, as it influences the outcome of investment games between multiple individuals caring for the same brood. We investigated the repeatability of provisioning behaviour and the potential for turn-taking among breeders and helpers in a cooperatively breeding bird, the rifleman *Acanthisitta chloris*. First we examined whether nest visit rate is an accurate measure of investment by assessing whether carers consistently bring the same size of food, and whether food size is related to nest visit rate. Our results support the use of visit rate as a valid indicator of parental investment. Next, we calculated the repeatability of visit rate and food size to determine whether these behaviours are fixed individual traits or flexible responses to particular contexts. We found that riflemen were flexible in visit rate, supporting responsive models of care over 'sealed bids'. Finally, we used runs tests to assess whether individual riflemen alternated visits with other carers, indicative of turn-taking. We found little evidence of any such coordination of parental provisioning. We conclude that individual flexibility in parental care appears to arise through factors such as breeding status and brood demand, rather than as a real-time response to social partners.

This manuscript was published in *Animal Behaviour* volume 125, pages 25-31. I have removed some detail of the methods from this version to avoid repeating Chapter 1; otherwise, the versions are the same.

Introduction

Parental investment is a critical component of most animal life histories, and understanding variation in parental investment is key to research in behavioural adaptation and life-history trade-offs, because of the importance of reproduction in determining individuals' inclusive fitness. Levels of investment observed in natural populations are expected to be products of coevolution between carers and dependent offspring (Trivers, 1972, 1974; Hatchwell, 1999; Hinde et al., 2010). In addition, individuals are expected to adjust their contributions to care in relation to the social and environmental context, if doing so can increase their reproductive success.

Theoretical work has explored a range of different assumptions about behavioural flexibility during parental care. Houston and Davies (1985) modelled parental investment as a fixed, per-individual 'sealed bid', optimised over evolutionary time. From this theoretical framework we would expect clear individual consistency in parental investment, persisting across multiple observations. Studies of house sparrows *Passer domesticus* have supported this prediction, especially in males (Schwagmeyer et al., 2002; Nakagawa et al., 2007). In contrast, more recent models incorporate behavioural plasticity through 'negotiation', in which individual parents vary their investment depending on the behaviour of their partner (McNamara et al., 1999; Johnstone, 2011). Johnstone et al. (2014) have shown that 'conditional cooperation', in which carers visit following their partners' visits, is a stable negotiation mechanism that maximises benefits to offspring. This response rule implies that carers should take turns visiting offspring, a prediction borne out in studies of provisioning great tits *Parus major* (Johnstone et al., 2014), chestnut-crowned babblers *Pomatostomus ruficeps* (Savage, 2014), long-tailed tits *Aegithalos caudatus* (Bebbington and Hatchwell, 2016) and acorn woodpeckers *Melanerpes formicivorus* (Koenig and Walters, 2016). The empirical support for both sealed bid and negotiation-based models suggests that both can provide evolutionary solutions to the organisation of parental care, with systems occupying different points along a continuum between complete inflexibility (sealed bids) and extremely responsive negotiation (Hinde and Kilner, 2007).

Plasticity in an individual's investment can also arise from factors other than their partners' behaviour. These may relate to an individual's own condition, characteristics of its partner or helpers, or extrinsic cues such as offspring demand, food availability, or predation pressure (Naef-Daenzer and Keller, 1999; Ghalambor et al., 2013; Brouwer et al., 2014). Such factors can generate noise when attempting to measure between-individual differences in behaviour. In cooperative breeders, species with non-parent contributors to care, behavioural flexibility may also take the form of 'load-lightening', wherein a parent's investment depends on the extent of provisioning by helpers (Crick, 1992; Hatchwell, 1999). Observed plasticity in parental care may therefore be a product of a number of factors, including social negotiation.

Robust measures of investment are required to investigate the coevolutionary processes underlying parental care strategies (Browning et al., 2012b). In birds, parental investment is commonly measured by counting provisioning visits made by carers to dependent offspring over a certain period. This 'visit rate' is used to quantify a parent's contribution to care, relative to the investment of its partner, helpers, or other parents in the population (e.g. Davies, 1986; Kilner et al., 2004; Nam et al., 2010). Visit rate is also useful for comparing the same individual across time, within or between breeding attempts. Despite the convenience of using visit rates as an index of investment, the value of food items that carers bring can also be important. For example, although consideration of food size has shown visit rate alone to be a robust measure of food delivery in house finches *Carpodacus mexicanus* (Nolan et al., 2001) and chestnut-crowned babblers (Browning et al., 2012b), higher visit rates in house sparrows (Schwagmeyer and Mock, 2008) and house wrens *Troglodytes aedon* (Bowers et al., 2014) correspond with parents bringing smaller food items, meaning that visit rate is largely unrelated to contributions to care. Visit rate alone is also a less meaningful measure if individual carers are consistent in the sizes of food they bring to offspring (e.g. individuals bringing relatively large food items have their contribution underestimated by visit rate). We might expect to observe these patterns because of between-individual differences in quality or foraging strategies (Dall et al., 2004; Smith and Blumstein, 2008; Bell et al., 2009). Food size is, therefore, a potentially important consideration when measuring investment during provisioning, but the effects of the social environment on both visit rate and load size have rarely been investigated in cooperative breeders.

We studied investment in offspring through observations of nestling provisioning by parents and helpers in riflemen *Acanthisitta chloris*. Riflemen are small (5-7 g) insectivorous passerines endemic to New Zealand. Pairs may breed up to twice in a season, laying 2-5 eggs in each breeding attempt. Chicks hatch on the same day and remain in the nest for c.24 days before fledging (Withers, 2013a). Brood sex ratios are apparently random with no evidence of departure from parity (Sherley, 1993). Riflemen are facultative cooperative breeders, with 2-6 individuals provisioning at nests observed in our study. Rifleman helpers are unusually variable, as they may be adult or juvenile, paired or unpaired, successful or unsuccessful breeders, and they do not necessarily share a territory with the breeders that they help; however, they are almost always close relatives of the nestlings they provision (Sherley, 1990a; Preston et al., 2013a). Nestlings attended by adult helpers receive more provisioning visits, and enjoy better survival prospects, than those in nests without helpers (Preston et al., 2016). Breeders are known to provision more than helpers, and male breeders more than females (Preston et al., 2013a), but finer-scale variation in individual provisioning has not yet been investigated. In this study, we tested whether a sealed bid or negotiation-based model of investment better explained variation in provisioning by riflemen. To test each model, we first needed to establish that visit rate was a reliable measure of investment by assessing whether individual carers consistently brought the same size of food items and whether food size was related to nest visit rate. We then investigated whether investment is repeatable, as envisaged by the sealed bid model, or flexible within individuals. Finally, we considered whether the observed variation in caring behaviour is a response to the investment of other carers, or simply dependent on factors such as brood demand.

Methods

Data collection

In this study we used provisioning data collected from footage of rifleman nests, recorded as described in Chapter 1. In total, data from 46 different individuals at 33 nests were used for this study; 15 (45%) of these nests were attended by parents and 1-4 helpers, with the remainder attended by parents only.

Videos were all transcribed by a single observer. For each visit in a video, the start and end time (accurate to one second), individual identity (recognised using colour-ring combination), sex, type of behaviour (brooding, successful/unsuccessful feeding, bringing/removing feathers, removing faecal sacs or unknown) and size of food brought for feeding visits were all noted. Food size was estimated relative to bill size (small = smaller than one third of bill size, medium = between one third and full bill size, large = larger than full bill size). Riflemen do not regurgitate food, and all food delivered to the nest is held in the bill. Nestlings are provisioned with small invertebrates, chiefly adult and larval moths, spiders, crickets and weta (Preston et al., 2013a). For the analyses presented here, non-feeding visits were removed from the data. All statistical analyses were implemented in R 3.2.2 (R Development Core Team, Vienna).

Testing the relationship between visit rate and load size

We examined the relationship between load size and visit rate using Spearman’s rank correlation test, by comparing the number of number of visits and the proportion of large food items brought by each individual for each recorded hour. If carers that infrequently visit compensate by bringing larger food, we would expect a negative relationship between these two variables. Repeatability tests on load size (see below) also informed our assessment of the validity of visit rate as a measure of food delivery.

Repeatability analysis

For this analysis, we first summarised provisioning data for each carer in each recorded hour during which it was observed, including the number of feeding visits of each size class, carer status (individual identity, parent/helper, sex, adult/juvenile) and context variables (date, time, brood size, nestling age, nest helped/unhelped). We retained data from four individuals who were observed during one observation period only, to contribute to estimating between-individual variation. We removed data from three nests at which nestlings were not sexed due to early mortality or inaccessibility, as brood sex ratio affects investment by carers (Chapter 3). We then calculated within-individual repeatability of visit rate and load size using a Bayesian generalised linear mixed-effects model (GLMM) approach in the MCMCglmm package (Hadfield, 2010). Visit rate was modelled using a Poisson error structure with number of feeding visits as the response variable. Load size was modelled using a binomial error structure with a two-column response variable: number of large food items brought and number of other food items brought (successes and failures respectively in statistical terms). This allowed the proportion of large food items to be examined with appropriate weight given to their total number of visits over the hour (Crawley, 2007). We concentrated on large food items because they were less likely to be misidentified than medium-sized food, and less likely to be missed altogether than small food.

Repeatability is calculated as the proportion of variance in a GLMM that is explained by the random effect of interest, in this case individual identity. To calculate agreement repeatability (R), we fitted no fixed terms other than the intercept, and included only the identity term as a random effect. We also calculated adjusted repeatability (R_{adj}), which controls for the effect of confounding factors on the response variable and is thus a more valid measure (Nakagawa and Schielzeth, 2010; Debeffe et al., 2015). We included number of nestlings (integer), nestling age (integer: in days), individual status (factor: parent or helper), nest status (factor: helped or unhelped), sex of carer (factor), brood sex ratio (continuous: proportion of males in the nest), date (integer: number of days since 1st September) and time (integer: number of hours since 0700 h) as fixed effects and territory identity as an additional random effect. We initially included season (2012-2013, 2013-2014 or 2014-2015) as an additional random effect, reasoning that it may have influenced the abundance and type of food available, but dropped this term from the model as it explained a negligible amount of variation that could not be accurately estimated, presumably because of similar climatic conditions across seasons. Continuous and integer predictors were scaled and centred. We extracted posterior mode and 50% and 95% credibility intervals (CIs) of repeatability from the models using the equations for GLMM-based repeatability outlined by Nakagawa and Schielzeth (2010). We present both R and R_{adj} here to illustrate differences between these measures.

Other factors affecting visit rate and load size

We assessed the contribution of factors beyond individual identity (brood size, nestling age, carer status, nest status, carer age, carer sex, brood sex ratio, date, time and territory) to the provisioning behaviour of carers using posterior modes and CIs from fixed effects in the same models used above to estimate R_{adj}.

Testing for alternation by carers

As well as the factors mentioned above, within-individual plasticity in parental care can arise from carers responding to the behaviour of their social partner(s) through real-time negotiation over care (Lessells and McNamara, 2012; Johnstone et al., 2014). We tested this hypothesis by looking for non-random patterns of alternation (turn-taking) between nest visits by different carers, which would indicate that carers are responding to each other. We only included provisioning data from day 12 onwards to avoid conflation of feeding visits with brooding. For this analysis, provisioning data collected from 22 nests between 2008 and 2011 were added, in which visits to the nest were recorded in the same way as in 2012-2015 but without information on load size.

We tested whether sequences of visits showed non-random patterns using a custom *k*-category runs test implemented in R, based on equations in Sheskin (2011). This computes whether a sequence shows more or fewer runs of the same value than expected by chance, in this case visits by an individual bird. The sequences we tested were the identity of carers at all feeding visits for each nest, recorded from day 12 onwards. This required concatenating data such that some consecutive data points did not correspond to true consecutive visits (e.g. the last visit on day 12 followed by the first visit on day 15). As these false steps occurred a maximum of 4 times per sequence, and sequences were on average 122 visits long, we assumed that they did not have a significant influence on our results.

Results

In total, we collected data from 355 observation periods of 46 unique carers provisioning at 33 nests between 2012 and 2015. This encompassed 301 observation periods of breeder provisioning and 54 observation periods of helper provisioning. Carers brought an overall mean of 10.84 ± 0.39 SE food items per hour, and 23% of all food items delivered were categorised as large. Of the 46 carers, 7 were recorded acting as breeders and helpers at different broods.

Relationship between visit rate and load size

We tested the relationship between visit rate and load size across all recorded observation periods. We observed a moderate positive relationship between the number of visits and the proportion of large food items brought by each carer in each observation period (Spearman's rank correlation test: $r_s = 0.32$, $n = 355$, $P < 0.001$; Figure 2.1). This indicates that provisioning riflemen do not trade off visiting frequently with bringing larger food items; in fact, those visiting more frequently also generally bring a greater proportion of large items.

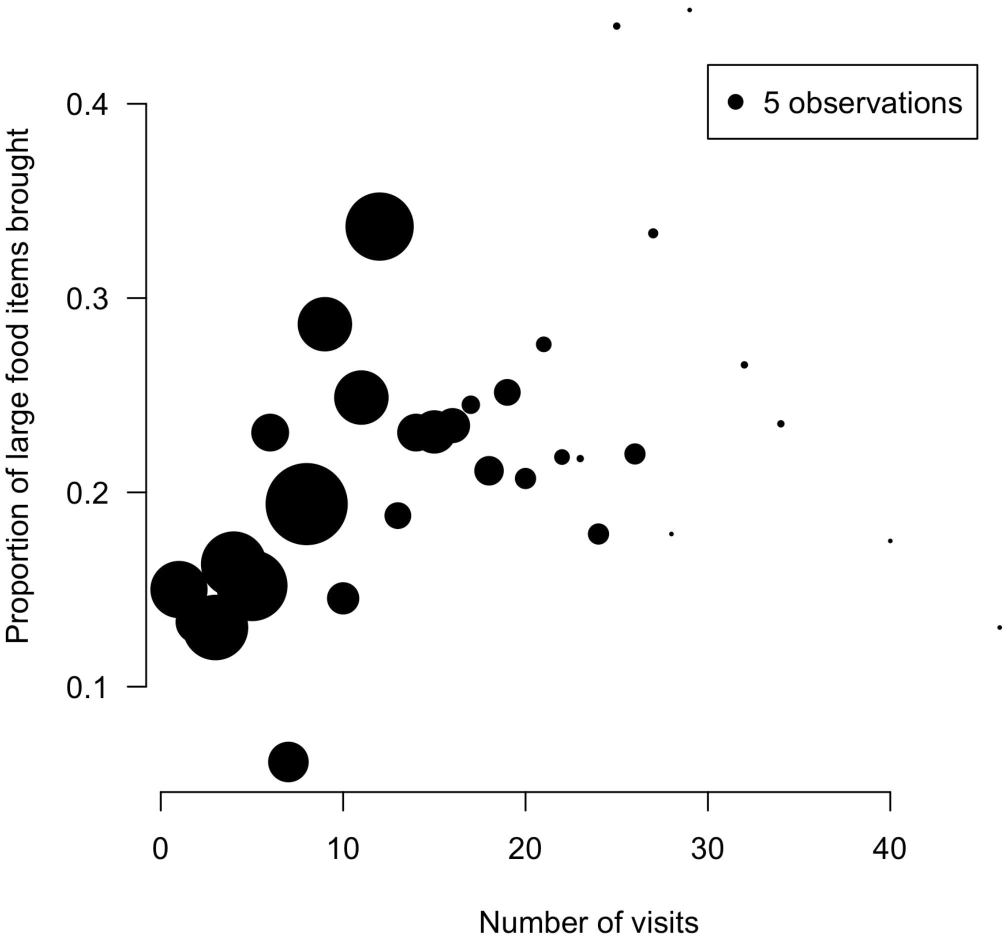

Figure 2.1 The relationship between the number of provisioning visits and the proportion of large food items brought during each recorded carer provisioning hour. Points represent the mean proportion of large food items brought for each number of visits, weighted by sample size.

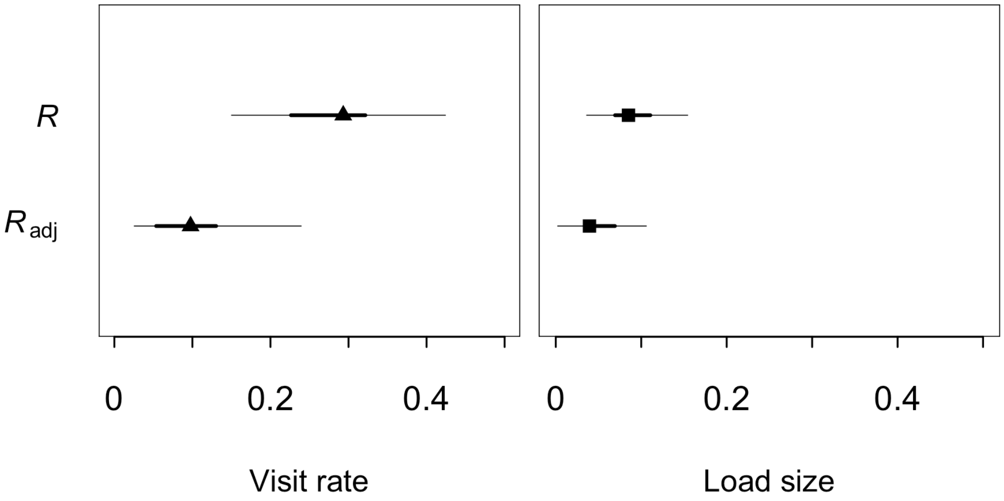

Figure 2.2 Estimates of agreement (*R*) and adjusted repeatability (R_{adj}) for visit rate and load size (the proportion of large food brought to nestlings) in provisioning riflemen, derived from MCMC generalised linear mixed-effects models. Points show the posterior mode of repeatability estimates, with bold lines spanning 50% credibility intervals (CI) and narrow lines spanning 95% CI. *R* was estimated from models including a random individual identity term and the population intercept as the only fixed term; R_{adj} was estimated from models including a number of confounding variables along with individual identity (see methods).

Repeatability of visit rate and load size

We calculated repeatability of visit rate and load size using data from a total of 338 observation periods for 46 unique individuals (26 males and 20 females) feeding broods on 14 different territories. *R* was moderate for visit rate and low for load size; however, adjusting for confounding variables gave lower estimates of repeatability for both parameters, notably visit rate (Figure 2.2).

Other factors affecting visit rate and load size

Fixed effect estimates from GLMMs evaluating the factors influencing visit rate and load size are summarised in Figure 2.3. Carers made more visits and brought larger food with increased brood size and nestling age, indicating a response to brood demand. Similarly, more visits were made to female-biased broods, which given the larger size of female nestlings is also likely to be a response to brood demand. Helpers made fewer visits to nests than breeders, and males tended to make more visits than females, but brought a lower proportion of large food (though 95% CIs for these estimates overlap zero). The proportion of large food loads decreased later in the day.

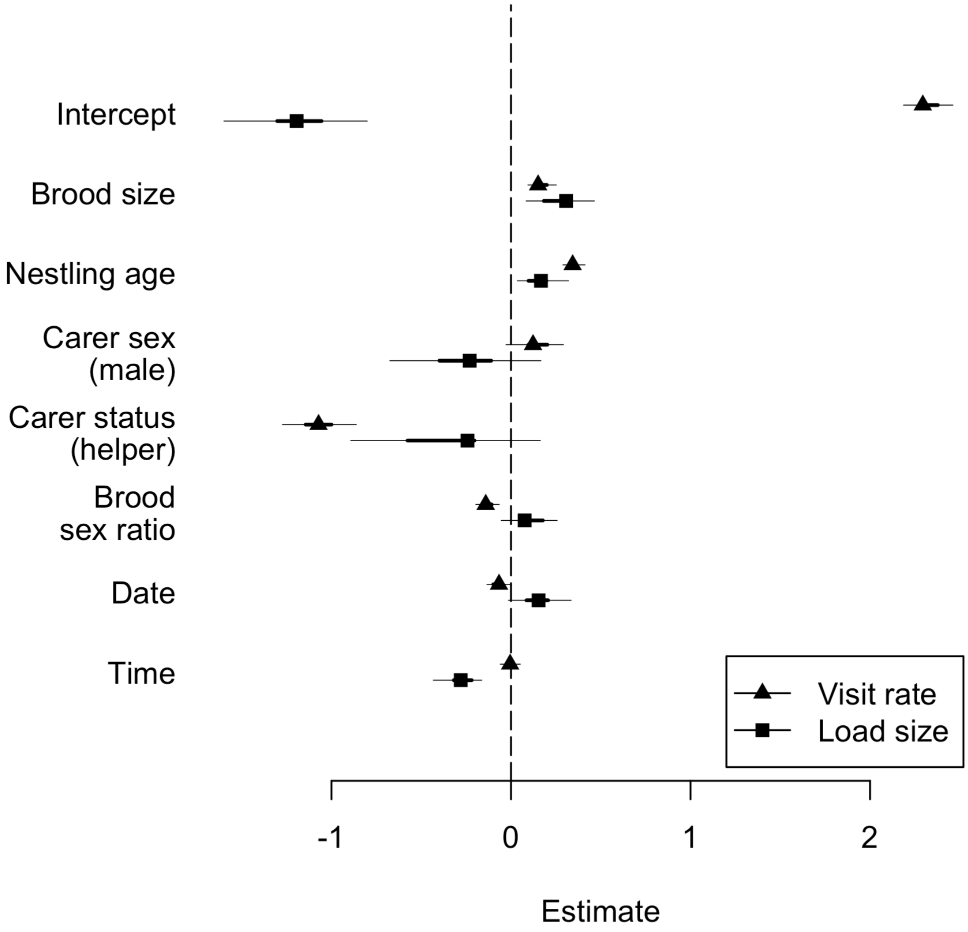

Figure 2.3 Fixed effect estimates from MCMC generalised linear mixed-effects models explaining variation in visit rate and load size (the proportion of large food brought to nestlings) in provisioning riflemen. Points show the posterior mode of parameter effect estimates, with bold lines spanning 50% credibility intervals (CI) and narrow lines spanning 95% CI. All estimates for visit rate and load size respectively are derived from the same models. Territory identity and individual identity were included as random effects in both models.

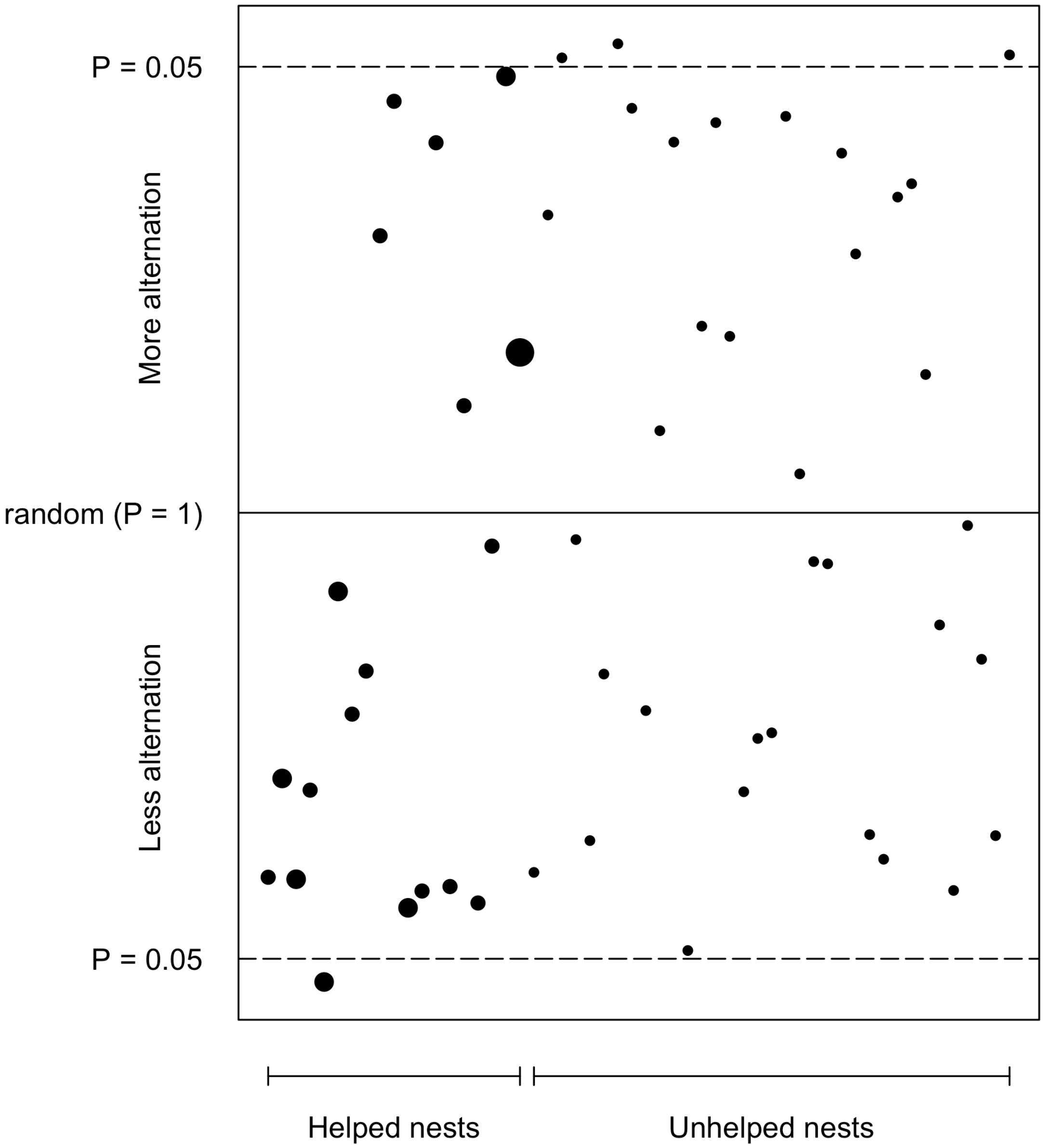

Figure 2.4 Results from runs tests for randomness carried out on sequences of rifleman visit data. Each point represents the probability for a given nest that visits occurred in a random order and therefore that sequences were not affected by carers responding to each other's provisioning visits. Points above the random line correspond to nests at which there were more runs by the same individual (more alternation) than expected by chance, and points below correspond to nests with fewer runs (less alternation). Point size represents the number of individuals provisioning at a nest (three, four or six at helped nests and two at each unhelped nest).

Alternation of nest visits

We analysed visit sequences at 54 nests (mean = 122 ± 8 SE visits per nest) using runs tests to assess patterns of randomness (Figure 2.4). There was little evidence for non-random visit sequences in either direction (carers taking turns more or less than expected): 23 nests showed a greater tendency for alternation than expected by chance (3 statistically significant at the 0.05 level), while 31 showed a lower tendency for alternation than expected by chance (1 statistically significant). The handful of 'significant' results are likely to represent false positives caused by multiple testing.

Discussion

Our results indicate that visit rate is a valid measure of carer investment in riflemen, as it correlated positively with the proportion of large food delivered to nestlings. This suggests carers visiting more made a genuinely greater contribution to food delivery. Visit rate and load size showed low within-individual repeatability when we accounted for confounding variables. Both also increased with brood demand (nestling age and brood size), although only visit rate was higher in female-biased clutches. Despite the flexibility indicated by the low repeatability of visit rate, carers showed little evidence of responding to each other's visits.

We assessed the validity of visit rate as a measure of parental investment in riflemen by examining its relationship with load size. Visit rate is the most commonly used measure of parental investment in nesting birds, but its value as a measure may be compromised where carers compensate for making fewer visits by bringing larger food items (Schwagmeyer and Mock, 2008). We found no evidence for this in riflemen; in fact visiting more often was positively correlated with bringing a greater proportion of large food items.

Another potential confound of calculating carer investment from visit rate arises if carers consistently bring food items of the same size: those consistently bringing larger food would have their contribution underestimated by visit rate alone. Sherley (1990a) found little evidence for non-random patterns of load sizes in provisioning riflemen, but did not assess between-individual differences. Here, we found repeatability of load size in provisioning riflemen to be low ($R_{\text{adj}} = 0.04$). Taken together, our results strongly support visit rate as a useful measure of parental investment in riflemen. In this regard riflemen align with house finches, in which visit rate almost perfectly predicts weight gain in a nest (Nolan et al., 2001), and chestnut-crowned babblers, in which visit rate is the best predictor of the total amount of food provided (Browning et al., 2012b).

