

**Cooperative breeding in the Noisy Miner
(*Manorina melanocephala*): The role of
genetic relatedness, sex, extra-pair
paternity and acoustic signals**

A Dissertation submitted by

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For the degree of Doctor of Philosophy

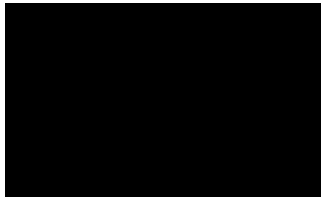


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Declaration

I certify that the ideas, experimental work, results, analyses, software and conclusions reported in this dissertation are entirely my own effort, except where otherwise acknowledged. I also certify that the work is original and has not been previously submitted for any other award, except where otherwise acknowledged.

Ahmad Barati



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Summary

Vertebrates use different reproductive strategies and consequently, diverse forms of parental care to improve offspring survival and thus their lifetime reproductive success. A relatively rare form of reproduction and parental care, which is known as cooperative breeding, evolves when individuals other than breeders (i.e. helpers) provide alloparental care to offspring. Given that any form of parental care is likely costly, helping to raise the offspring of others appears to be against the expectations of natural selection theory. Hence, factors that underpin the evolution and maintenance of cooperative breeding have attracted much research attention during the last few decades.

In this thesis, I describe my research on the dynamics of cooperative breeding and the importance of various determinants for its evolution in a native Australian passerine bird, the noisy miner (*Manorina melanocephala*). Why do some individuals assist in raising the offspring of others? Do breeding females choose their nest site to maximise the amount of aid received from helpers? How does a helper's sex or the presence of extra-pair (EP) mating influence the dynamics of helping? Does acoustic communication allow helpers to aid young in pathways that don't involve provisioning? These questions are some of the important aspects of cooperative breeding that were addressed using data collected from three years of field observations of colour-marked populations, combined with molecular analyses.

Female noisy miners tended to select areas close to open and fragmented patches for their nest site, a selection that consequently may facilitate more efficient foraging, nest defence and accessibility of nests for potential helpers. Overall, in this system, kinship or relatedness between individuals appeared to be the main reason why helpers aided offspring, as they increased their provisioning rate with increased genetic relatedness to breeding pairs. Therefore helpers primarily gain indirect fitness benefits through helping their relatives. Despite this, unrelated helpers also provisioned young, so other types of direct benefits, such as group augmentation,

might also be accrued by helpers and play a role in maintaining cooperatively breeding in this species.

In this system, helping behaviour seems to be closely associated with sex-related dispersal patterns and an overall male-biased population sex ratio, with helpers consisting of mainly philopatric males (93 % helpers). Given this, despite investment in male-biased broods appearing to offer higher fitness returns for both breeders and helpers, brood sex ratio did not influence the amount of food provided at a nest by either the breeding pair or their helper contingent. This finding could be due to an inability of provisioners to recognise nestling sex, or potential future costs that might be imposed upon philopatric males such as increased competition for resources and/or mating opportunities from offspring once they reach maturity.

Further, I examined the genetic mating system of this species and showed that: a) there is tendency to avoid copulation with genetically related individuals, with 86% of social breeding pairs being significantly less related to each other than the general population and b) 27% of broods contained extra-pair offspring and 14% of nestlings were sired by males other than the putative breeding male. However, extra-pair mating occurred independent of the degree of relatedness between members of the social breeding pair at a given nest. In addition, extra-pair mating did not lead to greater helper recruitment at nests, as successful extra-pair males did not provision at nests after they obtained paternity.

Finally, I show that noisy miner nestlings not only respond effectively to intraspecific alarm calls by ceasing vocalisation production, but also might have the ability to differentiate between terrestrial and aerial alarm calls of conspecifics, as nestlings suppressed begging signals for longer in response to terrestrial rather than aerial alarm calls broadcast near the nest. This demonstrates a possible novel form by which helpers aid offspring, as noisy miners routinely give different alarm calls to various threats and can warn nestlings of the presence and the type of danger.



Please be advised that this Thesis contains chapters which have been either published or submitted for publication:

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Chapters 1 and 7

Table of Contents

Declaration	i
Acknowledgements.....	ii
Summary.....	iv
Chapter 1 General Introduction: Cooperative breeding in avian systems....	1
Reproductive strategies and cooperative breeding.....	2
Different forms of cooperative breeding	5
Why do helpers help?.....	6
Study system.....	10
Study species.....	10
Breeding biology	11
Social organisation	11
Study objectives	14
Nest site selection and cooperative breeding.....	14
Direct and indirect benefits of helpers provisioning effort.....	15
Sex impacts on cooperative behaviour	16
Extra-pair mating and cooperative breeding.....	17
Warning offspring about danger as a form of helping behaviour?.....	18
Ethical notes	19
References.....	20
Statement of originality.....	27
Statement of Authors' contribution	28
Chapter 2 Fragmentation in eucalypt woodlands promotes nest-tree occupancy by a despotic species, the noisy miner (<i>Manorina melanocephala</i>)	29
Abstract.....	30
Introduction	31
Methods.....	34
Study area.....	34
Field data collection and habitat measurements.....	35
Statistical analysis.....	37
Results	38
Factors influencing nest tree selection in miner colonies.....	38
Factors influencing nest placement within trees by female noisy miners.....	40
Discussion	43
Preferred vegetation characteristics for nest tree selection by female noisy miners	43
Preferred characteristics of nest sites by female noisy miners	44
Habitat fragmentation, woodland avifauna and management considerations.....	45
Conclusion.....	47
References.....	48
Statement of originality.....	54
Statement of Authors' contribution	55
Chapter 3 Alloparental care in a highly social bird with a mixed- relatedness helper contingent.....	56
Abstract.....	57
Introduction	58
Methods.....	62
Study areas.....	62

Bird banding and general fieldwork	62
Quantifying nest attendant food delivery to broods	64
General molecular methods.....	65
Molecular sexing and genotyping.....	66
Parentage assignment.....	69
Genetic relatedness	70
Statistical analyses.....	72
Results	76
Disturbance effects on provisioning rate, prey size, type and biomass delivered... ..	76
Impact of brood age on provisioning behaviour	77
Helper age, sex and relatedness to the breeding pair	80
Does genetic relatedness influence helper provisioning rate?	81
Influence of status and age on total biomass provided to broods	83
Discussion	86
Provisioning rate, prey size and proportion of lerp increased with nestling age ...	86
Noisy Miner helpers are primarily adult males	87
Kin selection and potential mechanisms for kin discrimination.....	89
Group augmentation: a potential direct benefit for unrelated helpers	91
Conclusion.....	92
References.....	93
Statement of originality.....	101
Statement of Authors' contribution	102
Chapter 4 Does nestling sex influence helper effort in a sexually dimorphic bird with male-biased philopatry?.....	103
Abstract.....	104
Introduction	105
Methods.....	109
Study populations.....	109
Fieldwork and behavioural observations	109
Molecular methods and assignment of brood and adult sex ratio.....	111
Assignment of the breeding pair at each nest	112
Genetic relatedness	113
Statistical analyses.....	114
Results	117
Nestling and adult sex ratio in different colonies.....	117
Nestling body mass at hatching and subsequent development trajectories	118
Adult sexual dimorphism.....	122
Variations of provisioning rate according to brood sex ratio.....	122
Discussion	125
Offspring sex ratio.....	125
Helpers do not bias investment according to brood sex ratio, regardless of their relatedness to breeders.....	125
Breeding pairs do not preferentially provision broods according to offspring sex ratio.....	127
The costly sex and helper provisioning effort.....	128
Is there an acoustic cue that might facilitate nestling sex determination by either parents or helpers?.....	129
Conclusion.....	130
References.....	131
Statement of originality.....	136
Statement of Authors' contribution	137

Chapter 5 Extra-pair paternity and inbreeding avoidance in a cooperatively breeding bird, the noisy miner (<i>Manorina melanocephala</i>)	138
Abstract	139
Introduction	141
Methods.....	145
Study populations and general fieldwork.....	145
Observations of provisioning behaviour	145
Molecular methods and genetic relatedness	146
Parentage analysis and the identification of extra-pair (EP) mating	147
Statistical analysis.....	149
Results	151
Mate choice and genetic relatedness of breeders and male helpers	151
Genetic relatedness of breeders and helpers in relation to extra-pair mating	152
Helper attendance and age/sex structure according to the presence of extra-pair mating.....	154
Provisioning response to extra-pair mating according to nest attendant status	154
Discussion	156
Frequency of extra pair offspring in the noisy miner system.....	156
Inbreeding avoidance in noisy miners	158
No evidence for polyandry as a means by which females enhance help provided to broods.....	160
Response of cuckolded males to female EP mating	161
Conclusion.....	163
References.....	164
Statement of originality.....	173
Statement of Authors' contribution	174
Chapter 6 Nestlings reduce their predation risk by attending to predator-information encoded within conspecific alarm	175
Abstract	176
Introduction	177
Methods.....	180
Study population and general methods.....	180
Stimuli recording and preparation	181
Playback experiment design.....	182
Acoustic analysis of brood begging in response to stimulus presentation	184
Spectrographic cross-correlation to determine stimuli similarity.....	185
Statistical analyses.....	187
Results	189
Changes in begging rate in response to different playback stimuli	189
Changes in the spectral structure of begging vocalisations during playbacks.....	192
Similarity of stimulus signals across different vocalisation types.....	193
Discussion	194
Changes in the begging rate and suppression time in response to playback.....	194
Changes in begging acoustic structure in response to broadcast of different call types	196
Responses of nestlings to different interspecific calls.....	197
Conclusion.....	199
References.....	200
Statement of originality.....	205
Statement of Authors' contribution	206

Chapter 7 General Discussion	207
Overview and main findings.....	208
Nest-site selection may help facilitate cooperative breeding.....	208
Multiple benefits of helping drive noisy miner cooperative breeding.....	210
Helping irrespective of brood sex ratio, despite male offspring likely yielding greater benefits to helpers.....	211
No evidence for an adaptive function of extra pair copulation.....	213
Evidence for a new pathway for helpers to provide alloparental care?.....	214
Closing remarks and further directions.....	215
References.....	216
Statement of originality.....	220
Statement of Authors' contribution	221

General Introduction: Cooperative breeding in avian systems



Reproductive strategies and cooperative breeding

Natural selection theory predicts that individual behavioural traits are selected to maximise individual fitness benefits through increased genetic contributions to subsequent generations (Darwin 1859, Endler 1986, Fisher 1930). Hence, the reproductive behaviour and strategy of individuals evolves according to species-specific trade-offs between the associated costs and benefits of particular strategies, with a net result being a beneficial outcome in evolutionarily stable reproductive forms (Ricklefs 1977; Maynard-Smith 1978).

These trade-offs can be seen in reproductive strategies, with the two most extreme forms being known as the r- and k-selected strategies. When species live in highly unpredictable environments, in the absence of density effects and competition, organisms put maximum effort into producing as many progeny as possible, the r-selected strategy (Pianka 1970). At the other extreme, if density is maximal and available habitat is saturated with organisms, the optimal strategy in this high competition environment is to direct energy towards the production of fewer but more fit offspring (Pianka 1970; Stearns 1976). In this k-selected strategy, parents provide substantial postnatal care to the offspring. For example, in mammals and birds one or two parents provide care for offspring including nourishment of the developing embryo or providing food to the growing young prior to independence (Royle et al. 2012). Parental care is costly, therefore the evolution of parental care should only be favoured when the benefits of caring outweigh the associated costs on parental survival or future reproduction (Clutton-Brock 1991). Together with other environmental conditions, parental care leads to

relatively higher survival rates among offspring when compared to offspring of species that do not exhibit any form of parental care (Clutton-Brock 1991; Royle et al. 2012). Given this, parental care is a behavioural and evolutionary strategy that improves the fitness of offspring and thus the probability of their survival (Clutton-Brock 1991; Royle et al. 2012).

Parental care behaviour is very diverse among species, both in terms of the types of help provided, and the role of parents. Depending on the life-history traits and mating system of species, parental care might be provided by male, female or both parents (Clutton-Brock 1991; Westneat & Sherman 1993). A rarer form of reproduction and parental care is known as cooperative breeding, where individuals other than breeders (i.e. helpers) also provide parental care to the offspring (Brown 1987). Cooperative breeding occurs in most animal taxa, such as fish (Taborsky 1984), mammals (Solomon & French 1996) and is known to occur in 9% of bird species (Cockburn 2006, Jetz & Rubenstein 2011). Among all taxa that show any form of cooperative breeding, birds have received the greatest amount of research attention, with numerous studies investigating the factors that favour helpers providing aid in these systems (Box 1).

Despite a large amount of research into this reproductive strategy, a consensus on the precise benefits of helpers in many species of birds remains unclear (Koenig & Dickinson 2004, 2016). Some authors have argued that nestling provisioning by helpers is not adaptive, and could be driven by the same stimulus-response mechanisms that leads to parental feeding behaviour (Jamieson & Craig 1987). However, it has been shown that this argument is insufficient to explain high variability in the evolutionary significance of helping behaviour (Emlen et al. 1991) and its associated costs (Heinsohn & Legge 1999).

Box 1. Hypotheses proposed to explain the adaptive benefits of helping behaviour with example avian taxa species shown to support the hypothesis		
Hypothesis	Type of benefits	Example species
Kin selection theory (Hamilton 1964)	Increasing productivity of relatives	bell miner ¹ (<i>Manorina melanophrys</i>), long-tailed tit ² (<i>Aegithalos caudatus</i>), seychelles warblers ³ (<i>Acrocephalus echellensishaare</i>), chestnut-crowned babbler ⁴ (<i>Pomatostomus ruficeps</i>), florida scrub jay ⁵ (<i>Aphelocoma coerulescens</i>), green wood- hoopoe ⁶ (<i>Phoeniculus purpureus</i>)
Social Prestige (Zahavi 1974)	By investing more, helpers signal quality to potential future partner	arabian babblers ⁷ (<i>Turdoides squamiceps</i>), sociable weaver ⁸ (<i>Philetairus socius</i>) (but see the text below)
Parentage acquisition (Magrath & Whittingham 1997)	Helpers gain breeding position and obtain direct benefit	white-browed scrubwrens ⁹ (<i>Sericornis frontalis</i>), western bluebird ¹⁰ (<i>Sialia mexicana</i>)
Pay-to-stay (Kokko et al. 2002)	Helpers benefit staying in the territory but must 'pay rent' in the form of helping	common babbler ¹¹ (<i>Turdoides caudatus</i>) superb fairy-wrens ¹² (<i>Malurus cyaneus</i>)
Group augmentation (Kokko et al. 2001)	Increased group size will actively or passively benefit helpers	acorn woodpecker ¹³ (<i>Melanerpes formicivorus</i>) arabian babblers ¹⁴ (<i>Turdoides squamiceps</i>) ¹⁵ florida scrub jay ⁵ (<i>Aphelocoma coerulescens</i>),
References		
¹ Wright et al. 2010; ² Nam et al. 2010; ³ Komdeur 1994; ⁴ Browning et al. 2012; ⁵ Woolfenden & Fitzpatrick 1990; ⁶ Ligon & Ligon 1990; ⁷ Zahavi (1974); ⁸ Doutrelant & Covas (2007); ⁹ Magrath & Whittingham (1997); ¹⁰ Dickinson 2004); ¹¹ Gaston (1978); ¹² Mulder & Langmore (1993); ¹³ Stacey & Ligon. (1987); ¹⁴ Ridley (2012); ¹⁵ Woolfenden & Fitzpatrick (1978)		

The types of benefits that helpers gain through helping, have been a core topic for biologists since the discovery of this form of breeding (Cockburn 1998). Among all types of benefits that helpers might gain, kin selection and inclusive fitness theory (Hamilton 1964) is assumed to be the main driver of helping behaviour, and has received a high

amount of research attention (McDonald 2014). This theory implies that helpers gain indirect benefits by improving the fitness of their relatives or individuals with whom they share their genes (Hamilton 1964; Maynard-Smith 1964). Other benefits of help, however, are not well established and have not received such broad support, so determining what other benefits might be occurring is challenging, with a broad-scale, over-arching direct benefits hypothesis concerning why unrelated helpers help yet to be determined (McDonald 2014).

Different forms of cooperative breeding

As one of the rarer reproductive strategies, "cooperative" breeding occurs when more than two individuals of the same species direct care towards the rearing of young (Emlen 1986, Brown 1987). These help-providers (hereafter 'helpers'), boost reproductive success in many cooperatively breeding species, to the extent that some cannot breed successfully as unassisted pairs (Blackmore & Heinsohn 2007).

This breeding system is usually categorised into two different types. The first and most common type of cooperative breeding system is known as *nuclear families*, in which past offspring from previous breeding attempts stay in their natal territory and help their parents rear subsequent broods (Stacey & Koenig 1990). This type of cooperative breeding is usually referred to as a *helper-at-the-nest system* in which non-breeding individuals contribute to the helping behaviour without having a paternity or maternity share in the brood (Emlen 1986). However, helpers sometime have paternity or maternity in the brood and are not necessarily related to breeders. This scenario is the second most common form of cooperative breeding and is referred to as *communal breeders*, systems in which paternity share is the main driver of helping behaviour

(Emlen 1986). In these systems helpers at a given brood might have shared maternity, paternity, or both (Stacey & Koenig 1990), although a predominance of male helpers and shared paternity is the most common scenario, also known as *cooperative polyandry* (Brown 1987). For example in pukekos (*Porphyrio porphyria*), helping behaviour of unrelated males is positively linked with their paternity status in the brood (Jamieson et al. 1994). However, it worth noting that, further development in the molecular analysis of parentages and geographical expansions in the studies of cooperative breeding species, have revealed that cooperative breeding systems might be more complex and not limited to only the two main forms discussed above (Cockburn 1998).

Why do helpers help?

A core question when examining cooperative breeding is ‘why do helpers provide help?’. According to natural selection theory, individuals are expected to maximise their own reproductive success and survival (Darwin 1859, Endler 1986), therefore, the seemingly altruistic nature of helping others appears to be in contrast with this prediction (Emlen & Vehrenkamp 1983). The evolution of cooperative breeding remained largely unexplained until 1964, when the theory of kin selection (Hamilton 1964; Maynard-Smith 1964) provided an important solution and development for the understanding of cooperative breeding. According to the kin selection hypothesis, helpers accrue inclusive fitness benefits through aiding relatives with whom they share their genes (Hamilton 1964). Accordingly, helpers gain indirect fitness by increasing the reproductive success of their relatives and therefore the frequency of their own genes in subsequent generations. The frequency of shared genes could either be a result of direct descendants (e.g. parent-offspring) or even shared genes by chance at the population level, helping

either provides equivalent benefits regardless of the pathway by which common genes were inherited (Riehl 2013).

The theory of kin selection has been focus of a relatively large fraction of studies of cooperative (McDonald 2014). Given this, kin selection received clear support in some bird species such as the bell miner (*Manorina melanophrys*) (Wright et al. 2010), long-tailed tit (*Aegithalos caudatus*) (Nam et al. 2010) and seychelles warblers (*Acrocephalus echellensishaare*) (Komdeur 1994) (see more examples in Box 1) showing that helping effort is positively associated with kinship and the degree of genetic relatedness. In addition, recent meta-analyses demonstrated a broad cross-species patterns of the relationship between kinship and helping behaviour in cooperatively breeding bird species (Green et al. 2016).

Despite this support, and the important explanation for cooperative breeding, it is not a universal explanation for helping behaviour and is not able to explain helping behaviour provided between unrelated individuals. Perhaps surprisingly given the high level of support for kin selection theory, helping involving unrelated helpers to broods is common in cooperative breeding systems. For example in 44% of cooperatively breeding bird species, at least some helpers are unrelated to the breeders (Cockburn 1998; Riehl 2013). Thus many other, and usually more controversial (Clutton-Brock 2002, Koenig & Dickinson 2004, 2016), hypotheses have been proposed to account for help between non-relatives. These are collectively referred to as 'direct' benefit hypotheses, and cover a range of potential pathways by which helpers might gain fitness returns from provided aid (summarised in Box 1).

Among proposed direct benefits, the best-supported group of hypotheses concern ‘group augmentation theory’. Under this paradigm, when helpers benefit from an increased group size, individuals are expected to help raise young even they are unrelated to them as it ultimately leads to an increase in the helper's group size (Kokko et al. 2001). According to this hypothesis, helpers gain fitness benefit either by being aided in the future by the nestlings that they currently feed (Ligon & Ligon 1978), or through increasing the overall success of the group and thus the opportunities for future breeding and/or accessing a greater amount of resources (Brown 1987; Kokko et al. 2001). For example, in the white-winged trumpeters (*Psophia leucoptera*), females cannot breed successfully without being aided, therefore, unrelated females provide help and subsequently receive aid when they inherit the breeding position of dominant females when they perish (Sherman 1995).