While we calculated a moderate estimate of agreement repeatability for carers' visit rates ($R = 0.29$), this shrank to a much lower value ($R_{\text{adj}} = 0.10$) when adjusted for confounding variables. This illustrates that inflated estimates of R can arise as artefacts of brood size, status and sex, rather than differences between individuals in the character of interest. Measures of the repeatability of provisioning behaviour from previous studies are summarised in Table 2.1, illustrating both the surprising paucity of repeatability studies, and the variety of methods used to calculate R and R_{adj}, which makes comparison between studies challenging. Some high estimates of R could have resulted from a lack of confounding factors included in calculations (Freeman-Gallant and Rothstein, 1999; MacColl and Hatchwell, 2003). However, as a number of studies have controlled for confounds, it appears likely that there is a genuine continuum from highly repeatable, fixed-investment parental care such as that observed in male house sparrows (Schwagmeyer and Mock, 2003; Nakagawa et al., 2007), through the moderate between-individual variation of long-tailed tits (Adams et al., 2015), to species like riflemen in which repeatability is low and parental care highly flexible.

High repeatability of visit rate has been regarded as consistent with sealed-bid models of investment, in which investment is fixed over an individual's lifetime but subject to selection across generations (Houston and Davies, 1985; Nakagawa et al., 2007). At the opposite end of the continuum are systems in which individuals are highly flexible in their investment; specifically, their investment is strongly influenced by their social partners (McNamara et al., 1999; Schwagmeyer et al., 2002; Hinde and Kilner, 2007).

Table 2.1 Summary of studies investigating repeatability of parental care in provisioning birds. Repeatability of visit rate is presented unless stated otherwise. R is agreement repeatability, in which no confounding variables are controlled. R_{adj} is adjusted repeatability, where the factors controlled are given in the adjacent column. Asterisks denote estimates based on within-year data rather than across multiple years. Abbreviations for methods: ANOVA = analysis of variance; BLMM = Bayesian linear mixed-effects model; DEE = daily energy expenditure; LMM = linear mixed-effects model; MCMCglmm = Markov chain Monte Carlo generalised linear mixed-effects model. Abbreviations for adjusted factors: a = carer age; bs = brood size; d = date; #h = number of helpers at nest; mf = sex; na = nestling age; pa = partner age; pe = partner effort (visit rate); sr = brood sex ratio; st = status (breeder/helper); t = time.

Species	**Study**	**Method**	R	R_{adj}	**Adjusted factors**
Manx shearwater *Puffinus puffinus*	Gray et al. (2005)	One-way ANOVA on g/day (by pair)	0.02		
Savannah sparrow *Passerculus sandwichensis*	Freeman-Gallant and Rothstein (1999)	One-way ANOVA	♂ 0.60 ♀ 0.19		
House sparrow *Passer domesticus*	Schwagmeyer and Mock (2003)	ANOVA*	♂ 0.38 ♀ -0.06	♂ 0.44 ♀ 0.08	bs, d
	Nakagawa et al. (2007)	LMM*	♂ 0.58 ♀ 0.28	♂ 0.63 ♀ 0.27	bs
	Dor and Lotem (2010)	One-way ANOVA	♂ 0.51 ♀ 0.57		
	Cleasby et al. (2013)	BLMM		♂ 0.23 ♀ 0.33	a, bs, d, na, pa, pe
Long-tailed tit *Aegithalos caudatus*	MacColl and Hatchwell (2003)	One-way ANOVA	♂ 0.70 ♀ 0.37		
	Adams et al. (2015)	Animal model*		0.24	bs, #h, mf, na, st
European starling *Sturnus vulgaris*	Fowler and Williams (2015)	LMM	♀ 0.04	♀ 0.34	bs
Pied flycatcher *Ficedula hypoleuca*	Potti et al. (1999)	One-way ANOVA on DEE	♂ -0.21 ♀ 0.64		
Stitchbird *Notiomystis cincta*	Low et al. (2012)	LMM*		♂ 0.19 ♀ 0.02	bs, na
Rifleman *Acanthisitta chloris*	This study	MCMCglmm	0.29	0.10	bs, d, mf, na, sr, st, t

Recent theoretical and empirical work suggests that alternating visits to nestlings (taking turns) is a simple way by which negotiation over care can be regulated, and is associated with improved rates of food delivery and greater reproductive success (Johnstone et al., 2014; Bebbington and Hatchwell, 2016). Although theoretical predictions of alternation have so far been limited to biparental systems, models suggest that individual investment rules are lead to similar predictions when extended to cooperative groups (Johnstone, 2001; Savage et al., 2012, 2013, 2015), and data from cooperative groups of chestnut-crowned babblers show patterns suggestive of active turn-taking by carers (Savage, 2014). In contrast, our analysis did not support the hypothesis that rifleman carers take turns feeding nestlings, or visit in any other non-random pattern. This was the case for both helped nests and nests without helpers.

The lack of turn-taking in riflemen, despite its presence in other species, may be attributable to low levels of sexual conflict. Negotiation represents a stable solution to conflict over parental care, which arises from the divergent evolutionary interests of carers, who each fare better if others work harder. However, such conflict is diminished in riflemen, where there is no divorce (although adults will re-pair if their partner dies) and no recorded extra-pair paternity (Preston et al., 2013a). In a system without divorce, exploitation is a risky strategy because it will presumably be detrimental to breeding success in the event that a partner survives in poor condition. Riflemen thus appear closer to ‘true’ monogamy (with no conflict) than many comparable systems (Parker, 1985). Theoretical work to date has generally assumed conflict between partners, and focused on the resolution of this conflict (e.g Houston and Davies, 1985; McNamara et al., 1999; Johnstone et al., 2014). Further theoretical work is needed to examine how low conflict between carers affects the predicted behavioural outcomes of investment games.

In conclusion, we have combined analyses of repeatability, negotiation and other factors affecting parental behaviour to show that riflemen invest flexibly in offspring, but do not respond to each other’s investment by taking turns. Instead, individuals vary their provisioning in response to their brood’s demand and their own breeding status. We hypothesise that low sexual conflict might lead to species like riflemen exhibiting both low partner responsiveness and highly flexible investment, as there is less scope for exploitation. Our results demonstrate the range of questions that can be answered using provisioning data, and how some results inform our interpretation of others. We suggest that future studies should consider possible confounds before drawing conclusions from raw measures of visit rates, especially in species with highly variable social and environmental contexts of care.

SEX ALLOCATION IN COOPERATIVELY BREEDING BIRDS

3.

Cheaper, more helpful males are not overproduced by breeding riflemen

Nyil Khwaja, Stephanie A. J. Preston, James V. Briskie
and Ben J. Hatchwell

We studied patterns of parental investment and sex ratio allocation in a cooperatively breeding and sexually dimorphic bird species, the rifleman *Acanthisitta chloris*. We found that carers increased provisioning at broods in which females, the larger sex, made up a greater proportion of nestlings. This supports the assumption that daughters are more costly to raise than sons, and that at least some of these costs are shared by breeders and helpers during the provisioning phase. Sex allocation theory suggests that this should promote production of more sons, the cheaper sex. As the majority of helpers in this species are male, the repayment hypothesis also predicts that increased production of sons should be favoured. However, we found no evidence of a male bias in brood sex ratios at the population level, and no evidence for any facultative adjustment of brood sex ratios by breeding females. We discuss this result in light of the above hypotheses and suggest that selection for adaptive sex allocation may not be particularly strong in cooperative breeders in spite of the potential benefits of repayment.

A manuscript based on this chapter is currently in review (at the time of final submission).

Introduction

Theory predicts that parents should invest equally in sons and daughters (Fisher, 1930). All other factors being equal, this may be achieved by random sex allocation at a 1:1 ratio and a fixed level of investment per parent. However, a number of factors can promote biased investment towards a particular sex, especially where the sexes differ in behaviour, ecology or morphology. Biases are expected where one sex is more costly to produce, or where the adaptive value of producing each sex depends on context. For example, females in good condition should benefit from producing more of the sex with higher potential reproductive success, and females in dense populations should benefit from producing more of the dispersive sex to reduce competition (Trivers and Willard, 1973; Clark, 1978). To bias production in response to changing benefits relies on females evolving a mechanism of facultative sex determination. Patterns of investment may appear suboptimal if the costs of evolving such a mechanism is prohibitive, and even minor costs may negate the adaptive benefits of sex ratio control (Pen et al., 1999).

Despite the potential mechanistic constraints associated with sex determination, a number of studies on a variety of animal taxa have provided evidence for adaptive sex ratio adjustment (West, 2013). In some species, variation in sex ratio is related to the expected quality of offspring; for example, in red deer *Cervus elaphus* intense competition leads to highly skewed male reproductive success. Females of higher quality are more likely to produce males, which are a riskier investment with higher potential payoff (Clutton-Brock et al., 1984). Similarly in the polygynous lek breeding system of the lance-tailed manakin *Chiroxiphia lanceolata*, females are more likely to produce males from matings with less related partners, which are assumed to lead to higher-quality offspring (Sardell and DuVal, 2014). Alternatively, sex ratio may be adjusted in response to changes in the local environment. In Seychelles warblers *Acrocephalus sechellensis*, females on poor quality territories produce more males, which disperse further and thus do not compete for their parents' marginal local resources (Komdeur et al., 1997).

Cooperative breeders, in which parents are assisted in reproduction by non-breeding helpers, have been considered especially likely to evolve adaptive sex ratio adjustment (Emlen et al., 1986; Griffin et al., 2005). In most cooperative breeders, helpers are close relatives that enhance breeders' reproductive success, and they are more likely to be of one sex (Green et al., 2016). The 'repayment hypothesis' or 'local resource enhancement hypothesis' predicts that due to the benefits of having close relatives of the helping sex, it should pay mothers to bias the sex ratio of their offspring accordingly, especially when they do not already have help (Emlen et al., 1986; Griffin et al., 2005). However, these benefits may be compromised by increased competition for local resources (Pen and Weissing, 2000). It has also been suggested that cooperative breeding is especially likely to evolve in genetically monogamous lineages (Boomsma, 2009; Cornwallis et al., 2010; but see Kramer and Russell, 2014), in which case the reproductive skew that drives sex ratio adjustment in species like red deer and lance-tailed manakins is absent. Furthermore, the presence or absence of helpers may not be predictable at the time of sex determination. Therefore, while there appear sound theoretical reasons for cooperative breeders to adjust offspring sex ratios, it is uncertain whether they should be especially predisposed to do so.

The rifleman *Acanthisitta chloris* is a cooperatively breeding passerine bird endemic to New Zealand. Adult helpers are observed at c.25% of nests (Chapter 7) and are close relatives, most commonly previous offspring of the breeding pair, who enhance the recruitment of closely related young (Preston et al., 2013a, 2016). The majority of these helpers are male (72% recorded in our study, *n* = 32). While dispersal distances are short, helpers are established on their own territory rather than remaining with their parents, diminishing the cost of local resource competition with breeders (Preston et al., 2013a). Riflemen are also sexually dimorphic, with females larger than males and so likely to be more costly to produce. Therefore two factors may increase the value of rifleman males and promote a sex ratio skewed in favour of sons: (1) a higher likelihood they will be future helpers and hence repay parental investment, and (2) a lower cost of production.

The sex ratio of rifleman broods at Kowhai Bush has been previously studied by Sherley (1993), who found that brood sex ratios were 47% male and not significantly different from parity. Without genetic analysis, he was unable to assess the influence of early nestling mortality on this result. Here, we report nestling sex ratios over six years of study, including molecular sexing of nestlings that died early. We also investigate whether mothers adjusted their broods' sex ratios in response to a number of cues; principally among these, we might expect that a lack of helpers would stimulate a greater production of males. Sherley's (1993) study supported the assumption that daughters were more costly to raise than sons because they were heavier, and weighed less in unisex broods than mixed broods. We test this assumption further by modelling the effect of brood sex ratio on carer provisioning rates.

Methods

Data collection

For this study, data collected in 2008-2011 were added to those I collected in 2012-2015. The population ranged between 10 and 23 breeding pairs during the earlier period (overall range: 6-23 breeding pairs). Rifleman nestlings were sexed in the hand at day 15 (Chapter 1). The reliability of using morphological differences to sex birds was confirmed using the Z043B microsatellite marker (Dawson et al., 2016); this marker was also used to sex nestlings that died prior to day 15, from which tissue samples were collected. Footage was recorded from nests as described in Chapter 1, and transcribed to obtain carer provisioning rates following the protocol detailed in Chapter 2.

Data analysis

Statistical analyses for this study were carried out using R 2.12.0 (R Development Core Team, Vienna).

We used generalised linear mixed-effects models in the lme4 package (Bates et al., 2015) to investigate factors influencing sex allocation, and whether brood sex ratio influenced investment by carers. Brood sex ratio was modelled using a binomial error structure with a two-column response variable: number of males and number of females. This allowed the proportion of males to be examined with appropriate weight given to the total brood size (Crawley, 2007). We fitted population density (numeric: number of pairs breeding within 200 m of nest), brood order (factor: first or second), whether a brood was helped (factor), brood size (numeric), season (factor: 2008-2009, 2009-2010, etc.) and pairwise relatedness estimate between male and female parents (numeric) as explanatory fixed predictors. Pair identity nested within mother identity was fitted as a random effect. Father identity was not included as females are the heterogametic sex in birds, meaning males are unlikely to contribute directly to sex allocation (Rutkowska and Badyaev, 2008); fitting pair identity accounted for the potential effect of partner on female allocation decisions. Preston et al. (2013a) detected no extra-pair paternity in this population (including the broods studied from 2008-2011 that are used in the present study), so we assumed that social fathers sired all offspring in a brood.

To investigate carer responses to brood sex ratio, we modelled carer visit rates as a Poisson-distributed response variable, with sex ratio (numeric: proportion of males) as a fixed predictor along with potential confounds: number of nestlings (numeric), nestling age (numeric: in days), carer status (factor: parent or helper), sex of carer (factor), date (numeric: number of days from 1st September), time (numeric: number of hours from 0700 h) and season (as above). We also included the interactions between brood sex ratio and both carer status and sex. Individual identity and territory were fitted as random effects. Visit rate is an appropriate measure of investment by provisioning riflemen, as it does not trade off against load size as in some other systems (Chapter 2).

Results

Size dimorphism

Adult females were larger than males, and this dimorphism was also apparent when nestlings were weighed at day 15 (Figure 3.1). On average, females were 27% heavier than males as adults, and 14% heavier as nestlings.

Adult sex ratio

The adult rifleman population at Kowhai Bush displayed a slight, but non-significant bias towards males (Table 3.1).

Table 3.1 Numbers of adult male and female riflemen recorded in the Kowhai Bush population across six breeding seasons. The sex ratio was not significantly different from parity in any season or overall (binomial tests: all $P > 0.1$).

Season	Adult males	Adult females	Adult sex ratio
2008-2009	25	24	0.51
2009-2010	22	22	0.50
2010-2011	10	10	0.50
2012-2013	18	15	0.55
2013-2014	12	10	0.55
2014-2015	11	10	0.52
mean			0.52

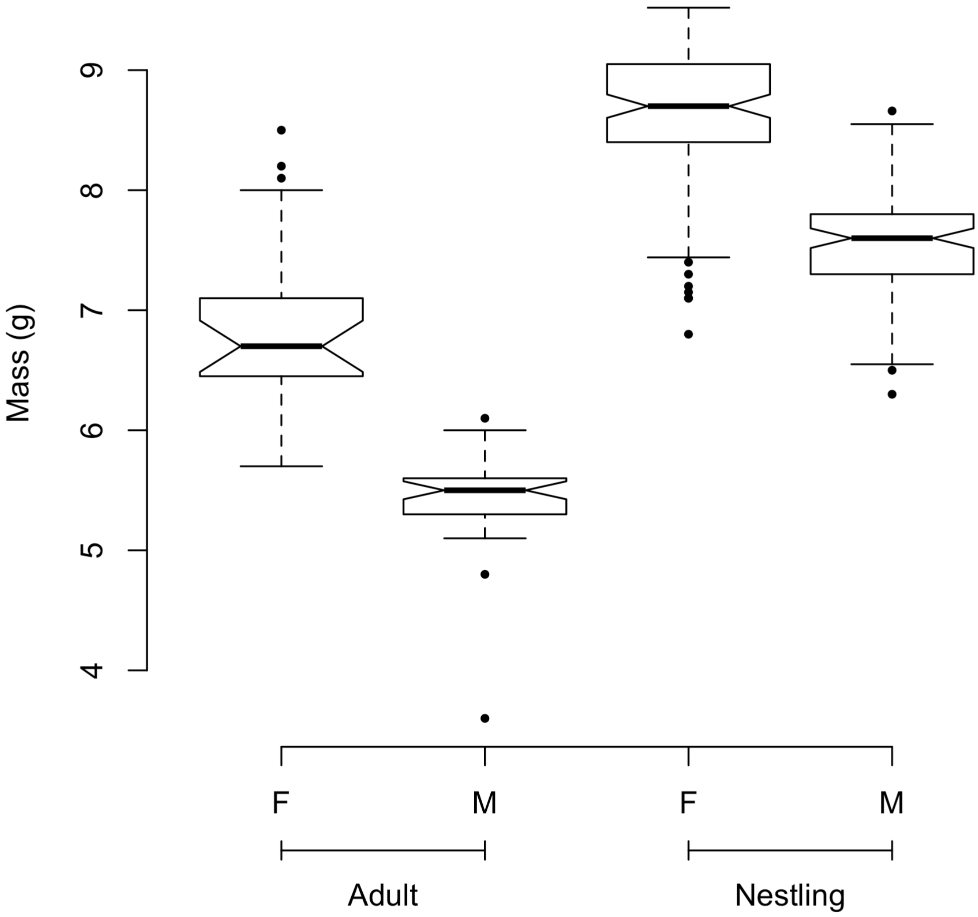

Figure 3.1 Differences in mass between female and male riflemen captured as adults (23 females and 40 males) and weighed as 15-day-old nestlings (111 females and 93 males), respectively. In both adults ($t = 8.94$, df = 29, $P < 0.001$) and nestlings ($t = 14.96$, df = 200, $P < 0.001$), the difference between the sexes was highly significant. This dataset was restricted to the 2012-2015 period to account for recorder effects.

Brood sex allocation

We determined the sex of 289 nestlings in 93 rifleman broods over 6 breeding seasons. We were unable to assign sex to 9 nestlings (3%) from 7 different broods, all of which died early in the nestling period. Of the 289 successfully sexed nestlings, 134 (46%) were male and 155 (54%) were female. The mean proportion of males across broods was 0.47 ± 0.03 SE (*n* = 93), which does not represent a significant deviance from parity (see intercept term in Table 3.2). There was no evidence for departure from the population sex ratio at the level of individual broods (χ^2 = 69.84, df = 91, P = 0.951). This was true when all 9 unsexed nestlings were assigned as males (χ^2 = 62.63, df = 85, P = 0.961) or females (χ^2 = 65.19, df = 85, P = 0.946). The nestling sex ratio was also not significantly different from the adult sex ratio (χ^2 = 1.42, df = 1, P = 0.233), even if all unsexed nestlings were assigned as females (χ^2 = 2.26, df = 1, P = 0.133). We found no evidence for non-random sex allocation with respect to any variables that we tested, although increasing population density had a marginal positive effect on the proportion of males produced (Table 3.2). In total, 37 sexed nestlings failed to survive to fledging, of which 16 were male and 21 female. Thus, out of 252 fledglings, 118 (47%) were male and 134 (53%) were female, a sex ratio that did not differ significantly from parity or from that for all nestlings (binomial and χ^2 tests: P > 0.1).

Table 3.2 Effect estimates on the logit scale from potential predictors of brood sex ratios in riflemen, modelled as fixed effects in a binomially-distributed generalised linear mixed-effects model, with the proportion of male offspring in a brood as the response variable (*n* = 80 broods). Pair identity (variance component < 0.01) nested within female identity (variance component < 0.01) was included as a random effect along with breeding season (variance component < 0.01). Second brood and helped are categorical predictors with first broods and unhelped nests as respective reference categories. All results were qualitatively equivalent when 13 more broods were included without estimates of mother-father relatedness (Table A.1, Appendix A), and when 9 unsexed nestlings, which we omitted from the model presented, were treated as all male (Table A.2, Appendix A) or all female (Table A.3, Appendix A).

Predictor	**Estimate ± SE**	*z*	**P**
Intercept	-0.58 ± 0.58	-1.01	0.314
Density (no. pairs within 200 m)	0.10 ± 0.06	1.76	0.078
Second brood	0.04 ± 0.38	0.11	0.911
Helped	0.21 ± 0.29	0.72	0.475
Brood size	< 0.01	0.03	0.973
Mother-father relatedness	0.26 ± 0.60	0.43	0.666

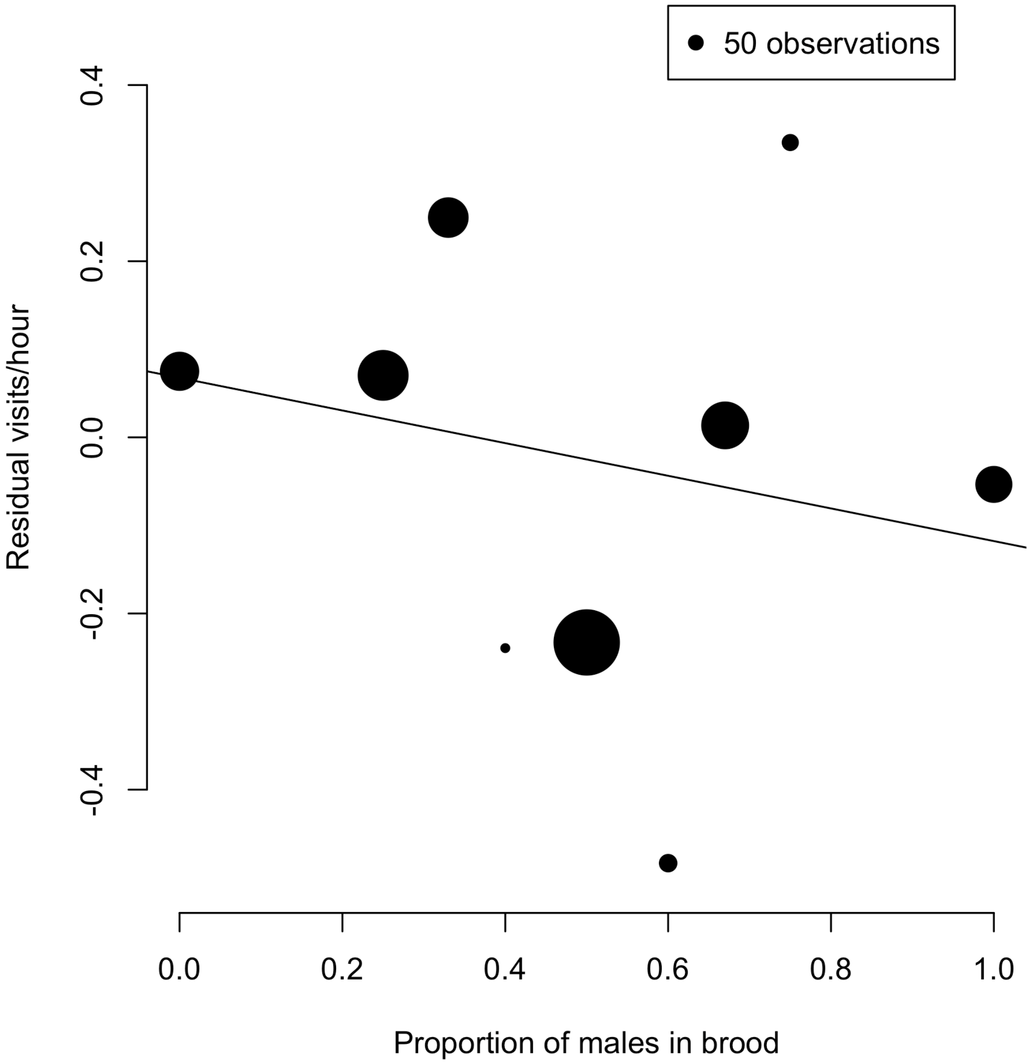

Figure 3.2 The effect of brood sex ratio on carer visit rates. Points show mean residuals on the logit scale from a Poisson-distributed generalised linear mixed-effects model in which all predictors detailed in the Methods were included, other than brood sex ratio, summarised for each observed proportion of males and scaled by sample size. The mean residual where proportion of males = 0.8 was -2.21; this is not included on the plot because it obscures the rest of the variation and it is based on 2 observations (total n = 1,124). The negative effect of proportion of males on provisioning rate was confirmed by including it in the full model (see Results and Table A.4, Appendix A).

Investment in relation to brood sex ratio

We analysed provisioning data from 1,124 observations of 122 different carers feeding 97 broods. Despite apparently random patterns of sex allocation, carers showed a significant response to brood sex ratio in their subsequent investment. Broods with a higher proportion of males received fewer provisioning visits during the nestling period (GLMM: effect estimate = -0.12 ± 0.05 SE, $z = -2.42$, P = 0.016; Figure 3.2). A number of the confounding variables we measured also had a significant influence on provisioning rate (Table A.4, Appendix A).

Discussion

We found that female nestling riflemen were significantly heavier than male nestlings and that rifleman carers invested more in broods that were more female-biased. However, despite this investment bias, which meant that daughters were more costly to produce than sons, we found no evidence that sex allocation in this system was either skewed towards the production of one sex, or responsive to any cues regarding the future value of offspring as potential helpers.

Particularly given their small size, riflemen show notably pronounced sexual size dimorphism, with female adults 27% larger than males. This is unusual among birds, where males are more commonly larger than females, and the majority of species are closer to monomorphism (Székely et al., 2007). Sherley (1985) suggested that female riflemen are unable to evolve to a more optimal smaller size because of the constraint of egg production, although this cannot explain why other small species do not also show similar levels of reversed sexual dimorphism. Size dimorphism and sexual dichromatism could also, or alternatively, represent adaptations to different foraging microenvironments (Hunt and McLean, 1993). Whatever the reason for the pattern in riflemen, size dimorphism carries clear implications for the cost of producing each sex.

Hamilton (1967) recognised the importance of differential costs to sex allocation theory, showing that where one sex is more costly to rear than the other, the evolutionarily stable sex ratio should be biased against it, as the additional costs mitigate the enhanced reproductive success enjoyed by the rarer sex. It is generally assumed that the larger sex is more costly to produce in dimorphic species, and that this influences optimum sex ratios and sex allocation (Benito and González-Solís, 2007). Sherley (1993) provided evidence supporting this assumption in riflemen by showing that nestling females in unisex broods weighed less than those in mixed broods, reasoning that this is likely to reflect an inability of carers to provision at optimal rates when all nestlings are female. Our finding that rifleman carers work harder when raising female-biased broods supports this assumption more directly and suggests that at least some of the additional costs of producing the larger sex are borne by all carers during the provisioning stage. It is possible that the correlative relationship between brood sex ratio and visit rate may be confounded by higher-quality parents, who are able to provision more, producing more of the costly sex. However, this is unlikely in riflemen given our lack of evidence for facultative sex allocation. Experimental studies indicate that parents provision the larger sex more in some species, such as brown songlarks *Cinclorhamphus cruralis* (Magrath et al., 2007), but not others, such as Eurasian kestrels *Falco tinnunculus* (Laaksonen et al., 2004). Riflemen appear to be an example of the former pattern. Other factors influencing rifleman provisioning, which we controlled in our model, are discussed in Preston et al. (2013a) and Chapter 2.

Consistent with the assumption of a costly larger sex, and with Hamilton’s (1967) predictions, are results of a comparative analysis showing that bird species with greater sexual size dimorphism exhibit both hatching and fledging sex ratios biased towards the smaller sex (Benito and González-Solís, 2007). In riflemen we found no evidence for a bias towards the cheaper sex (males), driven by differential production, and though more of the chicks that died in the nest between ringing at day 15 and fledging at day 24 were female, the sex ratio was still (non-significantly) female-biased at fledging. The observed brood sex ratio was remarkably similar to that which

Sherley (1993) recorded in the 1980s, suggesting that his results did not arise from differential early mortality. The mortality regime for rifleman nests at Kowhai Bush is not natural, as nestboxes afford almost full protection from predators, and predation at natural cavities is due entirely to introduced mammalian predators (Briskie et al., 2014). Nestbox protection is unlikely to mask female-biased mortality given that nest predation generally results in the total loss of all nestlings in a brood. It seems more likely that riflemen do not fit the theory and general pattern of differential allocation according to skewed costs of production.