While group augmentation is the most frequently invoked cooperatively breeding hypothesis concerning direct benefits in birds (McDonald 2014), other forms of direct benefits have also been proposed. For example, individuals may be selected to help in order to be allowed access to communal resources by breeders (the ‘pay-to-stay’ hypothesis; Kokko et al. 2002). Helpers are also proposed to gain social status or prestige that increases their future access to mates through helping (Zahavi 1995), although its importance has been questioned (Wright 2007) and unequivocal support for the hypothesis has not been found in any cooperatively breeding bird species to date (e.g. McDonald et al. 2008). Finally, if unrelated males help, then they may be more likely to achieve breeder status and mate with the female breeder when her social partner dies or in the case of divorce (Piper et al. 1995).

While cooperative breeding in birds might involve help accrued in a variety of contexts, the majority of studies have measured provisioning behaviour during the chick rearing stages (Cockburn 1998). Although provisioning nestling is probably the most obvious and important form of helping in avian systems, other forms of helping may also be critical. For example helpers can contribute to anti-predatory behaviour via mobbing potential predators (Arnold 2000; Arnold et al. 2005) or even participate in the antiparasite behaviour (Pacheco et al. 2008). Given this, some of these cooperative breeding hypotheses, can be untangled through careful choice of focal species, where cooperation is important and frequent, but is not limited to involving kin only and provisioning behaviour. Noisy miners (*Manorina melanocephala*) provide an excellent opportunity to explore some of the outstanding issues concerning cooperative breeding systems due to their highly complex social organisation (see below). In this thesis, I will first address patterns of nest placement and its implications for cooperative breeding and the ecological impacts of miner colonies on other taxa (**Chapter 2**). In the following chapters, three main aspects of the miner breeding system will be explored, including the importance of kinship on the provisioning effort of helpers (**Chapter 3**), the effect of brood sex ratio on helping decisions (**Chapter 4**) and the level of extra-pair fertilisations and their impact on this cooperative breeding system (**Chapter 5**). Finally, **Chapter 6** examines the mechanisms of acoustic communication between conspecifics and offspring in order to mitigate predation risk, and the possibility of another form of modality by which helpers might aid offspring that does not involve provisioning at the nest. Below I describe this study system and the outline of each chapter in more detail.

Study system

Study species

The focal species chosen for this study is the noisy miner, a honeyeater from the Meliphagidae family. Honeyeaters comprise about 160 species endemic to Australia, New Zealand, and the south-west Pacific region, of which, 66 species occur in Australia, including four from the genus *Manorina* (Higgins et al. 2001). All *Manorina* species including the yellow-throated miner (*M. flavigula*), bell miner (*M. melanophrys*), noisy miner and the endangered black-eared miner, (*M. melanotis*) breed cooperatively (Ford et al. 1988). The noisy miner is the most common *Manorina* species and is endemic to wooded country of south and eastern Australia (Higgins et al. 2001). This species is an cooperative breeder that lives in aggregations of up to several hundred birds. Colony members cooperate over various contexts, including the feeding of young (Higgins et al. 2001) or mobbing predators (Arnold 2000) in one of the most complex avian social systems known (see details below). Noisy miners vigorously defend their colony's territory not only against potential predators, but also against other small or medium-size birds by moving cooperatively to chase, attack and expel these from within the colony boundary (Dow 1977; Clarke & Oldland 2007). Given this important aspect of noisy miner behaviour, although noisy miners have been focus of numerous studies in the last two decades, most studies have examined their negative effect this aggression has on the native avifauna (Maron 2009), although there has been some previous research conducted on their cooperative breeding (e.g. Dow & Whitmore 1990).

Breeding biology

Noisy miners can potentially breed year-round and are flexible with regards to breeding season across much of their geographic distribution, breeding when conditions are apparently favourable, with egg-laying recorded from June through to April (Higgins et al. 2001). Only breeding females built the nest, which is deep and cup-shaped (Fig 1-2) and usually located on a eucalypt tree. Females develop an obvious brood patch before breeding, which can be used as an indication of breeding status and for sexing in the field (Fig 1-2). Hatching occurs asynchronously, usually with one-day intervals, with nestlings displaying typical altricial characteristics of a naked body and closed eyes at hatching (Fig. 1-2).

Social organisation

Noisy miners have a complex social system, occurring in stable, year-round colonies (Dow 1979). Within colonies, individuals form smaller groups known as 'coteries', which are composed of birds that involve in similar activities such as group foraging. However, when mobbing potential predators, they usually form 'coalitions' that can consist of individuals from multiple coteries or 'tribes' (Fig 1.1). Coalitions have flexible and dynamic membership that varies across time and space within a colony (Dow & Whitmore 1990). The sex ratio of noisy miners within a colony is typically male-biased as a consequence of female-biased dispersal, with females establishing and defending breeding territories within the colony (Dow & Whitmore 1990). Coalitions usually consist of males, but if they form within the activity space and territory of a female, then they may also join. As shown in Fig.1.1, coteries are the most stable unit of noisy miner

social organisation (Dow 1979). The activity space of females are distinguishable and usually do not overlap, especially during the breeding season when females are particularly territorial towards other females in the colony. In contrast, males do not defend a distinct spatial territory from other males (Dow & Whitmore 1990).

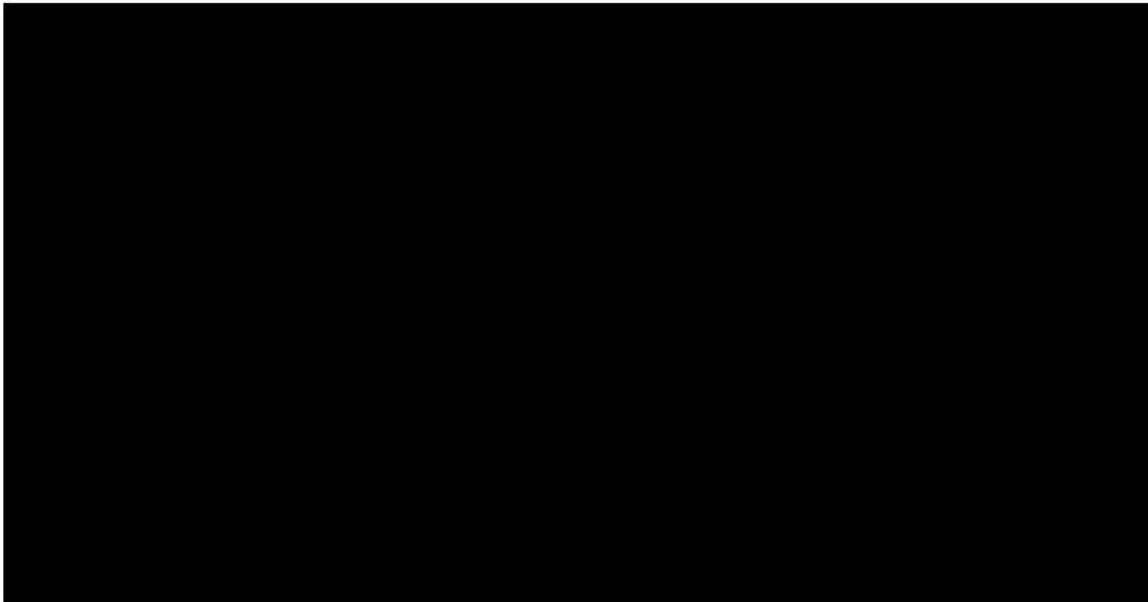


Fig 1.1. Schematic of the different levels of noisy miner social organisation (Source: Dow 1979).

Box 2. Study populations and fieldwork

Details of methods are presented in each relevant chapter. Briefly, the focal populations for this study were three colonies of noisy miner at the Newholme (Fig 1.2a.), Dumaresq Dam (Fig 1.2b) and Baker's Creek Travelling Stock Route, Armidale NSW. During 2013-2015, adult noisy miners ($n=288$) were captured using mist-nets (Fig 1.2c) or occasionally using a baited cage and were marked with a numbered metal band and a unique combination of coloured leg bands (Fig 1.2d-e). A small blood sample from all birds was taken for genetic analysis. Regular nest-searching were conducted from mid-August each year and once nests were found (Fig 1.2f), they were marked ($n=82$) with numbered plastic tags. Laying-, hatching- and fledgling dates, and breeding status were determined from regular nest checks. Nestlings were banded at the nest before fledging as per adults (Fig 1.2g). After hatching, behavioural observations were conducted at each nest from a hide placed in a distance of 25-50 m from the nest (Fig 1.2h) using binoculars or a telescope, with all nest observations audio and video-recorded for further analysis.



Fig. 1. 2. Example photos showing study sites and fieldwork including Newholme (a) and Dumaresq Dam (b) areas, mist nesting (c), banded noisy miners (d-e), noisy miner nest with egg and newly hatched nestlings (f), a banded nestling (g) and the observation hide (h). (Photos: A. Barati)

Study objectives

Nest site selection and cooperative breeding

An important determinant that might influence reproductive success in birds is the selection of a suitable nest site. This selection is a critical decision because it ultimately influences breeding success (Martin 1995). Nest site selection can primarily be influenced by factors such as protection from predators (Etterson et al. 2007), accessibility to foraging areas (Hafner & Fasola 1992) or protection from extreme weather conditions (Martin 1995). Among these, reducing predation risk is assumed to be the most important priority when birds decide upon a nest site (Martin 1995), although different strategies might evolve in birds to reduce the risk of nest failure, such as increased nest concealment to reduce predation risk (Collias & Collias 1984). However, in cooperatively breeding species, an ideal nest location must not only provide some protection against predation risk, but also facilitate nest accessibility to ensure that the helper cohort attends the nest and provisions offspring. For example, breeding females might select a nest site that is located in more open areas to maximise the probability that helpers will find and attend the nest (Dow & Whitmore 1990). In addition, for tree-nesting species that forage on the ground, nest location might be a compromise that maximises foraging efficiency and thus energy requirements of nest attendants over predation risk. Therefore, a decision on nest site location in cooperatively breeding species is likely a trade-off between concealment whilst maintaining some visibility of the surrounding area to facilitate helper attendance. Given this, **Chapter 2** tests nest placement and its potential implications for cooperative breeding by examining the

patterns of nest-site selection at a colony scale and the finer-scale structure of nest sites chosen.

Direct and indirect benefits of helpers provisioning effort

In birds, genetic relatedness has been found to be an important explanation for helping behaviour (Griffin & West 2003, Koenig & Dickinson 2004, Wright et al. 2010; Preston et al. 2013). However, many studies report substantial levels of helping by non-relatives (Wright et al. 2010) and have not found a positive relationship between genetic relatedness and levels of helper investment (Wright et al. 1999; Canestrari et al. 2005). These suggest that the indirect fitness benefits associated with aiding kin are not the only explanation favouring helping behaviour in some species. Thus, despite kin selection (Hamilton 1964; Maynard-Smith 1964) being a very important explanation of cooperative breeding in many species (see Box 1 for example species), other types of benefits may also be influential in the evolution and maintenance of helping behaviour. An understanding of the genetic benefits resulting from helping behaviour requires investigation of whether helpers gain direct benefits, indirect benefits or both. In **Chapter 3**, I examine how genetic relatedness to breeders shapes the provisioning efforts provided by helpers, and if other direct benefits might be accrued instead of, or in addition to, these. Thus, **Chapter 3** addresses both indirect and direct fitness benefits as drivers of helping behaviour in the noisy miner by quantifying the benefits that helpers accrue when providing aid.

Sex impacts on cooperative behaviour

In addition to genetic relatedness that predicts the contribution of helpers towards the care of offspring, the benefits of helping might also be influenced by the sex of both helpers and offspring. This is because sexes typically have very different dispersal patterns, with one sex typically providing both more aid, but also being more philopatric (Greenwood 1980). When sexes differ in their philopatry, then they may also experience differences in the costs and benefits of providing help. Although the indirect benefits of help accrue regardless of dispersal patterns, these factors are likely to heavily influence most forms of direct benefits. For example, the dispersive sex is less likely to access direct benefits that require an extended presence in the natal territory, such as group augmentation and future breeding opportunities (Wright et al. 2010). This leads to sex-related differences in the benefits of helpers providing aid, and also the probability of repayment of that aid from offspring in subsequent periods. For cooperatively breeding species, the most popular and intensely debated explanation for the possible effects of sex ratio variation in broods is the helper repayment hypothesis (Gowaty & Lennartz 1985). This hypothesis predicts that more investment is directed towards the more beneficial sex. In species with sex-biased philopatry, as is common in many cooperative breeders (Pusey 1987), there is evidence that adults prefer to invest in the philopatric sex, which commonly helps to raise future young (Doutrelant et al. 2004), or the dispersing sex when territory resources are limited (Ridley & Huyvaert 2007). Further, direct benefits such as group augmentation, should depend heavily on offspring sex (McDonald et al. 2010) because only the philopatric sex can provide direct fitness benefits through group augmentation. This is particularly relevant in noisy miners, as females disperse at a young age, whereas males stay in the natal colony their whole life (Higgins et al.

2001). Thus the sexes differ in their repayment potential to helpers and it is therefore anticipated that helpers would invest more heavily in the philopatric sex. **Chapter 4** examines how brood sex ratio influences helper efforts and if there is a tendency to provision the philopatric sex more often than the dispersing sex. The benefits that helpers gain from helping in male- or female-biased broods further depends upon the level of genetic relatedness between helpers and broods, so **Chapter 4** also examines if the level of kinship influences any sex-biased provisioning by helpers.

Extra-pair mating and cooperative breeding

Both males and females in many socially monogamous bird species engage in copulations outside of their social pair bond, a phenomenon known as extra pair (EP) mating. EP mating behaviour is widespread, with 90% of socially monogamous songbirds engaging in EP mating (Griffith et al. 2002). Despite a general assumption that alloparental care evolves in genetically monogamous species (Cornwallis et al. 2010), there are many examples of cooperative breeding species that show some extent of EP mating (Rubenstein 2007a-b). In cooperatively breeding species, EP mating might be challenging, as by engaging in EP mating, females would manipulate the average genetic relatedness of helpers to the resultant brood, particularly in species where helpers are retained offspring from previous broods, thereby reducing the relatedness of broods to helpers (Cornwallis et al. 2010). If cooperation is driven by inclusive fitness benefits (Hamilton 1964), then this reduced genetic relatedness is predicted to influence helper efforts and ultimately the evolution of cooperative breeding (Charnov 1981; Boomsma 2007, 2009). Therefore, in theory, a relationship between the evolution of a species mating system and cooperative breeding dynamics is expected. Given this, information on the level of EP mating and its function and consequences are necessary for a comprehensive understanding of cooperative

breeding in a given species, despite a relatively low amount of research in this area to date. **Chapter 5** examines this area in detail, determining relationships between EP mating and subsequent levels of aid provided by helpers of varying relatedness to broods.

Warning offspring about danger as a form of helping behaviour?

Although helpers can potentially provide various forms of help in cooperatively breeding birds, the majority of studies to date have focused on allofeeding (Emlen 1991; Cockburn 1998), with this form of helping further assumed to be the main form of helpers' contribution to cooperative systems (Stacey & Koenig 1990). However, other types of help may be equally important. For example, helpers contribute to antiparasite behaviour in the bell miner (*Manorina melanophrys*; Pacheco et al. 2008) and to the mobbing of potential predators in the siberian jay (*Perisoreus infaustus*) (Griesser & Ekman 2005). The fact that in most cooperatives species, helpers vary in the level of their provisioning effort (Cockburn 1998), raises the possibility that helper contributions to other tasks might balance the overall level of alloparental effort across individuals that is provided to a brood. Given this, complete understanding of the benefits that helpers might provide needs to be quantified across other potential pathways in addition to alloparental provisioning. One important non-provisioning contribution of helpers may occur in the form of anti-predatory behaviour (Griesser & Ekman 2005). This behaviour usually occurs via the 'mobbing' of predators, which is an assemblage of individuals around a potentially dangerous predator that cooperatively attack and/or harass a predator in order to expel them from the immediate area (Curio 1978). For example, in the noisy miner, this involves displays such as loud repetitive calling, swooping and bill clattering (Arnold, 2000). However, helpers can also indirectly warn offspring of the presence of nearby danger

using acoustic signals. The latter form of anti-predatory behaviour is especially important if offspring are highly vocal, as noise around the nest area can increase predation risk regardless of mobbing behaviour (McDonald et al. 2009). The evolution of this form of help requires a mechanism for the effective acoustic communication between helpers and offspring. This communication would particularly benefit from transmitting information on both the presence of a danger, and also its type and the subsequent urgency of any required response. **Chapter 6** examines these mechanisms of acoustic communication between offspring and helpers, using conspecific alarm calls as they can provide information on the potential threat to nestlings such as the presence of a predator. Noisy miners effectively use group-mobbing tactics to protect the nest (Arnold 2000), but they also give two distinct, functionally referential alarm signals to potential predators (Holt et al. 2016, Farrow et al. 2017). Terrestrial predators or perched raptors elicit the chur alarm call, whilst helpers produce aerial alarm calls in the presence of flying predators. Therefore, in **Chapter 6**, I test how effectively miner nestlings respond to these different types of alarm calls from nest attendants and familiar conspecifics.

Ethical notes

This study was conducted under protocols approved by University of New England Research Committee (Protocol no. AEC13-142). The project was also approved by New South Wales National parks and Wildlife Service (licence SL100314) and the Australian Bird and Bat banding Scheme (A2259). I minimised the time spent near the nest wherever possible to reduce disturbance.

References

- Arnold, K. E., Owens, I. P. F., Goldizen, A. W. (2005). Division of labour within cooperatively breeding groups. *Behaviour*, 142, 1577-1590.
- Arnold, K.E. (2000). Group mobbing behaviour and nest defence in a cooperatively breeding Australian bird. *Ethology*, 106, 385-393.
- Blackmore, C. J., Heinsohn, R. (2007). Reproductive success and helper effects in the cooperatively breeding grey-crowned babbler. *Journal of Zoology*, 273, 326-332.
- Boomsma, J. J. (2007). Kin selection versus sexual selection: why the ends do not meet. *Current Biology*, 17, 673-683.
- Boomsma, J. J. (2009). Lifetime monogamy and the evolution of eusociality. *Philosophical Transactions of the Royal Society B*, 364, 3191-3207.
- Brown, J. L. (1987). Helping and communal breeding in birds. Princeton University Press, Princeton.
- Browning, L. E., Patrick, S. C., Rollins, L. A., Griffith, S. C., Russell, A. F. (2012). Kin selection, not group augmentation, predicts helping in an obligate cooperatively breeding bird. *Proceedings of the Royal Society B*, 279, 3861-3869.
- Canestrari, D., Marcos, J. M., Baglione, V. (2005). Effect of parentage and relatedness on the individual contribution to cooperative chick care in carrion crows (*Corvus corone corone*). *Behavioral Ecology and Sociobiology*, 57, 422-428.
- Charnov, E. L. (1981). Kin selection and helpers at the nest: effects of paternity and biparental care. *Animal Behaviour*, 2, 631-632.
- Clarke M. F., Oldland J. M. (2007) Penetration of remnant edges by noisy miners (*Manorina melanocephala*) and implications for habitat restoration. *Wildlife Research*, 34, 253-61.
- Clutton-Brock, T. H. (1991). *The Evolution of parental care*. Princeton Univ. Press, Princeton, NJ.
- Clutton-Brock, T. H. (2002). Breeding together: kin selection and mutualism in cooperative vertebrates. *Science*, 296, 69-72.
- Cockburn, A. (1998). Evolution of helping behavior in cooperatively breeding birds.

- Annual Review of Ecology and Systematics*, 29, 141-177.
- Cockburn, A. (2006). Prevalence of different modes of parental care in birds. *Proceedings of the Royal Society B*, 273, 1375-1383.
- Collias, N. E., E. C. Collias. (1984). *Nest building and bird behavior*. Princeton University Press, Princeton, New Jersey.
- Cornwallis, C. K., West S. A., Davis, K. E., Griffin, A. S. (2010). Promiscuity and the evolutionary transition to complex societies. *Nature*, 466, 969-972.
- Curio, E. (1978). The adaptive significance of avian mobbing. I. Teleonomic hypotheses and predictions. *Zeitschrift für Tierpsychologie*, 48, 175-183.
- Darwin, C. (1859). *On the origin of species by means of natural selection*, or the preservation of favoured races in the struggle for life. London: John Murray.
- Dickinson, J. L. (2004). A test of the importance of direct and indirect fitness benefits for helping decisions in western bluebirds. *Behavioral Ecology*, 15, 233-238.
- Doutrelant, C., Covas R. (2007). Helping has signalling characteristics in a cooperatively breeding bird. *Animal Behaviour*, 74, 739-747.
- Doutrelant, C., Covas, R., Caizergues, A., du Plessis, M. (2004). Unexpected sex ratio adjustment in a colonial cooperative bird: pairs with helpers produce more of the helping sex whereas pairs without helpers do not. *Behavioral Ecology and Sociobiology*, 56, 149-154.
- Dow, D. D. (1977). Indiscriminate interspecific aggression leading to almost sole occupancy of space by a single species of bird. *Emu*, 77, 115-21.
- Dow, D. D. (1979). Agonistic and spacing behaviour of the noisy miner (*Manorina melanocephala*), a communally breeding honeyeater. *Ibis*, 121, 423-436.
- Dow, D. D., Whitmore, M. J. (1990). Noisy miners: variations on the theme of communality. In: Stacey, P. B., Koenig, W. D. (eds) *Cooperative breeding in birds*. Cambridge University Press, Cambridge, pp: 559-592.
- Emlen, S. T. (1986). *Cooperative breeding in birds and mammals*. In: Krebs, J. R., Davies, N. B. (eds) *Behavioural ecology*, 2nd edn. Blackwell, Oxford, pp 301-337.
- Emlen, S. T., Reeve, H. K., Sherman, P. W., Wrege, P. H. (1991). Adaptive versus non-adaptive explanations of behavior: the case of alloparental helping. *American Naturalist*, 138, 259-270.

- Emlen, S. T., Vehrenkamp, S. L. (1983). *Cooperative breeding strategies among birds*. In: Perspectives in ornithology. Brush, A. H., Clark, G. A. Jr. (eds). Cambridge: Cambridge University Press.
- Emlen, S.T. (1991). *Evolution of cooperative breeding in birds and mammals*. In: Behavioural ecology: an evolutionary approach (Krebs, J. R., Davies, N. B. eds.). Oxford: Blackwell Scientific, pp: 301–337.
- Endler, J. A. (1986). *Natural selection in the wild*. Princeton: Princeton University Press.
- Etterson, M. A., Nagy, L. R., Robinson, T. R. (2007). Partitioning risk among different causes of nest failure. *Auk*, 124, 432-443.
- Farrow, L., Doohan S., McDonald, P.G. (2017). Alarm calls are referential and elicit context-specific anti-predator behaviour among receivers in a highly cooperative bird, *Behavioral Ecology*, 28, 724–731.
- Fisher, R. A. (1930). *The genetic theory of natural selection*, 2nd edn. Oxford: Clarendon.
- Ford, H. A., Bell, H., Nias, R., Noske, R. (1988). The relationship between ecology and the incidence of cooperative breeding in Australian birds. *Behavioral Ecology and Sociobiology*, 22, 239-249.
- Gaston A. J. (1978). Ecology of the common babbler (*Turdoides caudatus*). *Ibis*, 120, 415-432.
- Gowaty, P. A., Lennartz, M. R. (1985). Sex ratios of nestling and fledgling red-cockaded woodpeckers (*Picoides borealis*) favor males. *American Naturalist*, 126, 347-353.
- Green, J. P., Freckleton, R.P., Hatchwell B. J. (2016). Variation in helper effort among cooperatively breeding bird species is consistent with Hamilton’s Rule. *Nature Communications* (DOI: 10.1038/ncomms12663).
- Greenwood, P. J. (1980). Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, 28,1140-1162.
- Griesser, M., Ekman, J. (2005). Nepotistic mobbing behavior in the Siberian jay, *Perisoreus infaustus*. *Animal Behaviour*, 69, 345-352.
- Griffin, A. S., West, S. A. (2003). Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. *Science*, 302, 634-636.

- Griffith, S. C., Owens, I. P. F., Thuman, K. A. (2002). Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology*, 11, 2195-2212.
- Hafner, H., Fasola, M. (1992). The relationship between feeding habitat and colonially nesting Ardeidae. In: Finlayson, C. M., Hollis, G. E., Davis, T. J. (Eds.), *Managing Mediterranean Wetlands and Their Birds*, vol. 20. International Wildlife Research Bureau, Special Publication, Slimbridge, UK, pp. 194–201.
- Hamilton, W. D. (1964). The genetical evolution of social behavior. *Journal of Theoretical Biology*, 7, 1-52.
- Heinsohn, R., Legge, S. (1999). The cost of helping. *Trends in Ecology and Evolution*, 14, 53-57.
- Higgins, P. J., Peter, J. M., Steele, W. K. (eds). (2001). *Handbook of Australian, New Zealand and Antarctic birds*, Oxford Univ. Press, Melbourne.
- Holt, J., Barati, A., McDonald, P. G. (2016). The acoustic repertoire of the noisy miner (*Manorina melanocephala*). *Emu*, in press.
- Jamieson, I. G., Craig, J. L. (1987). Critique of helping behaviour in birds: a departure from functional explanations. In *Perspectives in ethology*, vol. 7 (ed. P. Bateson & P. Klopfer), pp: 79-98. New York: Plenum Press.
- Jamieson, I. G., Quinn, J.S., Rose, P.A., White, B. N. (1994). Shared paternity among non relatives is a result of an egalitarian mating system in a communally breeding bird, the pukeko. *Proceedings of the Royal Society B*, 257, 271-277
- Jetz, W., Rubenstein, D. R. (2011). Environmental uncertainty and the global biogeography of cooperative breeding in birds. *Current Biology*, 21, 72–78.
- Koenig, W. D., Dickinson, J. L. (2004). *Ecology and evolution of cooperative breeding in birds*. Cambridge, UK: Cambridge University Press.
- Koenig, W. D., Dickinson, J. L. (2016). *Cooperative Breeding in Vertebrates: Studies of Ecology, Evolution, and Behavior*, Cambridge University Press.
- Kokko, H., Johnstone, R. A., Clutton-Brock, T. H. (2001). The evolution of cooperative breeding through group augmentation. *Proceedings of the Royal Society B*, 268, 187-196.
- Kokko, H., Johnstone, R. A., Wright, J. (2002). The evolution of parental and alloparental effort in cooperatively breeding groups: when should helpers pay to stay? *Behavioural Ecology*, 13, 291-300.

- Komdeur, J. (1994). The effect of kinship on helping in the cooperative breeding Seychelles warbler *Acrocephalus sechellensis*. *Proceedings of the Royal Society B*, 256, 47-52.
- Ligon, J. D., Ligon, S. H. (1978). Communal breeding in green woodhoopoes as a case for reciprocity. *Nature*, 276, 496-498.
- Ligon, J. D., Ligon, S. H. (1990) Green wood hoopoes: life history traits and sociality. In: Stacey, P. B., Koenig, W. D. (eds) *Cooperative breeding in birds*. Cambridge University Press, Cambridge, pp: 31-66
- Magrath, R. D., Whittingham, L. A. (1997). Subordinate males are more likely to help if unrelated to the breeding female in cooperatively breeding white-browed scrubwrens. *Behavioral Ecology and Sociobiology*, 41, 185-192.
- Maron, M. (2009). Nesting, foraging and aggression of noisy miners relative to road edges in an extensive Queensland forest. *Emu*, 109, 75-81.
- Martin, T. E. (1995). Avian life history evolution in relation to nest sites, nest predation, and food. *Ecological Monographs*, 65, 101-127.
- Maynard-Smith, J. (1964). Group selection and kin selection. *Nature*, 201, 145-147.
- Maynard-Smith, J. (1978). *The evolution of sex*. Cambridge: Cambridge University Press.
- McDonald, P. G., Ewen, J., Wright, J. (2010). Brood sex ratio does not affect helper effort in a cooperative bird, despite extreme sex-biased dispersal. *Animal Behaviour*, 79, 243-250.
- McDonald, P. G., Kazem, A. J. N., Clarke, M. F., Wright, J. (2008). Helping as a signal: does removal of potential audiences alter helper behavior in the bell miner? *Behavioral Ecology*, 19, 1047-1055.
- McDonald, P. G., Wilson, D. R., Evans, C. S. (2009). Nestling begging increases predation risk, regardless of spectral characteristics or avian mobbing. *Behavioral Ecology*, 20, 821-829.
- McDonald, P.G. (2014). Cooperative breeding beyond kinship: why else do helpers help? *Emu*, 114, 91-96
- Mulder, R. A. Langmore, N. E. (1993). Dominant males punish helpers for temporary defection in superb fairy-wrens. *Animal Behaviour*, 45, 830-833.
- Nam, K. B., Simeoni, M., Sharp, S. P., Hatchwell, B. J. (2010). Kinship affects investment by helpers in a cooperatively breeding bird. *Proceedings of the Royal Society B*, 277, 3299-3306.

- Pacheco, M. L., McDonald, P. G., Wright, J., Kazem, A. J. N., Clarke, M. F. (2008). Helper contributions to antiparasite behavior in the cooperatively breeding bell miner. *Behavioral Ecology*, 19, 558-566.
- Pianka, E. R. (1970). On r- and K-Selection. *The American Naturalist*, 104, 592-597.
- Piper, W. H., Parker, P. G., Rabenold, K. N. (1995). Facultative dispersal by juvenile males in the cooperative stripe-backed wren. *Behavioral Ecology*, 6, 337-342.
- Preston, S. A., Briskie, J.V., Burke, T., Hatchwell, B. J. (2013). Genetic analysis reveals diverse kin-directed routes to helping in the rifleman (*Acanthisitta chloris*). *Molecular Ecology*, 22, 5027-5039.
- Pusey, A. E. (1987). Sex-biased dispersal and inbreeding avoidance in birds and mammals. *Trends in Ecology and Evolution*, 2, 295-299.
- Ricklefs, R.E. (1977). On the Evolution of Reproductive Strategies in Birds: Reproductive Effort. *The American Naturalist*. 111, 453-478
- Ridley, A. R. (2012). Invading together: the benefits of coalition dispersal in a cooperative bird. *Behavioral Ecology and Sociobiology*, 66, 77-83
- Ridley, A. R., Huyvaert, K. P. (2007). Sex-biased preferential care in the cooperatively breeding arabian babbler. *Journal of Evolutionary Biology*, 20, 1271-1276.
- Riehl, C. (2013). Evolutionary routes to non-kin cooperative breeding in birds. *Proceedings of the Royal Society B*, 280, 20132245.
- Royle, N. J., Smiseth P. T., Kölliker, M. (2012). *The evolution of parental care*. Oxford Univ. Press, Oxford.
- Rubenstein D. R. (2007a). Female extrapair mate choice in a cooperative breeder: trading sex for help and increasing offspring heterozygosity. *Proceedings of the Royal Society B*, 274, 1895-1903.
- Rubenstein, D. R. (2007b). Temporal but not spatial environmental variation drives adaptive offspring sex allocation in a plural cooperative breeder. *American Naturalist*, 170, 155-165.
- Sherman, P.T. (1995). Social organization of cooperatively polyandrous white-winged trumpeters (*Psophia leucoptera*). *Auk*, 112, 269-309.
- Solomon, N. G., French, J. A. (1996). *Cooperative breeding in mammals*. Cambridge: Cambridge University Press.
- Stacey, P. B., Koenig, W. D. (1990). *Cooperative breeding in birds: long-term studies of ecology and behavior*. Cambridge: Cambridge University Press.

- Stacey, P. B., Ligon, J. D. (1987). Territory quality and dispersal options in the acorn woodpecker, and a challenge to the habitat saturation model of cooperative breeding. *American Naturalist* 130, 654-676.
- Stearns, S.C. (1976). Life-history tactics: A review of the ideas. *The Quarterly Review of Biology*, 51, 3-47.
- Taborsky, M. (1984). Broodcare helpers in the cichlid fish (*Lamprologus brichardi*): their costs and benefits. *Animal Behaviour*, 32,1236-1252.
- Westneat, D. F., Sherman, P. W. (1993). Parentage and the evolution of parental behavior. *Behavioral Ecology* 4, 66-77.
- Woolfenden, G. E. and J. W. Fitzpatrick (1978). The inheritance of territory in group-breeding birds. *Bioscience*, 28, 104-108.
- Woolfenden, G. E., Fitzpatrick, J. W. (1990) Florida scrub jays: a synopsis after 18 years of study. In: Stacey, P. B., Koenig, W. D. (eds) Cooperative breeding in birds. Cambridge University Press, Cambridge, pp: 239-266.
- Wright, J. (2007). Cooperation theory meets cooperative breeding: exposing some ugly truths about social prestige, reciprocity and group augmentation. *Behavioural Processes*, 76,142-148.
- Wright, J., McDonald, P. G., te Marvelde, L., Kazem, A. J. N., Bishop, C. (2010) Helping effort increases with relatedness in bell miners, but ‘unrelated’ helpers of both sexes still provide substantial care. *Proceedings of the Royal Society B*, 277, 437-445.
- Wright, J., Parker, P. G., Lundy, K. J. (1999). Relatedness and chick-feeding effort in the cooperatively breeding arabian babbler. *Animal Behaviour*, 58, 779-785.
- Zahavi, A. (1974). Communal nesting by Arabian babblers: a case of individual selection. *Ibis* 116, 84-87.
- Zahavi, A. (1995). Altruism as a handicap-the limitations of kin selection and reciprocity. *Journal of Avian Biology*, 26, 1-3.

Statement of originality

We, the Research PhD candidate and the candidate's Principal Supervisor, certify that the work displayed here including the text, figures and diagrams are the candidate's original work.

Name of candidate: Ahmad Barati

Name/title of Principal supervisor: Dr. Paul G. McDonald



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11/12/2016

Date



Principal supervisor

11/12/2016

Date

Statement of Authors' contribution

We, the Research Master/PhD candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

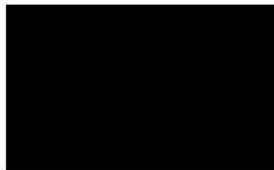
	Author's Name (please print clearly)	% of contribution
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Fragmentation in eucalypt woodlands promotes nest-tree occupancy by a despotic species, the noisy miner (*Manorina melanocephala*)



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Abstract.

The effects of habitat fragmentation as a threat to biodiversity are well known; decreased connectivity can potentially influence population processes and dynamics, resulting in smaller, isolated populations that may not function optimally. However, fragmentation may also increase the amount of edge or ecotone habitat available to open country species, benefiting their populations and enabling them to dominate remnant habitats. Noisy miners (*Manorina melanocephala*) are one such species, occupying eastern-Australian eucalypt woodlands. They are considered a ‘despotic’ species, in that their presence negatively impacts woodland avifauna biodiversity due to their aggressive exclusion of other taxa from occupied areas. Despite this well-known impact, little information exists on the patterns of nest-tree occupancy by noisy miners within eucalypt woodlands. In the current study, we explored the patterns of nest-tree occupancy by noisy miners across two successive years, aiming to identify preferences for breeding areas relative to vegetation structure. Our results show that both habitat fragmentation and the characteristics of individual eucalypt trees in an area influenced nest-tree occupancy. Noisy miners constructed nests in trees near the edge of woodland patches more often than expected. Moreover, the nest tree chosen was a eucalypt that was significantly smaller than randomly selected trees from the surrounding area. The results highlight the importance of habitat management measures that may reduce the suitability of woodland patches as nesting sites for this species, in order to mitigate the severe effects of this despotic edge specialist.

Keywords. Habitat fragmentation, eucalypt woodlands, noisy miner, avian biodiversity, nesting preferences.

Introduction

Habitat fragmentation is a process that divides connected areas into smaller patches, often arising from natural events such as fire (Wright 1974; Pickett & Thompson 1978) or high winds (Foster 1980). However, most habitat fragmentation is caused by the expansion and intensification of human land use, habitat transformation and deforestation (Burgess & Sharpe 1981). Habitat fragmentation and its numerous negative consequences upon biodiversity are well known, as decreased connectivity often impacts population processes and dynamics, typically resulting in isolated and smaller population sizes (Saunders et al. 1991; Harrison et al. 2012). Species affected by habitat fragmentation may experience declines in available habitat size or undergo changes in their biology, behaviour and interactions with other species. Thus, ameliorating the effects of fragmentation would likely benefit affected species by improving not only available habitat and/or habitat quality but also by improving the reproductive output of populations. Because the primary consequence of habitat fragmentation is a disruption and modification in spatial occupancy of individuals and populations, ameliorating the effect of habitat fragmentation requires understanding the spatial processes that underpin population dynamics (Wiens 1989).

When studying habitat fragmentation, it is often assumed that newly fragmented landscapes with subsequently smaller patches will be of a decreased suitability, leading to an overall reduction in available habitat and the area's importance or value for management (Johnson 2001). These effects may be further compounded by an increase in 'edge' habitat where woodland patches meet more open, cleared areas. Edge effects can be diverse and extreme, impacting the physical and biotic characteristics associated with patch boundaries (Murcia 1995; Ewers et al. 2007). Edge effects can also result in profound impacts on species diversity and structure,

community dynamics and ecosystem functioning, such as allowing open country avian species to move into and dominate remaining remnant woodland patches (Howell et al. 2007).

In south-eastern Australian eucalypt woodlands, these fragmented patches are typically colonized by noisy miners (*Manorina melanocephala*), a hyper-aggressive, despotic native honeyeater (family: Meliphagidae), which has been shown to have profound negative effects on woodland avifauna (Grey et al. 1997; Mac Nally et al. 2000; Major et al. 2001; Catterall 2004; Clarke & Oldland 2007; Maron 2007; Montague-Drake et al. 2011). The primary habitat for noisy miners is eucalypt woodland (Maron 2007; Higgins et al. 2001). Noisy miners have benefited from human-induced habitat modifications and habitat fragmentation throughout their range (Longmore 1991; Catterall et al. 1991; Low 1994; Grey et al. 1997), impacting avian biodiversity in cleared, fragmented landscapes but also more intact landscapes where increased road density and other disturbances (such as grazing/burning) provide open areas that facilitate noisy miner colonization of regions (Maron & Kennedy 2007; Eyre et al. 2009).

This is problematic for biodiversity management within fragments, as noisy miners are highly social birds, with a complex internal social structure that exists among birds in colonies that can consist of as many as several hundred birds (Dow 1979a, 1979b; Higgins et al. 2001). One important aspect of noisy miners' social behaviour is that they vigorously defend their colony's territory (hereafter 'colonies') against other small or medium-size birds. They move cooperatively to chase, attack, expel and even sometimes kill other avian taxa to remove them from the colony (Dow 1977; Catterall 2004; Clarke & Oldland 2007; Hannah et al. 2007; Maron 2007; Debus 2008). Given this despotic behaviour, noisy miners reduce avian biodiversity in areas that they

occupy throughout their range (Piper & Catterall 2003; Montague-Drake et al. 2011).

Fragmentation has affected approximately 80–90% of Australia's temperate woodland (Hobbs & Hopkins 1990; Robinson 1993; Major et al. 2001) with eucalypt woodland being affected more than many other ecosystems (Keith et al. 2009). This trend raises concerns about further loss of biodiversity because of the negative effects of despotic noisy miners increasing in abundance in an ideal habitat and highlights the necessity of management targeting noisy miner populations in fragmented landscapes to maximize remaining biodiversity (Montague-Drake et al. 2011; Maron et al. 2013). While numerous studies have highlighted the dominance of noisy miners in these habitats (reviewed in Maron et al. 2013), the patterns and mechanisms of nest-tree occupancy in relation to habitat fragmentation and eucalypt structure remain largely unexplored (but see Maron 2009). Gathering this information is an important aim, as understanding the factors that favour noisy miner fecundity, such as preferred nesting habitat (Martin 1995) is critical for biodiversity conservation.

In the current study, we therefore explored the patterns of nest-tree occupancy by noisy miners using nest site selection data for two consecutive breeding seasons. Specifically, we identified how this aggressive species occupied and selected nest sites in eucalypt woodland, based on nest tree structure and chosen vegetation structure relative to that of unused areas within the colony. We sought to determine the effect of changes in colony-level vegetation structure in nest distribution patterns and to predict how these patterns may influence avifaunal diversity in eucalypt woodlands. We aimed to test the following questions: (1) what microhabitat-scale factors are preferred by females when selecting nest sites? (2) Do breeding females prefer to nest near the edge or centre of woodland patches?