The absence of a sex allocation bias is more surprising viewed in light of the repayment hypothesis, which suggests that female cooperative breeders should produce more of the helping sex in order to maximise their future fitness (Emlen et al., 1986). In riflemen, most helpers (at least adult helpers, which are those that improve offspring recruitment and thus breeder fitness) are male (Preston et al., 2013a, 2016). However, as we have shown, there was no production bias towards this helpful sex. Empirical support for the repayment hypothesis is equivocal; high between-system variation in the fitness benefits available to the more and less helpful sexes makes it unclear whether it can yield generalisable predictions (Koenig and Walters, 1999). In particular, this hypothesis makes opposite predictions to the local resource competition hypothesis, which suggests instead that competition between breeders and the less dispersive sex (generally the more helpful sex in cooperative breeders) can promote enhanced production of the more dispersive sex (Clark, 1978). In Seychelles warblers, this apparent paradox is resolved by differential sex ratio adjustment according to territory quality, where helpful females are overproduced on good quality territories, and dispersive males overproduced on poor territories (Komdeur et al., 1997). This strategy is less applicable for riflemen at Kowhai Bush, because (1) dispersal distances do not vary significantly between the sexes (Preston, 2012) and (2) the site is a relatively homogeneous stand of woodland, in which riflemen forage on evenly spaced kanuka *Kunzea ericoides* trees and territory quality is unlikely to vary a great deal.

More pertinently, patterns of sex allocation in other cooperative breeders with ‘kin neighbourhood’ social systems, like riflemen, have not borne out the predictions of the repayment hypothesis. These systems are characterised by offspring dispersing short distances from their parents' territories and attempting to breed, and helping relatives once dispersed, either as an alternative strategy after pairing or nest failure, or alongside their own breeding attempts (Hatchwell and Dickinson, 2004). Two well-studied examples are the long-tailed tit *Aegithalos caudatus* and western bluebird *Sialia mexicana*, and neither exhibit nestling sex ratios skewed toward the helpful sex (Koenig and Dickinson, 1996; Nam et al., 2011). These systems are characterised by a lack of ecological constraint on offspring attempting to breed independently, and breeders may gain more fitness from their offspring in turn breeding successfully than the contributions they make as helpers. This weakens the adaptive basis for repayment.

We also demonstrated a lack of evidence for facultative sex ratio adjustment in riflemen with reference to a number of potential adaptive cues. This is perhaps unsurprising given the lack of any population-level trend. Riflemen have an equal adult sex ratio and are genetically monogamous, meaning there is effectively no difference in potential reproductive success between the sexes (Preston et al., 2013a). Most studies documenting sex ratio adjustment show that favourable conditions promote overproduction of males, in species where males have greater variance in reproductive success (e.g. Sheldon et al., 1999; Sardell and DuVal, 2014; Bonderud et al., 2016). This driver is clearly absent in riflemen and we found no indication that breeder condition had any influence on brood sex ratios. However, as cooperative breeders, riflemen may be expected to increase production of the helping sex (males) when they have no helpers (Griffin et al., 2005). We tested for this effect and found no response. One possible reason may be that breeding females have no reliable information on whether they will be helped at the time of sex determination, because their potential helpers have usually dispersed onto their own territories. Thus, they may be unable to make adaptive allocation decisions.

Although riflemen may adjust their brood sex ratio in response to cues not measured here, a more parsimonious interpretation of our results is that they are either unable to do so, or it is not adaptive. Facultative sex allocation is widely reported in birds, and is apparently adaptive in Seychelles warblers (Komdeur et al., 2002). Cooperative breeders are predicted to adjust their sex ratios when in need of help, but empirical evidence for this is mixed (Komdeur et al., 1997; Griffin et al., 2005; Kingma et al., 2011; Gressler et al., 2014). This discrepancy may be underlain by mechanistic constraint. Though mechanisms of sex ratio adjustment have been suggested, these remain unconfirmed (Rutkowska and Badyaev, 2008); the precise mechanism, and the costs associated with its evolution and implementation, may vary between species. Theoretical work suggests that the adaptive value of facultative sex allocation is quickly eroded by even small costs (Pen et al., 1999). Elucidating the mechanisms involved in avian sex allocation may improve our understanding of why adaptive patterns are inconsistent, and appear to be absent in species like riflemen.

4.

No cross-species support for the repayment hypothesis in birds

Nyil Khwaja, Ben J. Hatchwell, Robert P. Freckleton and Jonathan P. Green

The repayment hypothesis predicts that dominant females in cooperative breeding systems overproduce the helping sex. Thanks to well-documented examples of this predicted sex ratio bias, repayment has been considered an important driver of variation in sex allocation patterns. Here we test this hypothesis using data on population brood sex ratios and facultative sex allocation from 26 cooperatively breeding bird species. We find that biased sex ratios of helpers do not correlate with production biases in brood sex ratios, contrary to predictions. We also test whether females facultatively produce the helping sex in response to a deficiency of help (i.e. when they have fewer, or no helpers). Although this is observed in a few species, it is not a significant trend overall, with a mean effect size close to zero. We conclude that, surprisingly, repayment does not appear to be a widespread influence on sex ratios in cooperatively breeding birds. This is unusual in the field of sex allocation, in which theory and data generally marry well. We discuss possible explanations for our results, and encourage further theoretical examination of the repayment model.

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Introduction

Sex allocation theory has become an extremely successful branch of evolutionary biology, based on the premise that the allocation of a parent's offspring to one sex or the other can be non-random and subject to natural selection (West, 2009). In this framework sex allocation is interpreted as an adaptive decision made in the context of parental investment, with the relative costs and benefits of producing each sex being key to this decision. In cooperative breeders, one benefit that has attracted a great deal of attention is the fitness gain that parents receive from offspring who help them in future breeding attempts (Malcolm and Marten, 1982; Koenig and Walters, 1999; Griffin et al., 2005). Help is usually more likely to come from one sex than the other (Komdeur, 2004). The 'repayment hypothesis' predicts that parents should invest more in this helpful sex, because of the greater chance that their investment will be repaid through future help (Emlen et al., 1986; Lessells and Avery, 1987).

The repayment hypothesis yields intuitive predictions about offspring sex ratios in cooperative breeders, and long-term studies of bird populations provide valuable datasets on which to test these. The first general prediction is that offspring sex ratios should be biased towards the helping sex at the population level, because of the extra payoff associated with producing this sex. For example, brood sex ratios in bell miners *Manorina melanophrys* are consistent with this prediction: helping is extremely male-biased, and 58% of hatchlings are male (Clarke et al., 2002). The second prediction is that breeders with no, or fewer helpers, should facultatively adjust the sex ratio of their broods to be more biased towards the helping sex, as they have more to gain from doing so. Again there is some empirical support, especially from Seychelles warblers *Acrocephalus sechellensis*: helping is female-biased in this species, and breeders without helpers produce more females when their territory is of sufficient quality to support the extra philopatric offspring (Komdeur et al., 1997).

Despite these examples, the status of repayment as a widespread driver of sex ratio skew is called into question by a number of negative results. For example, in purple-crowned fairy-wrens *Malurus coronatus* and white-banded tanagers *Neothraupis* *fasciata*, there is no significant bias toward production of the helping sex at the population level, and no evidence for facultative adjustment of brood sex ratios in response to any cues (Kingma et al., 2011; Gressler et al., 2014). While the predictions of the repayment hypothesis are intuitively appealing, the adaptive value of producing each sex will also depend on difficult-to-measure components of the direct fitness of both parents and offspring. Long-term data on red-cockaded woodpeckers *Picoides borealis* have been used to estimate direct fitness differentials between the sexes, and incorporate them into a repayment model; however, the model still failed to predict brood sex ratios at the population level (Koenig and Walters, 1999). A meta-analysis by Griffin et al. (2005) suggests that individuals are more likely to adjust their offspring's sex ratio in systems where the benefits of help are high. This provides some explanation for failures to observe adjustment, but such negative results have proliferated since Griffin et al.'s (2005) study, perhaps because authors realise they run counter to the prevailing view of adaptive sex allocation (Cockburn and Double, 2008).

Here we use a comparative analysis and meta-analysis to test the key predictions of the repayment hypothesis, using data from previously published studies of 26 cooperatively breeding bird species spanning 17 families. We then discuss the usefulness and generality of the concept of repayment, in light of our results.

Methods

We collected data from relevant studies identified using the search term '(brood) sex ratio birds' in Web of Science and Google Scholar, as well as from Koenig and Dickinson (2016), Komdeur (2004) and references within. We included all available brood sex ratios from bird species with kin-based cooperative breeding systems, as production is only related to future help in these cases. Where multiple studies were published for the same species, we chose that with the largest sample size, or in the case of Seychelles warblers and eclectus parrots *Eclectus roratus*, that which came from a natural population (Komdeur et al., 1997; Heinsohn et al., 2011). Helper sex ratios were obtained from Green et al. (2016) or directly from the literature, where possible from the same population as brood sex ratios.

We investigated the effect of helper sex ratio on log-transformed brood sex ratio across 26 species, controlling for phylogeny using a phylogenetic generalised least squares (PGLS) model implemented in R 3.2.2 (R Development Core Team, Vienna). We applied this model across 1,000 trees downloaded from birdtree.org using the Hackett et al. (2008) backbone (Jetz et al., 2012; Figure 4.1). Following Green et al. (2016) we included data quality as a variance component in the model, using categories of weak, medium or strong assigned to each study. When assigning these scores, we considered the sample size, length of study, sample size of helper sex ratio estimate and point at which brood sex ratio was measured (with closer to primary sex ratio being considered better quality).

For 17 studies that tested for facultative sex allocation in cooperatively breeding bird species, we used a meta-analysis to investigate the hypothesis that breeders without helpers, or with fewer helpers, show greater production of the helping sex. This is the strongest prediction made by the repayment hypothesis in the context of facultative allocation. We extracted effect sizes of this relationship using equations from Lajeunesse (2013), except for three studies where we obtained effect sizes from Griffin et al. (2005). Brood sex ratio was treated as the response, and the predictor was either whether a nest was helped (categorical, 8 studies), or number of helpers (integer, 9 studies). We derived confidence intervals (CI) and sample weights for each study using equations from Lipsey and Wilson (2001), and calculated a weighted mean and 95% CI using the Hmisc package in R (Harrell, 2016).

Results

We collected data on helper and brood sex ratios for 26 species of cooperatively breeding bird. The mean brood sex ratio (proportion of males) across species was 0.50 ± 0.01 SE (median = 0.50); this value ranged from 0.37 in broods of the black-eared miner *Manorina melanotis*, to 0.58 in broods of the apostlebird *Struthidea cinerea*. The mean helper sex ratio across species was 0.76 ± 0.06 SE (median = 0.87), ranging from exclusively female helpers in white-browed tit-warblers *Leptopoecile sophiae*, to exclusively male helpers in nine species (Table B.1, Appendix B).

There was effectively no phylogenetic signal to brood sex ratio (PGLS: $\lambda < 0.01$; Figure 4.1). As an illustrative example, the two most closely related species we included were the black-eared miner and bell miner, whose broods contained the lowest and fourth-highest proportion of males respectively (Figure 4.1). As expected, studies with a data quality score of weak accounted for more variance ($1.54\lambda^{23}$) than those scoring medium ($1.41\lambda^{22}$), and those scoring strong accounted for the least ($1.80\lambda^{20}$) . Once these effects were controlled, we found no relationship between helper sex ratio and brood sex ratio (PGLS: $R^2 < 0.01$, $t = 0.19$, $P = 0.849$; Figure 4.2). With phylogeny accounting for such little variation, a simple linear model without phylogenetic or data quality controls produced equivalent results (ANOVA: $R^2 < 0.01$, $F_{1, 24} < 0.01$, $P = 0.885$).

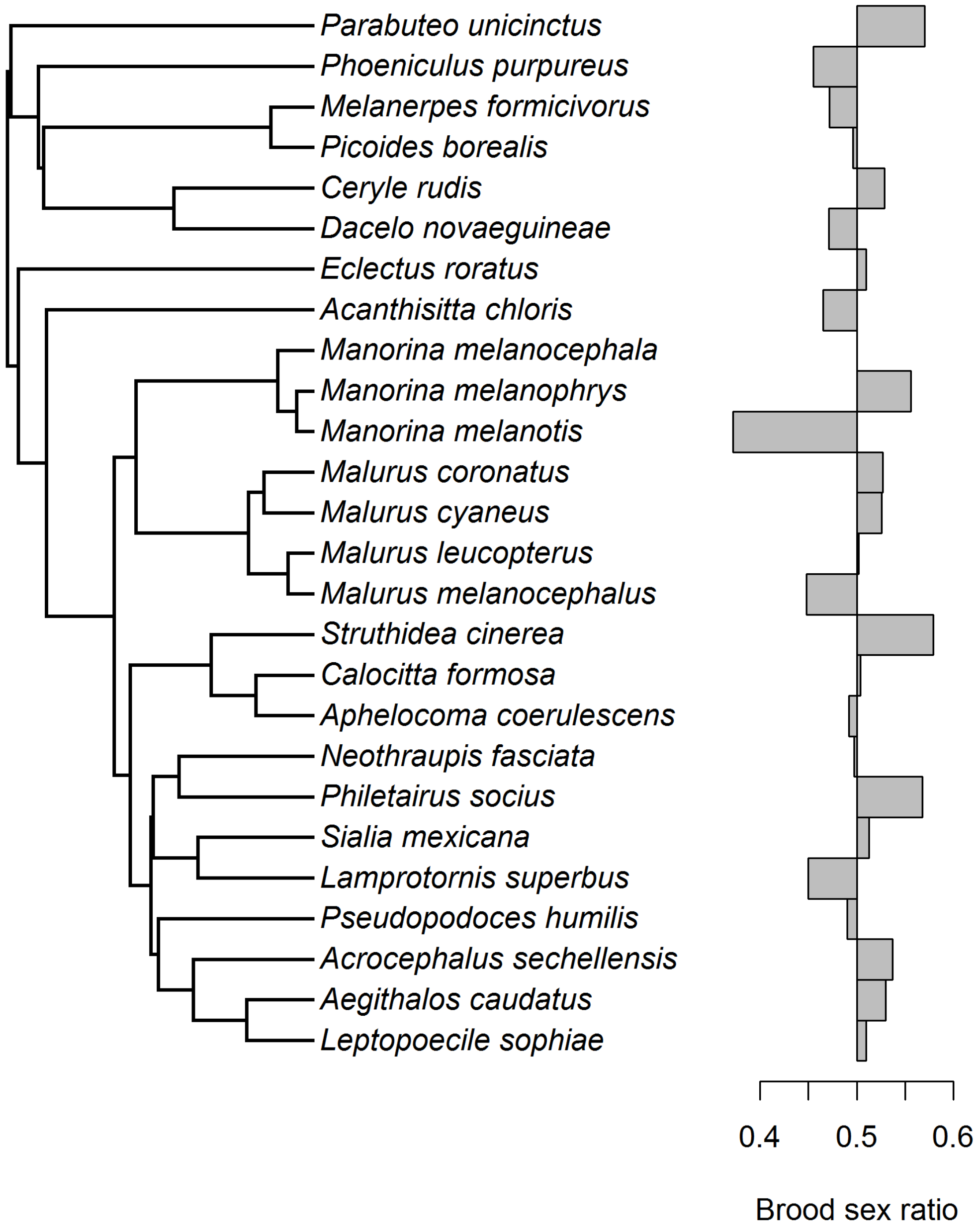

Figure 4.1 Phylogeny showing relationships between the 26 species used in our comparative analysis, with sample branch lengths from 1 of 1,000 equiprobable trees downloaded from birdtree.org (Jetz et al., 2012). Brood sex ratio for each species is expressed as the mean proportion of males in broods from a studied population. Brood sex ratio is randomly distributed with respect to phylogeny (see Results).

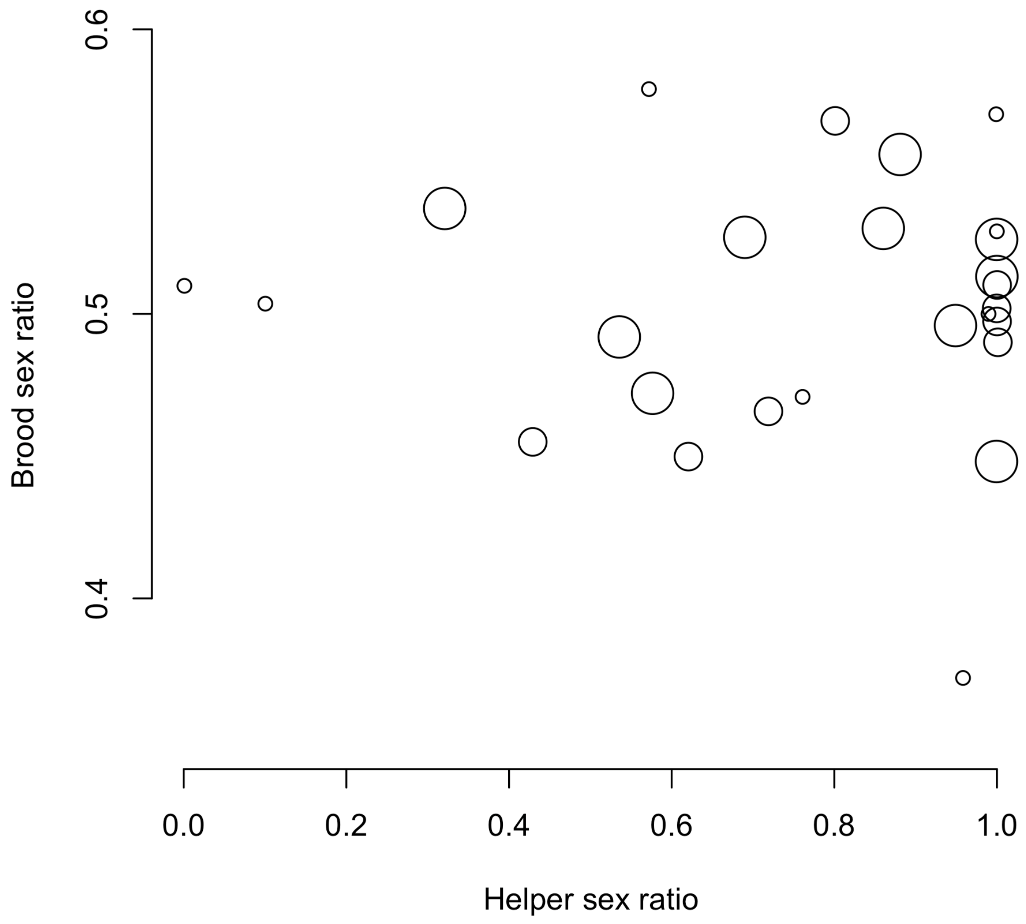

Figure 4.2 Observed sex ratios of helpers and broods across 26 cooperatively breeding bird species, expressed as proportions of males. There is no relationship between these two variables (see Results), in contrast to the predictions of the repayment hypothesis. Point sizes represent our assessment of data quality (see Methods).

We obtained effect sizes of the relationship between number of helpers or being helped, and brood sex ratios, from 17 studies of different cooperatively breeding bird species (Figure 4.3; Table B.2, Appendix B). Nine of these effects were in the expected direction, with more of the helping sex produced by unhelped females or those with fewer helpers. Six were in the opposite direction, and the directions of two small effects were not reported. The weighted mean of the 15 effect sizes whose direction was reported was 0.01 (95% CI = -0.17-0.36) in the direction expected. This result was the same (to two decimal places) when the two excluded effect sizes were treated as both positive or both negative. Effect sizes have tended to be closer to zero in more recent studies (ANOVA: $R^2 = 0.23$, $F_{1,15} = 4.47$, $P = 0.052$; Fig. 4), notably since the publication of Griffin et al.'s (2005) meta-analysis of sex ratio adjustment in cooperative breeders.

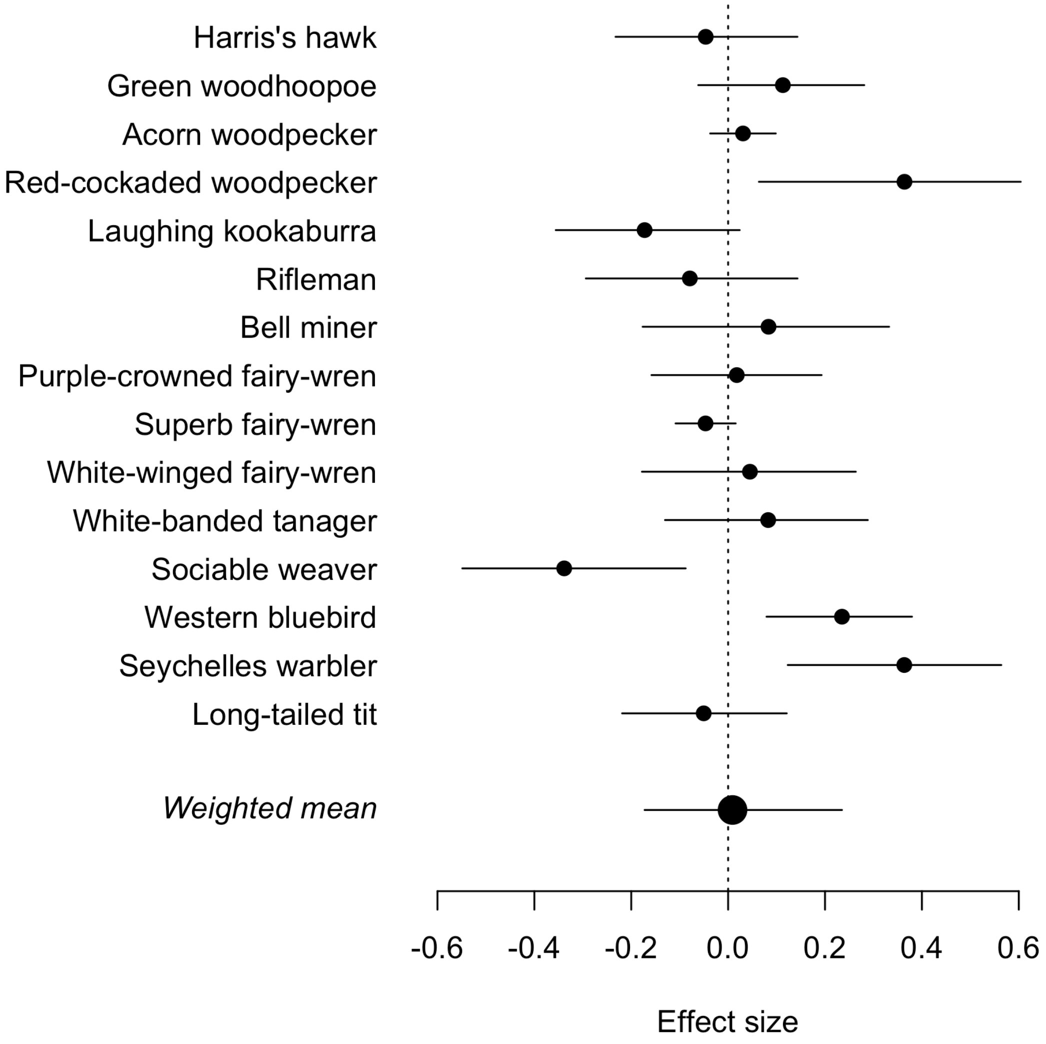

Figure 4.3 Effect sizes and confidence intervals from 15 studies investigating facultative production of the helping sex in cooperatively breeding birds. Positive effect sizes are in the direction expected from theory: females producing more of the helping sex when deficient of help. The effect sizes of two studies (on apostlebirds and superb starlings) are not shown because their directions were not reported (see Results).

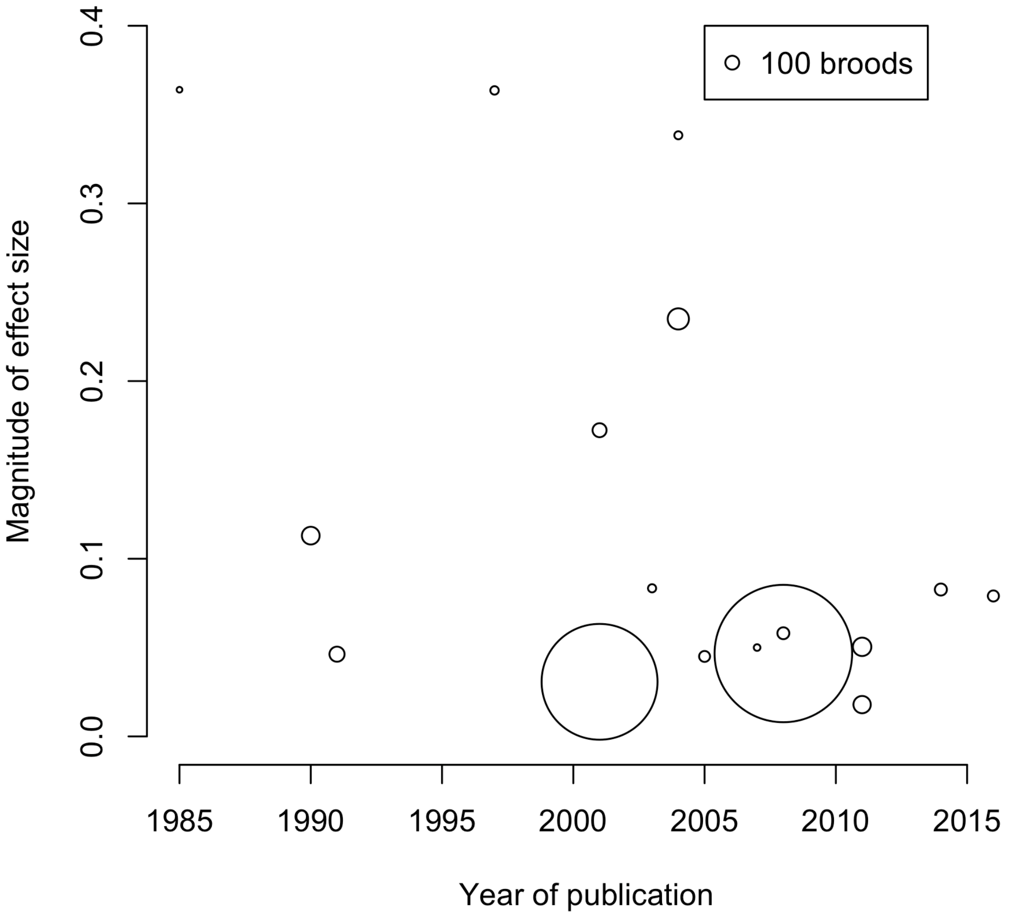

Figure 4.4 The relationship between the magnitude of effect sizes from studies investigating facultative sex allocation in cooperative breeders, and their year of publication (see Results). The direction of the effects are not shown: this plot represents their distance from zero.

Discussion

We investigated two general predictions attributed to the repayment hypothesis. The first is that in kin-based cooperative breeders, females produce broods biased towards the helpful sex, because the future fitness contribution made by helpers repays some of their investment. Across 26 bird species, we found no evidence that this was generally the case, despite a lack of phylogenetic signal indicating that brood sex ratio is a labile trait. The second prediction was that females in need of help adjust their brood's sex ratios further in favour of the helping sex. We drew together tests of this prediction from 17 studies, and although it is borne out in a minority of cases, there was not a significant directional trend across species. Earlier studies tended to report greater effect sizes, which may have led to an overestimation of the prevalence of adaptive sex allocation in the literature.

No overall bias to producing the more helpful sex

A predicted global sex-ratio skew toward the helping sex in cooperative breeders is the most direct interpretation of the repayment model; in their introduction to the original model, Emlen et al. (1986) proposed that 'the sex ratio should achieve an equilibrium in which there is an overproduction of the helper sex'. There are two levels at which this prediction may falter: proper accounting of the costs and benefits of producing each sex, and empirical observation. Where sexes differ in their tendency to help, they are also likely to differ in other key life-history traits. Although one sex may 'repay' investment by helping, and thus improving their parents direct fitness, this could feasibly be counteracted by enhanced reproductive success in the non-helping sex improving parents' indirect fitness. This was identified by Koenig and Walters (1999), who incorporated sex differences in survival and reproductive success into an extended repayment model, to predict optimal brood sex ratios in red-cockaded woodpeckers. Their results were interesting in two respects: (1) the predicted sex ratio in a species with male-biased help was still male-biased, and little affected by considering differences in the direct fitness achieved by male and female offspring, and (2) they were significantly different from observed sex ratios, which were close to parity. Here, we are unable to account for the influence of direct fitness on expected sex ratios, as few (if any) species in our sample have been studied in as much detail as the red-cockaded woodpecker. Instead, we used helper sex ratio, the key driver of expected brood sex ratio bias in Koenig and Walters (1999), and similarly found that observed brood sex ratios do not fit expectation. This does not necessarily mean that the extended repayment model as applied to red-cockaded woodpeckers also fails in other systems; it is still possible that this species is exceptional, and that in most cases including sex differences in direct fitness may better explain patterns. It does mean that females biasing production towards the helping sex, which is the most intuitive prediction of repayment models, is not a valid generalisation.

The repayment hypothesis makes opposite predictions to the local resource competition hypothesis, which suggests that females may benefit from overproducing the dispersive (generally non-helping) sex, to reduce competition for resources (Clark, 1978). Costs of competition could explain the discrepancy between our predictions and results, though there are reasons to doubt this interpretation. Firstly, in the majority of systems these costs do not compensate fully for the benefits of help, as helped nests receive more provisioning visits (reviewed in Hatchwell, 1999). Further, if competition provided the main constraint to adaptively biasing the sex ratio, we would expect biases towards the helping sex to be especially pronounced in species like riflemen *Acanthisitta chloris*, where most helpers had previously dispersed from their natal territory, the sexes do not differ significantly in their dispersal distances, and males more commonly help (Preston, 2012). It is noteworthy, therefore, that the observed sex ratio is not male-biased in this species (Chapter 3). Nevertheless, competition may limit the adaptive value of biased sex allocation in other species.

A third possible explanation is simply that biasing brood sex ratios is constrained or costly, though again this has little support. Mechanisms of biased sex allocation are currently unknown, and some suggestions rely on potentially taxon-specific characteristics such as size dimorphism in the centromeres of sex chromosomes (Rutkowska and Badyaev, 2008). However, our models showed a complete lack of phylogenetic constraint on brood sex ratios. Although across species these average around parity, some studies with convincingly large sample sizes show significantly biased population brood sex ratios (Clarke et al., 2002; Cockburn and Double, 2008). There may be a mechanistic barrier to biasing sex ratios in some species, but as this is clearly not global, and phylogenetically random, it is not a well-supported interpretation (West and Sheldon, 2002). While a combination of the three explanations we have suggested may underlie our results, none are especially convincing for the reasons discussed. We would welcome a theoretical re-evaluation of the repayment model, which may make sense of its uneasy fit with empirical data.

Rarity of sex-ratio manipulation

Seychelles warblers are a species in which biased sex allocation apparently comes with little cost (Komdeur et al., 2002). Here, females show extraordinarily sophisticated control of their broods' sex ratios, with females (the more helpful sex) overproduced on good quality territories without existing helpers (Komdeur et al., 1997). This does not result in an overall population-level bias towards the production of females though; in this case, an unbiased population sex ratio is consistent with the repayment hypothesis because females across different contexts adaptively allocate their offspring's sex. Komdeur et al.'s (1997) influential study has been cited over 400 times, and inspired similar work on at least 13 cooperatively breeding bird species since, as identified in our meta-analysis. Although western bluebirds *Sialia mexicana* also show evidence of facultative control (Dickinson, 2004), by and large this body of work shows Seychelles warblers to be the exception rather than the rule (with an average effect size close to zero). There is no general tendency across species to produce the helping sex when breeders are deficient of help. This picture emerges strongly with the inclusion of more recent publications documenting negative results, providing a contrast to the mainly positive findings published by the time of Griffin et al.'s (2005) previous meta-analysis, and leading us to somewhat different conclusions (Figure 4.4). We hope this encourages publication of negative results, which are essential for the validity of comparative work. Our findings raise the question of why helpers are apparently not facultatively produced in response to need elsewhere, where there is a plausible benefit to doing so; or what is exceptional about Seychelles warblers? We outline four suggestions below.

Unpredictable environments. Adaptive sex allocation in Seychelles warblers relies on assessment of environmental quality. Helpers are produced only on territories with sufficient food (Komdeur et al., 1997). Cooperatively breeding birds are commonly associated with unpredictable environments, with extra care thought to buffer against stochasticity (Jetz and Rubenstein, 2011). Species living in such conditions may lack adaptive cues for sex allocation.

Unpredictable help. In species that breed in kin neighbourhoods, such as riflemen and long-tailed tits *Aegithalos caudatus*, helpers are not permanently affiliated with the breeding pair (Hatchwelland Dickinson, 2004). Respectively, helpers in these species begin the breeding season dispersed onto their own territories, or attempting to breed themselves, and they start helping at a nest partway through the provisioning phase (Preston et al., 2013; Hatchwell, 2016). In long-tailed tits this is contingent on nest failure. As a result, breeding females may not have good information on the amount of help they will receive. If helpers are only related to the male in a pair (usually the case in long-tailed tits), they may not even be recognised by the female (Nam et al., 2011). This limits her potential to make adaptive sex allocation decisions based on need.

Other cues influencing allocation decisions. Sociable weavers *Philetairus socius* display a surprising sex allocation pattern, in which females with helpers produce relatively more rather than fewer males, the helping sex (Doutrelant et al., 2004). Clearly in this species the need for help is not a key driver of allocation decisions; the authors suggest the inheritance of within-colony rank by philopatric males may select for overproduction of males by high-ranking breeders, which would inevitably correlate with levels of help. In white-winged fairy-wrens *Malurus leucopterus* and superb starlings *Lamprotornis superbus*, sex allocation is consistent with the Trivers-Willard hypothesis, where females with better reproductive prospects overproduce males, who have higher potential fitness (Trivers and Willard, 1973; Rathburn and Montgomerie, 2005; Rubenstein, 2007). In these species, allocation decisions based on rank and breeding condition presumably carry more benefit than those based on a need for help.

Insufficient benefits of help. Griffin et al. (2005) found that the benefit of help was key to the extent of facultative production of the helping sex. They used helpers' effect on productivity as a measure of this benefit. There are issues with this approach, as in some species helpers improve downstream recruitment rather than breeding productivity, and in others they have less effect on productivity but provide direct benefits to breeders through load-lightening (Hatchwell, 1999; Hatchwell et al., 2004; Preston et al., 2016). Nevertheless, in some species such as Harris's hawks *Parabuteo unicinctus*, the effect of help may be insufficient to select for facultative adjustment of brood sex ratios (Bednarz, 1987).

Conclusion

The repayment hypothesis fits well with certain 'textbook' examples in cooperatively breeding birds, but our study shows that its predictions do not explain variation across species. On a generalised level, its importance is marginal in driving both sex ratio biases and facultative sex allocation. Though we suggest a number of confounds that may have diminished its importance, it is surprising that such an intuitively appealing theory does not fit observed patterns. This contrasts with the majority of work on sex allocation, in which theoretical predictions have matched empirical results with a great deal of success (West, 2009). We conclude that repayment is an occasional, but not widespread, influence on sex allocation in cooperatively breeding birds.

TESTING POTENTIAL KINSHIP CUES IN RIFLEMEN

5.

Potential chemical cues to relatedness in cooperative riflemen

Nyil Khwaja, Ben J. Hatchwell, James V. Briskie and Laura Azzani

Riflemen *Acanthisitta chloris* are cooperatively breeding birds in which patterns of helping and mate choice indicate an ability to discriminate kin from non-kin. We investigated the potential for olfactory cues to perform this function, using uropygial secretions (preen wax) as a measure of odour. The chemical composition of preen wax samples taken from nestling and adult riflemen was assessed using gas chromatography. Preen wax composition differed significantly in both major principal components between nestlings and adults. In nestling dyads, there was no effect of relatedness on chemical similarity, but in the preen wax of adults, female-female dyads were more similar in the first principal component when they were more closely related (male-male dyads showed the same directional trend, but this was not statistically significant). We conclude that the chemical structure of preen wax contains some kinship information in this species. We then report the results of a field experiment, in which we tested whether riflemen responded to a manipulated olfactory cue. To do so we introduced foreign and native nest material into active nests, and compared the subsequent behaviour of carers. Our results indicated differences in provisioning behaviour based on treatment, although not in the way that we predicted. We suggest that this behavioural response, coupled with the kinship information present in preen wax, opens the possibility that riflemen recognise kin by olfaction.

Introduction

Indirect fitness benefits are an important driver of the evolution of helping behaviour in many cooperatively breeding birds (Green et al., 2016; Koenig and Dickinson, 2016). In such cases, help should be reliably directed towards kin in order to be adaptive (Emlen, 1997), but the strength of selection on a mechanism of active kin discrimination varies among species according to their social system (Cornwallis et al., 2009). Where cooperative breeding is characterised by delayed dispersal, helpers can potentially gain indirect benefits by caring for any young on their natal territory (which are likely to be close relatives) without the need for a mechanism of kin recognition. Arabian babblers *Turdoides squamiceps* and laughing kookaburras *Dacelo novaeguineae* are both cooperative species where helpers delay dispersal and helping appears to be indiscriminate (Wright et al., 1999; Legge, 2000). Kinship is less spatially predictable in colonial species, where colonies are shared with non-kin, and in kin neighbourhoods, where individuals disperse away from their natal territory before becoming helpers (Hatchwell, 2009; McDonald et al., 2016). The strongest evidence for kin discrimination in helping behaviour has emerged from these types of system: in the colonies of bell miners *Manorina melanophrys* and the kin neighbourhoods of long-tailed tits *Aegithalos caudatus*, helping is closely correlated with relatedness (Russell and Hatchwell, 2001; Nam et al., 2010; Wright et al., 2010). In both species, there is evidence that individuals are able to recognise the vocalisations of their relatives, which may be used to allocate help adaptively and maximise helpers' indirect fitness (Sharp et al., 2005; McDonald and Wright, 2011).

The rifleman *Acanthisitta chloris* is a facultative cooperative breeder endemic to New Zealand, whose social system is best described as a kin neighbourhood. Riflemen may breed twice in a season, and juveniles from a first brood sometimes remain with their parents to help provision the second, although their contributions are equivocal. More effective help is provided by adult helpers that have previously dispersed away from their natal territory but return to help, in some cases while breeding simultaneously. Adult helpers are observed at around 25% of nests (Chapter 7). They are close relatives of the broods they help, and these broods show enhanced recruitment rates, indicating that helping provides indirect fitness benefits (Preston et al., 2013a, 2016). This pattern of helping is unlikely to arise through indiscriminate helping, suggesting that riflemen use some mechanism of kin recognition, an interpretation supported by evidence that they actively avoid inbreeding (Preston, 2012).

Here we investigate the hypothesis that olfactory cues function as a kinship signal in riflemen. There is burgeoning interest in avian olfaction (Bonadonna and Mardon, 2013), and recent studies across a range of taxa have suggested a role for chemical cues in the recognition of potential mates, familiar relatives, and conspecifics (Amo et al., 2012; Krause et al., 2012; Mihailova et al., 2014). Chemical signals have the potential to function as simple indicators of genetic relatedness, without necessarily requiring familiarity or associative learning (Leclaire et al., 2012; Breed, 2014; Riehl and Stern, 2015). In spite of this they have been little studied in cooperatively breeding birds, where the focus has been on vocalisations instead (e.g. Sharp et al., 2005; Akçay et al., 2013; Crane et al., 2015). The evolutionary history of riflemen makes them a particularly interesting species in which to address this gap in understanding. Birds on oceanic islands without natural mammal predators, like New Zealand, are less likely to have experienced selection to mask their odours, and perhaps because of this have generally more volatile (smellier) uropygial secretions (Thierry, 2014; Azzani, 2015). In light of this, we might expect olfaction to be a more important component of these species' communication systems.

We first examine the potential for olfactory cues to signal relatedness, using the chemical composition of uropygial gland secretions, or preen wax, as a proxy for odour. Preen wax is a substance rich in volatile compounds that is used to coat a bird's feathers and is considered to be a key determinant of avian body odour (Hagelin and Jones, 2007; Campagna et al., 2012). Following this analysis, we present the results of a field experiment, in which we tested whether manipulation of the olfactory environment of nests affected the behaviour of provisioning riflemen.

Methods

Sample collection and processing

We collected preen wax samples from 15-day-old rifleman nestlings, which we removed from the nest (see Chapter 1), and from adults which we caught by mist-netting near to known nests, when their nestlings were at least 9 days old. Blood samples were collected at the same time and genotyped using the procedure detailed in Chapter 1.

We placed birds in clean paper bags before collecting preen wax samples, and handled them using clean latex gloves, to reduce the risk of contaminating the wax with oils from our hands or other birds. We collected preen wax samples by gently massaging a bird's uropygial gland with wax-coated forceps. When successful, this technique elicits a drop of wax, which we collected on a stainless steel inoculation loop. This was placed inside a sealed glass vial, which we kept inside a coolbox containing ice packs while in the field, and then stored in a freezer at -20 C until processing.

We analysed the content of preen wax samples using gas chromatography at the University of Canterbury's Department of Chemistry, New Zealand, following the protocol of Azzani et al. (2016). We aligned peaks by retention time and gave them ordinal names so that they could be compared across samples. In total, we detected peaks representing 160 different compounds. Although compounds were matched across samples, we did not use mass spectrometry and so they were not explicitly identified. We converted each peak's area for a sample to a proportion of that sample's total peak area, so that the quantity of preen wax in a sample (which could not be standardised) did not influence results. Proportions exceeding 0.001 (0.1%) of a sample's total area were retained for analysis.

Statistical analysis of chemical data

We used R 3.3.2 (R Development Core Team, Vienna) for all statistical analyses. Our method draws on that used in Leclaire et al.'s (2012) study of genetic relatedness and the odour profiles of black-legged kittiwakes *Rissa tridactyla*. We summarised variation across the 160 peaks of chemical profiles using principal component analysis. Sample scores for the first two principal components (PC1 and PC2) were used for analysis. Together, these explained 71.2% of the variation in chemical profiles, with PC1 explaining 47.6% of the variation and PC2 explaining 23.6%; the remaining principal components each explained less than 7%. We examined the effects of age and sex on chemical profiles using analyses of variance with PC1 and PC2 scores as response variables and age (adult/juvenile) and sex (male/female) as categorical predictors, along with their interaction.

We calculated the difference in PC1, the difference in PC2, and the absolute Euclidean distance between profiles, for each sample-sample dyad in our dataset. We then created separate matrices with each of these distances for adult and juvenile male-male (MM) and female-female (FF) dyads respectively. We created equivalent genetic distance matrices using each dyad's relatedness estimate subtracted from one. We tested each measure of chemical distance's relationship with genetic distance by comparing the appropriate matrices using Mantel tests, implemented in the vegan package (Oksanen et al., 2011). This calculates a correlation coefficient (*r*) that accounts for the non-independence of multiple distance measurements including the same individual, and generates a P-value using permutation tests, for which we ran 5,000 permutations. Significantly positive values of *r* indicate that more genetically similar dyads have more similar chemical profiles. The Mantel test cannot be used on non-square matrices and therefore was inappropriate for testing opposite-sex dyads. We instead used a Pearson's correlation test on male-female (MF) dyads. This is a liberal test, as it does not control for the non-independence of data points, and so significant results should be interpreted with caution.

For juveniles, we tested for a nest effect on chemical structure by calculating ANOVA-based repeatabilities (*R*) of PC1 and PC2 by nest, using the rptR package

(Schielzeth and Nakagawa, 2013). Although ANOVA-based repeatability tests are problematic in datasets that require correction for confounding variables, they are advantageous when looking solely at the effect of one factor because *R* is not bounded by zero, making the calculation of meaningful P-values straightforward (Nakagawa and Schielzeth, 2010).

Experimental design and analysis

We investigated whether a change in olfactory environment influenced the behaviour of riflemen by testing the responses of provisioning birds exposed to unfamiliar nest material. Evidence from zebra finches *Taenopygia guttata* suggests that nest material contains olfactory signatures, which can be used by birds to recognise kin (Krause et al., 2012; Kohlwey et al., 2016). Recording the behaviour of breeding birds at a nest allows experimental manipulation and behavioural observations on wild individuals in situ, as they return regularly to the nest area. This has formed the basis of previous successful olfactory experiments by Mihailova et al. (2014).

We sewed pouches from clean muslin cut into approximately 20 cm × 20 cm squares. These pouches were only handled using clean latex gloves and stored in clean zip-lock plastic bags to avoid contamination. On day 17 (where hatching = day 0), we took two small samples (c.4 cm across) of nest material from each nest's entrance. These were placed in separate muslin pouches, which were then replaced in their zip-lock bags, stored in a cool box with ice packs while in the field, and then transferred to a freezer at -20 C. One sample was subsequently used as a 'native' treatment in its nest of origin, and another as a 'foreign' treatment in another nest. We carried out experimental treatments on days 18, 19 and 20 of the nestling period. We placed a pouch within the nestbox but outside the nest entrance (riflemen build contained nests within nestboxes, and so there is an inner entrance beyond the hole in the box). This pouch contained either no nest material (control), material from that nest (native) or material from another nest (foreign). In this way we could estimate the effects of both introducing nest material into the environment, and of that material being foreign. We systematically shuffled the order in which treatments were presented between nests.

We filmed each nest for a 30-minute period following placement of the pouch, and removed the pouch once this period had elapsed.

Videos were transcribed by a single observer, who was blind to treatment. For each visit to a nest, the following information was recorded: time, the provisioning individual's identity (colour-ring combination) and sex, whether it fed the nestlings, and its latency to enter the nestbox or to feed the nestlings if this occurred from outside the nestbox (recorded as an integer in seconds).

If riflemen used the odour of their nests as a behavioural cue during provisioning, we expected to observe (1) a lower visit rate and (2) more cautious or aberrant behaviour (i.e. a lower proportion of feeding visits and a longer latency to feed), during the foreign treatment, as this was the only treatment in which a non-kin or unfamiliar rifleman scent was presented to provisioning carers.

For analyses, we modelled carers' visit rates and proportion of feeding visits across an observation period, and latency for each visit where a carer entered the nestbox and/or fed the nestlings, using mixed-effects models in the lme4 package in R (Bates et al., 2015). Visit rate (number of visits) was modelled using a generalised linear mixed-effects model with Poisson-distributed error structure. Proportion of feeding visits (number of feeding visits | number of non-feeding visits) was modelled as a generalised linear mixed-effects model with binomially-distributed error structure. Latency was log-transformed for normality: we used a Gaussian linear mixed-effects model fitted to the log of (latency + 1), because latency was recorded as 0 for visits where feeding or entry was instantaneous. In each model, treatment (factor: control, native or foreign), sex (factor) and status (factor: parent or helper) of provisioning individual, time (numeric: number of hours since 0700 NZST) and date (integer: number of days since 1st September) were included as fixed effects. Time and date were scaled and centred. The identity of the provisioning individual nested within a nest identity term was fitted as a random effect. We tested the significance of the effect of experimental treatment and other variables using type II Wald χ^2 tests implemented in the car package in R (Fox and Weisberg, 2011), and carried out pairwise comparisons within significant categorical predictors using Tukey tests implemented in the multcomp package (Hothorn et al., 2008).

Results

Chemical composition

We analysed the composition of 46 preen wax samples from 32 nestlings from 11 nests (10 males and 22 females), and 13 adults (7 males and 6 females), collected between 2012 and 2015. One adult male was sampled twice, so the total included 8 adult male samples overall. We ran the following analyses using data from one of this male's samples to avoid pseudoreplication; all were repeated and were qualitatively equivalent when this was substituted for the other sample.

We detected a significant difference between the preen wax composition of adults and nestlings as described by PC1; there was no significant effect for PC2, and no significant difference between the sexes in either principal component (Table 5.1; Figure 5.1; Figure 5.2). The lack of a significant age effect on PC2 appears to be driven by an outlying juvenile with a score of -0.54, which dwarfs the rest of the variation in this axis (all other scores range between -0.02 and 0.06); the effect of age becomes significant when this outlier is removed (Table 5.1).

Table 5.1 Analyses of variance testing the effects of age and sex on the chemical composition of 45 rifleman preen wax samples, as described by the first two principal components. The third test is on a dataset where one outlying juvenile female was removed (see Results). Results are equivalent when one sample from a resampled adult male is substituted for the other (see Results).

	PC1			**PC2**			**PC2 (outlier removed)**		
	df	**F**	**P**	**df**	**F**	**P**	**df**	**F**	**P**
Age (adult/juvenile)	1	145.72	< 0.001	1	0.24	0.624	1	56.20	< 0.001
Sex	1	0.05	0.818	1	0.34	0.563	1	0.16	0.696
Interaction	1	0.28	0.599	1	0.13	0.725	1	0.27	0.609
Residual	41			41			40		

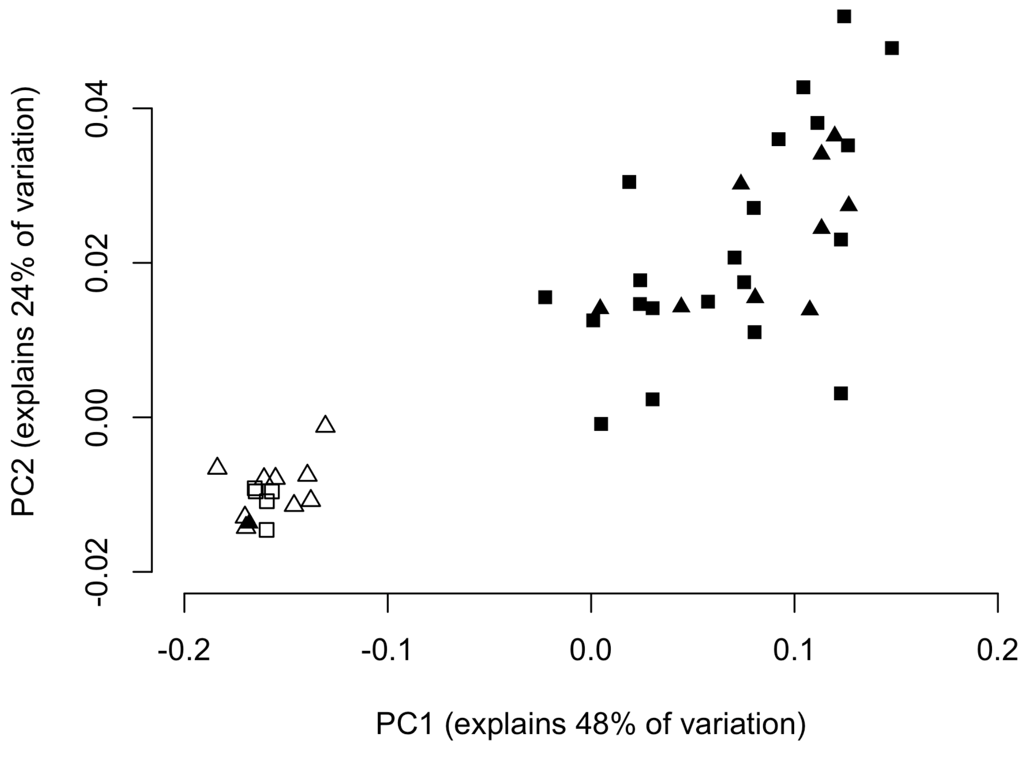

Figure 5.1 First two principal component scores from the chemical profiles of adult (open points) and nestling (filled points) riflemen. Males are represented by triangles and females by squares. One female nestling is not shown as her PC2 score of -0.54 obscures the rest of the variation in that axis (PC1 score = 0.15). Differences in PC1 are significant by age but not sex; differences in PC2 are significant by age only when the outlying nestling is removed, and not by sex in either dataset (see Table 5.1).

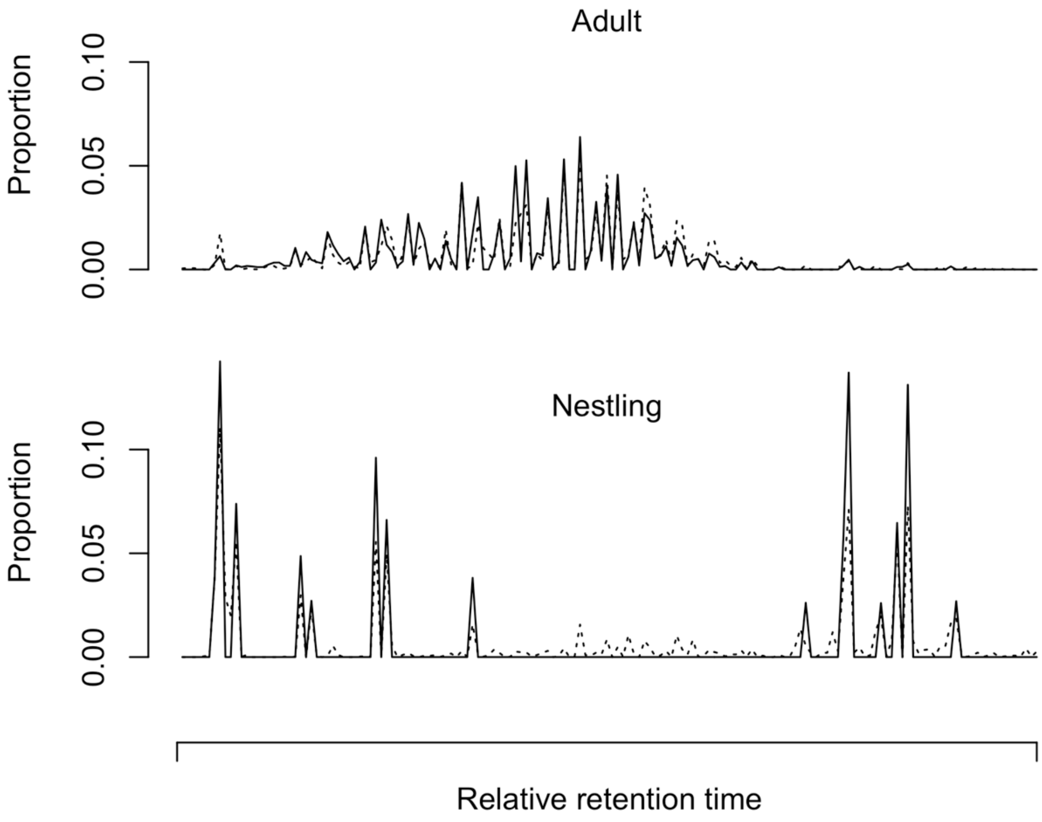

Figure 5.2 Example chemical profiles of the preen wax composition of adult and nestling riflemen. Each peak represents a different compound, which are ordered by retention time along the *x*-axis. Mean peak areas (from proportion of total peak area for each sample), for all adults and nestlings respectively, are shown with dotted lines. One female sample was excluded from the mean nestling profile due to its disproportionate effect on mean proportions (see Results).

In adults, differences in PC1 showed a significant, strongly positive correlation with genetic distances in FF dyads (Mantel test: $r = 0.62$, P = 0.018; Figure 5.3). A weakly positive correlation for the equivalent relationship in MM dyads was not statistically significant (Mantel test: $r = 0.19$, P = 0.164). There was no relationship between difference in PC1 and genetic distance in MF dyads (Pearson's correlation test: $r = -0.03$, $n = 40$ dyads, P = 0.832). Differences in PC2 and overall Euclidean distances between chemical profiles showed no significant relationship with genetic distance in any type of dyad (Mantel and Pearson's correlation tests: all P > 0.05).