Methods

Study area

This study focused on a colony of noisy miners breeding at the Newholme Field Research Station of the University of New England (UNE) ($30^{\circ} 25' 24''\text{S}$; $151^{\circ} 38' 84 38''\text{E}$, Fig. 2.1); a total area of 1942 ha located 12 km north-west of Armidale, NSW, Australia over two successive breeding seasons (summer of both 2013 and 2014). The study area is characterized as temperate with average maximum and minimum temperatures of around 26°C and 13°C in January, and 12.5°C and 1°C , respectively in July, with average rainfall of 760mm (Reid et al. 1994). The vegetation types of Newholme range from tall grassy forest in the higher elevations, through to grassy forest and tall woodland on the lower slopes where noisy miners breed (Hobbs & Jackson 1977). The most common canopy species is New England peppermint (*Eucalyptus nova-anglica*) (Andrews & Reid 2000), with sparse understorey vegetation dominated by introduced pasture grasses, consistent with the typical habitat of noisy miners (Higgins et al. 2001).

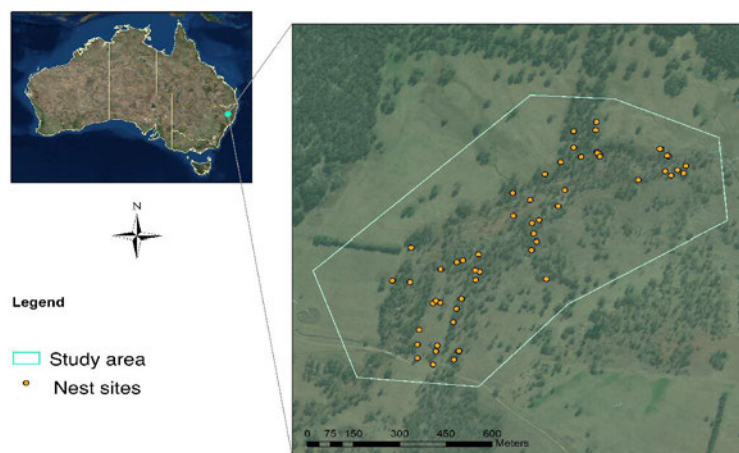


Fig. 2.1. Location of the focal noisy miner colony near Armidale, Australia and the distribution of nests within the colony during 2013-2014 breeding seasons.

Field data collection and habitat measurements

Although noisy miners can potentially breed throughout the year, most breeding occurs from July to November (austral spring); however, the peak breeding time is subject to local environmental conditions and the suitability of habitat for raising young (Dow 1978). In the focal population, nest-building and mating typically begins in early August and the peak nesting period occurred in October–November (Chapter 3). Regular visits to search for nests were made from late June until nesting had ceased each year. Nests were built in the fork of the foliage of a tree, so a ladder was used to access the nest contents. Trees containing newly built nests were individually marked with a numbered tag ($n = 49$) attached to the bottom of the tree. Nests were visited daily to record egg-laying dates. At the nest-site, we measured the nest characteristics at two scales, which of the nest tree to determine nest microhabitat preferences, and the surrounding vegetation in an area comprising a 25-m radius around the nest, a distance previously defined as the biological edge region for noisy miners (Clarke & Oldland 2007). These data allowed colony scale preferences for nest site location to be assessed and compared with an additional 49 random plots of the same size. These were selected based on the following approach: first, we divided the colony into 60m by 60m grids and sampled plots with 25-m radius randomly selected inside these grids. The distances between random plots were measured using a GPS (Garmin Etrex 10). At each plot, the distance of the plot centre to the edge of a woodland patch was measured with a tape. We defined edges of woodland patches with open areas that were largely devoid of trees as regions where the average diameter of the open area exceeded 100m. The number of trees at the plot was also recorded as an index of tree density. For each plot, we estimated canopy cover as the proportion of ground cover by the vertical projection of the tree crowns (Jennings et

al. 1999; Verma et al. 2014), using the eight-point crown projection method suggested by Fleck et al. (2011).

For each nest tree (n= 49), and a randomly selected tree within random plots (an additional n = 49), we measured four structural parameters: tree height, canopy diameter, canopy depth and diameter at breast height (DBH) (Table 2.1). Tree height and canopy depth were measured using an extendable pole. Canopy depth was measured in a similar manner to tree height. We defined the canopy depth (or crown depth) as the beginning of the lowest main branch to the top of the tree. Following Fleck et al. (2011), we defined main branches as those where the branch made up at least approximately 10% of foliage cover at the same height. Measuring the canopy diameter or crown projection area from the ground involved measuring the crown projection across different angular segments of the canopy (Verma et al., 2014). We followed the methods of Hemery et al. (2005) who concluded that two, orthogonal diameter measurements (4 radii) are suitable for computing crown diameter from which crown projection area can be subsequently derived.

We measured each diameter of the canopy using the same extendable pole as aforementioned. First, we measured the largest diameter and then the other diameter perpendicular to the first measurement, calculating the mean for these separate measurements. The DBH of individual nesting and random trees were derived from the measured trunk circumference at 1.3m above local ground level (Verma et al., 2014). For trees with multiple main trunks at 1.3m above the ground, the DBH of each trunk was measured, and tree DBH calculated as mean value of multiple trunks. Nest concealment was estimated visually for each nest based on the estimated visibility of the nest from an aerial predator's directly overhead view – if a nest was fully covered with leaves and branches, the concealment was scored as 100%, if

completely bare, then 0% concealment was recorded. Nest height was measured using the same method applied to determine tree height. We also measured two other nest placement factors: distance to the main trunk and distance to the branch edge (distance to the end point of longest branch at the same height). We undertook nest-site and random plot sampling and measurements after fledging (December–April) in order to minimize potential disturbance to the breeding birds.

Statistical analysis

We first checked the data to ensure models did not violate assumptions of independence in the data (Quinn & Keough 2002). We tested for multi-collinearity using the Variance Inflation Factor (VIF) method based on a $VIF > 5$ (Rogerson 2001; Zuur et al. 2010). To investigate which tree and plot characteristics were predictors of nest site location, we ran generalized linear mixed models (GLMMs). The response variable was defined as presence/absence of a nest in trees or plots and had a binomial distribution pattern. Plot characteristics modelled as fixed factors were distance to open areas (i.e. the edge of patches), tree density within plots and canopy projection within plots. For nest tree characteristics, fixed factors included were nest tree height, canopy diameter, crown depth and tree DBH. To avoid the possibility of non-independent data, we included breeding female and the year as random effects during analysis. We applied the information-theoretic approach for comparing different plausible models explaining noisy miner nest-site selection at both the plot and colony scale. In order to identify the most parsimonious of all possible models, we considered second-order Akaike's information criterion values that were corrected for small sample size (AICc) (Quinn & Keough 2002; Burnham & Anderson 2004). Smaller AICc values show a more parsimonious model fit, relative to the number of parameters fitted to each model. We then selected best models among all models based

on the differences between AICc value of that particular model and that of the best model ($\Delta AICc_i = AICc_i - AICc_{min}$, where $AICc_{min}$ is the lowest AICc value in the model set). If the difference of AICc was approximately 2, the model was listed as a competitive model. It has been suggested that if there is not a clear best model (based on model weight >0.9) then coefficients should be obtained through model-averaging (Grueber et al. 2011). We applied a natural average method to obtained model-averaged coefficients (Burnham & Anderson 2004; Grueber et al. 2011).

Within-microhabitat differences in selected nest trees and in random trees were compared with a paired t-test and percentages of occurrence of nest with regard to fixed covariates (distance to open areas) were assessed using a chi-square test. Analysis and statistical tests were performed in the R statistical language and environment (R Core Team 2015). We used lme4 package (Bates et al. 2014) to perform GLMM modelling, and the AIC table was generated using the AICmodavg package (Mazerolle 2015). We also used the MuMIn package (Bartoń 2015) to perform model-averaging after ranking and selecting the top model for each analysis. Predictions for the effects of main factors were generated using `sjp.glmer` function in the package `sjPlot` (Lüdecke 2015).

Results

Factors influencing nest tree selection in miner colonies

Mean cover for nest plots was 30.21 ± 1.93 (mean \pm SE) and nests were located at a mean distance of 38.48 ± 3.96 m from each other, at a height of 4.33 ± 0.23 m above ground. A number of models were within two AICc points of the most preferred model assessing the colony-scale factors shaping nest site selection (Table 2.2). All of these had distance to the edge of a patch as a factor, highlighting its importance. Tree

density and plot cover were the other factors that received some, albeit less, support (Tables 2.2 and 2.3). Of these three factors, only distance to the edge of a patch had an estimated coefficient \pm error that did not encompass zero (Table 2.3). There was a tendency for females to select plots with shorter distances to patch edges (Fig. 2.2), with the majority of noisy miner nests occurring at a distance of 0–10m from open areas (Fig. 2.3). Nesting probability slightly decreased with increasing canopy cover of plots; however, the strength of the model-averaged coefficient for this term was comparatively low (Table 2.3).

Table 2.1. Descriptions of variables measured for trees either used for nesting by noisy miners or randomly selected from within the area occupied by noisy miners.

Variables	Descriptions
Tree	
Tree height (m)	Tree height from ground to the highest point of the tree
Canopy depth (m)	Height of the crown from first stem to the top of the tree
Canopy diameter (m)	Mean distance between two ends of the tree canopy (see Methods for more detail)
Tree DBH (cm)	Girth of the nesting or random tree at breast height
Plot	
Tree density	Number of trees taller than 2m within a 25 m radius
% Canopy cover	Estimation of percentage canopy cover of the plot
Distance to edge (m)	Distance from centre of plot to nearest open area (ie, the edge)

Table 2.2. GLMM results for modelling nest-site selection of the noisy miner. AICc indicates the Akaike's Information Criterion for small samples; Δ AICc the scaled AICc relative to the top model; logL, log-likelihood; w_i the Akaike model weight; k the number of parameters included in models, Intercept-only: null models with only random effects.

Models	k	AICc	Δ AICc	w_i	logL
Plot scale					
Distance to patch edge + Density	5	118.85	0.00	0.31	-54.08
Distance to patch edge + Canopy cover	5	119.08	0.23	0.28	-54.19
Distance to patch edge	4	119.10	0.26	0.28	-55.32
Distance to patch edge + Canopy cover + Density	6	120.55	1.70	0.13	-53.79
Distance to patch edge	4	134.1	15.25	0	-62.82
Density	4	135.92	17.08	0	-63.73
Canopy cover + Density	5	136.3	17.46	0	-62.81
Intercept-only	3	139.3	20.46	0	-66.52
Nesting trees					
Height + Canopy diameter	6	134.42	0.00	0.59	-60.74
Height + Canopy diameter + Canopy depth	6	136.26	1.84	0.24	-61.66
Height	4	139.07	4.65	0.06	-65.31
Intercept-only	3	139.3	4.89	0.05	-66.52
Canopy diameter	4	140.98	6.56	0.02	-66.27
DBH	4	141.31	6.9	0.02	-66.44
Canopy depth	4	141.48	7.06	0.02	-66.52

Factors influencing nest placement within trees by female noisy miners

All 49 nests in this study were placed on New England peppermint trees. Tree height and canopy diameter were the most important determinants for within-plot nest tree occupancy based on AICc values. The top model was the model with tree height and canopy diameter as factors, with the second best model selected containing tree height, canopy diameter and crown depth (Table 2.2). Tree height and canopy diameter were the most important covariates explaining the occurrence of noisy miner nests at the plot or colony scale. There was a tendency to select trees smaller than the average height within the plots and trees with smaller canopy size for nests (Fig. 2.3).

Within plots, nest-tree occupancy appeared to be less affected by the crown depth of nest trees. We performed a paired t-test to test for variations between nesting trees and random trees inside the same sampling plots; Eucalyptus trees selected for nest placement had a lesser height ($t = -2.48$, $df = 46$, $P = 0.016$) and smaller canopy diameter than randomly selected trees in the plot ($t = -2.23$, $df = 46$, $P = 0.03$). Canopy depth and DBH did not vary significantly between nesting and random trees ($P > 0.05$).

Comparison of eucalypt features for selected and random trees is presented in Fig.

2.4.

Table 2.3. Model-averaged coefficient and standard error from GLMMs exploring nest site selection of noisy miners.

	Coefficient	SE	Z	<i>p</i>
Plots				
Intercept	1.68	0.65	2.53	0.01
Distance to patch edge	-0.06	0.02	3.46	0.001
Density	-0.04	0.06	0.62	0.54
Canopy cover	-0.01	0.02	0.56	0.58
Trees				
Intercept	3.31	1.75	1.88	0.06
Height	-0.45	0.17	2.57	0.01
Canopy Diameter	-0.58	0.46	1.25	0.21
Canopy depth	0.12	0.20	0.57	0.57

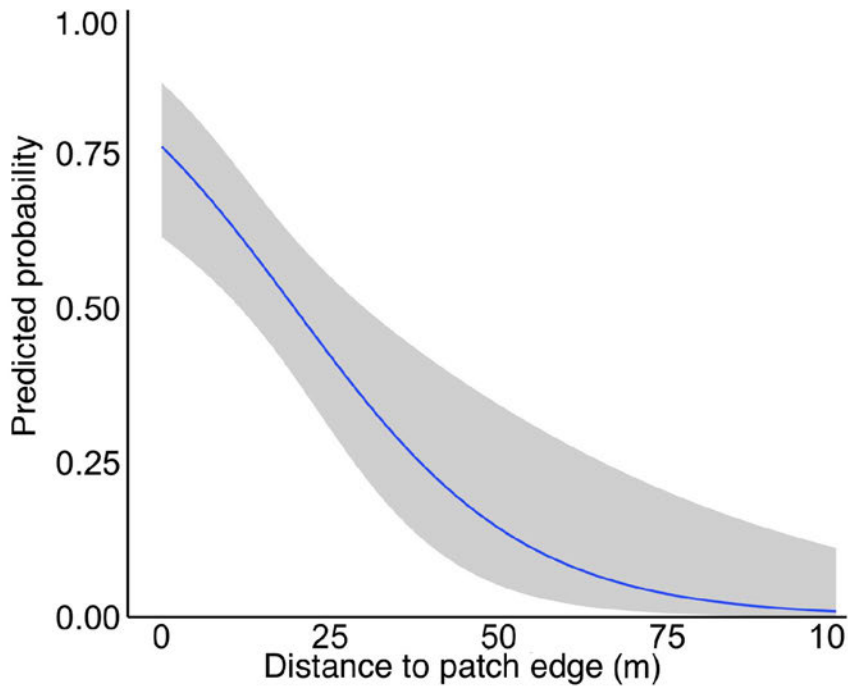


Fig. 2.2. Predicted probabilities (solid line) with confidence intervals (grey) of generalized linear mixed effects models showing the probability of a tree being used as a nest site and its distance from the edge of a patch (m)

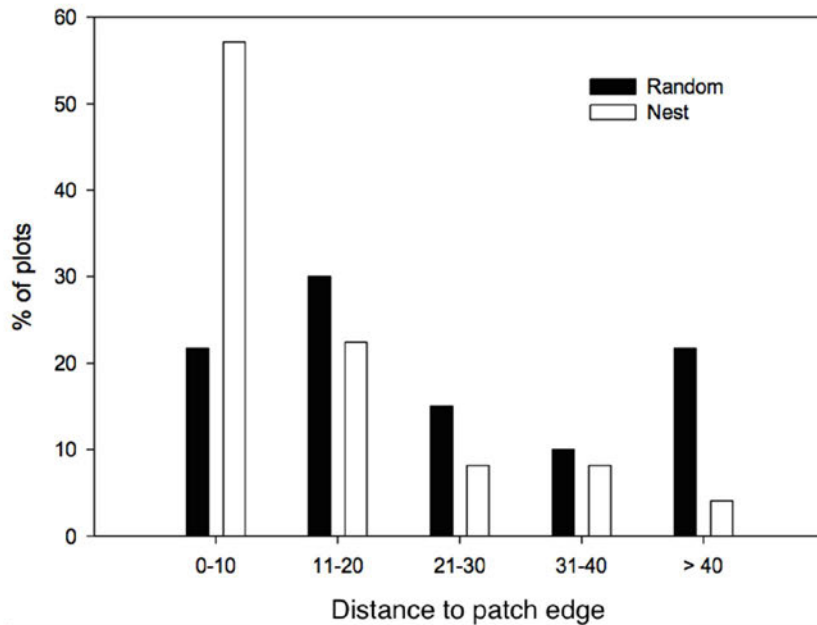


Fig. 2.3. Percentage of occurrence of nesting and random plots with respect to distance to patch edges bordering open areas devoid of trees.

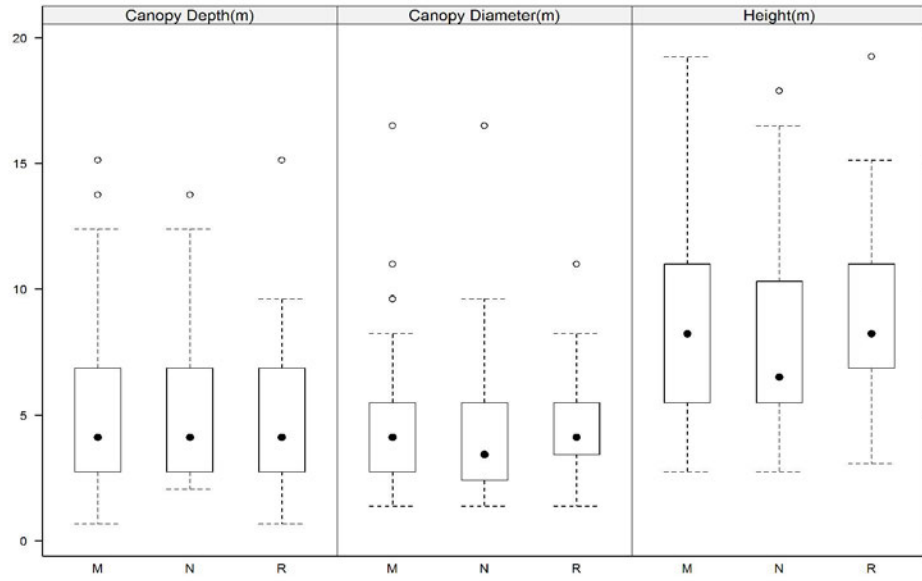


Fig. 2.4. Mean eucalypt parameters throughout site (M), trees used for nesting (N) and randomly (R) selected trees within the focal colony of noisy miners near Armidale, New South Wales, Australia.

Discussion

Preferred vegetation characteristics for nest tree selection by female noisy miners

The characteristics of nest sites chosen were assessed at different levels of habitat selection. At the colony scale, the distance of a nest tree to a patch edge appeared to be the most important determinant that influenced the likelihood of noisy miners selecting the area for a nest site, with females preferring to build nests in areas closer to open areas than further away from the edge. Nest-site selection is a critical decision that affects fitness (Martin 1995), and females likely choose areas that maximize protection from both predators and detrimental climatic factors (Etterson et al. 2007). However, other factors may also influence nest site placement, particularly for cooperative breeders such as noisy miners, where accessibility of nests to potential helpers may also be important. In this species, a female may nest in more exposed

locations to ensure potential helpers that will feed her offspring are able to locate the nest (Dow 1979a, 1979b; Dow & Whitmore 1990). Equally, selecting nest sites at the edges of fragments may facilitate better defence of nest sites if it enables miners to readily observe the approach of intruders and more effectively mob them so that they leave the colony area (Taylor et al., 2008). Further, as noisy miners spend a large amount of time feeding in open areas by foraging on the ground (Maron 2009), nest tree preferences may facilitate greater feeding efficiency and reduce travel distances between young and food sources.

While the presence of noisy miners has been shown to be strongly influenced by eucalypt density in some Australian woodlands (Maron 2007), we found little support for eucalypt density within an area of 25m of the nest tree being important for nest site selection. This suggests that noisy miners can probably tolerate a larger range of vegetation density for nesting than previously acknowledged.

Preferred characteristics of nest sites by female noisy miners

Mixed models exploring the factors that best explained variation in nest site selection found two main structural vegetation characteristics to be important – nest tree height and canopy diameter. Female noisy miners selected eucalypts with lower heights than those randomly selected from the study site, perhaps as a strategy to avoid nest predators that are primarily aerial in nature and flying overhead at the higher height of the average canopy (Barati, personal obs.). Sieg and Becker (1990) suggested that taller surrounding trees play a role in maximizing concealment and security of nests, especially from avian predators, and this may further explain why nests were placed lower than expected.