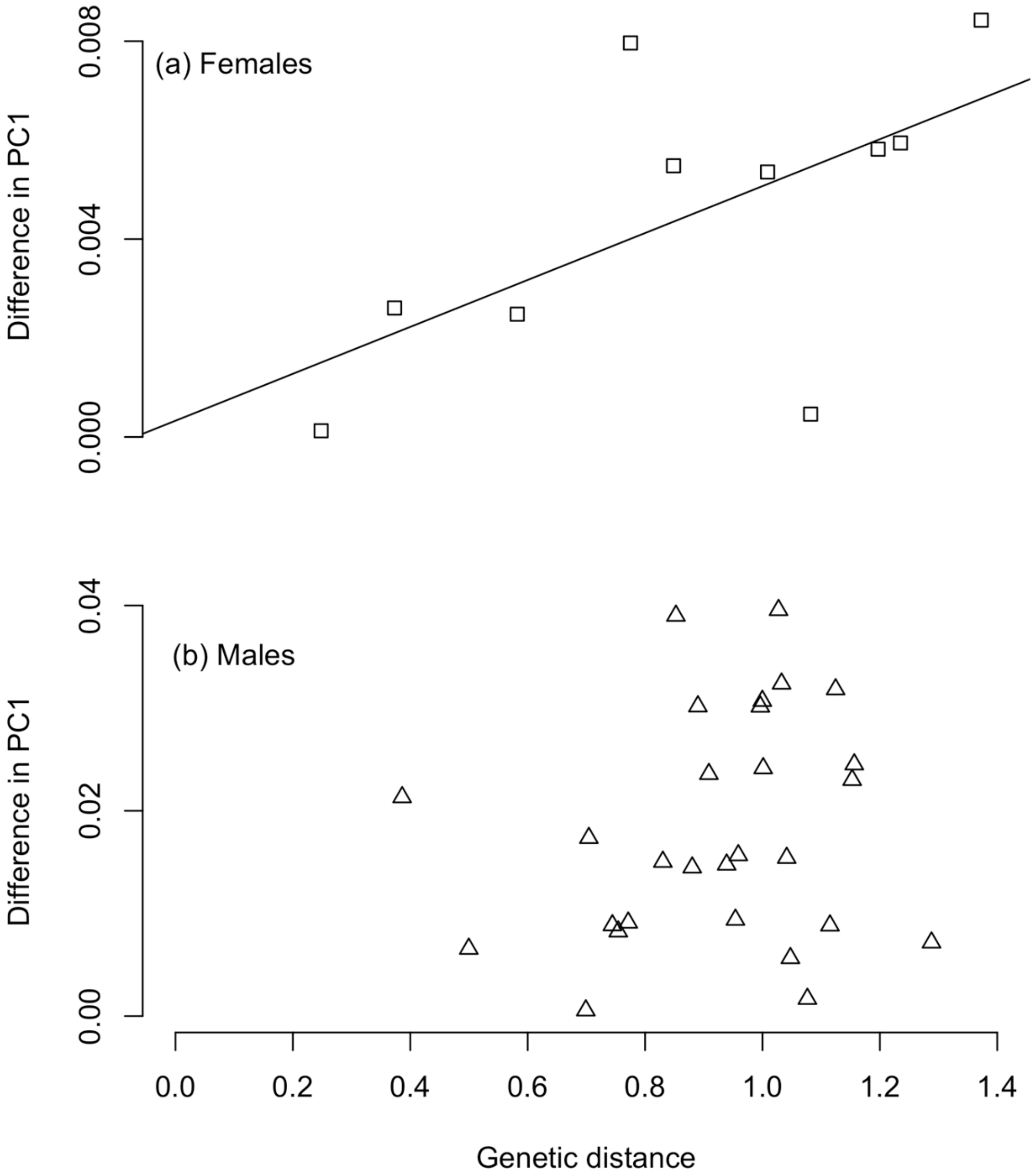

Figure 5.3 Relationship between genetic distance and difference in PC1 in the chemical profiles of adult female (a) and adult male (b) riflemen. The correlation is significant in females, but not in males (see Results for statistical tests).

In nestlings, genetic distance showed no relationship with differences in PC1 or PC2, or Euclidean distance (Mantel and Pearson's correlation tests: all $P > 0.2$). There was no significant nest effect on PC1 (ANOVA-based repeatability: $R = -0.13$, $n_{\text{nestlings}} = 32$, $n_{\text{nests}} = 10$, $P = 0.832$). A significant nest effect on PC2 (ANOVA-based repeatability: $R = 0.98$, $n_{\text{nestlings}} = 32$, $n_{\text{nests}} = 10$, $P = 0.011$) was driven by the inclusion of the outlying female nestling, without which the effect was not significant (ANOVA-based repeatability: $R = 0.13$, $n_{\text{nestlings}} = 31$, $n_{\text{nests}} = 9$, P = 0.223).

Manipulation of nest odour

We conducted our experiment on nine nests during the 2014-2015 breeding season. Each nest was attended by two parents, none had adult helpers and one nest had juvenile helpers (two). Thus in total we recorded the behaviour of 20 provisioning carers, once for each treatment, giving 60 observations for visit rate. Visit rate was 0 for 2 of these observations, so we used 58 observations to analyse proportion of feeding visits. In total, we recorded 262 visits in which carers entered the nestbox and/or fed the nestlings, which we used in our analysis of latency.

Carers visited an average of 6.32 ± 0.47 times during a half-hour observation period. Visit rate was unaffected by experimental treatment; carer status was the only significant predictor of visit rate (Table 5.2a), with parents visiting more than helpers (Tukey contrast: estimate = 0.91 ± 0.35, $z = 2.59$, P = 0.010), in line with the results of other studies (Preston et al., 2013a; Chapter 2).

On average, carers fed nestlings on 65% ± 5% of visits. Treatment had a significant effect on the likelihood of carers feeding (Table 5.2b). Carers fed on a lower proportion of visits in the native nest treatment, compared to both the foreign treatment (Tukey contrast: estimate = -1.44 ± 0.48, $z = -3.03$, P = 0.007) and the empty muslin control (Tukey contrast: estimate = -1.15 ± 0.44, $z = -2.61$, P = 0.024).

Latency to enter or feed ranged from 0-55 seconds (mean = 2.72 ± 0.30 seconds). Treatment had a significant effect on latency (Table 5.2c). Carers took longer in the native nest treatment to enter the nest or feed the chicks, compared to both the foreign treatment (Tukey contrast: estimate = 0.38 ± 0.11, $z = 3.41$, P = 0.002) and the empty muslin control (Tukey contrast: estimate = 0.34 ± 0.11, $z = 3.19$, P = 0.004). Latency was lower later in the day (model estimate = -0.12 ± 0.05, $t = -2.57$, P = 0.011).

Table 5.2 Significance tests from linear and generalised linear mixed-effects models assessing the effect of odour treatment on visit rate, proportion of visits where food was brought, and latency to enter the nest, for observations of 20 provisioning riflemen carers at 9 nests. Carer sex and status (breeder/helper), date and time were included as potential confounds. The three treatments were an empty muslin control, a muslin pouch containing native nest material and a muslin pouch containing foreign nest material. Carer identity nested within nest identity was fitted as a random effect in all models to control for repeated measures. Significant results are shown in bold.

Predictor	**Response**								
	(a) Visit rate			**(b) Proportion visits fed**			**(c) Latency to enter**		
	χ^2	**df**	**P**	**χ^2**	**df**	**P**	**χ^2**	**df**	**P**
Treatment	0.62	2	0.730	**9.94**	**2**	**0.007**	**13.35**	**2**	**0.001**
Sex	1.40	1	0.237	0.07	1	0.789	0.11	1	0.740
Status	**6.70**	**1**	**0.010**	2.35	1	0.125	0.02	1	0.884
Date	0.10	1	0.750	0.98	1	0.323	1.25	1	0.264
Time	< 0.01	1	0.951	0.24	1	0.623	**6.61**	**1**	**0.010**

Discussion

We present evidence that the chemical profile of rifleman preen wax is significantly different in adults relative to nestlings. Our results suggest that similarity in some important elements of the chemical profile, those captured by the first principal component, covaries with relatedness in FF dyads of adult riflemen. This implies candidacy as a kinship signal. No relatedness effects or nest effects were detected in any component of the preen wax profiles of nestlings. We used a field experiment to test whether provisioning riflemen modified their behaviour in response to the presence of foreign nest material, which we assumed would change the olfactory environment. We found, surprisingly, that carers fed on a lower proportion of visits, and took longer to enter the nestbox, when they were presented with material from their own nest.

The chemical composition of preen wax in nestling birds has been little studied. One notable exception is Azzani (2015), who investigated the ontogeny of preen wax production in five species, including riflemen, and confirmed that birds begin to produce preen wax as nestlings, generally in the medium to late term of nestling development (median = 58% of time through nestling phase, rifleman = 56%). Here we showed that rifleman nestlings produce preen wax at 15 days old (63% of time through nestling phase). The striking difference in the chemical composition of preen wax produced by nestlings and adults could be a simple consequence of developmental constraints dictating that the chemical structure of preen wax changes as birds mature. Our samples were collected just a few days after preen wax production started, so it is perhaps not surprising that profiles differed from those of adults, although it is interesting that they appeared to do so in consistent ways, including compounds that were both more volatile as well as of higher molecular weight (Figure 5.2).

The alternative explanation is that preen wax differences between nestlings and adults are adaptive. Native passerines in New Zealand lack natural mammalian nest predators, which makes avoidance of predators using olfactory ‘repellents’ or odour-masking an unlikely adaptive explanation for this difference (barring rapid evolutionary response to the introduction of mammals). Distinct olfactory signals have also been suggested to play a role in sibling competition, though such conflict may be low in riflemen, where nestling starvation is rare (Preston et al., 2016). Alternatively, if adaptive, the compounds present in nestling preen wax may protect against parasites present in the nest environment (Moyer et al., 2003). More longitudinal sampling at different stages of development, as well as tests of function, are required to resolve this issue.

We found no evidence of a nest effect on nestling preen wax composition, nor any indication that chemical similarity correlates with genetic relatedness in nestlings. This is not inconsistent with olfactory signatures functioning as kinship cues in riflemen, given that preen wax structure appears to change significantly as individuals mature. The presence of reliable kinship cues should provide a clear benefit in adult riflemen, where they can be used to avoid inbreeding and allocate help (Preston, 2012; Preston et al., 2013a). Preston (2012) and subsequent data have shown no evidence of extra-pair paternity or intraspecific brood parasitism in this population. There is therefore little corresponding benefit to nestling kinship cues, as nestlings are reliably full siblings and carers do not have potential to provision nepotistically within broods.

Our results indicate that genetic distance correlates with chemical distance (as described by difference in PC1) in adult same-sex dyads, unlike nestlings. This result is only significant in FF dyads, and further data are required to test whether the lack of a significant relationship in males arises from the power limitations of our small sample size, or the genuine absence of an effect. Our results suggest that the relative abundance of compounds associated with PC1 could plausibly carry kinship information. Although the non-relationship in MF dyads seems to preclude assessment of kinship by self-referent matching in opposite-sex interactions, similarity to known opposite-sex kin such as mothers or fathers could still provide a kinship cue. A similar relationship between genetic and chemical distance in same-sex dyads has been reported in black-legged kittiwakes (Leclaire et al., 2012). As with riflemen, this is only significant within one sex (in this case males), and is mediated through differences in a principal component rather than absolute Euclidean distances between profiles. These results support Hurst and Benyon's (2010) prediction that 'it is likely that many of the constituent compounds [of a semiochemical profile] will be "silent" in kin recognition', and that variation in a minority of molecules is key to functional chemical diversity. On the other hand, more recent work by Leclaire et al. (2014) has documented a correlation in black-legged kittiwakes between Euclidean chemical distances and differences in proteins associated with the major histocompatibility complex (MHC). MHC-compatibility has been shown to function in mate choice, and so may determine odour where olfactory kin recognition is used in this context (e.g. Strandh et al., 2012). It would be interesting to investigate whether MHC-relatedness correlates with chemical distance in riflemen and other cooperative breeders, where kinship cues are also likely to be used to make helping decisions.

In our behavioural experiment we translocated material between nests, which we assumed would manipulate their odour, to test whether this affected the behaviour of provisioning riflemen. Our results supported this hypothesis, but the effect ran counter to our predictions: while there was no effect of treatment on the number of visits carers made, they fed on a lower proportion of these visits and took longer to do so when presented with their *own* nest material, as compared to foreign nest material and an empty muslin control. It is understandable that native nest material was the treatment that elicited a different response, as the muslin control and foreign material treatment were both previously unfamiliar odours. A functional explanation for the direction of this response is less clear. In crimson rosellas *Platycercus elegans*, similarly a more familiar odour was associated with a negative response at the nest, but as the treatments were consubspecifics and heterosubspecifics, this was attributed to the greater competitive threat posed by consubspecifics (Mihailova et al., 2014). If competition were driving the behavioural responses of provisioning riflemen, we would expect (as we predicted) a negative response to the foreign, rather than native treatment. Perhaps the native treatment reinforced the nest’s odour such that nestlings appeared healthier and less in need of food, or the unfamiliar odour of the foreign and empty muslin treatments was perceived as a threat and carers reduced the time they spent at the nest each visit as a result. These explanations are clearly speculative and do not align with our predictions. Nevertheless, the different responses we observed suggest that provisioning riflemen modify their behaviour according to olfactory cues. The effect of genetic distance on chemical similarity may therefore have adaptive value in kin recognition in this cooperatively breeding species.

6.

Playback of calls containing kinship information in cooperative riflemen

Nyil Khwaja, James V. Briskie and Ben J. Hatchwell

Vocalisations are the most well-studied form of communication in birds. Here we investigate their potential to act as recognition cues in the kin-based cooperative breeding system of the rifleman *Acanthisitta chloris*. We identified a contact call that is regularly used in affiliative interactions, including between partners from outside to inside of the nest, which we considered likely to encode information on individual identity. We recorded these calls at the nest from 18 adult riflemen and found that measurements of call characteristics were individually repeatable, and assigned to the correct individuals using discriminant function analysis more often than expected by chance. In addition, relatedness information could be conveyed by these calls because dyadic call similarity was significantly correlated with relatedness among males. Thus, these contact calls could act as individual signatures and indicate kinship, allowing helpers to recognise familiar relatives, who are almost always the recipients of their help. To test this hypothesis, we conducted a playback experiment in which we broadcast the calls of a relative and a non-relative as separate treatments. Focal birds rarely responded aggressively or affiliatively, and their tendency to do so was unrelated to the treatment. We conclude that despite the individuality of contact calls and their correlation with kinship (at least in males), the mechanism of kin recognition in this species remains unknown.

Introduction

Vocalisations are the dominant sensory modality in the avian communication literature (Bradbury and Vehrencamp, 2011). In part, this undoubtedly owes to the relative ease with which researchers can record, replicate and even manipulate vocal cues. Nevertheless, simple ornithological observation reveals regular use of calls and songs by a majority of species, which are likely to function in communication. Experimental studies have confirmed roles for vocalisations in such diverse contexts as parent-offspring communication (Burford et al., 1998), sibling recognition (Nakagawa and Waas, 2004), kleptoparasitism (Flower et al., 2014), mate attraction (Catchpole and Slater, 2008) and male-male competition (Leedale et al., 2015). In cooperatively breeding species, vocalisations are the only cues to date that have been demonstrated to function in kin recognition (Crane et al., 2015).

Differential responses to playback of the vocalisations of kin as opposed to non-kin, or members versus non-members of a cooperative group, have been demonstrated in at least seven cooperatively breeding bird species (Crane et al., 2015). Such results suggest that vocal cues are likely to play a role in recognition of relatives when individuals make helping decisions in species where helping provides indirect fitness benefits (e.g. Sharp et al., 2005). In most cases, vocal cues appear to be learned associatively as belonging to familiar kin or group members, although in bell miners *Manorina melanophrys*, similarity in ‘mew’ calls closely matches genetic similarity, and individuals are thought to use these calls to recognise even unfamiliar relatives through self-referent matching (McDonald and Wright, 2011). In long-tailed tits *Aegithalos caudatus*, evidence from cross-fostering experiments show that vocalisations that can be used to discriminate familiar kin are learned by chicks in the nest from provisioning adults, rather than being fixed by genotype (Sharp et al., 2005). More widely, learning is known to play a key role in the development and diversity of vocalisations across the Passeri or songbirds (Catchpole and Slater, 2008), the clade including all of the seven cooperative species where vocal recognition has so far been demonstrated (Crane et al., 2015).

Riflemen *Acanthisitta chloris* are outsiders to this clade (Jetz et al., 2012), being part of Acanthisittidae, the ancient New Zealand wren family that forms an outgroup (Acanthisitti) to all other passerine birds (Passeri and Tyranni). Riflemen are facultatively cooperative, and show strong signals of kin discrimination in nepotistic helping and in inbreeding avoidance (Preston, 2012). Spatial cues alone do not provide reliable kinship information in this system, because riflemen live in kin neighbourhoods in which helpers may have both related and unrelated potential recipients, a situation that is likely to select for effective kin recognition (Cornwallis et al., 2009). However, the mechanism by which they recognise kin is unknown, and here we test the potential role of vocalisations.

Vocal communication in riflemen has been the subject of three recent studies. Withers (2013b) identified three elements, which she termed the ‘zip’, ‘chuck’ and ‘pip’, that make up the repertoire of rifleman contact calls. She showed that recorded parameters from these calls varied significantly between separate subpopulations of riflemen on the North Island, but that adult birds did not respond differently to played back calls from their own population, as compared with those from other populations. Krull et al. (2009) demonstrated that adult contact calls (specifically one zip and one chuck-like element) and a nestling begging call contained ultrasonic components. They suggested these may play a role in communication or foraging; alternatively they could exist as an adaptively neutral epiphenomenon. Preston (2012) also used a playback experiment, in this case testing whether breeding riflemen recognised the calls of their own juvenile offspring, or other adult kin in the population, using two pairwise experiments. Like Withers (2013b), she found no difference in behavioural responses to different playback treatments.

Here we focus on the zip call. The pip is usually produced in a moderately distressed context (e.g. as a precursor to alarm calls), and chuck calls generally constitute quiet and variable ‘punctuation’ between louder zips, so we considered the zip to be the most likely candidate call to encode information about kinship (N. Khwaja, pers. obs.). Furthermore, zip calls are uttered by adults before entering the nest (e.g. prior to replacing their partner during incubation) at a time when the signaller and receiver are unable to see each other, so they may encode information on individual identity (S. Withers and N. Khwaja, pers. obs.). This behaviour continues into provisioning, making it plausible that nestlings learn the zips of their parents in the nest. We used a standardised procedure to record zips from breeding birds visiting the nest during incubation. We analysed these calls to assess their potential for encoding information regarding individual identity and relatedness, and tested breeding birds' responses to the calls of kin and non-kin using a playback experiment.

Methods

Fieldwork for this study was carried out between September 2014 and January 2015, when the known rifleman population at Kowhai Bush numbered 23 mature individuals (11 pairs and 1 unpaired male).

Recording calls

We used a standardised procedure to record zip calls from nesting riflemen while they were incubating eggs. We waited until neither parent was in the nest, and placed an Olympus ME15 tie-clip microphone inside the nestbox, clipped to the entrance of the nest chamber (riflemen build fully enclosed nests within nestboxes, rather than simply lining the box). The microphone was attached to an Olympus WS-812 recorder, which we left on top of the nestbox to record for c.1 hour. We started the recording simultaneously with a video camera mounted on a tripod, which filmed the nest entrance from c.10 m away. We watched the footage to determine when adults arrived at the nest to start an incubation bout. This often coincided with the utterance of a zip call, which we confirmed by checking a spectrogram of the audio recording generated using the program RavenPro 1.5 (Cornell Lab of Ornithology, Ithaca). We isolated those calls that were unambiguously attributable to one individual (i.e., produced by an adult arriving when its partner was known not to be in the nest), and used them in the following analysis.

Call individuality

Example spectrograms of rifleman zip calls are shown in Figure 6.1. We used RavenPro's selection tool to measure zip call parameters. For each call, we measured the peak frequency (frequency at maximum amplitude); first quartile, central and third quartile frequencies (frequency at time-point when 25%, 50% and 75% of the energy in the call has been produced, respectively); interquartile frequency range (difference between the first and third quartile frequencies), and interquartile duration (time between the first and third quartiles). We did not use the measurements of minimum or maximum frequency, total frequency range, or total duration, because these measurements were hard to define: calls generally faded from their lowest frequency at the beginning to their highest frequency at the end (Figure 6.1), so it was difficult to standardise maximum and minimum frequencies. By focusing on quartile-based measurements we circumvented this issue, as the faded areas in question contained a negligible proportion of the call's total energy.

Some of these variables were expected to correlate with each other, notably the frequency measurements, so we simplified further using a principal component analysis in R 2.12.0 (R Development Core Team, Vienna), after scaling and centring each variable. The first two principal components (PC1 and PC2) explained 88% of the variation in the data, and were used in further analysis (Table 6.1).

We checked for an effect of sex on the structure of calls by fitting two linear mixed-effects models (LMMs) using the nlme package (Pinheiro et al., 2010). PC1 and PC2 scores were the respective response variables. Sex was fitted as a fixed effect with individual identity as a random effect. There was no effect of sex on PC1 score (LMM: $F_{1,16} = 1.09$, $P = 0.311$), and a marginally non-significant effect on PC2 score (LMM: $F_{1,16} = 4.25$, $P = 0.056$).

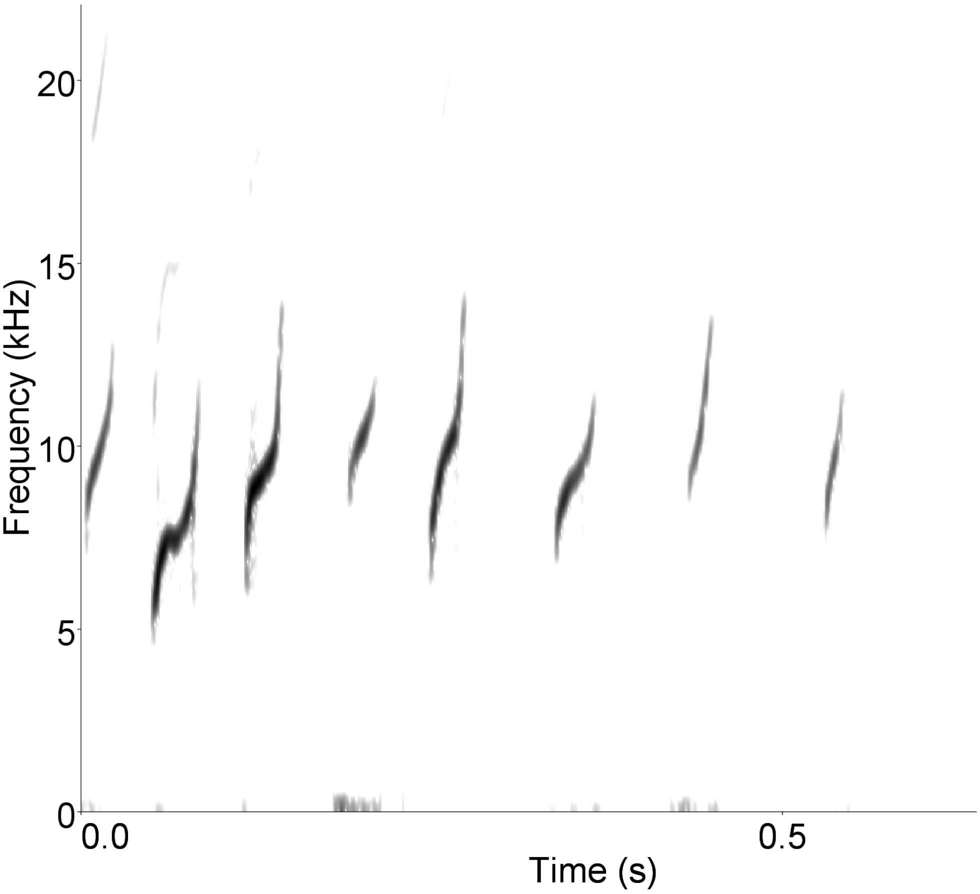

Figure 6.1 Example spectrograms of rifleman zip calls recorded from eight different individuals. The leftmost shows a trace of the call's ultrasonic harmonic. Most calls show the tail at the lowest and highest frequencies, which meant that measuring minimum and maximum frequency was problematic in noisier recordings.

We assessed whether zip calls could encode information on individual identity by calculating ANOVA-based repeatability (R) for each principal component in the rptR package (Schielzeth and Nakagawa, 2013). Significantly positive values of R indicate that there is individual consistency in the character of interest. Because of the lack of a significant sex effect on principal component scores, we analysed all data together; though because this non-significance was marginal for PC2, we checked the validity of R by calculating an LMM-based adjusted repeatability (R_{adj}) accounting for the effect of sex (Nakagawa and Schielzeth, 2010). We used a discriminant function analysis in the MASS package (Venables and Ripley, 2002), to calculate the proportion of calls that could be assigned to the correct individuals based on their PC1 and PC2 scores. To test whether these calls were assigned correctly more often than would be expected by chance, we shuffled the observed principal component scores 1,000 times such that they were randomly assigned to individuals. We ran the analysis on each of these simulated datasets and derived a 95% confidence threshold from the distribution of the proportions.

Call similarity and kinship

We compared the calls described above using a dynamic time warping algorithm implemented in the program Luscinia (available: http://rflachlan.github.io/Luscinia/). This technique optimally aligns two signals to calculate a dissimilarity score based on specified parameters (Lachlan et al., 2010); we compared the durations and dynamic fundamental frequencies of calls to obtain this score. Luscinia then provides an overall dissimilarity score between two individuals based on the pairwise comparisons between their individual calls. We used these scores as measures of acoustic distance between the individuals we studied.

We created acoustic distance matrices using these measures for male-male (MM) and female-female (FF) dyads respectively. We created equivalent genetic distance matrices using the procedure described in Chapter 5, and as in that study compared the two using Mantel tests implemented in the vegan package (Oksanen et al., 2011). We used 5,000 permutations to obtain a P-value for the correlation coefficient (r). Significantly positive values of r indicate that more closely related individuals have more similar calls, accounting for the non-independence of repeated comparisons using the same individuals. The Mantel test cannot be used on non-square matrices, and so we used a Pearson's correlation test to examine the relationship between genetic and acoustic distance in opposite-sex (MF) dyads. This technique does not account for non-independence as the Mantel test does, and therefore significant results should be treated with caution.

Playback experiment

We used the zip calls recorded and analysed as above to create short playback tracks using the program Audacity 2.0.6 (available: http://audacity.sourceforge.net). These tracks consisted of a single representative zip call for an individual repeated every 10 seconds, with generated silence in between.

For each individual tested, we conducted two treatments: one playback of a first-order relative (mother, father, sibling or offspring), and one playback of an unrelated individual. We carried out each treatment on separate days for each individual, with a maximum of one treatment per day at each nest. The order in which individuals were presented with the kin and non-kin treatments was alternated between experiments. Individuals were tested while they were provisioning nestlings, between day 13 and day 23 of a breeding attempt (where day 0 is the day of hatching). In each treatment, an experimenter erected a pop-up camouflage tent within view of a nest, when neither adult was present. When the focal individual returned to the area on its own, the experimenter started the playback track, which was played from a laptop using Audacity, connected to a pair of speakers placed outside the tent. The track was repeated until the individual left the area, or 10 minutes had elapsed. The following aspects of the individual's behaviour were noted during this time: whether it moved towards the speaker; its starting distance from, and closest approach to, the speaker; whether it called in response to the recording, and if so the type of call, and whether it changed its behaviour from one type to another (categorised as foraging, preening, calling, staying still, approaching the speaker or flying away).

In contrast to species where responses to playback are uniformly aggressive approaches to the speaker, which vary in severity or duration (e.g. Akçay et al., 2013), in our experiment riflemen sometimes approached the speaker aggressively, sometimes affiliatively, and often responded neutrally or appeared not to respond. This meant that responses such as closest approach to the speaker, or time spent in close proximity to it, were inappropriate directional metrics of response. Instead, we gave responses a score: 1 for aggression (e.g. approaching speaker and mobbing, or producing pip calls), 2 for a response that was not obviously aggressive or affiliative (e.g. staying still, preening or continuing previous behaviour), and 3 for an affiliative response (responding to calls with zips or chucks). We had insufficient sample size to use a multinomial response or McNemar's test, so we used a paired Wilcoxon rank sum test to evaluate the hypothesis that individuals would score higher on this affiliation index when presented with playbacks of their kin.

Results

We recorded and measured 156 zip calls from 18 adult riflemen (10 males and 8 females). The mean, minimum and maximum values for each measurement are given in Table 6.1.

Individuality of calls

Repeated measures within individuals were more similar than expected by chance for both PC1 (ANOVA-based repeatability: $R = 0.38 \pm 0.10$ SE, $P < 0.001$) and PC2 (ANOVA-based repeatability: $R = 0.18 \pm 0.08$ SE, $P < 0.001$), as well as the raw measurements these scores were based on (Table 6.1). Using an LMM approach to account for the marginal effect of sex on PC2 gave an equivalent value (LMM-based repeatability: $R_{\mathrm{adj}} = 0.14 \pm 0.07$ SE). This implies the potential for zip calls to encode information on individual identity. Discriminant function analysis assigned 27% of calls to the correct individual. This was significantly more than expected by chance (randomisation test: $P < 0.001$; Figure 6.2). When calls were subset by sex, the proportion assigned correctly for males (38/95) and that assigned correctly for females (18/61) did not differ significantly (proportion test: $\chi^2 = 1.35$, df = 1, $P = 0.245$).

Table 6.1 Parameters measured from 156 rifleman zip calls. ANOVA-based repeatability is shown for each, along with its statistical significance in brackets (*** < 0.001, ** < 0.010, * < 0.050). Contributions to each of the two major principal components are provided as percentages. These respectively accounted for 65% and 23% of the total variation in the measured parameters.

Parameter	Mean ± SE	Min.	Max.	Repeatability	% loading	
					PC1	PC2
Q1 frequency (kHz)	8.84 ± 0.08	6.03	11.03	0.39 (***)	22	3
Central frequency (kHz)	9.24 ± 0.07	6.72	11.20	0.39 (***)	22	6
Q3 frequency (kHz)	9.67 ± 0.07	7.02	11.53	0.41 (***)	21	16
Peak frequency (kHz)	9.18 ± 0.08	5.86	11.20	0.32 (***)	22	2
IQ frequency range (kHz)	0.83 ± 0.03	0.17	2.24	0.20 (***)	5	40
IQ duration (ms)	7.20 ± 0.24	2.90	14.51	0.10 (*)	8	34

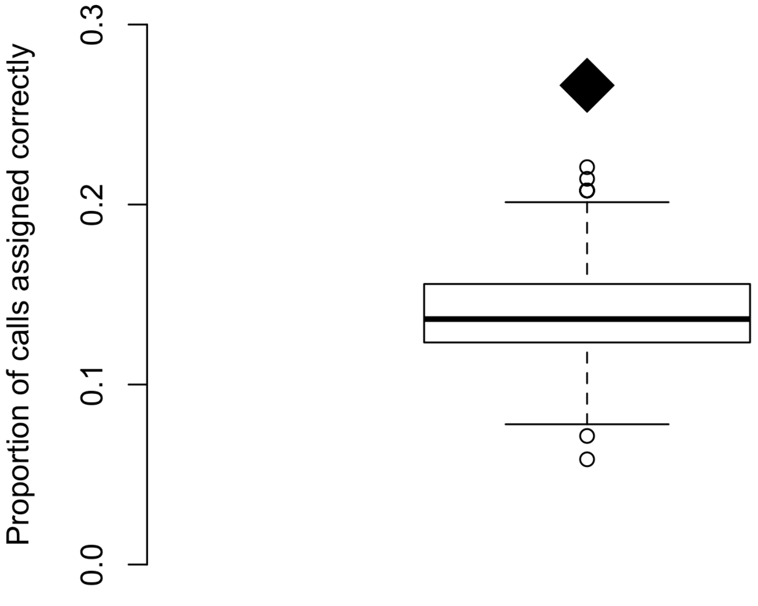

Figure 6.2 Proportion of rifleman calls assigned to the correct individual using a discriminant function analysis on their two major principal component scores. The boxplot shows the distribution of expected proportions from 1,000 randomisations of the data; the filled diamond at 0.27 shows the observed proportion assigned correctly.

Call similarity and kinship

Acoustic and genetic distances were significantly positively correlated in MM dyads (Mantel test: $r = 0.26$, P = 0.042; Fig. 3). There was no equivalent relationship in FF dyads (Mantel test: $r = -0.01$, P = 0.535), and a marginally non-significant relationship in MF dyads (Pearson's correlation test: $r = 0.20$, df = 78, P = 0.069), despite use of a liberal statistical test (see Methods).

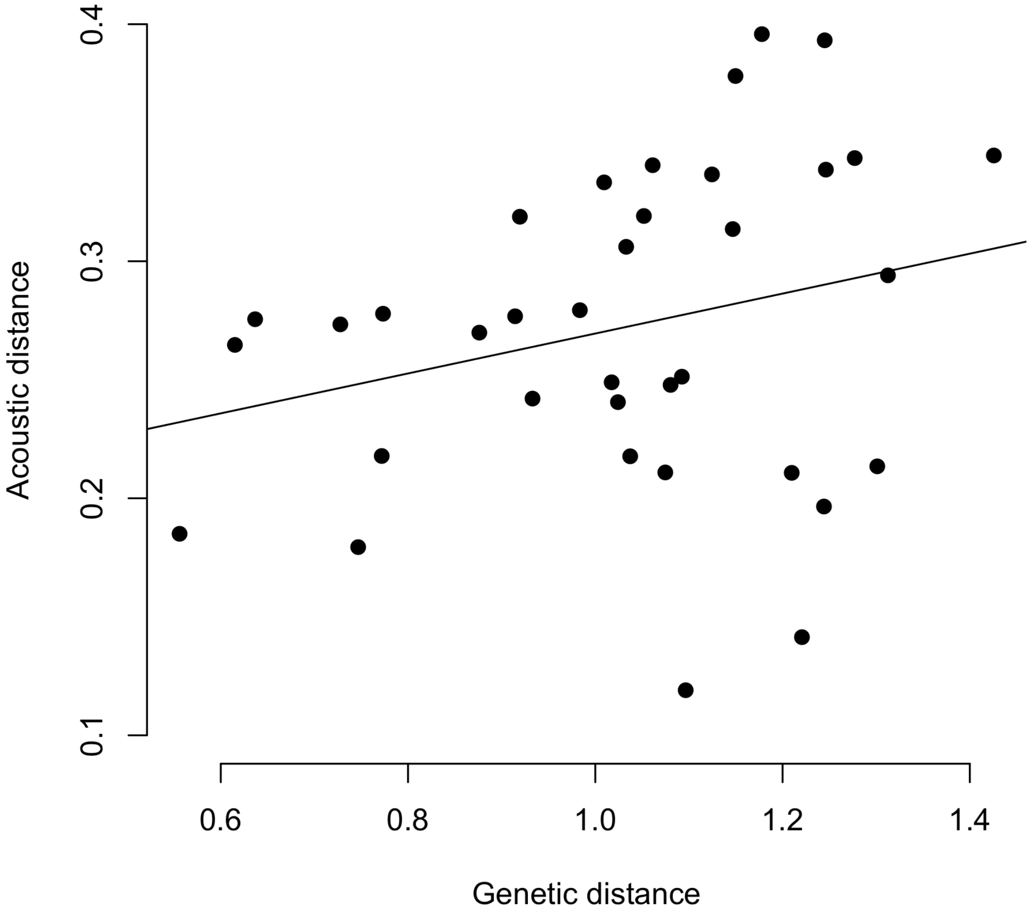

Figure 6.3 Relationship between genetic distance and difference in zip call structure in adult male riflemen. There was no correlation between these measures in females.

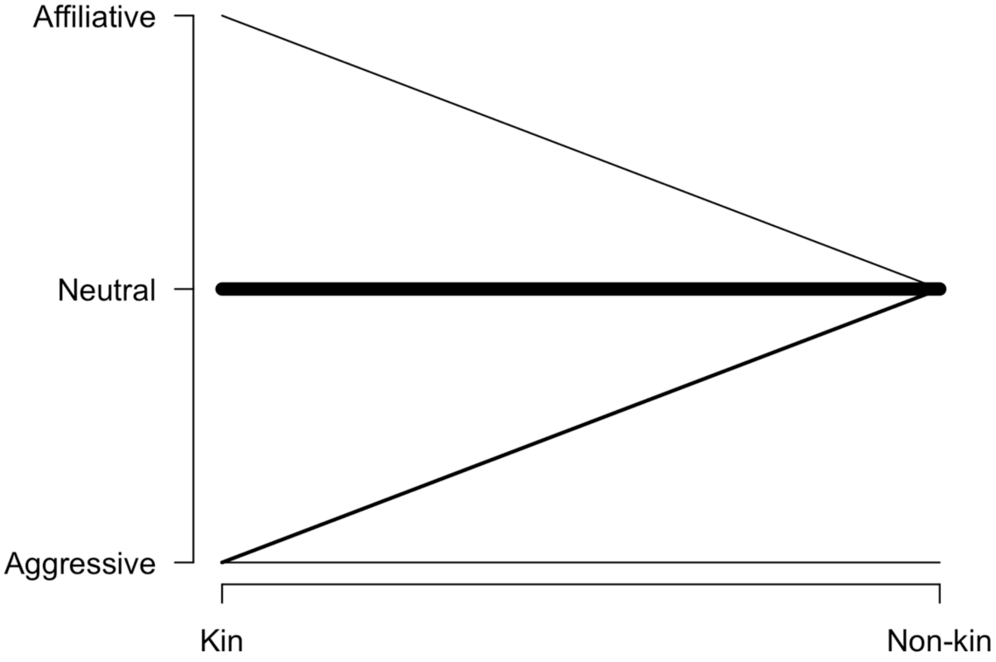

Figure 6.4 Paired responses of adult riflemen to playback of the zip calls of first-order relatives and unrelated individuals. Lines connect the responses of the same individuals to respective treatments; line weights represent the number of individuals for whom the combination of responses was the same (minimum = 1, maximum = 7).

Playback experiment

We carried out both treatments of our playback experiment on 11 individuals (4 males and 7 females). We carried out one treatment on a further 3 males (2 with a non-kin treatment and 1 with a kin treatment). We used only the first 11 in the Wilcoxon test. In the kin treatment, 2 individuals responded affiliatively, 3 responded aggressively and 7 responded neutrally. In the non-kin treatment, 1 individual responded affiliatively, 1 responded aggressively and 12 responded neutrally. Individuals were not significantly more affiliative towards kin (paired Wilcoxon test: V = 2, *n* = 11 paired responses, P = 0.807; Figure 6.4). It is noteworthy that the only affiliative responses we observed were from individuals exposed to kin (twice), and that the most aggressive response we observed (mobbing of the speaker) was from an individual exposed to non-kin, although two individuals did respond to kin with agitated pip calls. Nevertheless there was little evidence of a general preference for kin overall, with most birds responding neutrally to the experiment.

Discussion

We tested the potential role of the zip call in rifleman kin recognition. This is a contact call that appears to be readily used by familiar birds in communication with one another. By measuring components of zip calls, we confirmed that they contained individual characteristics that enabled automated recognition at a rate that was significantly better than random. We also demonstrated that relatedness and call similarity correlated positively in male dyads. We used a playback experiment to test whether provisioning riflemen responded differently to the calls of their kin and non-kin. Although the most affiliative responses were observed in the kin treatment, and the most aggressive response observed in the non-kin treatment, there was not a general tendency for riflemen to behave more affiliatively when exposed to kin than non-kin.

For a cue to be used to recognise individuals, repeated measures from the same individual must be more similar to one another than expected by chance (Falls, 1982). We confirmed that this was the case with repeatability tests and discriminant function analysis, using measured parameters of zip calls. Individual-specific call characteristics have been commonly reported in birds, including in other cooperative breeders (Sharp and Hatchwell, 2005; Crane et al., 2015), and non-songbirds (Wanker and Fischer, 2001). However, they are not universal: for example, in contrast to ‘churr’ calls, the ‘tut’ calls of long-tailed tits are considered unlikely to be appropriate recognition cues because of a lack of between-individual variation (Sharp and Hatchwell, 2005). The results of our analysis supported our inference from observing rifleman behaviour that zip calls were appropriate candidate cues to use to investigate recognition.

Although discriminant function analysis assigned significantly more calls to the correct individuals than expected by chance, this was still only successful in 27% of cases. This is considerably less than the c.50% reported for the ‘churr’ call of long-tailed tits (Sharp and Hatchwell, 2005), not to mention the 100% reported from a study of short-toed treecreepers *Certhia brachydactyla* (Bauer and Nagl, 1992). The measurements we were able to take would not have captured all of the variation present in rifleman zips, particularly given that we omitted some measurements because of concerns over their accuracy (see Methods). The success rate is perhaps unsurprising in light of a study of riflemen on the North Island, in which discriminant function analysis assigned only 47% of zip calls to the correct subpopulation (Withers, 2013b); it fits that this level of differentiation is greater than that between individuals, given the isolation of these subpopulations. The relative simplicity of zip calls may make classification by acoustic analysis more difficult. It is feasible that misclassification-type errors in riflemen’s perception of the calls may underlie the rare occasions (c.10%) in which adult helpers provision at unrelated broods (Chapter 7).

In riflemen, where there is no recorded extra-pair paternity (Preston et al., 2013a), offspring, nestmates, social parents and parents’ future offspring could all be reliably assigned as kin using an individually distinct cue such as the zip call. The hypothesis that riflemen learn cues through these relationships, and use these to recognise kin to help, is consistent with our observations: all helpers with a known history from our six-season study have held one of the above social relationships to at least one member of the pair they helped (Chapter 7). Interestingly this includes one juvenile who was cross-fostered into another brood helping at the second brood of his social parents (Preston et al., 2013a). It is less clear whether being able to identify only familiar (usually first-order) kin could underlie patterns of inbreeding avoidance in riflemen, where relatedness within a pair is significantly lower than would be expected under models of random mate choice given the kin structure of the population (Preston, 2012). As our kinship analysis showed a trend for call similarity to reflect relatedness in MF dyads, this may also inform mate choice decisions.

We showed that males' zip calls were more similar to those of more closely related males. There was also a non-significant trend in this direction for MF dyads, but surprisingly (given these two results) no trend for FF dyads. These results echo McDonald and Wright's (2011) study of bell miners, which showed that vocal similarity closely matched genetic relatedness between helpers and male, but not female, breeders. The mechanistic basis of such a sex-biased effect is unclear, and it would be interesting to test how widespread it is among birds, cooperatively breeding or otherwise. In bell miners, the result fits neatly into an adaptive explanation of the species's cooperative breeding system, where males limit dispersal and females are immigrants to colonies. Helpers (usually male) thus vary in their relatedness to breeding males, but are unrelated to all breeding females except their mothers. This makes call similarity a useful indicator of the indirect fitness gains available from helping a particular male. In riflemen, helping is also male-biased, but the adaptive basis for a male-specific effect is less obvious. Males and females disperse similar distances, and equivalent fitness gains are available to females helping their sisters and mothers, as males helping their brothers and fathers (Preston, 2012); however, we have not recorded any helping events where a female helper was related only to the female breeder, so observed patterns are not inconsistent with the use of call similarity as a cue to helping.

Despite the potential for zip calls to function in kin recognition, our playback experiment did not support the hypothesis that riflemen responded differently to the calls of kin and non-kin. Given the results of our kinship analysis, it would have been ideal to use only male calls during our experiment; unfortunately due to limited available kin within the population, we were not able to standardise this, potentially influencing our results. Birds were often unresponsive to the experiment, and their behaviour was difficult to interpret: of 25 experimental trials, only 6 responses were clearly either affiliative or aggressive. Previous studies of other cooperative bird species have found that speakers were approached more closely, or more often, when broadcasting non-kin calls (e.g. Payne et al., 1988; Hatchwell et al., 2001; Keen et al., 2013). In these cases, it appears that sustained approach is a symptom of aggressive behaviour. This interpretation was inappropriate in our experiment. Riflemen moved towards the speaker in 13 of the 25 trials (7/12 kin treatments, 6/13 non-kin treatments), but these approaches ranged from the affiliative, in which the focal individual responded to broadcast zips with zips of their own, through seemingly neutral responses, to an aggressive approach which culminated in the focal individual mobbing the speaker. Because of this variation, we did not report tendency to or proximity of approach as a measure of response (though neither of these differed significantly between treatments either; data not shown). Another potential symptom of aggression, the production of pip calls, was only observed three times, two of these in response to the kin treatment.

These results indicate that the playback as a simulated territorial intrusion did not generally provoke alarm from focal riflemen. Riflemen are mostly considered weakly territorial; we have witnessed disputes at territory boundaries and a tendency to remain within the confines of a territory, but aggression between conspecifics was rarely observed over the course of our study. Similar trends have been reported by other authors studying this population (Hunt and McLean, 1993; Sherley, 1994). Crane et al.'s (2015) experiments on another weakly territorial species, the chestnut-crowned babbler *Pomatostomus ruficeps*, were also notable for their lack of aggressive responses by focal birds (but see Sharp et al., 2005). They demonstrated a differential response by simultaneously broadcasting calls from group members and outsiders. In this scenario, the focal group moved affiliatively towards the call of their group member. Using a similar protocol in riflemen may be a more appropriate test for any future studies, given that responses to a single broadcast did not differ according to relationships, and rarely provoked aggression.

This study adds to two previous playback experiments on riflemen with negative results (Preston, 2012; Withers, 2013b). It is possible that this has arisen from limitations in study design, perhaps because the low responsiveness of riflemen has made successful experiments challenging to implement. Other explanations are that vocalisations alone are insufficient stimuli for riflemen to behave naturally towards conspecifics, or that they are unable to recognise the calls of their kin. As discussed above, although zip calls were individually identifiable more often than expected by chance, we only confirmed this in 27% of cases, which may make the calls suboptimal cues relative to alternatives. Differences in odour signatures appear to correlate with genetic distances in riflemen, and these may communicate relatedness more accurately even between unfamiliar birds (Riehl and Stern, 2015; Chapter 5). The potential for riflemen (or to our knowledge any cooperatively breeding birds) to recognise one another visually has not yet been explored. Though most birds have strong visual systems, experimental study in this area has been neglected owing to the practical difficulties of systematically manipulating visual cues (Nakagawa and Waas, 2004). Addressing this gap represents one important challenge for the future of kin recognition research. Another is to design experiments that replicate the key decisions that rely on successful kin discrimination. In riflemen these are the decision to help and the selection of a mate, rather than the tolerance of breeding birds to manipulations. The difficulties of this approach are clear, but it may bring us closer to understanding the mechanisms that drive and constrain kin-based cooperative breeding.

SYNTHESIS

7.

Cooperative breeding in riflemen: indirect fitness in a kin-structured neighbourhood

The rifleman *Acanthisitta chloris* is a tiny, wren-like bird, endemic to New Zealand, that has attracted scientific attention since the late 20th century for its phylogenetic position at the base of the passerine radiation (Ericson et al., 2002); the energetic importance of its small size (Sherley, 1985; Lill, 1991); the causes and consequences of its sexual dimorphism (Hunt and McLean, 1993; Sherley, 1993; Chapter 3), and in particular its unusual cooperative breeding system (Sherley, 1990a; Preston et al., 2013a, 2016). Here I focus on the latter, bringing together work towards this thesis and that carried out by Preston (2012) and previous authors, to synthesise our current understanding of rifleman cooperation following the approach used in Koenig and Dickinson (2016). ‘Our research/study’ refers to pooled data from Preston (2012) and my own fieldwork unless otherwise stated; Preston et al.’s (2013a, 2016) publications are based on the former. The picture that emerges is of a system in which helpers are relatively unconstrained by ecological factors, and likely to gain predominantly indirect fitness benefits from their help. However, our understanding of how these benefits are realised is still incomplete.

Ecology and life history

Riflemen are insectivorous birds that obtain food by gleaning invertebrates from the forest canopy and probing beneath the bark of tree-trunks. Most of the population occurs in mature forest across the higher-altitude wilderness areas of both major New Zealand islands (Gill, 2004). Some also occur in scrub and secondary regrowth in the lowlands, such as the seral forest of kanuka *Kunzea ericoides* at Kowhai Bush where our research, and the bulk of previous work on the species, has been carried out.

Riflemen are socially and sexually monogamous (Preston et al., 2013a), and form long-term pair bonds: we have not recorded a single divorce event during six seasons of study. Therefore birds begin a breeding season in September sharing a territory with their partner from the previous season, unless this partner has died (adult survival ≈ 50%). First-year birds also disperse onto their own territory and pair with other first-years or ‘bereaved’ adults. Dispersal distances are short: most birds breed less than 200 m from their natal nest (maximum recorded = 655 m). In years when the adult sex ratio is uneven, some birds (usually males) remain unpaired; when the population density is low these individuals range more widely over unoccupied territories than do members of pairs.

Pairs build nests together in natural cavities or nestboxes. The male makes a greater contribution to nest-building (Sherley, 1994); often, multiple nests are built around the territory before eggs are laid in one. The male provisions the female in ‘courtship-feeding’ bouts before and while she lays a clutch. Females lay 3-5 eggs in their first clutch of a season, each of which makes up almost 20% of their body weight (Sherley, 1989). They incubate the eggs in partnership with the male, who makes the greater contribution (Sherley, 1990b). Incubation lasts 18-21 days. Once the eggs hatch, nestlings are fed by their father, who provisions at the highest rate; their mother, and for a significant minority of broods (26% over the course of our study) one or more additional adult birds, who provision at a lower rate than parents. These helpers arrive partway through the nestling period. Nestlings fledge after 22-28 days (usually 24 or 25).

Rifleman pairs make a maximum of two successful reproductive attempts in a season; rarely, more than two clutches may be laid if one or more are unsuccessful. We define ‘second broods’ as only those following a successful reproductive bout; those following failed attempts are still considered first broods. Females lay 2-4 eggs in their second clutch. The progress of second broods occurs similarly to that of first broods, but surviving juveniles from pairs’ first broods often remain on their natal territory and help to feed second-brood nestlings, in addition to the other carers (occurring at 65% of second broods over the course of our study).

Juveniles disperse from their natal territory before the onset of winter. Riflemen generally remain in their territories during the non-breeding season, when their daily activity differs little from levels observed while breeding (Sherley, 1985; Lill, 1991).

Helper demography and relatedness

One of the most striking features of rifleman cooperative breeding identified by Preston et al. (2013a) was the diversity of helpers' life-history contexts. This has continued to be the case in subsequent seasons (Table 7.1). As mentioned above (see Ecology and Life History), helpers can be split into two broad categories: adult and juvenile. Adult helpers may help at either a pair's first or second brood in a season. Juvenile helpers are fledged offspring from first broods who remain with their parents to help raise siblings at second broods. While the juvenile route to helping is consistent, the status of adult helpers varies considerably. The majority (61%) of adult helping events have involved unpaired birds, perhaps unsurprisingly as these individuals have no potential to obtain direct fitness through breeding in a given season. These unpaired birds have been male in all cases, reflecting a generally male-biased adult sex ratio (Chapter 3). Birds making either successful or unsuccessful breeding attempts may also act as helpers at other nests. We have recorded this occurring concurrently with feeding nestlings or fledglings, and after nest failure. As adult helpers provide the majority of help (see Helper Contributions) and have previously dispersed from their natal territory (Preston et al., 2013a), the social system is best considered a kin neighbourhood (Dickinson and Hatchwell, 2004).

Table 7.1 Age and breeding status of helpers at rifleman nests in 2008-2011 and 2012-2015, adapted and updated from Preston et al. (2013). Each record represents a separate helping event, meaning that some individuals and broods are represented multiple times. Unringed helpers are excluded.

Age	**Status**	**First broods**		**Second broods**		**Total**
		Male	**Female**	**Male**	**Female**	
Adult	Unpaired	17	0	2	0	19
	Failed breeder	3	3	0	0	6
	Successful breeder	2	3	0	2	7
	Total	21	6	2	2	31
Juvenile	-	-	-	8	12	20

Despite the diversity of helpers' backgrounds, they are almost universally close genetic relatives of the broods they help (Figure 7.1). All recorded juvenile helping events have involved individuals provisioning at their own parents' second broods (as opposed to other pairs in the population). As we have recorded no extra-pair paternity in the population, this implies full-sibling relatedness to broods in each case. Adult helpers similarly exhibit high levels of kinship to the broods they provision and the parents of those broods, and all those with a known pedigree have, like juvenile helpers, been social relatives of their recipient broods (Table 7.2).

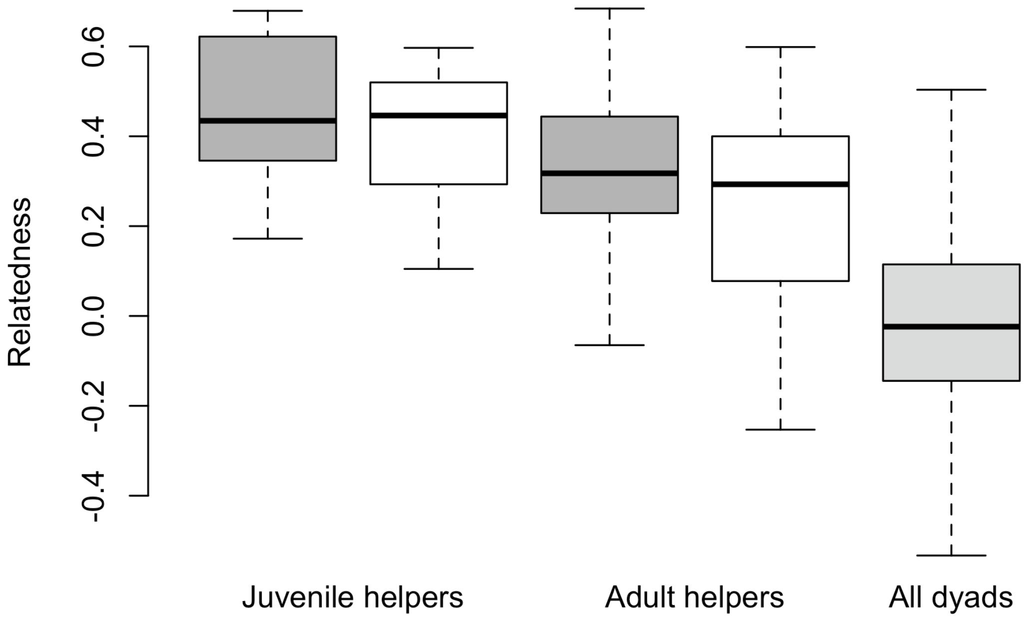

Figure 7.1 Queller-Goodnight relatedness (*r*) between helpers and the broods they provisioned (shaded boxes), and the parents of those broods (white boxes), with the distribution of relatedness in all dyads in the population shown for reference. Adapted and updated from Preston et al. (2013a). The data from helpers are mean *r* values to all nestlings in a brood, and the mother and father of that brood, respectively. Outliers are omitted: these only occurred for 'all dyads', and ranged between -1 and 1.

Table 7.2 Summary of pedigree relationships between helpers and the recipients of their help. Relationships were reconstructed using genetic data from 16 microsatellite markers in the program Colony (Jones and Wang, 2010). The number of relationships known from ringing records and fieldwork are given in brackets. 'Half uncle' refers to males who are half siblings to one of a brood's parents. Those of unknown relationship were not classified by Colony, but were not always of low genetic relatedness to the brood ($r < 0.1$ in three cases; r to brood and each parent all < 0.1 in only one case). Colony does not assign relationships at the level of cousins, which may explain the failure to classify some of these dyads.

Helper's relationship to brood	**Social relatedness**		**Frequency**
	To brood	**To parents**	
Adult helpers			
Full sibling	0.5	0.5 & 0.5	14 (6)
Half sibling	0.25	0.5 & 0	3 (0)
Grandfather	0.25	0.5 & 0	1 (0)
Uncle	0.25	0.5 & 0	3 (2)
Half uncle	0.125	0.25 & 0	2 (0)
Unknown			8 (23)
Juvenile helpers			
Full sibling	0.5	0.5 & 0.5	20 (20)
Unknown			0 (0)

Kin discrimination

Active kin recognition

For helping to confer indirect fitness benefits it must be directed towards kin. Rifleman helpers are especially successful in this regard (Figure 7.1). If relatives are reliably structured in space, help may be allocated adaptively using simple spatial rules such as 'feed any brood on my natal territory'. This rule has been fulfilled for all 'natural' juvenile helpers, who may have provisioned broods based entirely on following their parents within the territory (but see discussion of one cross-fostered juvenile below). However, our observations over six seasons suggest it is not sufficient to explain patterns of helping by adults. Firstly, these helpers do not always provision on their natal territory: one male helped at his brother's first broods in 2013-2014 and 2014-2015, on a territory more than 500 m from their natal nest. Secondly, no potential helpers with known history have returned to their natal territory to provision non-relatives or even half-siblings (though the latter is likely to have occurred for helpers of unknown history, see Table 7.2), despite opportunities to do so. For example in the 2014-2015 season, any of 20 breeding adults could have become helpers on their natal territory; the only one that did so was also the only one whose parents both remained on that territory. This suggests an ability for birds to assess the relatedness of potential recipients of help before deciding whether or not to provide it. Results suggesting that riflemen avoid inbreeding strengthen the case that kin recognition is active and have stimulated research on possible mechanisms (Preston, 2012).

Potential mechanisms

Work towards this thesis has identified two types of cue that riflemen may plausibly use to identify their kin. We demonstrated that a regular contact call shows significant individual consistency, and therefore could be used to recognise familiar individuals who are known to be kin. This contact call also contained some information on genetic relatedness, though perhaps not enough to be useful: more closely related males had more similar calls, but this trend was non-significant in male-female dyads and completely absent between females (Chapter 6). Chemical cues also contained some relatedness information: more closely related females had more similar uropygial secretions as described by their major principal component (Chapter 5). We were unable to test for individually distinct chemical signatures as we only successfully resampled one individual; results from other bird species are mixed (e.g. Thomas et al., 2010; Azzani et al., 2016). Although they may be a suitable innate cue to relatedness, it is therefore uncertain whether individual scents are sufficiently distinct to be learned associatively between known kin. The possible cues we have identified are far from perfect: there is considerable variation around even statistically significant correlations, and discriminant function analysis only assigned 27% of calls to the correct individuals. On the other hand, allocation of help may also be imperfect (adult helpers help unrelated broods c.10% of the time and we have documented no direct fitness benefits of helping, see Fitness Consequences of Help), and riflemen's regular social interactions are likely to mean that potential recognition errors based on snapshots of cues are less problematic. Experimental manipulations of these cues suggested that provisioning riflemen do respond to changes in their olfactory environment (Chapter 5), but do not react differently to zip calls of their kin (Chapter 6); whether either cue is used to make helping or mate choice decisions remains unconfirmed.