In contrast, we found that an increasing area of crown decreased the chance of a tree being selected as a nest tree by noisy miners, and nests were mainly located in

close proximity to branched edges (<1m). These could be explained as a measure to improve visibility around the nest and thus facilitate more effective mobbing, and thus reduce predation risk of both young and brooding adults alike. Placement of the nest at the basal crown height may reflect the relatively unobstructed view of the ground afforded in this position, allowing nesting birds to spot potential nest predators approaching, while simultaneously providing them with greater protection from an overstorey canopy and possible aerial predators.

We found that noisy miner nests have around 30% nest concealment in our study area. When leaves cover a nest from overhead, it could improve nest survival by decreasing the probability of being detected by potential predators. However, it may also reduce the visibility of the surrounding area for incubating or brooding birds, making them more vulnerable to predation, as well as making the nest more difficult to find for potential non-breeding helpers that cooperatively provision young in this species. Nest coverage and its position on the tree is therefore likely a trade-off between concealment while maintaining some visibility of the surrounding areas and thus approaching predators by incubating or brooding females (Götmark et al., 1995; Cousin 2008). Communication of nest location to potential helpers appears to be further enhanced by breeding females during nest building and while feeding nestlings, as they use a ‘headup’ posture when approaching or leaving the nest in this period (Dow 1978), while young are notoriously easily detected acoustically in this species (Higgins et al. 2001).

Habitat fragmentation, woodland avifauna and management considerations

Many studies have documented the relationship between the abundance of noisy miners and the diversity and density of small passerines in Australian woodlands (Catterall 2004; Hannah et al. 2007; Maron 2007; Debus 2008). However, little is

known about the preferred nesting habitat of noisy miners, an important consideration for understanding the reproductive output of this species. We identify that both characteristics of patches occupied by miners and nest tree traits can influence the location of nests by female noisy miners. Females prefer to nest near patch edges overlooking open areas, consistent with Major et al. (2001) who characterized these birds as ‘edge specialists’. Positive consequences of habitat fragmentation in terms of both occupancy (e.g. Maron et al. 2013) and now breeding position (this study) are an issue of concern in terms of protection small woodland avifauna species. Noisy miners are considered to be a threat to woodland birds through aggressively excluding them from occupied areas (Grey et al. 1997 and Grey et al. 1998; Piper & Catterall 2003). This effect persists regardless of patch size (Major et al. 2001) and can lead to reductions in eucalypt tree health and seed dispersal (Grey et al. 1997; MacDonald & Kirkpatrick 2003; Southerton et al. 2004; Montague-Drake et al. 2011), thereby further reducing habitat quality for other taxa. This effect has also recently been documented in a congener the yellow throated miner (*Manorina flavigula*) (O’Loughlin et al. 2015) and is well known in the bell miner (*M. melanophrys*; Loyn et al. 1983).

Our study suggests that noisy miners prefer to nest on the edge of open patches. Given that there is on-going landscape modification, it has previously been predicted that noisy miners will likely colonize most parts of woodlands in the eastern Australia (Maron et al., 2013). Based on these general patterns, management measures are required and should be taken to prevent further exclusion of woodland avifauna and decline in tree condition across large areas. Habitat restoration measures should be conducted to control the dominance and occupancy of noisy miners in high conservation value habitat. Montague-Drake et al. (2011) showed that increasing

structural complexity of remnant woodland patches could reduce the suitability of the area for noisy miners. Habitat restoration and revegetation is a possible measure for mitigating the negative effects of noisy miners (Maron 2007); however, when planning for habitat restoration, it is important to choose suitable species to replant. For example, habitats dominated by species such as *Callitris* have been shown to be less suitable for noisy miners than eucalypt-dominated areas, which potentially could improve the abundance of small birds in some regions (Major et al. 2001; Maron & Kennedy 2007; Eyre et al. 2015). Other measures could focus on the removal of noisy miners from habitat where they are causing severe negative effects. Grey et al. (1997, 1998) showed that the removal of a small or high percentage of noisy miners can lead to a major change in avifaunal diversity in a short time and that experimental removal of these aggressive birds can also improve tree condition. When noisy miner abundance is substantially reduced, even very small remnants of woodland (<10 ha) can support a significant diversity of small woodland avifauna (Grey et al. 1997, 1998; Piper & Catterall 2003).

Conclusion

Our survey shows that noisy miners are benefiting from increased fragmentation of eucalypt woodlands throughout their range by the availability of greater numbers of potential nest sites. Habitat simplification and fragmentation further facilitate the early detection of potential competitors by miners (Maron et al. 2013) and can therefore also increase the aggressive behaviour shown towards other species. Noisy miner aggressiveness is enhanced as a result of group nest defence (Arnold 2000), so the chance of exclusion of other birds increases in areas occupied by miners during the breeding season. Currently, little is known regarding variation in aggressive behaviour in relation to breeding activities and further investigation is needed in this

area. The fact that noisy miners have also relatively recently been able to colonise many cities – so effectively that they are characterized as urban adaptors (Lowry et al. 2011) – is a further cause for concern. This colonisation is mostly a consequence of anthropogenic activities and the availability of food resources in urban areas. Noisy miners are able to occupy patches that lack a shrub layer and urban areas are suitable habitat for these birds as a result. The domination of this species in an urban setting may further result in a decline in urban bird diversity. Finally, as many of the affected species have already undergone severe declines (Montague-Drake et al. 2011; Barrett et al. 1993; Barrett 2003), a greater understanding of both occupation and breeding habitat preferences of noisy miners will allow land managers to make more informed decisions to ensure that miners are not attracted to key biodiversity regions.

References

- Arnold K. E. (2000). Group mobbing behaviour and nest defence in a cooperatively breeding Australian bird. *Ethology*, 106, 385-393.
- Andrews S. P., Reid, N. (2000). The user guide to Newholme: A Dictionary of natural resources information for Newholme field laboratory. University of New England, Armidale.
- Bartoń, K. (2015). MuMIn: Multi-Model Inference. R package version 1.13.4. <http://CRAN.R-project.org/package=MuMIn>.
- Bates, D., Maechler, M., Bolker, B., Walker, S. (2014). lme4: Linear mixed-effects models using Eigen and S4_. R package version 1.1-7, <http://CRAN.R-project.org/package=lme4>.
- Barrett, G. (2003). The New Atlas of Australian Birds, Birds Australia (Royal Australasian Ornithologists Union), 824 pp.
- Barrett, G. W., Ford, H. A., Recher, H. F. (1993). Conservation of woodland birds in a fragmented rural landscape. *Pacific Conservation Biology*, 1, 245-56.
- Burgess R. L. & Sharpe D. M. (1981) Forest Island Dynamics in man-Dominated Landscapes. Springer, New York.
- Burnham K. P., Anderson, D. R. (2004). Multimodel inference: understanding AIC

- and BIC in model selection. *Sociological Methods & Research*, 33, 261-304.
- Catterall, C. P. (2004). Birds, garden plants and suburban bushlots: where good intentions meet unexpected outcomes. In: *Urban Wildlife: More than Meets the Eye* (eds S. Burgin & D. Lunney) pp: 21-31. Sydney, Royal Zoological Society of NSW.
- Catterall, C. P., Green, R. J., Jones, D. N. (1991). Habitat use by birds across a forest-suburb interface in Brisbane: implications for corridors. In: *Nature Conservation 2: the Role of Corridors* (eds D. A. Saunders & R. J. Hobbs) pp: 247-258. Surrey Beatty & Sons, Sydney.
- Clarke, M. F., Oldland J. M. (2007). Penetration of remnant edges by noisy miners (*Manorina melanocephala*) and implications for habitat restoration. *Wildlife Research*, 34, 253-61.
- Cousin, J. A. (2008). Nest site selection by the western yellow robin (*Eopsaltria griseogularis*) in wandoo woodland, Western Australia. *Corella*, 2009, 33, 30-34.
- Debus, S. J. S. (2008). The effect of noisy miners on small bush birds: an unofficial cull and its outcome. *Pacific Conservation Biology*, 14, 185-90.
- Dow, D. D. (1977). Indiscriminate interspecific aggression leading to almost sole occupancy of space by a single species of bird. *Emu*, 77, 115-21.
- Dow, D. D. (1978). Breeding biology and development of the young of *Manorina melanocephala*, a communally breeding honeyeater. *Emu*, 78, 207-22.
- Dow, D. D. (1979a). Agonistic and spacing behaviour of the noisy miner (*Manorina melanocephala*), a communally breeding honeyeater. *Ibis*, 121, 423-36.
- Dow, D. D. (1979b). The influence of nests on the social behaviour of males in *Manorina melanocephala*, a communally breeding honeyeater. *Emu*, 79, 71-83.
- Dow, D. D., Whitmore, M. J. (1990). Noisy Miners: variations on the theme of communality. In: *Cooperative Breeding in Birds: Long-Term Studies of Ecology and Behavior* (ed Stacey & Koenig) pp. 561-592. Cambridge University Press, Cambridge.
- Etterson, M. A., Nagy L. R., Robinson T. R. (2007). Partitioning risk among different causes of nest failure. *Auk*, 124, 432-443.
- Ewers, R. M., Thorpe S., Didham R. K. (2007). Synergistic interactions between edge and area effects in a heavily fragmented landscape. *Ecology*, 88, 96-106.

- Eyre, T. J., Maron M., Mathieson M. T., Haseler, M. (2009). Impacts of grazing, selective logging and hyper-aggressors on diurnal bird fauna in intact landscapes of the Brigalow Belt, Queensland. *Austral Ecology*, 34, 705-16.
- Eyre, T. J., Ferguson D. J., Kennedy, M., Rowland, J., Maron, M. (2015). Long term thinning and logging in Australian cypress pine forest: Changes in habitat attributes and response of fauna. *Biological Conservation*, 186, 83-96.
- Fleck, S., Mölder, I., Jacob, M., Gebauer, T., Jungkunst, H. F., Leuschner, C. (2011). Comparison of conventional eight point crown projections with LIDAR-based virtual crown projections in a temperate old-growth forest. *Annals of Forest Science*, 68, 1173-85.
- Foster, R. B. (1980). Heterogeneity and disturbance in tropical vegetation. In: Conservation Biology. An Evolutionary-Ecological Perspective. Sinauer (eds M. E. Soule & B. A. Wilcox) pp: 75-92, Sinauer Associates, Inc., Sunderland, MA.
- Götmark, F., Blomqvist, D., Johansson, O. C., Bergkvist, J. (1995). Nest site selection: a trade-off between concealment and view of the surroundings? *Journal of Avian Biology*, 26, 305-12.
- Grey, M. J., Clarke, M. F., Loyn, R. H. (1997). Initial changes in the avian communities of remnant eucalypt woodlands following a reduction in the abundance of noisy miners (*Manorina melanocephala*). *Wildlife Research*, 24, 631-48.
- Grey, M. J., Clarke, M. F., Loyn, R. H. (1998). Influence of the noisy miner (*Manorina melanocephala*) on avian diversity and abundance in remnant Grey Box woodland. *Pacific Conservation Biology*, 4, 55-69.
- Grueber, C. E., Nakagawa, S., Laws, R. J., Jamieson, I. G. (2011). Multimodel inference in ecology and evolution: challenges and solutions. *Journal of Evolutionary Biology*, 24, 699-711.
- Hannah, D., Woinarski, J. C. Z., Catterall, C. P., McCosker, J. C., Thurgate, N. Y., Fensham, R. J. (2007). Impact of clearing, fragmentation and disturbance on the bird fauna of eucalypt savanna woodland in central Queensland, Australia. *Austral Ecology*, 32, 261-76.
- Harrison, K. A., Pavlova, A., Amos, J. N., et al. (2012). Fine-scale effects of habitat loss and fragmentation despite large-scale gene flow for some regionally declining woodland bird species. *Landscape Ecology*, 27, 813-27.

- Hemery, G. E., Savill, P. S., Pryor, S. N. (2005). Applications of the crown diameter-stem diameter relationship for different species of broadleaved trees. *Forest Ecology and Management*, 215, 285-94.
- Higgins, P. J., Peter, J. M., Steele, W. K., eds (2001). Handbook of Australian. In: New Zealand and Antarctic Birds. Oxford Univ. Press, Melbourne.
- Hobbs, J. E., Jackson, I. J. (1977). Climate. In: An Atlas of New England, Vol. 2, (eds D. A. M. Lea, J. J. J. Pigram & L. M. Greenwood). The Commentaries, Department of Geography, University of New England.
- Hobbs, R. J., Hopkins, A. J. M. (1990). From frontier to fragments: European impact on Australia's vegetation. *The Proceedings of the Ecological Society of Australia*, 16, 93-114.
- Howell, C. A., Dijak W. D., Thompson, R. R. (2007). Landscape context and selection for forest edge by breeding brownheaded cowbirds. *Landscape Ecology*, 22, 273-84
- Jennings, S. B., Brown, N. D., Sheil, D. (1999). Assessing forest canopies and understorey illumination: canopy closure, canopy cover and other measures. *Forestry*, 72, 59-73.
- Johnson, D. H. (2001). Habitat fragmentation effects on birds in grasslands and wetlands: A critique of our knowledge. *Great Plains Research*, 11, 211-31.
- Keith, D. A., Orscheg, C., Simpson, C. C., et al. (2009). A new approach and case study for estimating extent and rates of habitat loss for ecological communities. *Biological Conservation*, 142, 1469-79.
- Longmore, W. (1991). *Honeyeaters and Their Allies of Australia*. Angus & Robertson, UK.
- Loyn, R. H., Runnalls, R. G., Forward G. Y., Tyers, J. (1983). Territorial bell miners and other birds affecting populations of insect prey. *Science*, 221, 1411-3.
- Low, T. (1994). Invasion of the savage honeyeaters. *Australian Natural History*, 24, 26-33.
- Lowry, H., Lill, A., Wong, B. B. M. (2011). Tolerance of auditory disturbance by an avian urban adapter, the noisy miner. *Ethology*, 117, 490-7.
- Lüdecke, D. (2015). Sj Plot: Data Visualization for Statistics in Social Science_. R package version 1.6.9, <http://CRAN.R-project.org/package=sjPlot>.
- Mac Nally, R., Bennett, A. F., Horrocks, G. (2000). Forecasting the impacts of habitat fragmentation. Evaluation of species specific predictions of the impact of

- habitat fragmentation on birds in the box–ironbark forests of central Victoria, Australia. *Biological Conservation*, 95, 7-29.
- MacDonald, M. A., Kirkpatrick, J. B. (2003). Explaining bird species composition and richness in eucalypt-dominated remnants in subhumid Tasmania. *Journal of Biogeography*, 30, 1415-26.
- Major, R. E., Christie, F. J., Gowing, G. (2001). Influence of remnant and landscape attributes on Australian woodland bird communities. *Biological Conservation*, 102, 47-66.
- Maron, M., Grey, M. J., Catterall, C. P., et al. (2013). Avifaunal disarray due to a single despotic species. *Diversity and Distribution*, 19, 1468-79.
- Maron, M. (2007). Threshold effect of eucalypt density on an aggressive avian competitor. *Biological Conservation*, 136, 100-7.
- Maron, M. (2009). Nesting, foraging and aggression of noisy miners relative to road edges in an extensive Queensland forest. *Emu*, 109, 75-81.
- Maron, M., Kennedy, S. (2007). Roads, fire and aggressive competitors: determinants of bird distribution in subtropical production forests. *Forest Ecology and Management*, 240, 24-31.
- Martin, T. E. (1995). Avian life history evolution in relation to nest sites, nest predation, and food. *Ecological Monographs*, 65, 101-27.
- Mazerolle, M. J. (2015). AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 2.0-3. <http://CRAN.R-project.org/package=AICcmodavg>.
- Montague-Drake, R. M., Lindenmayer, D. B., Cunningham, R. B., Stein J. A. (2011). A reverse keystone species affects the landscape distribution of woodland avifauna: a case study using the noisy miner (*Manorina melanocephala*) and other Australian birds. *Landscape Ecology*, 26, 1383-94.
- Murcia, C. (1995). Edge effects in fragmented forests: implications for conservation. *Trends in Ecology and Evolution*, 10, 58-62.
- O'Loughlin, T., O'Loughlin, L. S., Clarke, M. F. (2015). Influence of the yellow-throated miner (*Manorina flavigula*) on bird communities and tree health in a fragmented landscape. *Wildlife Research*, 41, 537-44.
- Pickett, S. T. A., Thompson, J. H. (1978). Patch dynamics and the design of nature reserves. *Biological Conservation*, 13, 27-37.
- Piper, S. D., Catterall, C. P. (2003). A particular case and a general pattern: hyper

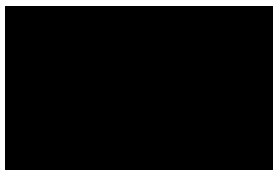
- aggressive behaviour by one species may mediate avifaunal decreases in fragmented Australian forests. *Oikos*, 101, 602-14.
- Quinn, J. P., Keough, M. J. (2002). *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.
- R CoreTeam. (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Reid, N., Yan, Z., Fittler, J. (1994). Impact of mistletoes (*Amyema miquelii*) on host (Eucalyptus blakelyi and Eucalyptus melliodora) survival and growth in temperate Australia. *Forest Ecology and Management*, 70, 55-65.
- Robinson, D. (1993). Vale Toolern Vale: the loss of our woodland birds. *Wingspan* 9, 1-3.
- Rogerson, P. A. (2001). *Statistical Methods for Geography*. Sage, London.
- Saunders, D. D., Hobbs, R. J., Margules, C. R. (1991). Biological consequences of ecosystem fragmentation: a review. *Conservation Biology*, 5, 18-32.
- Sieg, C. H., Becker, D. M. (1990). Nest-site habitat selected by Merlins in south eastern Montana. *Condor*, 92, 688-94.
- Southerton, S. G., Birt, P., Porter, J., Ford, H. A. (2004). Review of gene movement by bats and birds and its potential significance for eucalypt plantation forestry. *Australian Forestry*, 67, 44-53.
- Taylor, R. S., Oldland, J. M., Clarke, M. F. (2008). Edge geometry influences patch-level habitat use by an edge specialist in south-eastern Australia. *Landscape Ecology*, 23, 377-89.
- Verma, N. K., Lamb, S.W., Reid, N., Wilson, B. (2014). An allometric model for estimating DBH of isolated and clustered Eucalyptus trees from measurements of crown projection area. *Forest Ecology and Management*, 326, 125-32.
- Wiens, J. A. (1989). Spatial scaling in ecology. *Functional Ecology*, 4, 385-97.
- Wright, H. E. Jr. (1974). Landscape development, forest fire and wilderness management. *Science*, 186, 487-95.
- Zuur, A. F., Ieno, E. N., Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, 1, 3-14.

Statement of originality

We, the Research PhD candidate and the candidate's Principal Supervisor, certify that the work displayed here including the text, figures and diagrams are the candidate's original work.

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11/12/2016

Date



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11/12/2016

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Statement of Authors' contribution

We, the Research Master/PhD candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

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Alloparental care in a highly social bird with a mixed-relatedness helper contingent



This chapter will be submitted for publication as a standard paper.

Abstract.

Cooperative breeding is a relatively rare breeding system in which offspring receive care not only from their parents, but also from other individuals known as helpers. This helping behaviour is clearly costly to attendants; however, one of the means by which helpers can override these costs is through preferentially directing aid towards kin. I studied cooperative breeding dynamics and the effect of genetic relatedness on the provisioning behaviour of helpers in the noisy miner (*Manorina melanocephala*), a common Australian honeyeater, which lives year-round in large, complex colonies that contain both kin and non-kin. I show that in this cooperatively breeding system, there is a strong sex-bias in helper effort, with 93% of helpers being male, compared to 69% of the population. While helpers were also mainly adult birds (76%), when they did provide aid, first year birds gave equivalent levels of care to that of older birds. Although some nests remained unassisted by helpers, the number of helpers and their provisioning rate increased with nestling age, and helpers brought progressively larger prey with a higher proportion of lerp (a food source rich in carbohydrates) as broods increased in age. Both related and unrelated birds provisioned broods; however, genetically related helpers provisioned the broods substantially more often than unrelated helpers, with helper effort being positively related to an individual's genetic relatedness to breeding pair. Based on these results, there is a clear sex difference in helping behaviour in this species, with the majority of help being provided by related males who were most likely retained offspring from previous years. Kinship therefore appears to be an important driver of helping behaviour in noisy miners, although given that unrelated helpers also provisioned young, other types of direct benefits, such as group augmentation, might also be accrued by helpers and play a role in maintaining cooperatively breeding in this species.

Keywords. Noisy miner, cooperative breeding, *Manorina melanocephala*, provisioning effort, kin-selection

Introduction

Cooperative breeding occurs when individuals other than the parents provide parental care to a single clutch of offspring. This breeding system is known to occur in approximately 9% of the world's bird species (Cockburn 2006; Jetz & Rubenstein 2011). The most commonly studied form of alloparental care is brood provisioning, a behaviour that is presumably costly to participants (Clutton-Brock 1991). Given this, a number of different hypotheses have been proposed to explain the evolution of cooperative breeding in vertebrates. Overall, these hypotheses suggest that helpers might accrue indirect benefits through improvement in fitness of their relatives or direct benefits such as enhanced survivorship as a result of increased group size (Clutton-Brock 2009, 2002; Hatchwell 2009). Amongst this myriad of suggested hypotheses, kin selection serves as the main explanation for helping behaviour and implies that helpers gain indirect benefits through improving the breeding success and fitness of their relatives and others with whom they share genes (Hamilton 1964; Brown 1987; Emlen 1997; Stacey & Koenig 1990; Koenig & Dickinson 2004, 2016). According to kin selection theory and Hamilton's rule (Hamilton 1964), an increase in the relatedness between a helper a recipient of the help would result in a higher probability of helping behaviour and investment by helpers (Hamilton 1964; Russell & Hatchwell 2001; Griffin & West 2003; Cornwallis et al. 2009).

Although kin selection is usually proposed to be the main driver of cooperative breeding (Hamilton 1964) and empirical evidence supports this hypothesis across a range of species of birds (Russell & Hatchwell 2001, Nam et al. 2010; Wright et al. 2010, Browning et al. 2012), fish (Taborsky 1984) and mammals (Solomon & French 1996), others have questioned the importance of indirect fitness benefits to helping decisions (Cockburn 1998; Clutton-Brock 2002; Griffin & West 2002). For example,

some studies have emphasised the lack of relationship between helping effort and the genetic relatedness to the recipient of the help (Magrath & Whittingham 1997; Clutton-Brock et al. 2000). The criticisms for the importance of kin selection and indirect benefits are generally categorised into two main groups.

First, some argue that in most cooperative species, individuals live in family groups that usually consist of closely related members (Hatchwell 2009) and kin-based helping behaviour could be a consequence of living in such groups (Clutton-Brock 2009, 2002; Cockburn 1998). In other words, kin selection is a consequence of demographic viscosity and is not necessarily driven by indirect fitness benefits and active selection of kin by helpers (Cockburn 1998; Clutton-Brock 2009, 2002; Hatchwell 2010). Thus, to confirm that helpers actively direct their efforts towards their kin, it is expected that even when helpers live in groups with variable levels of genetic relatedness, helpers should adjust their helping efforts according to the level of genetic relatedness to breeders that they help. This relationship provides a more compelling case concerning the importance of kinship in helping decisions, although, in cooperatively breeding birds, it has been largely restricted to a few studies to date (Nam et al. 2010; Wright et al. 2010; Browning et al. 2012).

Second, the kin selection hypothesis fails to explain substantial care that is often provided by unrelated helpers (Cockburn 1998; Clutton-Brock 2002; Wright et al. 1999; Clutton-Brock et al. 2001; Dickinson 2004; Canestrari et al. 2005; Wright et al. 2010). When unrelated helpers provide substantial care, other forms of benefits are required to explain this behaviour. Many hypotheses have been proposed to explain how help between non-relatives might occur. One of the more likely hypotheses is referred to as 'group augmentation' (Kokko et al. 2001; Clutton-Brock 2002; Wright 2007). According to group augmentation theory, helpers gain direct fitness benefits

through an increase in the focal group size, with larger groups improving the fitness of helpers through greater access to resources and/or mates (Kokko et al. 2001).

Separating the effect of genetic relatedness and the importance of group augmentation, however, may be confounded by the effect of helper sex. Both sexes help in many bird species (e.g. Wright et al. 2010; Browning et al. 2012), but sexes may differ in both their genetic relatedness to breeding birds and their dispersal patterns. These might lead to sex-differences in the costs and benefits of helping behaviour (Cockburn 1998; Heinsohn & Cockburn 1994). For instance, in most cooperatively breeding birds, females tend to disperse, whereas males tend to be philopatric (Cockburn 1998). In such a condition, both sexes may gain indirect benefits if they help (Wright et al. 2010) but the philopatric sex is more likely to gain additional direct benefits from pathways such as group augmentation, as the dispersing sex leaves the natal colony prior to accessing these benefits. Thus, sex differences in natal dispersal patterns and philopatry can provide predictable differences in the benefits that individuals can accrue through group augmentation (Wright et al. 2010). If the sexes are similar in their philopatry patterns, equal numbers of male and female helpers should be expected or at least the sex ratio of helpers might be same as the population sex ratio. Conversely, if there is strong sex-biased dispersal, helping is expected to be provided mainly by the philopatric sex. In this scenario, the philopatric sex could gain indirect (e.g. kin selection), direct (e.g. group augmentation) or both benefits (e.g. kinship + group augmentation) depending on their genetic relatedness to breeders.

These difficulties in separating the kin selection and group augmentation hypotheses can be readily examined in a cooperative breeding system in which: a) there is a complex social system where both kin and non kin actively contribute to

helping behaviour; b) related helpers consist of individuals with different level of relatedness to breeders and c) there is a sex-biased philopatry. In this system, the possibility of both kin selection and group augmentation can be assessed through examining the genetic relatedness and sex structure of helpers and their influence on the amount of care that each helper provides. The social system of the noisy miner (*Manorina melanocephala*) provides just such a cooperatively breeding system ideal for examining the importance of kin selection relative to group augmentation theory. Noisy miners are common Australian honeyeaters that live in large, complex colonies containing both kin and non-kin year-round (Higgins et al. 2001). Noisy miners are cooperative breeders that form large colonies comprising many non-breeding helpers (Higgins et al. 2001). In this system, male offspring remain in their natal colony but females usually disperse (Chapter 4, Higgins et al. 2001) to find new breeding territories. As a consequence, there is a male-biased sex ratio at the colony level (Chapter 4). Given this, in this chapter I aimed to test how genetic relatedness of helpers predicts the extent of helper's investment and if helping behaviour is consistent with group augmentation. I predicted that 1) helping effort would increase with increasing relatedness of helpers to breeders, 2) related helpers of the philopatric sex (males) would provide more help than the dispersive sex (females) and unrelated helpers of the philopatric sex.

Methods

Study areas

Newholme Field Research Station. Newholme Field Research Station (hereafter 'Newholme') is a working rural property owned by the University of New England (UNE) located 12 km north-west of Armidale, NSW (30° 25' 24''S; 151° 38' 84 38''E, Chapter 1). It covers a total area of 1942 hectares and the climate is characterised as temperate, with average maximum and minimum temperatures of around 26°C and 13° C in January, and 12.5° C and 1° C respectively in July, with average rainfall of 760 mm (Reid et al. 1994). The vegetation types of Newholme range from tall grassy forest in the higher elevations, through to grassy forest and tall woodland on the lower slopes (Hobbs & Jackson 1977). The most common canopy species is New England peppermint (*Eucalyptus nova-anglica*) (Andrews & Reid 2000), with sparse understorey vegetation dominated by introduced pasture grasses, which is ideal typical habitat for noisy miners (Higgins et al. 2001).

Dumaresq Dam Public Reserve. This area is located ca. 12 km northwest of Armidale (30°30'S, 151°40'E; Fig. 2.1). The noisy miner colony at this site is situated near the Dumaresq Dam Public Reserve (hereafter 'Dumaresq Dam'). The area is located approximately 5 km from Newholme and has a similar vegetation assemblage, although some areas also have pine (*Pinus* spp.) present.

Bird banding and general fieldwork

Adult birds were caught and banded from August 2012 until late November 2015. Adult birds were captured with mist nets typically, but at Dumaresq Dam some birds were occasionally captured with baited, walk-in cage traps. When a bird was caught, it was kept in a calico bag in a cool environment, before being banded with a

combination of 3 plastic colour bands and a metal band stamped with a unique number (supplied by the Australian Bird and Bat Banding Scheme). The position (e.g. right leg/ left leg; upper/lower) of metal bands was changed between colonies to increase the number of available unique colour combinations. A small Passive Integrated Transponder (PIT) Tag (Nano Tag, Biomark, Boise USA) was glued to the outside of one of the colour the bands. Nestlings were also caught at the nest by hand at around 10-14 days post-hatch, and these were fitted with colour and PIT tag bands as per adults. Free-flying birds were aged based on the alula tip shape; those with sharp tips were categorised as adults (i.e. age of 1+ years), whereas those with a round tip were yet to undergo a moult and were thus less than one year of age (Higgins et al. 2001). The reliability of this aging method on this population was confirmed with 35 known age birds recaptured throughout the study (Barati, personal. obs.). From all birds, approximately 70 μ L of blood was collected from the alar vein via venepuncture, placed in 70% ethanol and then stored at the Avian Behavioural Ecology Laboratory, UNE, at -3°C for future analysis. After banding, birds were measured and weighed before being released at the same location within the colony.

To locate nests, study sites were surveyed every 2-3 days. I also noted any other signs of nesting activity, such as females carrying nest material. Once nest sites had been found, they were marked with numbered cattle ear tags (Allflex, Australia) attached to a nearby tree (at least 2 m metres away to prevent possible attraction of predators). Nest contents were checked via direct observation (using a ladder) or by a mirror attached to the end of a 10-meter pole. These observations were used to determine when the clutch was complete and incubation was initiated. We noted chick-hatching date for each nest, and monitored clutch progress at least every other day. Nests were occasionally in high and very thin branches of trees, which were too

dangerous to access. For these nests, hatch date and nestling age was estimated after fledging occurred using average durations for the nestlings and incubation periods for the given colony.

Quantifying nest attendant food delivery to broods

Once a nestling hatched (hatch date termed day 0) behavioural observations were carried out to quantify provisioning behaviour and other activities at the nest. Nest observations took place from 13 September 2013 until 30 October 2015 during peak breeding times when colonies contained active nests. A total of 174.6 h of observation, spread across 38 different nests (10, 16 and 12 nests in 2013, 2014 and 2015 respectively) were undertaken. Experimental manipulations were performed on some nests during this period (see Chapter 6), but this chapter reports on data collected during control observations only, when nests were not manipulated in any way. Each observation lasted approximately 1 h (mean \pm se: 1.1 hrs \pm 0.03 SE, N=163) and was conducted from a hide (Grizzly Blinds, USA) placed either near (15-29m) or far (30-40m) from the nest, the average placement being 28.2 m \pm 0.38 SE, N=163). These distances were chosen to quantify and minimise any disturbance to normal provisioning behaviour in this species (McDonald et al. 2007). For all observations, data collection only commenced 15 minutes after entering the hide to allow for any disturbance effects of my passage through the colony to dissipate. Vocalisations of nestling and adult birds were recorded at 44.1kHz with a 16-bit accuracy using a portable Professional Solid State Recorder (Marantz PMD661, USA) and a Sennheiser shotgun microphone (ME67, USA) protected by a fur windshield (Rycote Softie, UK). In addition, a lapel microphone (Sony ECM – 44B, Australia) was used to simultaneously dictate observations on the second channel of the stereo recording, including; bird identity, prey type, load size (estimated as a percentage of

prey item size relative to bill size) as well as observation time, date and location to the same recorder whilst viewing behaviours through either a telescope (Gerber Montana 15-45x) or binoculars (Monarch 7, 10x42, Nikon, Japan; LF). A camera (2013-2014: analogue Hi8 camcorder, Sony, Japan; 2015: digital Panasonic HC-V270, Korea) was placed on a tripod 3-10 m ($5.5 \text{ m} \pm 0.13 \text{ SE}$, $N=163$) from the nest to videotape nest events for later analysis if field notes were incomplete. Birds normally arrived at the nest after pausing briefly at several branches in the nest tree, providing ample time to identify the bird based on leg band colours, as well as the type and size of prey it was carrying. Observations were carried out between 0800 and 1700 hours, although midday hours (1200-1400) were typically avoided due to low levels of activity around the nest. I used the sum of load size delivered to the brood per hour as an index of biomass delivered rates to broods. Visit rate at each observation session was defined as all nest visits by birds including those leading to a feeding event (e.g. provisioning rate) and those visits that was not associated with feeding nestlings. Overall the nest observations yielded a total of 5122 nest visits, of which 96% were by conducted by banded birds with a known breeding and genetic status (131 birds) and only 4% (228 visits) were by unbanded birds of an unknown sex or relatedness.

General molecular methods

DNA extractions were completed in the Molecular Ecology Laboratory, UNE. From each blood sample, around 50-100 μL of blood in 70% ethanol was transferred to a 1.5 mL extraction tube. First, I added 240 μL of Digsol (20mM EDTA, 120mM NaCl, 20mM Tris, 1% SDS) and 10 μL of 20 mg/mL Proteinase K into each extraction tube. Samples were incubated at 50°C either overnight or for at least 3 hours, and 190 μL of 5 M ammonium acetate were added to each tube in order to

precipitate cell debris. Samples were centrifuged for 10-15 minutes at 15,000 rpm, supernatants were transferred to new tubes and 1 mL of 100% ethanol added. Samples were centrifuged for 15 minutes at 15,000 rpm and the supernatant discarded. After an additional wash in 700 μ L of 70% ethanol, the residual pellet was dried in a 50°C oven for 15 minutes and 50-150 μ L TE buffer added. Samples were stored at -3°C before polymerase chain reactions (PCRs) were undertaken.

Molecular sexing and genotyping

Individual blood samples were sexed molecularly using a PCR-based method focused on two Chromo-Heli-case-DNA (CHD) binding genes that are found on avian sex chromosomes (Griffiths et al. 1998). I used two primers (P2 and P8) to simultaneously amplify the homologous parts of the CHD-W and CHD-Z genes. The following step down thermal profile was used: 95 degrees at 15 min; seven cycles each of 94 degrees at 30 seconds, (50, 48, 46, 43, 40) degrees at 60 seconds and 72 degrees at 60 seconds; and a final extension step of 72 degrees at 10 minutes. PCR products were separated by electrophoresis at 110 V for about 40 minutes on a 2% Agarose gel containing Sybr-Safe dye (ThermoFisher, Australia) at the manufacturer's recommended concentration and visualized under UV illumination. To evaluate the molecular sexing method, I then compared the results of molecular sexing with previous assignment of a birds' sex based on their observed behaviour (e.g. only females build nests in this species; Higgins et al. 2001) and molecular results were entirely consistent with field observations (N=58, 29 females and 29 males).

In order to analyse the genetic relatedness of individuals and perform paternity tests, I further genotyped individuals using 20 (Table 3.1), microsatellite markers previously isolated and characterized in noisy miners (Kopps et al. 2013; Abbott et.al.

2002; Painter et al. 1997). PCRs were conducted at the Molecular Ecology Laboratory, UNE, following the method described in Kopps et al. (2013). In total 320 individuals from three colonies were successfully genotyped. Of the 20 loci used, 6 deviated from Hardy-Weinberg equilibrium (Table 3. 1). The number of alleles per loci ranged from 3 to 15 (mean \pm SE: 7.55 \pm 0.64 for all 20 loci and 7.64 \pm 0.63 for 14 loci that did not deviate from Hardy-Weinberg equilibrium; Table 3. 1) with a mean heterozygosity of 0.63 \pm 0.04. For each locus the probability of maternal and paternal exclusion, i.e., the probability of exclusion of a randomly chosen male or female as parent of the offspring, is provided based on allele frequencies.

Table 3. 1. Characteristics of 20 microsatellite loci used for parentage and relatedness analysis in analysis in noisy miners. *k*: number of alleles per locus; *N*: number of individuals genotyped; *H_o*: observed heterozygosity; *H_e*: expected heterozygosity; *PIC*: mean polymorphic information content; *NE-1P*: nonexclusion probability for first parent; *NE-2P*: nonexclusion probability for second parent; *NE-SI*: nonexclusion probability for sibling identity, *P_{HWE}* (test for deviance from Hardy–Weinberg equilibrium, *NS*: not significant, * : significant deviation from expectations, *P* < 0.05); Use, RP: Parentage and relatedness analysis, P: only parentage analysis.

Locus	<i>k</i>	<i>N</i>	<i>H_o</i>	<i>H_e</i>	<i>PIC</i>	<i>NE-1P</i>	<i>NE-2P</i>	<i>NE-SI</i>	<i>P_{HWE}</i>	Use	Range (bp)
Mame11	5	254	0.60	0.60	0.54	0.80	0.65	0.50	NS	RP	87–95 ¹
Mame09	8	293	0.39	0.60	0.54	0.80	0.65	0.50	*	P	151–161 ^k
Mame24	8	294	0.83	0.79	0.77	0.56	0.38	0.36	NS	RP	160–185 ^k
Mame08	10	292	0.75	0.76	0.73	0.60	0.42	0.38	NS	RP	193–223 ^k
Mame31	4	313	0.39	0.41	0.34	0.91	0.81	0.64	NS	RP	228–234 ^k
Mame46	6	309	0.75	0.70	0.65	0.71	0.53	0.43	NS	RP	206–212 ^k
Mame12	8	307	0.72	0.71	0.66	0.69	0.52	0.42	NS	RP	90–105 ^k
Mame47	15	301	0.77	0.85	0.84	0.44	0.28	0.33	*	P	103–140 ^k
Mame05	8	295	0.81	0.84	0.82	0.48	0.31	0.34	NS	RP	160–184 ^k
Mame13	8	309	0.80	0.78	0.75	0.59	0.41	0.37	NS	RP	172–187 ^k
Mame16	6	270	0.48	0.63	0.57	0.78	0.62	0.48	*	P	166–172 ^k
Mame39	7	245	0.62	0.71	0.66	0.70	0.52	0.42	*	P	225–229 ^k
Mame32	8	313	0.80	0.79	0.76	0.58	0.40	0.37	NS	RP	246–281 ^k
6-30	11	258	0.75	0.72	0.70	0.64	0.45	0.41	NS	RP	120–159 ^A
8-50	10	311	0.84	0.81	0.79	0.53	0.35	0.35	NS	RP	140–179 ^A
PCA2	3	271	0.01	0.02	0.02	1	0.98	0.97	NS	P	231–240 ^D
BMC5	8	317	0.71	0.70	0.65	0.70	0.53	0.43	NS	RP	109–121 ^P
BMC2	10	315	0.61	0.59	0.55	0.79	0.62	0.50	NS	RP	169–205 ^P
Mame44	5	307	0.56	0.64	0.57	0.78	0.63	0.47	*	P	153–166 ^K
Mame36	3	319	0.51	0.50	0.38	0.87	0.80	0.59	NS	RP	172–176 ^K

^K Kopps et al. 2013; ^AAbbott et al. 2002; ^D Dawson et al. 1997; ^P Painter et al. 1997

Parentage assignment

I genotyped 320 individuals at all 20 loci with three to 15 alleles per locus (mean \pm SE: 7.55 \pm 0.64) and a mean observed heterozygosity H_o of 0.63 \pm 0.04 across loci. See Table 1 for loci characterisation and exclusion probabilities.

I used the software CERVUS 3.0.7 (Kalinowski et al 2007) to determine parentage of all nestlings in our study. Maternity was initially identified in the field based on maternal behaviours restricted to breeding females, such as nest construction, incubation and a greater number of visitations to the nest than other birds in the first two days post-hatch and the only bird that brooded nestlings. I confirmed the sex of these adults by the presence of a brood patch (N=8) and the female-specific genotype at the CHD-W and CHD-Z genes. Field identification of maternity was supported in all cases (N=29) by an absence of mismatching genotypes between the brooding female and her nestlings.

Paternity was assigned using a combination of genetic and behavioural approaches. I conducted independent analyses each year and included all adult males present as possible candidate sires, including offspring born the previous year. Therefore, the number of candidate sires varied depending on the sampling year (2013: N=72; 2014: N=82; 2015: N=106). For each offspring, CERVUS calculates the likelihood of paternity of that candidate sire relative to a randomly chosen male in the population, while considering the contribution from the maternal genotype. I estimated that 85% of the adult population had been sampled to account for un-banded adults present, and estimated a genotype error rate of 1%. Paternity was assigned to candidate sires if there was not more than one allele mismatch between the candidate and the offspring and the probability of parentage was \geq 80%. In the two cases when CERVUS could not distinguish between the two most likely candidates and both candidates were

observed provisioning that brood (N=2), I assigned paternity to the male that provisioned the brood most often.

Genetic relatedness

I also used CERVUS to test our genotype data for deviations from Hardy-Weinberg equilibrium and found significant deviations in six of the 20 loci tested (see Table 1). While these deviations do not impact our assessment of paternity, as CERVUS simply excludes candidates based on mismatching genotypes, these deviations may be more problematic when inferring population-wide pairwise estimates of relatedness. Therefore, I excluded these six loci from subsequent analyses.