Genetic or learned cues

Kinship cues may be of two types: those that are individually distinct and learned associatively from known relatives, and those in which similarity between cues correlates with genetic relatedness; the two are not necessarily mutually exclusive. The latter type has the advantage of allowing kinship assessment between unfamiliar individuals, but such genetic cues may be unstable when they drive cooperative behaviour because of positive frequency-dependent selection (Crozier, 1986; Rousset and Roze, 2007). Disassortative mating such as that driven by inbreeding avoidance provides a potential solution to this issue, but evidence for the use of genetic cues in cooperative behaviour remains scarce, especially in vertebrates (Holman et al., 2013; Riehl and Stern, 2015; but see McDonald and Wright, 2011). We have shown that zip calls have some potential to function as both genetic and learned cues (Chapter 6), and that uropygial chemistry is also informative as a genetic cue (Chapter 5). Cues learned through association should be accurate indicators of kinship in riflemen due to the absence of extra-pair paternity and conspecific brood parasitism in their breeding system. Without systematic cross-fostering we are unable to provide definitive support for the allocation of help based on either genetic or learned cues, though it is interesting that in one isolated example a cross-fostered juvenile male helped at the second brood of his foster parents (Preston et al., 2013a). This provides some suggestion that kinship cues are learned, especially given that he was returned to his genetic parents' nest before fledging, ruling out simple spatial cues as an explanation.

Also consistent with this interpretation are patterns of helping by individuals of known history: all have been familiar kin to the breeders they helped, although this may not have been the case for all individuals of unknown history (Table 7.2). In summary, helping patterns are mostly consistent with learned kinship cues, which should accurately reflect relatedness in riflemen's social system; contact calls represent a plausible individual-specific basis for this. Nevertheless we have identified candidate genetic cues to kinship, which may complement or act instead of learned signals.

Helper contributions

Helpers begin to help during the nestling period (none have been recorded incubating eggs). They vary in their time of appearance: some have been observed provisioning as early as day 3, but one was not recorded until day 24 (median = day 12; hatch date = day 0). When in the vicinity of the nest they contribute to its defence, e.g. responding aggressively when nestlings are removed for ringing. We were unable to consider the importance of this because the vast majority of nests at Kowhai Bush are built in nestboxes, which afford almost full protection from predators (Briskie et al., 2014); even natural nests suffer an unnatural predation regime thanks to the recent introduction of terrestrial mammals to New Zealand (Innes et al., 2010).

Helpers' principal contribution is to provision dependent offspring, and this continues into the post-fledging period. Helpers provisioned nestlings at lower rates than breeders, with juvenile helpers contributing less than adults (Preston et al., 2013a; Chapter 2). As juveniles are all full siblings of the broods they help, we could not investigated whether their provisioning effort correlated with relatedness. Among adult helpers, higher provisioning rates tended to be observed where helper-brood relatedness was greater, but there were insufficient data to support this statistically (Figure 7.2).

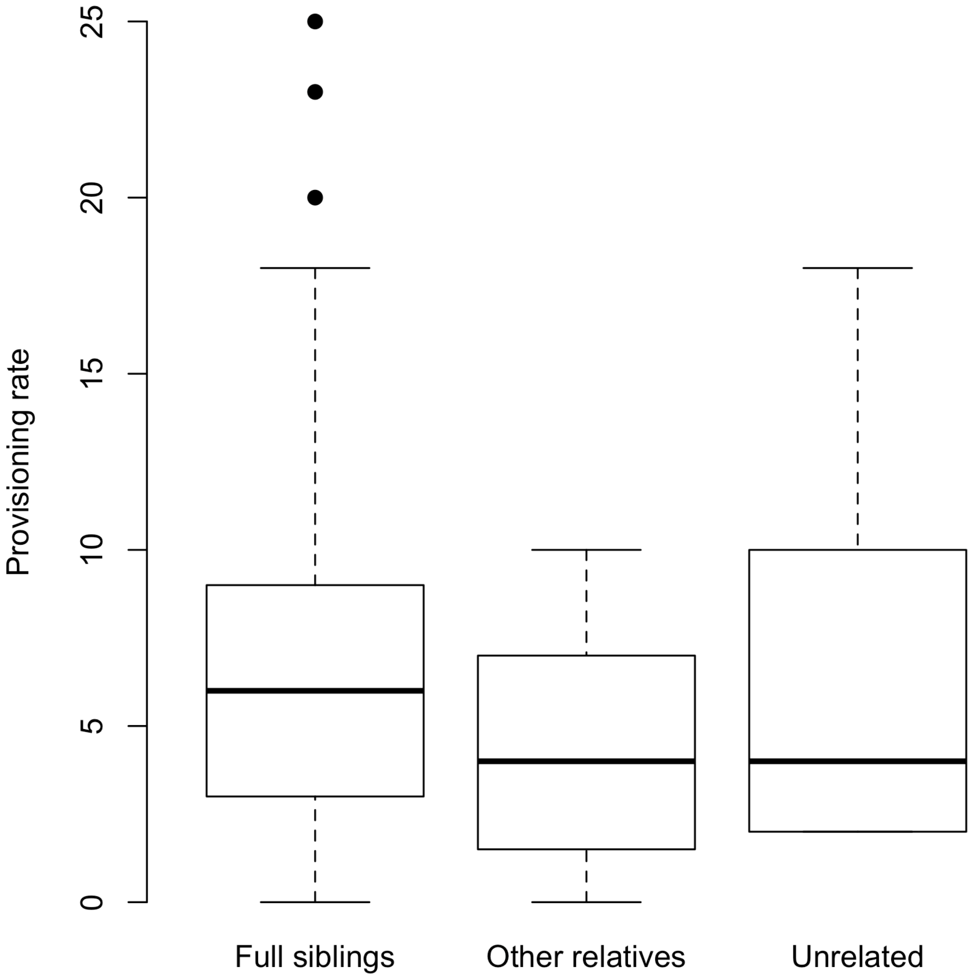

Figure 7.2 The effect of helper-brood relatedness on the provisioning rate (visits/hour) of adult helpers. Full siblings were known from ringing records or assigned using the program Colony (Jones and Wang, 2010). Other relatives had an average relatedness > 0.1 to the brood they were helping. Significance was tested using a Poisson-distributed generalised linear mixed-effects model of provisioning rate implemented in the R package lme4 (Bates et al., 2010), in which brood size, nestling age, date (number of days since 1st September), time (number of hours since 0700 NZST), helper sex, brood sex ratio and mean helper-brood relatedness were fitted as fixed effects, and helper identity was fitted as a random effect. Numeric predictors were scaled and centred. The effect of relatedness was non-significantly positive (GLMM: effect estimate = 0.81 ± 0.68 SE, $z = 1.20$, P = 0.231).

Fitness consequences of help

Indirect fitness benefits

Due to the high relatedness between helpers and the breeders and broods they help, there is clear potential for helpers to gain indirect fitness benefits, by reducing the workload and thus improving the fitness of related breeders, and/or increasing the recruitment and fitness of related offspring.

Preston et al. (2016) found no overall trend for breeders to reduce their provisioning rate when helped, but did observe a significant sex difference in this effect: females tended to ‘load-lighten’ more than did males. My data from the following three seasons also showed no overall tendency to load-lighten, but again there was a significant sex effect. This time, males showed the greater tendency than females to reduce their effort when helped. When data were pooled, there was no overall effect of help, and the sex differences observed over the two periods cancelled one another out. The differences between the two time periods illustrate that although there is not a general tendency for breeders to reduce their visit rate when helped, there is considerable variation in their responses (Figure 7.3): more of the breeders who reduced their effort were female in Preston et al. (2016), and more were male in the later dataset. A random slopes model allowing for individual variation in breeders’ response to help fits the data significantly better than a simpler model assuming a uniform response (Figure 7.3). This suggests that the lack of an overall trend is masking a tendency for some breeders to take advantage of help by load-lightening. It would be interesting to explore the factors that drive this variation, with sex apparently not important overall.

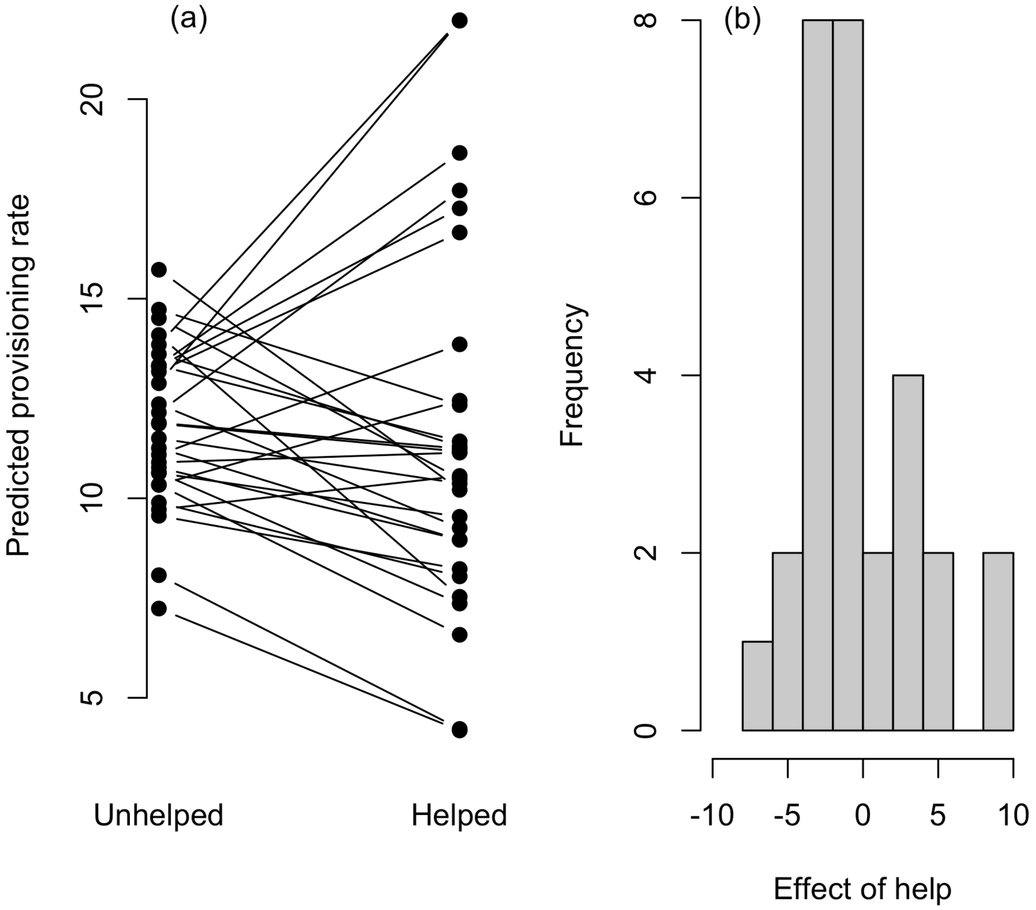

Figure 7.3 Individual-level effects of being helped on breeder provisioning rates (visits/hour), back-transformed from a Poisson-distributed mixed effects model with random slopes. (a) shows individual intercepts from the model ('unhelped') ± individual-specific effects of help ('helped'). (b) shows the distribution of these effects: most are negative, but there is considerable variation and no significant global effect (GLMM: helped effect = -0.02 ± 0.06 SE, $z =$ 0.38, P = 0.703). Individuals with no helped observations are omitted from both plots because their modelled effects are simply the average of those shown. The model was fitted in lme4 (Bates et al., 2010), and included provisioning data from breeders at first broods only because of the uncertain effect of juvenile help on breeder behaviour, following Preston et al. (2016). Brood size; nestling age; date (number of days since 1st September); time (number of hours since 0700 NZST); brood sex ratio; breeder sex, and whether a nest was helped, were fitted as fixed effects, along with the interaction between the final two terms. Identity was fitted as a random intercept along with territory. The effect of being helped was fitted as a random slope in response to identity; incorporating this led to a better fit than a simpler model (ANOVA model comparison: $\chi^2 = 55.07$, df = 1, P < 0.001). Breeder sex did not affect the response to help (GLMM: helped × male interaction = -0.02 ± 0.07 SE, $z =$ 0.31, P = 0.755).

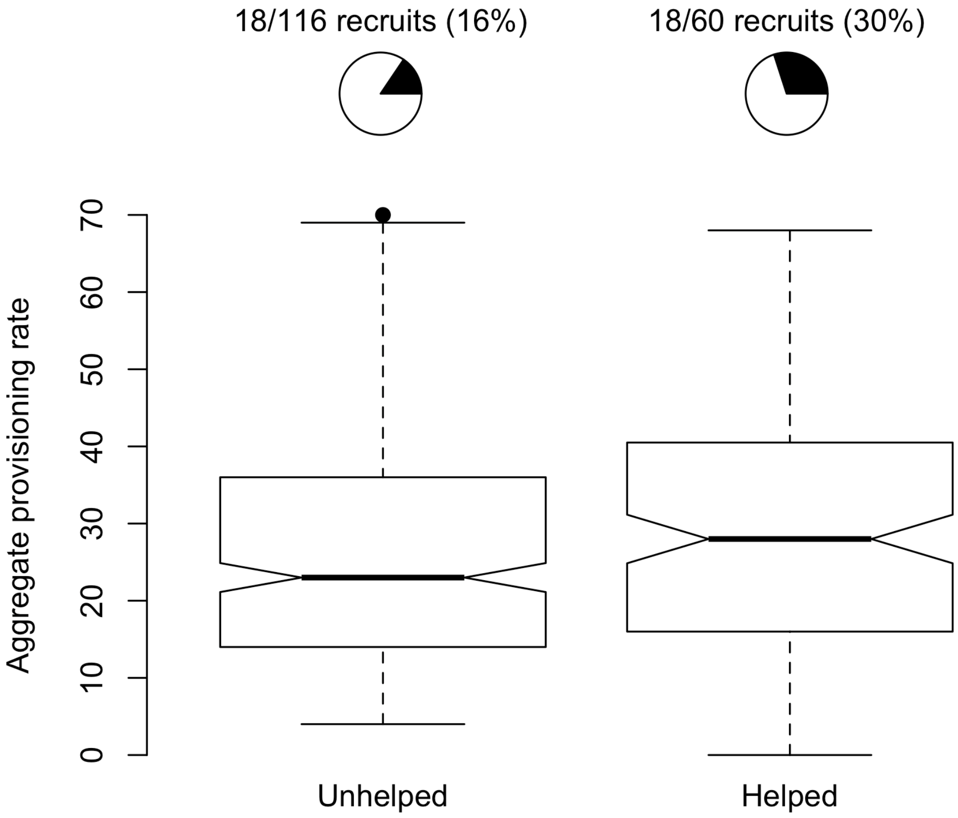

Figure 7.4 Effect of help on total rates of food delivery to broods (all carer's provisioning visits aggregated over the course of an hour of footage). Significance was tested using a Poisson-distributed generalised linear mixed-effects model of aggregate provisioning rate implemented in the R package lme4 (Bates et al., 2010), in which brood size, nestling age, date (number of days since 1st September), time (number of hours since 0700 NZST), brood sex ratio and whether a nest was helped were fitted as fixed effects, and nest identity was fitted as a random effect. Numeric predictors were scaled and centred. The effect of help was significantly positive (GLMM: effect estimate = 0.09 ± 0.04 SE, $z = 2.08$, P = 0.038).

Rather than lightening the load of breeders, Preston et al. (2016) found that the main effect of helpers was to increase the total rate of food delivery to broods. This did not affect productivity, presumably because of the low starvation rate of rifleman nestlings (starvation occurs in < 10% of broods and usually involves just one nestling in those broods). Helped nestlings instead showed enhanced rates of recruitment relative to their unhelped counterparts. This trend has been substantiated by adding three more seasons of data (Figure 7.4). Therefore although breeders vary in their response to help (Figure 7.3), and this is likely to have an influence on whether its benefits accrue to them or their offspring, evidence suggests that nestlings are the principal beneficiaries of help in riflemen and that it confers indirect benefits to helpers.

Direct fitness benefits

The interpretation that indirect fitness benefits have been an important driver of the evolution of helping in riflemen is strengthened by an absence of plausible sources of direct fitness. In cooperative breeding systems with delayed dispersal, philopatric helpers may gain various direct fitness benefits such as access to suitable or high-quality habitat (the ecological constraints and benefits of philopatry hypotheses), and group augmentation where this improves foraging success or the avoidance of predation (Emlen, 1982; Stacey and Ligon, 1987; Heinsohn, 1991; Kokko et al., 2001). In riflemen's dispersed kin neighbourhood system, these fitness benefits are unavailable to helpers because they do not join the breeding pair's 'group' or territory. It has also been suggested that helping may evolve as a reciprocal trait, though empirical evidence for this is scant in natural systems (Clutton-Brock, 2009). We have not recorded a single helping event in which the breeder had previously helped the helper, ruling out reciprocity as a key fitness benefit of help in riflemen. Gaining skills to improve future parenting is a potential source of fitness for juvenile helpers, but one that we were unable to test: 13 of the 20 juvenile helpers we recorded were in the 2010-2011 and 2014-2015 breeding seasons, in which there was no follow-up fieldwork the following season, and only 1 of the remaining 7 recruited into the population. Although this would be an interesting hypothesis to investigate, it seems that doing so would require many years of study given the generally low rate of recruitment (Figure 7.4).

One further potential source of direct fitness has had an important influence on the history of rifleman study: the possibility that helpers help because it improves their access to mating opportunities. Sherley (1990a) studied riflemen at Kowhai Bush in the 1980s, without the aid of genetic tools. He recognised that some helpers, such as juveniles, were previous offspring of the pairs they helped, but considered these 'casual' helpers. He suggested that most help was provided by unpaired males who did so to gain access to females, specifically pairing with female offspring that they helped. Following this work, riflemen were considered essentially unique in having a cooperative breeding system of this kind (Cockburn, 2004), but this interpretation has not been substantiated by our research. The generally high relatedness between helpers and the broods they help is likely to make them unsuitable partners, especially given that riflemen tend to avoid inbreeding (Preston, 2012). We have only observed one instance of a genuine helper-recipient pairing from our six years of study: a male born in the 2012-2013 season who helped at his parents' first brood in 2013-2014 was paired with one of the offspring from that brood (his full sibling) in 2014-2015. Perhaps unsurprisingly, this pair did not reproduce successfully, with the female not even laying eggs. Therefore we have recorded no fitness returns derived from helpers rearing future mates. It is possible that this strategy that was more common during Sherley's (1990a) study period than ours; the three unrelated helpers we observed were all unpaired males, who may have been hopeful reproductives, though if so they were unsuccessful. However, as Sherley's (1990a) conclusions were based on post-fledging observations of 'pair bonds' from the same season as helping events, we consider it more likely that these were in fact close carer-recipient associations, as helpers continue to provision offspring after they have fledged (Preston et al., 2013a).

Costs of help

Unlike cooperative breeders exhibiting delayed dispersal and independent reproduction, there is no opportunity cost to helping in riflemen, because helpers are either unable to breed (juveniles and unpaired adults), failed breeders, or breeding simultaneously. On the other hand, helping is likely to incur some energetic costs. The overall fitness cost of the behaviour to helpers is difficult to determine, because the majority of helpers were unpaired birds and all documented unpaired birds became helpers, meaning that we were unable to compare unpaired birds that helped with those that did not. The six helpers who were simultaneously provisioning their own nestlings had a mean brood size of 1.83 ± 0.40 SE (maximum 3), compared with the population mean brood size of 3.20 ± 0.11 SE. This suggests that only breeders with a relatively low workload become helpers. Perhaps because of this, among paired individuals there was no negative effect of helping on survival to the following breeding season (8/10 paired helpers survived, overall survival rate of paired birds = 50%).

Sex allocation

Across 10 seasons of rifleman research, including 4 from an earlier study in the 1980s, the adult sex ratio of riflemen at Kowhai Bush has been generally biased towards males, though not significantly different from parity in any individual season (Sherley, 1993; Chapter 3). There are two reasons why we might expect this to result from biases at the production stage. The first is male offspring being more likely to 'repay' investment by helping, because helping is male-biased. This repayment should effectively make producing males a cheaper investment (Emlen et al., 1986). The second reason is that males are physically smaller even as nestlings and therefore actually cheaper at the time of production, with carers having to provision relatively more in more female-biased broods (Chapter 3).

In light of these predictions, it is surprising that brood sex ratios are not significantly biased towards males in this species. More surprising still, they are female-biased (c.47% male), and pooling our data with Sherley's (1993) very similar results causes this bias to approach statistical significance (binomial test: n = 768 nestlings, P = 0.052). This result is difficult to explain from an adaptive perspective: it appears evolutionarily unstable because breeders producing more male-biased sex ratios should benefit from reduced energetic expenditure and a greater likelihood of future help. A comparative analysis suggests that riflemen are not alone among cooperatively breeding birds in failing to confirm adaptive sex allocation predictions (Chapter 4).

Conclusion

The rifleman cooperative breeding system does not follow the pattern associated with delayed dispersal helping-at-the-nest, in which offspring remain on their natal territory and help their parents in subsequent reproductive bouts. Although this is true of juvenile helpers, adult helpers (which are both more common and more effective) disperse prior to helping. The social system is better described as a kin neighbourhood (Dickinson and Hatchwell, 2004). Although cooperation is not mediated by delayed dispersal, *limited* natal dispersal of both sexes appears to be important in promoting the conditions for kin-directed helping. This generates a kin structure in the population whereby individuals are spatially close to their relatives, and can move easily between territories to help them (Preston, 2012). Sexual fidelity also drives up the inclusive fitness available to helpers and is likely to favour cooperative breeding (Cornwallis et al., 2010). Whether this fidelity itself has an adaptive basis is unclear: the most plausible adaptive hypothesis in riflemen is that females are constrained from seeking extra-pair mating because they require high investment from their social partner (Mulder et al., 1994). This is consistent with the high rates at which breeder males provision, and may also directly favour cooperative breeding if offspring stand to benefit substantially from extra care (Dillard and Westneat, 2016). Alternatively, a number of non-adaptive hypotheses put forward to explain variation in promiscuity may underlie its absence in riflemen (Forstmeier et al., 2014). Explaining such variation has interesting implications for understanding cooperative breeding generally, given that it should be favoured in sexually monogamous systems like riflemen, but also in highly promiscuous systems where paternity uncertainty means that maternal siblings are a male's most reliable genetic relatives, over and above his social offspring (Kramer and Russell, 2015).

Studies of species where cooperative breeding occurs outside the rigid family structure generated by delayed dispersal have arguably provided the strongest evidence available that helpers accruing indirect fitness can be a sufficient basis for the evolution of cooperation, because sources of direct fitness such as philopatry benefits and group augmentation are unavailable. In long-tailed tits *Aegithalos* *caudatus*, a substantial effect of help on recruitment coupled with a lack of opportunity costs means that helping by failed breeders can satisfy Hamilton's rule even when relatedness is low (Hatchwell et al., 2014). In riflemen, relatedness is generally high, which may explain why it is adaptive for helpers to help across a wider variety of life-history contexts. With an absence of divorce, no recorded extra-pair paternity, and little constraint on the behaviour of helpers, the rifleman breeding system seems especially low in conflict, with the fitness interests of all carers closely aligned (Chapter 2). Comparison with species in which the opposite statement could be made in each case, such as superb fairy-wrens *Malurus cyaneus* (Cockburn et al., 2016), illustrates the remarkable diversity of avian cooperative breeding systems, and the challenges inherent in providing unifying explanations for their occurrence.

8.

General discussion

In this thesis, I have investigated patterns of offspring care, investment in relation to offspring sex and potential cues for kin recognition, in the fascinating cooperative breeding system of New Zealand's rifleman *Acanthisitta chloris*. I hope that my results are of general interest to researchers in the fields of behavioural and sensory ecology; some have interesting implications for our current understanding of these fields, and future directions within them. In this chapter I first summarise my findings, and then discuss three general themes that have emerged from them. The role that coauthors have played in the preceding chapters is acknowledged elsewhere in this thesis (Acknowledgements and chapter title pages); I would like to do so again here to excuse the use of 'I' throughout this discussion.

Summary of results

In Chapter 2, I examined carer provisioning behaviour using data from filmed observations at rifleman nests that were made regularly throughout their nestling periods. This study yielded a number of important results. Firstly, I employed two methods to evaluate the accuracy of provisioning rate as a measure of food delivery to offspring (see Implications and Future Directions below). Both of methods supported the use of provisioning rate in riflemen, and so I continued to use this measure in the rest of the chapter and other parts of the thesis (Chapter 3, Chapter 7). Secondly, I showed that rifleman carers were flexible in their provisioning effort, with a low estimate of within-individual repeatability compared to other species. Thirdly, I tested whether this flexibility was a result of carers responding to each other's investment by taking turns to feed nestlings. This is a form of real-time negotiation between carers that is suggested to be a stable mechanism of organising parental care (Johnstone et al., 2014). This hypothesis was not supported, in contrast with observed patterns from four other recently-studied bird species (Johnstone et al., 2014; Savage, 2014; Bebbington and Hatchwell, 2016; Koenig and Walters, 2016). I speculated that this discrepancy might result from a relative absence of conflict in the rifleman breeding system, lessening the need for negotiation between carers.

Female riflemen are over 25% heavier than males as adults, and the sexes are already size-dimorphic in the nest, the difference averaging 14% when nestlings are 15 days old. In Chapter 3, I used a large dataset to show that carers worked harder when provisioning female-biased broods: evidence that larger female offspring are a more expensive investment than males. Fisher's (1930) theory that each sex should receive equal investment thus predicts that offspring production should be biased towards males in this species. Emlen et al.'s (1986) repayment hypothesis also predicts a male bias to rifleman sex ratios, because helping is male-biased, meaning that sons are more likely to repay investment in the future and are thus an effectively cheaper investment. These two theories often generate opposite predictions (e.g. Koenig et al., 2001; Kingma et al., 2011), and riflemen therefore provided an ideal opportunity to test a strong directional sex allocation hypothesis in a cooperatively breeding species. Surprisingly, but similarly to Sherley (1993), I instead observed more female offspring produced than males, with a mean brood sex ratio not significantly different from parity. I also found no evidence for facultative sex-ratio adjustment according to any metrics of breeder context. Chapter 4 used a comparative approach to show that studies of cooperatively breeding bird species do not in general support the predictions of the repayment hypothesis, another surprising result which suggests that explanations for my findings may not be specific to riflemen.

Chapter 5 and Chapter 6 focused on the question of how riflemen identify their kin. This provides an important context to understanding helping behaviour, because help is directed towards relatives on a scale that would be highly unlikely if it were indiscriminate or relied on spatial cues alone (Cornwallis et al., 2009; Preston et al., 2013; Chapter 7). I focused on two candidate mechanisms: olfactory detection of chemical signatures in uropygial secretions, and recognition of the 'zip' calls that are regularly used by adults in benign social interactions. I demonstrated that similarity in both of these cues correlated with kinship in certain same-sex dyads. I also found that zip calls contained information that allowed them to be successfully assigned to the individuals they were recorded from more often than expected by chance. Field experiments suggested that riflemen respond to manipulations in their olfactory environment, albeit in a surprising way, with the presentation of material from their own nest appearing to elicit a negative response. In contrast, I found no evidence from playback experiments that riflemen respond differently to the vocalisations of their relatives.

In Chapter 7, I outlined how these results fit into our current understanding of the rifleman cooperative breeding system. Below, I discuss their importance in relation to three more general themes with wider implications in the field.

Implications and future directions

Measuring parental investment

In studies of breeding birds provisioning rate is used almost ubiquitously as a measure of parental investment. It is easy to see why: even using traditional methods, this generates large quantities of data, and the adoption of automated techniques such as those based on passive integrated transponder tags has increased this by orders of magnitude for some long-term studies (e.g. Dickinson et al., 2016; Russell, 2016). In addition, the data are readily analysed using generalised linear mixed-effects models (GLMMs) to assess causes of variation. Nevertheless, there may be issues with its universal use as a measure of investment.