I used the software KINGROUP v2 (Konovalov et al 2004) to estimate pairwise relatedness (Goodnight and Queller 1999) for all individuals within each colony separately. Pairwise estimates provide relatedness values between individuals that are not connected through our limited parentage analyses. To test if two individuals were significantly related or unrelated I performed a kinship test, which calculated the significance of genetic relatedness for two individuals based on background allele frequencies. Two individuals could be significantly 'related' ($r=0.5$ i.e. full siblings), or significantly 'unrelated' ($r=0$) based on the likelihood ratio required to exclude 95% of 1000 simulated pairwise comparisons (Goodnight & Queller 1999). As some individuals were intermediate and not significantly different from unrelated ($r=0$) and related ($r=0.5$) individuals, they were placed in third group and referred to herein as 'unresolved' and considered to be approaching $r=0.25$ (i.e. half-siblings) (Queller & Goodnight 1989). When two individuals were classified as significantly related, r values varied between 0.2 and 0.89 ($0.43 \pm 0.01SE$, $N=2,502$), whereas r values for significantly unrelated dyads ranged from -0.42 to 0.18 ($-0.004 \pm 0.001SE$, $N=40,576$)

and finally for those dyads that genetic relatedness remained unresolved, r values ranged from 0.16 to 0.47 ($0.29 \pm 0.01\text{SE}$, $N=902$). Mean values of r therefore approximated those theoretically expected, providing confidence in the relatedness assignment methodology.

For each breeder (both female and male), helpers were placed into three groups with regards to the outcome of these tests: 1) helpers that were significantly related, indicating a parent-offspring relationship (assigned an r of 0.5); 2) helpers that were significantly unrelated (assigned an r of 0) and 3) ‘unresolved helper’ (assigned an r of 0.25). Since benefits to helpers could occur via relatedness along either maternal lineages, paternal lineages, or a combination of both (McDonald et al. 2008a-b), the genetic relatedness of helpers was assessed relative to each breeder separately, as well as the mean relatedness to the breeding pair as a proxy for relatedness to the brood. I calculated mean relatedness to the breeding pair for each helper based on the average value of comparisons to the breeding female and male. For example, if a bird was significantly related to the breeding female ($r=0.5$) but significantly unrelated to the breeding male ($r=0$), then relatedness to the breeding pair was calculated as $(0.5+0)/2=0.25$; leading to five possible values for relatedness to the breeding pair for each bird (0, 0.125, 0.25, 0.375, 0.5).

Statistical analyses

First, the impact of observation distance on nest attendant behaviour was assessed by comparing overall visit and provisioning rate, load size, prey type and total biomass delivered relative to observer distance from nests, with observations carried out with the observer in the hide being classified as either Near ($25.18 \text{ m} \pm 0.1 \text{ SE}$) or Far ($31.48 \text{ m} \pm 0.58 \text{ SE}$) from nests. For each variable, two Generalized Linear Mixed Models (GLMMs) were compared using a Likelihood Ratio Test (LRT) assessing the significance of removing the only fixed factor term of observer distance (Near or Far). Nest identity and observation order were random effects in both models (e.g. Response \sim distance class + (1| nest identity) + (1| observation order)). A subset of 14 nests that had both near and far observation categories was included in analysis regarding the effect of observation distance.

To examine if visit rate, provisioning rate, prey type and size, total biomass delivered and the number of helpers attending a nest change with brood age, I fitted separate Generalized Linear Mixed Models (GLMMs). In each model, the fixed effect was nestling age and random effects were nest identity and observation order. Similarly, I tested different nestling ages to detect if there was a period where these variables did not change with the age. I ran GLMMs with this subset of nestling ages (e.g. 11-15 days posthatch) and included 'age' as fixed effect with nest identity and observation order as random effect. The significance of the models were determined by comparing these models to those containing the random term and intercept only using a LRT, after first confirming that model fit was acceptable through plotting residual distributions. I used contingency tables to examine the distribution of helpers in categories of age, sex and relatedness to breeders and a t-test to compare the relatedness values of helpers with male and female breeders.

I performed GLMMs to test for the effects of genetic relatedness and the age of helpers on their provisioning rate. As fixed effects, I included only the most important factors that were of primary interest and biologically relevant to avoid overparameterization, following Harrell (2001). Because the primary prediction of interest was to investigate if helpers' relatedness status predicted their helping behaviour, I fitted a global model with bird 'status' (7 levels, breeding female, breeding male and helpers of $r=0$, 0.125, 0.25, 0.375 and 0.5 to breeders) as a fixed effect. As helping rate may vary between birds of different ages, I added the helpers' age (two levels: helpers of up to 1 year of age and helpers age ≥ 1) to the global model. I then generated submodels from this global model (Table 3. 2) following the method described in Grueber et al. (2011). The response variable (provisioning rate) was square root transformed to normalise its distribution and to reduce residual variance. In this cooperatively breeding system, female helpers were rare (~7% of helpers, N=7 female helpers); therefore, I excluded female helpers from the analysis regarding the effects of genetic relatedness and age. In addition, the analysis was limited to the periods when provisioning rate was stable (11-15 days post hatch), to control for the effect of nestling age. In the models, I also included bird identity and nest identity as random effects to control for non-independence of data collected from multiple observations of the same individuals and the same nests. Brood size and the order of observations were also included as random effects to control for any potential effect of number of nestlings and observation order at a given nest. Given that any effect of 'status' could arise from the effect of parental provisioning behaviour, I repeated the models excluding parents and examining the provisioning behaviour of helpers alone with the same model structures. In order to identify the most parsimonious of all possible models, I considered second-order Akaike's information

criteria corrected for small sample size (AICc) (Burnham & Anderson 2004), in which smaller AICc show higher model fit, taking into account the number of parameters. I then selected subset of models for model averaging if the difference of AICc was approximately 2 from the best model (Grueber et al. 2011). For each model, average conditional R^2 (R^2_c) and average marginal R^2 (R^2_m) were calculated (Nakagawa and Schielzeth 2013), in order to obtain information on the overall variance explained by each model (R^2_c) and to compare amongst models including different fixed effects (R^2_m). I applied the natural average method to obtained model-averaged coefficients (Burnham & Anderson 2004; Grueber et al. 2011). Where there was an effect, I evaluated the importance of different factor levels based on the estimated effects sizes and 95% CIs (Nakagawa and Cuthill 2007). As such, the effect of each level was considered to be biologically relevant if 95% CIs of effect size did not overlap with zero. All analyses were performed in the R statistical language and environment (R Core Team 2014). I used *lme4* package (Bates et al. 2014) to perform GLMM modelling and the AIC table was generated using the *AICmodavg* package (Mazerolle 2015). I also used the *MuMIn* package (Bartoń 2015) to obtain R^2_c and R^2_m ('r.squaredGLMM' function) and to perform model-averaging after ranking and selecting the top model for each analysis. Distribution families used in the models were either Gaussian (link=identity) for continues responses (e.g. visit and provisioning rates) or Poisson (link=log) for categorical responses (e.g. prey type and size). Means are present \pm 1 SE throughout this chapter.

Table 3.2. List of models to explain provisioning rate by breeder and helpers.

Model number	Fixed effects	Random effects
1	Intercept-only	Nest+ Order+ Individual ID+ Brood Size
2	Status +Age	Nest+ Order+ Individual ID+ Brood Size
3	Status	Nest+ Order+ Individual ID+ Brood Size)
4	Age	Nest+ Order+ Individual ID+ Brood Size

Results

Disturbance effects on provisioning rate, prey size, type and biomass delivered

Overall, I recorded 3101 nestling provisioning events by 123 birds during 131.3 h of nest observation across 38 nests. The distance of observation did not significantly influence either visit rates or feeding rates at nests (GLMM, visit rate: $\chi^2_1=1.12$, $p=0.28$; feeding rate: $\chi^2_1=1.59$, $p=0.20$, $N=14$; Fig. 3.1). Observation distance also did not influence mean prey size delivered to broods (GLMM, $\chi^2_1=0.11$, $p=0.73$), prey type (GLMM, $\chi^2_1=0.001$, $p=0.99$) nor total biomass delivered (GLMM, $\chi^2_1=1.68$, $p=0.19$).

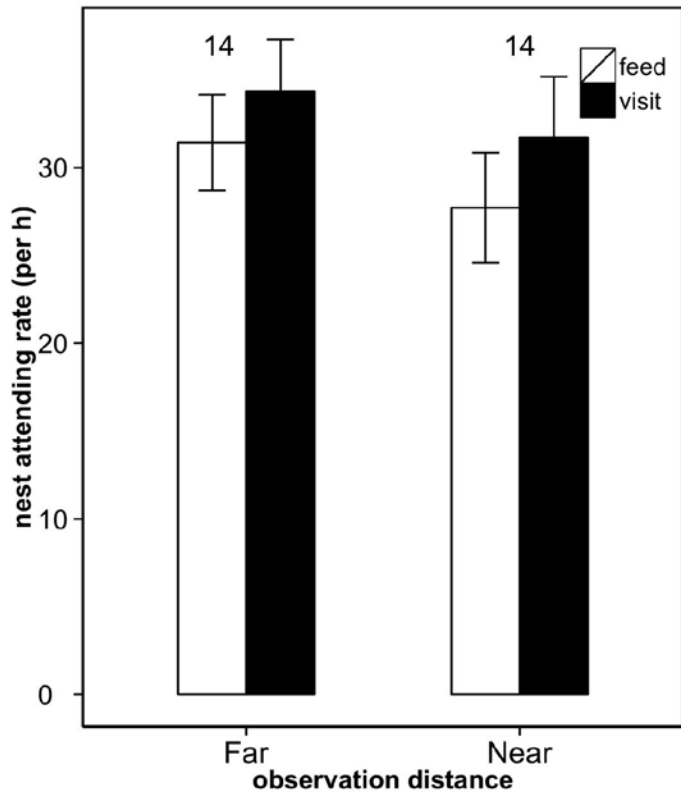


Fig. 3.1. Mean \pm SE of nest visit and provisioning rates by breeders and helpers in relation to observation distance. Visit rates include all visits made by birds to the nest and feed rates are the subset of visits when nest attendants provided food. Numbers of broods are given on the bars.

Impact of brood age on provisioning behaviour

Provisioning rate per nestling per hour increased with increasing nestling age (GLMM, $\chi^2_1=16.39$, $p<0.001$; Fig. 3.2). Similarly, average prey size, total biomass delivered and the proportion of lerp in loads also increased with nestling age (GLMM, prey size: $\chi^2_1=16.71$, $p<0.05$, total biomass: $\chi^2_1=15.17$, $p<0.01$; proportion of lerp: $\chi^2_1=28.01$, $p<0.01$; Fig. 3.3). The number of helpers observed each day at a given nest also increased significantly with brood age (GLMM, $\chi^2_1=6.08$, $p<0.01$; Fig. 3.4).

Provisioning rate increased from hatching to 11 days post hatch but appeared to level off from this age and remained consistent until nestlings fledged (Fig. 3.2). Subsequent analysis confirmed that nestling age did not significantly influence provisioning rate when the subset of data for broods aged 11-15 days post hatch was tested (GLMM, $\chi^2_1=0.12$, $p=0.72$). Again, when the data were restricted to ages 11-15 days post-hatch, brood age did not influence prey size (GLMM, $\chi^2_1=0.30$, $p=0.58$), total biomass delivered per hour (GLMM, $\chi^2_1=0.30$, $p=0.57$) and the proportion of lerp delivered in each load (GLMM, $\chi^2_1=0.37$, $p=0.54$). The number of helpers observed each day also did not differ significantly between 11-15 days post hatch (GLMM, $\chi^2_1=0.30$, $p=0.56$). Therefore, in subsequent analyses and modelling of provisioning rate, data from the 11-15 days post-hatch period only were collated and used.

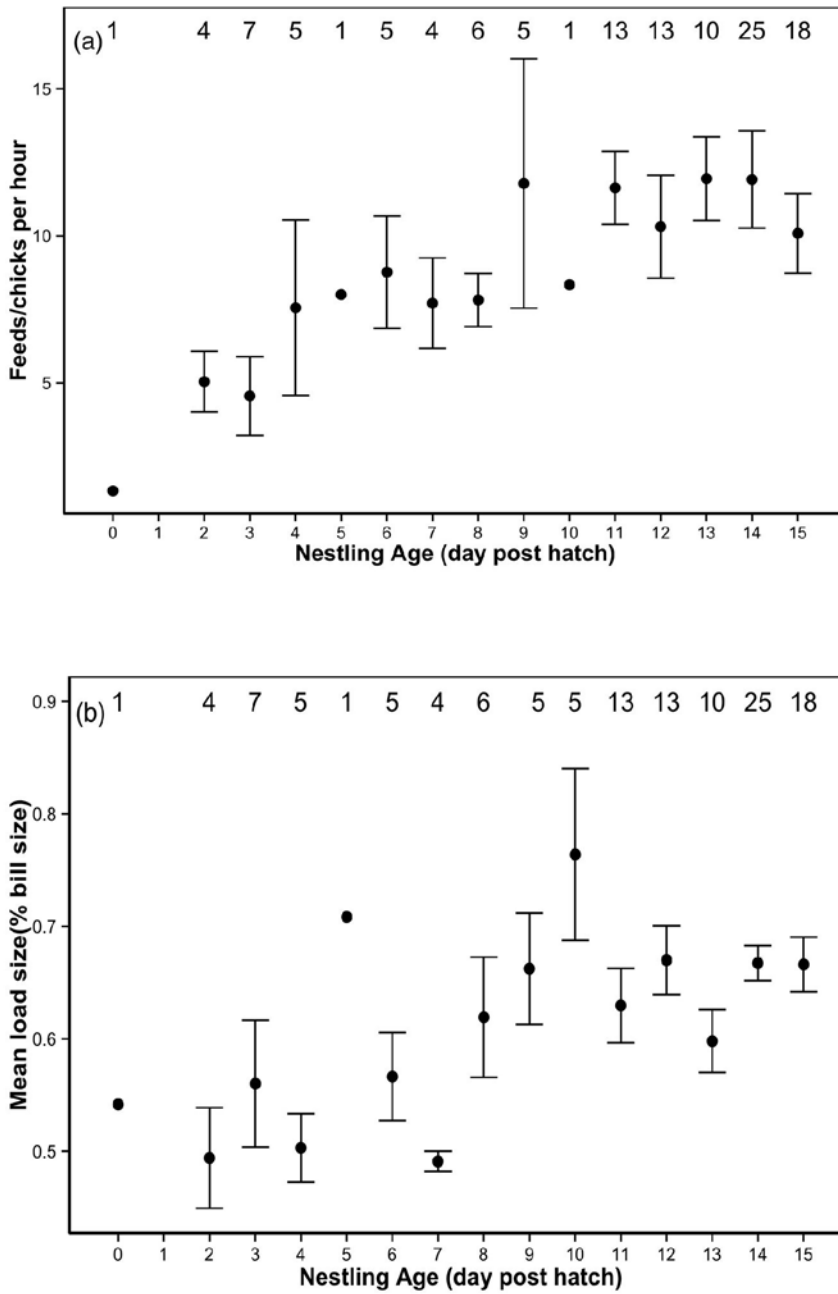


Fig. 3.2. Mean \pm SE variations of provisioning rate (a) and load size (bill size %) (b), with nestlings' age. Sample sizes for each age group are presented above corresponding error bar.

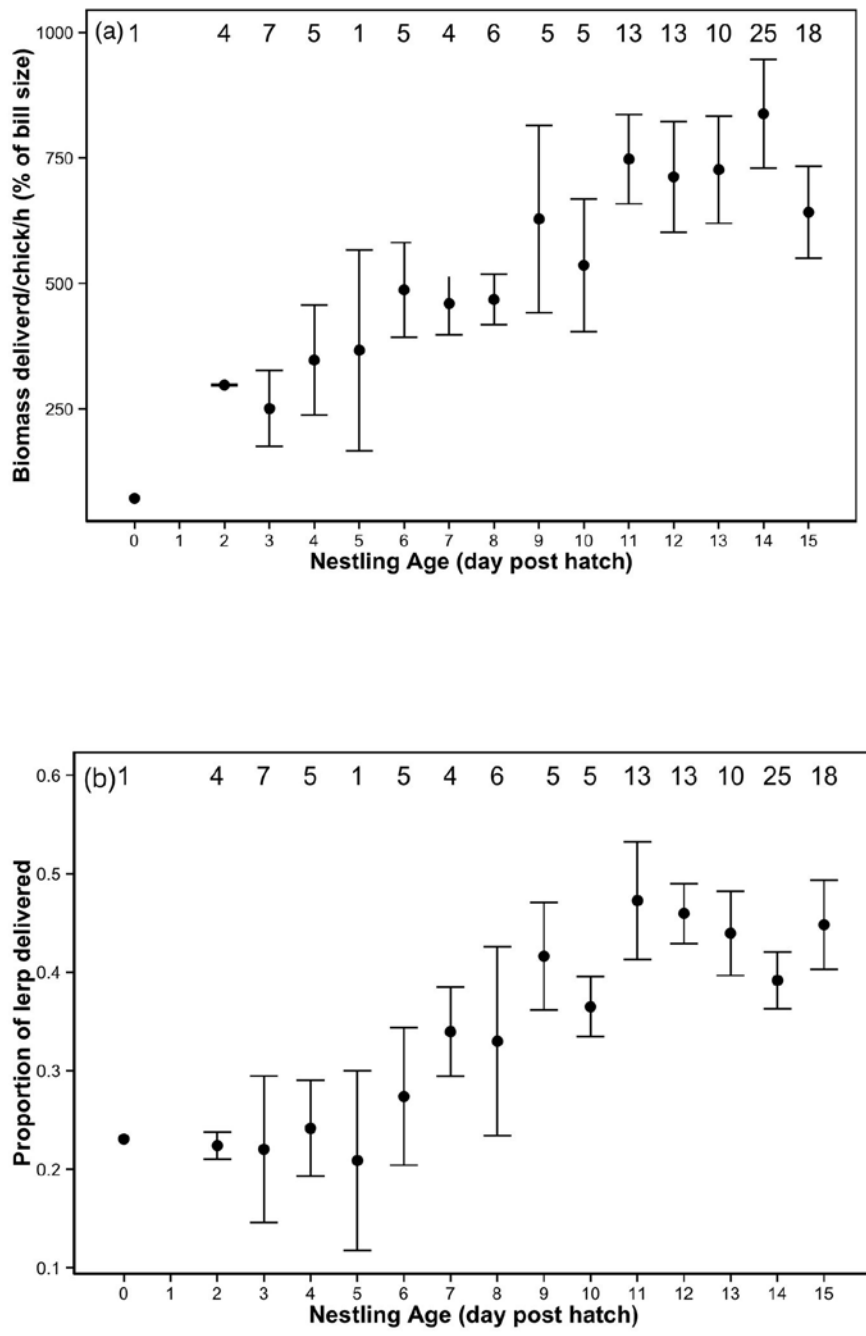


Fig. 3.3. Mean \pm SE variations of biomass delivered (a), and proportion of lerp (b) with nestlings' age. Sample sizes for each age group are presented above corresponding error bar.

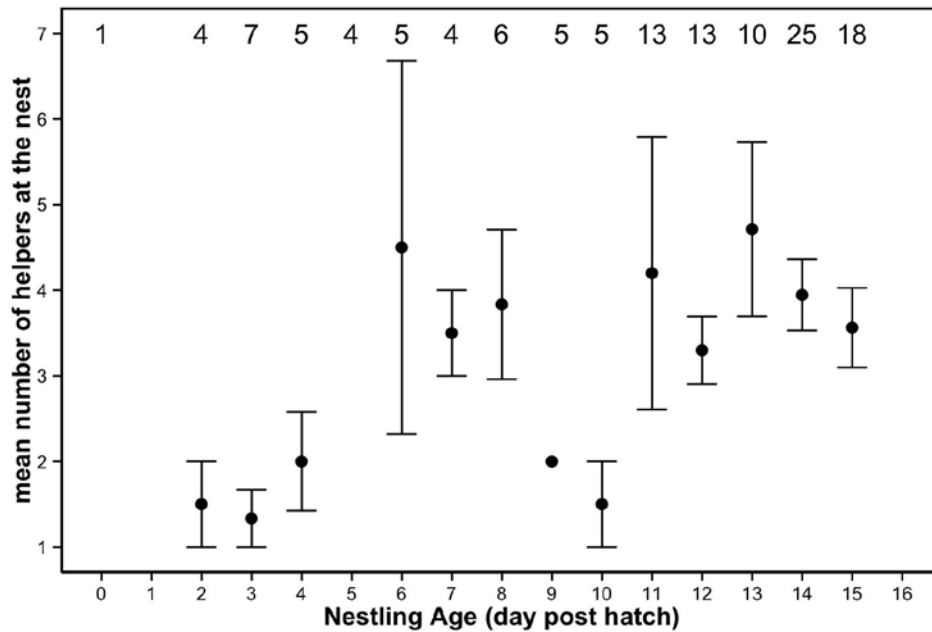


Fig. 3.4. Mean \pm SE variations of number of helpers with nestlings' age. Sample sizes for each age group are presented above corresponding error bar.