One of these issues is dealt with in Chapter 2: the concern that provisioning rate may not accurately describe the amount of food brought to nestlings, because of variation in the loads that carers bring on provisioning visits. This has been considered in previous studies, some of which concluded that visit rate was an accurate measure of food delivery (Nolan et al., 2001; Browning et al., 2012). However, Schwagmeyer and Mock (2008) found that this was not the case in their population of house sparrows *Passer domesticus*, because of a tradeoff between visit rate and load size (less frequent visitors compensated by bringing larger loads). I tested this relationship in riflemen, and found a positive correlation (i.e. carers visiting more often, brought a greater proportion of large loads), supporting the validity of visit rate as a measure of food delivery. I also used the data on load size to perform a novel test of its repeatability (*R*) reasoning that if carers consistently bring the same sized loads, their contribution is likely to be underestimated or overestimated from their visit rate. This was not the case, and I concluded that visit rate was a valid measure of food delivery in riflemen. Rather than generalise from this result and suggest that this is likely to be true across species, I would encourage similar checks to be applied in other studies that use provisioning rates. This should make these conclusions more robust and increase the value of a widely useful measure. Guidelines for calculating *R* are available in Nakagawa and Schielzeth (2010).

Another issue, and one that is a limitation of this thesis, is that investment in offspring is a multi-stage process that is not restricted to the delivery of food to nestlings. Although provisioning rate in riflemen seems to describe investment *at this stage* successfully, it does not equate to a full measure of parental investment across a breeding attempt. For example it is inaccurate to extrapolate from provisioning rates and conclude that males make greater reproductive investments than females, without accounting for the energetic expenditure involved in egg-laying; Sherley’s (1989, 1990b, 1994) papers provide an excellent account of investment at the pre-hatching stage in riflemen, which is high for both sexes. The value of considering investment holistically is exemplified by Russell et al.’s (2007) remarkable finding that in superb fairy-wrens *Malurus cyaneus*, mothers reduce their investment when helpers are present by laying low-quality eggs. In riflemen, it is likely that the fitness consequences of help are realised during provisioning, because this is the point at which helpers appear. Nevertheless, considering all stages of investment together can provide a fuller understanding of how parental and cooperative care operates, than looking at provisioning alone (Savage et al., 2012).

Development of evolutionary theory

A number of recent theoretical papers have improved our understanding of how investment is expected to evolve in cooperative breeding systems. For example, Savage et al. (2012, 2013) have shown that mothers' early investment patterns are likely to coevolve with later investment by their partners and helpers; the outcomes of this coevolution depend on breeder-helper relatedness and constraints on offspring production. Johnstone's (2011) extension of negotiation models of parental care to cooperative breeders, and Johnstone et al.'s (2014) subsequent formulation of a novel negotiation model based on turn-taking, have been influential in recent studies of provisioning. While the latter modelled biparental systems, observed patterns of turn-taking in two species have invoked its occurrence in cooperative groups (Savage, 2014; Koenig and Walters, 2016).

Chapter 2 shows riflemen to be a notable exception to this trend, with little evidence for turn-taking whether helpers are present or absent. A putative explanation for this is that there is little conflict between carers in riflemen, because there is no divorce or extra-pair paternity in the breeding system, while helpers are relatively unconstrained ecologically and usually of high relatedness to recipient broods. Such an absence of conflict might make negotiation (a process modelled to explain how evolutionary conflicts are resolved) redundant. This is a hypothesis that could probably be tested only by comparative analysis, but it illustrates an interesting gap in theories of parental investment. These generally assume conflict, and focus on its resolution. However, conflict is reduced where individuals are interdependent, as in pair bonds without divorce where the future fitness of one partner is closely correlated with that of the other (Roberts, 2005). Including a term for the strength of conflict (or interdependence) might widen the diversity of care patterns predicted by theoretical models.

The results of Chapter 3 and especially (given that it concerned multiple species) Chapter 4, also failed to confirm theoretical predictions, this time in relation to Emlen et al.'s (1986) repayment hypothesis. One of Chapter 4's results suggests that facultative adjustment of offspring sex ratios according to a need for help is rare among cooperative breeders. Across species there are a number of reasons why this might be the case, which I discuss in the chapter. Pen et al. (1999) showed that small mechanistic costs should erode the adaptive benefits of this adjustment, and so its rarity is understandable in the context of theory. The finding that there is no general tendency across species for females to produce more of the more helpful sex (consistently rather than facultatively) is seemingly more problematic. Although the initial formulation of the repayment theory has been considered incomplete, each update to the model has concluded in turn that biased sex ratios favouring the more helpful sex are expected to occur in cooperative breeders (Lessells and Avery, 1987; Koenig and Walters, 1999; Pen and Weissing, 2000). Further, the observed variation in sex ratios was phylogenetically random, and a number of them differed significantly from parity, which provides little support to the suggestion that an equal sex ratio is the consequence of a mechanistic constraint on sex allocation. In this case, the discrepancy between theory and evidence is more difficult to explain. In riflemen, relatively low survival might underlie the result: adults have only a 50% chance of surviving to the next breeding season, and helpers rarely help to raise half-siblings (Chapter 7). This means that the probability of a breeder being in a position to receive help in year $n + 1$, from an offspring produced in year n, is c.25% (assuming the offspring in question survives). For the breeder this diminishes likely fitness returns gained through repayment relative to those gained through that offspring's direct fitness. Testing repayment models under low survival probabilities might alter expected outcomes of the theory.

Opportunities and challenges in avian sensory ecology

In Chapter 5, I demonstrated that similarity in chemical cues contained within rifleman preen wax could provide information on relatedness. This has been shown in recent studies of two other bird species: a gull (Leclaire et al., 2012, 2014) and a songbird (Slade et al., 2016). Its extension to a third major avian clade here suggests it might be a widespread pattern among birds, and to my knowledge is the first time it has been tested in a cooperative breeder. It is an interesting avenue for future research in kin recognition, especially as the cited studies provide evidence linking this relationship to genes for the major histocompatibility complex (MHC). As MHC genes are likely to be under strong negative frequency-dependent selection (Holman et al., 2013), this represents a possible solution to Crozier's (1986) paradox of genetic kin recognition, in which genetic cues for cooperation are considered inherently unstable because the most common should spread rapidly to fixation. Field experiments suggested that riflemen responded to an altered olfactory environment in the nests they were provisioning, although this had the opposite effect to that we predicted (see Chapter 5). This joins other studies suggesting that a diverse array of bird species respond to olfactory manipulations (Bonadonna and Nevitt, 2004; Krause et al., 2012; Mihailova et al., 2014), and suggests that riflemen could recognise their kin by smell. A recent growth of interest in this field promises exciting new results that will improve our understanding of avian communication (Bonadonna and Mardon, 2013).

My study of vocal communication in riflemen provided no evidence for kin discrimination using an experimental approach (Chapter 6). Improvements could have been made to the experiments, for example using a simultaneous pairwise design or standardising the sex of playback individuals (though the latter would have required a larger population). Several studies of cooperatively breeding birds have showed discrimination based on vocalisations, although so far this has been limited to songbirds (Crane et al., 2015). Even so, there are challenges to conducting these studies. One is to make experimental treatments as realistic as possible. Crane et al.'s (2015) work on chestnut-crowned babblers *Pomatostomus ruficeps* introduced a novel method of doing this: circumventing 'expectancy violation' by removing playback individuals from the groups being tested. Where this is not controlled, focal individuals may react unnaturally because of the apparent presence of the playback individual in two places at once (Townsend et al., 2012). Future studies of vocal recognition in group-living species, such as many cooperative breeders, are likely to benefit from following this method. A second challenge is in replicating the decision to help when testing individuals' responses to treatments. In previous studies, aggressive responses to cues from non-kin have been used to infer their importance in affiliative interactions with kin (e.g. Sharp et al., 2005; Akçay et al., 2013). I found this to be inappropriate in riflemen, where focal individuals rarely responded aggressively to playback. Implementing more direct tests of what affects helping decisions is a challenge for future research. Counterintuitively, non-experimental studies can shed new light on this: McDonald and Wright (2011) used recordings of bell miner *Manorina melanophrys* provisioning calls to show that call similarity between potential helpers and breeders predicted their likelihood of helping, more so than genetic relatedness (though the two were correlated). This powerful test of what affects helping decisions would be interesting to replicate in other species, though it relies on call similarity, rather than associatively learned individual signatures, being the source of relatedness information.

Finally, visual recognition has been treated as something of a black box in the literature on avian cooperative breeding (Nakagawa and Waas, 2004); attentive readers will have noted that this thesis is no exception. The obvious reason is that experiments manipulating subtle individual visual signals are difficult even to conceive, let alone implement, but this is does not mean that these cues are non-existent or unimportant in avian kin recognition. Modern imaging techniques have shown that visual characteristics of eggs play a key role in the arms races between brood parasites and their hosts (Spottiswoode and Stevens, 2010). Applying these methods to whole organisms has the potential to address key questions across diverse strands of evolutionary biology (Stoddard and Prum, 2008; Cooney et al., 2017). Though variation at an intraspecific scale will be especially challenging to quantify, developments in this field would likely bring important advancements to our understanding of kin recognition in birds.

APPENDICES

A.

Supplementary model results from study of rifleman sex allocation

Table A.1 Effect estimates on the logit scale from potential predictors of brood sex ratios in riflemen, modelled as fixed effects in a binomially-distributed generalised linear mixed-effects model, with the proportion of male offspring in a brood as the response variable (*n* = 93 broods). Breeding season was included as a random effect, but explained no variation. Second brood and helped are categorical predictors with first broods and unhelped nests as respective reference categories.

Predictor	**Estimate ± SE**	*z*	**P**
Intercept	-0.36 ± 0.49	-0.73	0.468
Density (no. pairs within 200 m)	0.07 ± 0.05	1.44	0.150
Second brood	-0.02 ± 0.36	-0.05	0.964
Helped	0.16 ± 0.26	0.62	0.533
Brood size	> -0.01	-0.08	0.937

Table A.2 Effect estimates on the logit scale from potential predictors of brood sex ratios in riflemen, modelled as fixed effects in a binomially-distributed generalised linear mixed-effects model, with the proportion of male offspring in a brood as the response variable (*n* = 85 broods). Nine unsexed nestlings were assumed to be male. Pair identity (variance component < 0.01) nested within female identity (variance component < 0.01) was included as a random effect along with breeding season (variance component < 0.01). Second brood and helped are categorical predictors with first broods and unhelped nests as respective reference categories.

Predictor	**Estimate ± SE**	*z*	**P**
Intercept	-0.27 ± 0.52	-0.52	0.601
Density (no. pairs within 200 m)	0.10 ± 0.06	1.72	0.087
Second brood	-0.06 ± 0.37	-0.17	0.864
Helped	0.26 ± 0.27	0.94	0.352
Brood size	-0.06 ± 0.13	-0.44	0.663
Mother-father relatedness	0.15 ± 0.58	0.26	0.795

Table A.3 Effect estimates on the logit scale from potential predictors of brood sex ratios in riflemen, modelled as fixed effects in a binomially-distributed generalised linear mixed-effects model, with the proportion of male offspring in a brood as the response variable (*n* = 85 broods). Nine unsexed nestlings were assumed to be female. Pair identity (variance component < 0.01) nested within female identity (variance component < 0.01) was included as a random effect along with breeding season (variance component < 0.01). Second brood and helped are categorical predictors with first broods and unhelped nests as respective reference categories.

Predictor	**Estimate ± SE**	*z*	**P**
Intercept	-0.28 ± 0.52	-0.52	0.588
Density (no. pairs within 200 m)	0.10 ± 0.06	1.76	0.079
Second brood	-0.09 ± 0.37	-0.25	0.807
Helped	0.27 ± 0.28	0.97	0.331
Brood size	-0.08 ± 0.13	-0.59	0.557
Mother-father relatedness	0.19 ± 0.59	0.33	0.745

Table A.4 Effect estimates on the logit scale from potential predictors of carer provisioning rate in riflemen, modelled as fixed effects in a Poisson-distributed generalised linear mixed-effects model (*n* = 1,124 observations). Carer identity (variance component = 0.07), territory (variance component = 0.02) and breeding season (variance component < 0.01) were included as random effects. Second brood and helped are categorical predictors with first broods and unhelped nests as respective reference categories. Brood size, nestling age, date (number of days since 1st September) and time (number of hours since 0700 NZST) were scaled and centred. Carer status, carer sex and second brood are categorical predictors with breeder, female and first broods as respective reference categories.

Predictor	**Estimate ± SE**	*z*	**P**
Intercept	2.37 ± 0.06	37.76	< 0.001
Proportion of males in brood	-0.12 ± 0.05	-2.42	0.016
Brood size	0.27 ± 0.02	17.29	< 0.001
Nestling age	0.31 ± 0.01	28.24	< 0.001
Carer status (helper)	-1.01 ± 0.05	-20.40	< 0.001
Carer sex (male)	0.14 ± 0.05	3.03	0.002
Time	-0.03 ± 0.01	-3.69	< 0.001
Date	-0.09 ± 0.02	-3.44	< 0.001
Second brood	0.09 ± 0.06	1.45	0.146

B.

Tables of data used for comparative analysis and meta-analysis

Table B.1 Brood sex ratio (BSR) and helper sex ratio (HSR), expressed as proportion of males, for 26 cooperatively breeding bird species. Source references are specificed for helper SR where these differ from the source for brood SR.

Species	**BSR**	**Source**	**HSR**	**Source**
Harris's hawk *Parabuteo unicinctus*	0.57	Bednarz and Hayden (1991)	1.00	Mader (1975)
Green woodhoopoe *Phoeniculus purpureus*	0.46	Ligon and Ligon (1990)	0.43	
Acorn woodpecker *Melanerpes formicivorus*	0.48	Koenig et al. (2001)	0.58	Koenig et al. (2016b)
Red-cockaded woodpecker *Picoides borealis*	0.50	Koenig and Walters (1999)	0.95	Lennartz et al. (1987)
Pied kingfisher *Ceryle rudis*	0.53	Reyer (1990)	1.00	
Laughing kookaburra *Dacelo novaeguineae*	0.47	Legge et al. (2001)	0.76	Legge and Cockburn (2000)
Eclectus parrot *Eclectus roratus*	0.51	Heinsohn et al. (2011)	1.00	
Rifleman *Acanthisitta chloris*	0.47	Chapter 3	0.72	
Noisy miner *Manorina melanocephala*	0.50	Arnold et al. (2001)	0.99	
Bell miner *Manorina melanophrys*	0.56	Clarke et al. (2002)	0.88	Wright et al. (2010)
Black-eared miner *Manorina melanotis*	0.37	Ewen et al. (2001)	0.96	
Purple-crowned fairy-wren *Malurus coronatus*	0.53	Kingma et al. (2011)	0.69	Kingma et al. (2009)
Superb fairy-wren *Malurus cyaneus*	0.53	Cockburn and Double (2008)	1.00	
White-winged fairy-wren *Malurus leucopterus*	0.50	Rathburn and Montgomerie (2005)	1.00	
Red-backed fairy-wren *Malurus melanocephalus*	0.45	Varian-Ramos et al. (2010)	1.00	
Apostlebird *Struthidea cinerea*	0.58	Woxvold and Magrath (2008)	0.58	Woxvold et al. (2006)
White-throated magpie-jay *Calocitta formosa*	0.50	Berg (2004)	0.10	
Florida scrub-jay *Aphelocoma coerulescens*	0.49	Fitzpatrick and Bowman (2016)	0.54	Woolfenden and Fitzpatrick (1984)
White-banded tanager *Neothraupis fasciata*	0.50	Gressler et al. (2014)	1.00	Manica and Marini (2011)

Species	BSR	Source	HSR	Source
Sociable weaver *Philetairus socius*	0.57	Komdeur (2004)	0.80	Covas et al. (2006)
Western bluebird *Sialia mexicana*	0.51	Koenig and Dickinson (1996)	1.00	Dickinson et al. (1996)
Superb starling *Lamprotornis superbus*	0.45	Rubenstein (2007)	0.62	
Ground tit *Pseudopodoces humilis*	0.49	Do and Lu (2010)	1.00	Lu et al. (2011)
Seychelles warbler *Acrocephalus sechellensis*	0.54	Komdeur et al. (1997)	0.37	Richardson et al. (2002)
Long-tailed tit *Aegithalos caudatus*	0.53	Nam et al. (2011)	0.86	Hatchwell et al. (2004)
White-browed tit-warbler *Leptopoecile sophiae*	0.51	Lu et al. (2009)	0.00	

Table B.2 Effect sizes (*r*) for the relationship between a female being helped^1 or her number of helpers^2, and her brood's sex ratio, across 17 cooperatively breeding bird species. Positive effect sizes are in the direction expected from theory: greater production of the helping sex when a female is unhelped or has fewer helpers; ? denotes effect sizes of unknown direction. Species marked * are those where helping is female-biased; it is male-biased in all others.

Study	**Species**	***r***	**Calculated from**
Bednarz and Hayden (1991)	Harris's hawk *Parabuteo unicinctus*	-0.05	Comparison of proportions^1
Ligon and Ligon (1990)	Green woodhoopoe *Phoeniculus purpureus**	+0.11	Obtained from Griffin et al. (2005)^2
Koenig et al. (2001)	Acorn woodpecker *Melanerpes formicivorus*	+0.03	Comparison of proportions^1
Gowaty and Lennartz (1985)	Red-cockaded woodpecker *Picoides borealis*	+0.36	Obtained from Griffin et al. (2005)^2
Legge et al. (2001)	Laughing kookaburra *Dacelo novaeguineae*	-0.17	GLMM^1
Chapter 3	Rifleman *Acanthisitta chloris*	-0.08	GLMM^1
Ewen et al. (2003)	Bell miner *Manorina melanophrys*	+0.08	GLM^2
Kingma et al. (2011)	Purple-crowned fairy-wren *Malurus coronatus*	+0.02	GLMM^2
Cockburn and Double (2008)	Superb fairy-wren *Malurus cyaneus*	-0.05	GLMM^2
Rathburn and Montgomerie (2005)	White-winged fairy-wren *Malurus leucopterus*	+0.05	GLM^2
Woxvold and Magrath (2008)	Apostlebird *Struthidea cinerea*	?0.06	GLMM^2
Gressler et al. (2014)	White-banded tanager *Neothraupis fasciata*	+0.08	GLMM^1
Doutrelant et al. (2004)	Sociable weaver *Philetairus socius*	-0.34	GLMM^1
Dickinson (2004)	Western bluebird *Sialia mexicana*	+0.24	Obtained from Griffin et al. (2005)^2
Rubenstein (2007)	Superb starling *Lamprotornis superbus*	?0.05	GLM^2
Komdeur et al. (1997)	Seychelles warbler *Acrocephalus sechellensis**	+0.36	G-test^1
Nam et al. (2011)	Long-tailed tit *Aegithalos caudatus*	-0.05	GLM^1

C.

Eurasian blackbird *Turdus merula* nest parasitised by song thrush *T. philomelos*

Nyil Khwaja and David J. Lloyd-Jones

This short communication describes an observation made while carrying out fieldwork for this thesis. It was published in *Notornis* volume 62, pages 41-44. Only the formatting has been changed in this version.

Brood parasitism, in which a female lays her eggs in the nest of another individual, is best known as a driver of intricate coevolution between parasites such as cuckoos and their host species (Davies, 2000). Facultative brood parasites are those that do not rely on their hosts to complete their life cycle, but occasionally lay eggs in the nests of others, usually of the same species. This has been recorded in over 200 bird species worldwide (Yom-Tov, 2001). Here we report an unusual observation of a facultative brood parasite laying its eggs in the nest of a different, though closely related species. We also describe the subsequent behaviour of the host and the progress of the nest until its desertion.

The Eurasian blackbird *Turdus merula* and song thrush *T. philomelos* were introduced to New Zealand from Britain during the 1860s and 1870s (Gill et al., 2010) and both species are now common and widespread throughout the country (Robertson et al., 2007). Their breeding biology is similar: females of both species make similarly sized cup-shaped nests, lay 3-5 eggs and incubate them for 12-14 (blackbird) or 10-17 (song thrush) days, before provisioning nestlings in partnership with the male (Higgins et al., 2006). Blackbirds (84-120 g) are larger than song thrushes (58-85 g) and have larger eggs on average (blackbird: 29 × 21 mm; song thrush: 27 × 20 mm). The eggs of the two species also differ in colour and pattern: blackbird eggs are blotchy blue-green and red-brown, while song thrush eggs are bright blue, and flecked with black (Higgins et al., 2006).

Conspecific brood parasitism has been reported at low rates (< 5%) in both species, including a New Zealand population of song thrush (Samas et al., 2014). Experimental work has shown that both species tend to reject non-mimetic eggs added to their nests, both in their native range (Davies and Brooke, 1989) and in New Zealand (Hale and Briskie, 2007). Egg rejection in these species is thought likely to be an evolutionary response to past parasitism by the common cuckoo *Cuculus canorus*, an obligate brood parasite sympatric with their British source populations (Davies and Brooke, 1989). Thus parasitism between these two species, with their different eggs, might be expected to lead to egg rejection by the host female. To our knowledge there are no published records of it occurring.

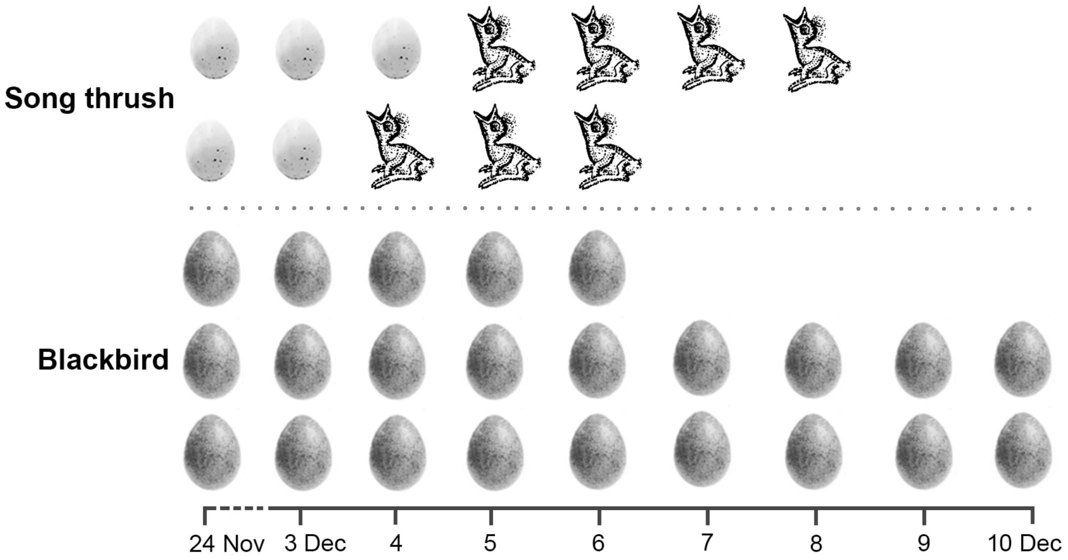

Figure C.1 Daily observations made at the blackbird nest following parasitism by a song thrush in November-December 2014. Illustrations represent the eggs and chicks of each species present in the nest with the date given below. The female blackbird deserted the nest on 10th December.

On 24 November 2014 we found a blackbird nest at Kowhai Bush, Kaikoura (173° 37’ E, 42° 23’ S), containing 3 blackbird eggs and 2 song thrush eggs. The female blackbird did not reject the song thrush eggs and over the next 10 days incubated all 5 eggs. Figure C.1 illustrates observations made on the following days. The first song thrush egg hatched on 4th December (Figure C.2) and the second egg hatched the next day. The two song thrush chicks were observed begging on our subsequent visits to the nest. They appeared to be growing but were ejected separately on 7th and 8th December, respectively. One blackbird egg was also ejected, on 6th December. The nest and remaining two blackbird eggs were deserted by the female on 10th December.

Figure C.2 Photograph of the blackbird nest taken on 4th December 2014, showing a song thrush chick on the day it hatched, one song thrush egg (far right) and three blackbird eggs.

On 7th December, with one song thrush chick and two blackbird eggs remaining, we video recorded nest activity from 1445-1814 h. A Sony Handycam video camera (Sony Inc., Tokyo) was attached to a tree 11 m from the nest, at a height of 3.5 m. The brooding female left the nest seven times during this period and returned with food at least six times (the bird's bill was not visible on the final visit). Time away from the nest averaged 426 s (sd = 526 s, *n* = 7). From the video recording, we could not determine whether the chick ate any food brought to the nest; on two occasions the female clearly attempted to feed it but after apparently failing, ate the food herself. We suspect that this may have occurred more than twice. From this brief observation period, we established that the female was both incubating her remaining eggs and attempting to feed the song thrush chick, but that she was unable to feed it on at least some attempts.

Our observation of a blackbird nest parasitised by a song thrush yielded a number of interesting questions. Firstly, why did a song thrush lay eggs in the blackbird's nest? Laying eggs in other nests can be adaptive in a variety of situations (Lyon and Eadie, 2008). For example, a female may enhance her fitness by passing the costs of parental care onto another, especially if she is in poor condition. Alternatively, a female whose nest is predated while laying may make the best of a bad job by laying her remaining eggs in another nest. Either of these could have driven the parasitism we observed. The song thrush's decision to use a blackbird nest could have resulted from either a recognition error or a lack of available conspecific nests. Given the high rates of foreign egg rejection by blackbirds in this population (Hale and Briskie, 2007), and the unsuccessful feeding and eventual ejection of the chicks we later recorded, an adaptive basis for choosing a blackbird host seems unlikely. Another song thrush would likely have made a better surrogate.

Hale and Briskie (2007) found that most blackbirds rejected non-mimetic model eggs of New Zealand's obligate brood parasites, the shining bronze-cuckoo *Chrysococcyx lucidus* and long-tailed cuckoo *Eudynamys taitensis*. They interpreted this behaviour as an evolutionary relic of selection to reject foreign eggs in response to parasitism by British common cuckoos, as there are no records of blackbird nests parasitised by either New Zealand species of cuckoo. In light of their study, it was a surprise to observe this blackbird accepting and incubating song thrush eggs. If other blackbirds behave similarly it would suggest that Hale and Briskie's (2007) model eggs were rejected not because they were foreign but specifically because they were cuckoo eggs. This conclusion cannot be drawn from our one observation, but tests on more blackbird nests would be illuminating. A more likely explanation is that the blackbird host observed here represented one of the minority (16%) of individuals that Hale and Briskie (2007) found were acceptors, and that most would have rejected the song thrush eggs. Potential observers would be unlikely to notice this, which might explain the lack of similar records in the literature. This interpretation is further supported by recent evidence that blackbirds are often able to recognise and reject even the foreign eggs of other blackbirds (Samas et al., 2014).

Having accepted the song thrush eggs, the blackbird incubated them until each hatched, on 4th and 5th December. After this, on 6th December, only two blackbird eggs were found in the nest. We did not find the missing blackbird egg around the nest and can only speculate on reasons for its disappearance. While hatchling cuckoos are known to eject the eggs of their hosts (Davies, 2000), song thrushes are not specialist brood parasites and the chicks are unlikely to have been capable of this. It may have been depredated, but this also seems unlikely as the remaining eggs and two song thrush chicks were unharmed. This suggests it was removed by the female, perhaps because of damage or because the chick hatched and then died.

Each song thrush chick went missing from the nest at three days old. Again, we did not find them around the nest, but consider predation an unlikely explanation as they were lost separately; more probably they died and were removed by the female. It is possible that at three days old, they had grown sufficiently to be recognised as foreign chicks. Some birds are able to recognise parasitic chicks and evict them from the nest (Sato et al., 2010; Tokue and Ueda, 2010). As far as we know this ability has not been tested in blackbirds. Alternatively, the chicks may have been ejected because they had died. Our footage showed the female had difficulties provisioning the chicks, perhaps due to a mismatch in feeding cues or dietary requirements between the species. Song thrush chicks usually remain still and silent when approached; our observations of hungry chicks begging when we visited the nest support the interpretation that the nestlings starved and were subsequently removed by the blackbird as she would her own dead offspring.

The female blackbird's desertion of her remaining two eggs at first glance may seem odd. However, it is likely that the chicks hatching stimulated her to stop incubating. Their subsequent death may have been the cue for this desertion: the death of a nest's only chicks generally constitutes a nest failure, making it adaptive for a parent to abandon that breeding attempt and conserve resources or nest again.

This rare record of parasitism of a blackbird by a song thrush has illustrated many of the adaptively puzzling behaviours associated with brood parasites. A better explanation of these events would only be possible with a properly replicated study, investigating whether blackbird females consistently accept song thrush eggs and how they respond to song thrush chicks. Nonetheless, our observations provide evidence that parasitism between these two species can occur in the wild, and thus could play some role in shaping the evolution of their breeding behaviour.

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