Helper age, sex and relatedness to the breeding pair

In addition to the breeding male and female, on average 3.8 ± 0.4 (mean \pm SE) helpers provisioned nestlings (range: 0-12, N=29). Helpers that were at least 1 year of age were significantly more common at nests than first year helpers (76%, $\chi^2_1 = 19.05$, $p < 0.001$). Helpers that provisioned at the nest had a broad range of values of relatedness to the breeding pair at nests, having a mean relatedness of $r = 0.15 \pm 0.01$ (N=67). Where genetic relatedness to both breeders was known for the helpers that I used in the analysis of provisioning, mean relatedness of helpers to the breeding male was $r = 0.18 \pm 0.02$ (range: -0.18, 0.70, N=90), and slightly higher compared to the breeding female ($r = 0.13 \pm 0.02$, range: -0.21, 0.7, N=106). However, the difference in relatedness of the helpers to breeding males versus females were not significant ($t = 1.31$, $df = 185.5$, $p = 0.18$, 95% CI of differences: -0.02, 0.11). Helpers were not

distributed equally among different categories of relatedness to breeders. The proportion of helpers unrelated to both breeders was significantly greater than other groups of helpers ($\chi^2_3 = 30.95$, $p < 0.001$; Fig 3.3). Male helpers were significantly more abundant than female helpers (93% male and 7% female helpers, $\chi^2_1 = 41.55$, $p < 0.001$) and female helpers were all unrelated to breeders (Fig 3.5). Therefore in further analysis regarding the effects of genetic relatedness, female helpers were excluded from the analysis for model simplicity and to avoid rank deficiency.

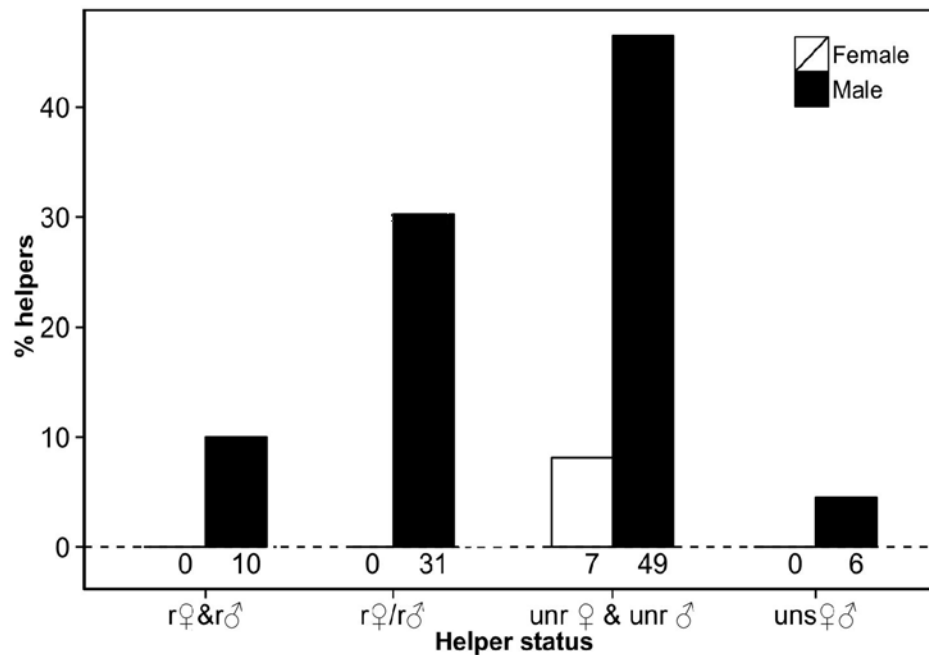


Fig. 3.5. Percentage of male and female helpers in different categories. Helpers were grouped as related to both breeders ($r_{\text{♀}} \& r_{\text{♂}}$), related only to one breeder ($r_{\text{♀}}/r_{\text{♂}}$), unrelated to both breeders ($unr_{\text{♀}} \& unr_{\text{♂}}$) and unsolved ($uns_{\text{♀♂}}$). Number of helpers of each category is presented below corresponding bar.

Does genetic relatedness influence helper provisioning rate?

Table 3.3 shows the modelling results for the effects of different factors on helper provisioning rate. A model with only ‘status’ had the highest support and lowest AIC_c.

value, although there was some support for the second model with status and age as fixed effects ($\Delta AICc = 2.2$ relative to best model; $w_i = 0.25$, Table 3. 3). Given this, I averaged these top two models that contained status and status plus age as predictors of provisioning rate (Table 3.3). Effect sizes and 95% CI of the effect sizes for status as well as for age are given in Table 3. 4. On average, the breeding female provisioned the nestlings more often than all other groups of birds, followed by the breeding male (Table 3. 4, Fig. 3.6a). There was a strong effect of genetic relatedness on the provisioning effort by helpers (Table 3. 4, Fig. 3.6a). Average helper provisioning rate increased with genetic relatedness to the breeding pair, and thus the brood (Table 3.3, Fig. 3.6a). Based on model averaged coefficients, helpers with $r = 0.25$, $r = 0.375$ and $r = 0.5$ provisioned nestlings more often than unrelated helpers ($r = 0$) (Table 3.4). However, no difference was found for the provisioning rate among unrelated helpers and helpers with $r = 0.125$ (Table 3.4).

To examine the variations within helpers alone, I then excluded the breeding pair and re-ran the same models with only helpers included. A model containing only bird status was still the best model explaining variations in provisioning rate by helpers alone (Table 3. 3). The second best model was a model with status and the birds' age, which received and had lower support ($w_i = 0.26$). To obtain the effect size for each explanatory variable, I averaged these two models. Based on the coefficients effect sizes presented in Table 3. 4, helpers with $r = 0.25$, $r = 0.375$ and $r = 0.5$ provisioned nestlings significantly more than unrelated helpers (Table 3. 4) and mean provisioning rate by helpers increased with their relatedness to breeders (Fig.3.6a). In addition, helper age did not influence the mean provisioning rate (Table 3.4). Therefore, whether or not the breeding pair was included or excluded from analysis, an individual attendant's relatedness status was the most important predictor of variation in the provisioning rate and there was little support for the effect of helper's age.

Table 3.3. GLMM results for modelling nest provisioning rate by breeders and helpers noisy miners. AICc indicates the Akaike’s Information Criterion for small samples; Δ AICc the scaled AICc relative to the top model; w_i the Akaike model weight; K the number of parameters and Cum w , cumulative model weight, R^2_m average marginal R^2 , R^2_c average conditional R^2 .

Models	K	AICc	Δ AICc	w_i	Cum w	R^2_m	R^2_c
All birds							
Status ^a	12	734.52	0	0.75	0.75	0.49	0.74
Status +Age ^b	13	736.72	2.2	0.25	1	0.49	0.74
Intercept-only	6	861.65	127.13	0	1	0.00	0.67
Only helpers							
Status	10	454.77	0	0.74	0.74	0.24	0.65
Status +Age	11	456.85	2.08	0.26	1	0.24	0.66
Intercept-only	6	486.12	31.35	0	1	0.00	0.49

a Status of birds grouped based on genetic relatedness to breeders.

b Helpers and breeders age grouped as 1st year or ≥ 2 years of age birds.

Influence of status and age on total biomass provided to broods

Similar to provisioning rate, total biomass provided to the nestlings varied significantly among birds of different status (GLMM, $\chi^2_6=66.38$, $p<0.001$). The breeding female provided the highest volume of biomass to nestlings in comparison to other birds (Fig.3.6b). When breeders were excluded, genetic relatedness influenced the total biomass provided to broods (GLMM, $\chi^2_4=9.357$, $p<0.05$). Overall, total biomass provided to nestling increased with relatedness of helpers to the breeders ($\beta=0.38 \pm 0.14$, $p=0.008$). Helper age again did not have a meaningful impact on the mean biomass provided to nestling (GLMM, $\chi^2_1=0.59$, $p=0.44$).

Table 3.4. Estimated effect sizes and 95% CI around the mean of predictors of the provisioning rate at the nest by breeders and helpers. Estimates of final model terms were calculated using a maximum likelihood approach. Only male helpers are included in the modelling.

	All birds				Only helpers			
	β	SE	95%L	95%U	β	SE	95%L	95%U
Fixed-effects								
Intercept	0.91	0.20	0.51	1.31	0.94	0.22	0.51	1.37
Status ($r=0.125$) ^a	-0.51	0.36	-1.21	0.20	-0.38	0.37	-1.10	0.35
Status ($r=0.25$)	0.60	0.19	0.23	0.97	0.74	0.19	0.38	1.11
Status ($r=0.375$)	1.11	0.29	0.54	1.68	1.49	0.31	0.87	2.10
Status ($r=0.5$)	1.24	0.27	0.72	1.76	1.23	0.28	0.67	1.78
BF	2.28	0.20	1.88	2.68				
BM	2.04	0.20	1.65	2.43				
Age ^b	0.001	0.22	-0.44	0.44	-0.10	0.22	-0.54	0.34
Random Effect								
	Variance	SD			Variance	SD		
Individual identity	0.35	0.59			0.30	0.55		
Nest identity	0.06	0.25			0.18	0.43		
Observation order	0.03	0.17			0.02	0.13		
Brood Size	0.02	0.16			0.02	0.16		
Residual	0.48	0.69			0.45	0.67		

a Helper with $r=0$ is considered reference level

b 1st year helpers are considered as reference level

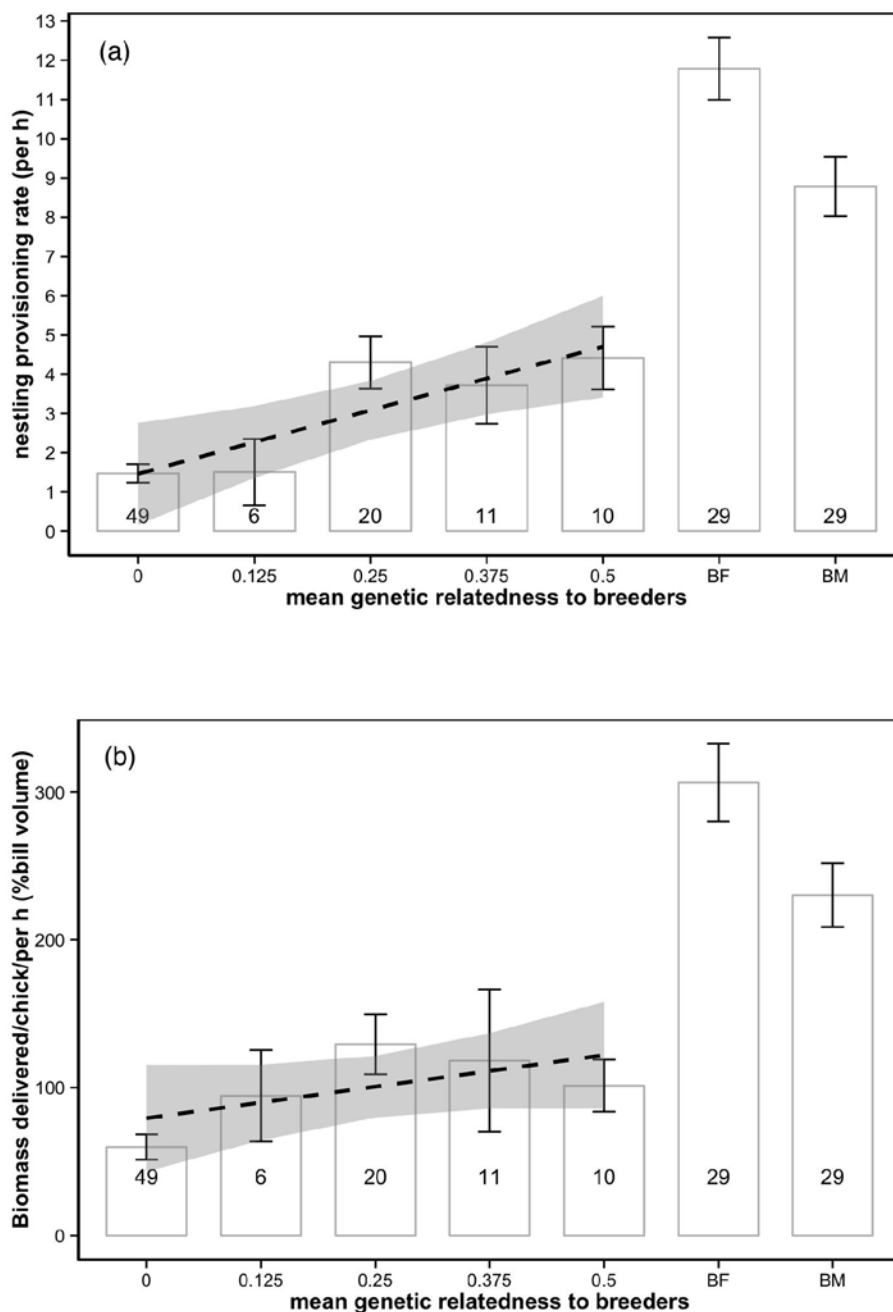


Fig 3.6. Mean \pm SE of (a) provisioning rate and (b) total biomass delivered by helpers and breeders to the brood. Helpers are grouped based on their mean genetic relatedness to breeders. BF: breeding female and BM: breeding male. Sample sizes for each group of relatedness class are displayed at the top of corresponding bar. In each graph, dashed line shows the best linear fitted line that represents relationship between genetic relatedness and provisioning rate (a) or biomass delivered (b) by helpers. Shaded areas display 95% confidence intervals around the fitted line.

Discussion

Results of this study suggest that helping behaviour in the noisy miner is not a simple response, but is selected for and entails different fitness benefits to helpers. There was a clear positive relationship between the provision rate of helpers and their genetic relatedness to the breeding pair and thus offspring. Given this, helping in this system is consistent with the kin selection hypothesis (Hamilton 1964) indicating that helpers gain indirect fitness benefits from aiding their relatives. However, in addition to indirect fitness benefits, helpers could also gain benefits through direct pathways such as group augmentation, a mechanism that explains the assistance provided by unrelated helpers. Helpers in this cooperatively breeding system were dominantly philopatric males, thus, direct benefits could be accrued by these males through more efficient mobbing and colony defence with increased group size for example, or if aided offspring subsequently reciprocated help later in their life. Therefore, both types of pathways, indirect and direct fitness benefits, that have been suggested to be important in the evolution and maintenance of cooperative breeding (Clutton-Brock 2009, 2002; Hatchwell 2009) appear to shape helping decisions in the noisy miner.

Provisioning rate, prey size and proportion of lerp increased with nestling age

Overall, provisioning rate and total biomass provided increased as nestlings aged. This is not surprising, given that the nutritional requirements of broods likely also increases with age. Both the size and type of prey provided to nestlings influenced nestling condition in the bell miner (*M. melanophrys*), a congener of the Noisy Miner (te Marvelde et al. 2009). Beyond size, the types of prey provided to nestlings also changed with brood age, with the proportion of lerp in prey provided also increasing. This may have been driven by two different factors. First, given that lerp consists

mostly of complex carbohydrates, improvements in the ability of nestlings to digest carbohydrates similar to other frugivorous species might appear with increased age, making lerps a more nutritional food type for older offspring (Breitwisch et al. 1984; Frith & Frith 2001). Second, the increasing proportion of lerp might reflect the increased nutritional demands of nestlings. In response, provisioners may favour this abundant food resource, which can be more simply gathered than other types of food, such as larvae or adult insects (te Marvelde et al. 2009). In addition, given that nestlings start thermoregulating while in the nest, energy-rich food should always be useful (Breitwisch et al. 1984). However, details on the availability of lerps relative to other prey types provided to nestlings are required for this to be fully tested. Similarly, the number of helpers present also increased with nestling age, potentially as a result of increased nestling demand.

Noisy Miner helpers are primarily adult males

The number and composition of helpers differed substantially between nests and ranged from 0 through to 12 different attendants in addition to the breeding pair. Interestingly, despite noisy miners being considered obligate cooperative breeders (Dow and Whitmore 1990), at some broods (N=3) no helper was seen provisioning offspring, and the breeding pair alone successfully reared nestlings. Several factors could influence the number of helpers at the nest in the noisy miner breeding system. For example the relatedness status of breeders to helpers, nest location and the position of nest relative to coterie area have all been suggested to influence helper number (Higgins et al. 2001, Barati et al. 2016). Environmental conditions might also be a factor, for example if helpers are nutritionally stressed they may reduce or forgo aid completely given it is costly (Heinsohn & Legge 1999).

However, perhaps surprisingly, the number of helpers did not seem to influence

nestling growth patterns (Chapter 4). This is most likely due to the highly variable provisioning efforts between different types of helpers, such as related versus unrelated individuals, in that the number of helpers does not necessarily closely reflect the volume of food delivered to broods, with the genetic relatedness of the helper contingent to the breeding pair being far more important (Fig. 3.4).

The majority of helpers in this system were philopatric males, and consequently philopatric males might be expected to gain more direct fitness benefits from aid than dispersive females (Wright et al. 2010). This female-biased dispersal pattern is a typical characteristic of many cooperatively breeding birds (Clarke et al. 2002, Wright et al. 2010, but see Komdeur 1994). It is not clear at what age female noisy miners disperse from natal colonies, however it is interesting to note that in the bell miner, females disperse at around 8 months of age, but still contribute to helping behaviour. More details are needed on the pattern of female dispersal and aid in the noisy miner system as it is not apparent from this dataset why females largely do not feed offspring as they do in bell miners.

Helpers started provisioning in their first year of life, although two fledglings occasionally fed broods (Barati, personal obs). Helpers in this system were mainly adult birds (76% of helpers), but first year and adult helpers of the same sex provided a similar amount in terms of their provisioning effort. In most cooperatively breeding species, first years helpers invest less than adult birds (e.g. Heinsohn & Cockburn 1994; Komdeur 1996; Langen 1996; Seddon et al. 2003). One primary explanation for the lower provisioning rate of first year helpers could be a higher cost of helping due to the poor body condition of yearling birds, which has been observed in different species (Brown et al. 1982; Brown 1987; Woxvold et al. 2006). For example, in apostlebirds (*Struthidea cinerea*), first year helpers are about 10% lighter than adults

and provision the broods 35% lower than adult helpers (Woxvold et al. 2006). The lower care provided by first year helpers could also be associated with lack of efficient foraging skill (Brown 1987). For example in the white-winged chough, (*Corcorax melanorhamphos*), juveniles require up to 4 years to develop foraging skills (Heinsohn et al. 1988). Given this, the finding that provisioning rate was similar between first year and adult helpers in noisy miners is inconsistent with other studies and could be explained in two different ways. First, juvenile and adult helpers did not differ significantly in their body mass (Chapter 4), suggesting that a relatively higher cost of helping due to the poor body condition of young birds is less likely to be relevant for first year noisy miners. Second, juvenile miners gain nutritional independence in their first year, much earlier than other species (e.g. white-winged chough, Heinsohn & Cockburn 1994). Consequently, this may lead to a rapid development of foraging skills in first year noisy miners and enable them to meet the costs of helping as effectively as adult birds.

Kin selection and potential mechanisms for kin discrimination

As expected, breeders provisioned the broods at a higher rate than helpers, a result previously reported for this species (Poldmaa et al. 1995). Helpers showed a clear increase in the provisioning rate and amount of biomass delivered to broods with whom they shared higher genetic relatedness. Therefore, helping behaviour in noisy miners did not conform to suggestions that helpers simply fed any begging nestling in their vicinity (Brown & Brown 1980; Jamieson & Craig 1987). Similarly, despite nests being attended by a genetically complex array of attendants that included both kin and non kin, helping behaviour appeared to be an active selection by individual helpers according to the level of relatedness to recipients. Given this, the helping strategy in noisy miners is consistent with the kin selection hypothesis, and helping

behaviour is likely to yield considerable benefits to attendants through the indirect fitness benefits of kin selection (Brown 1980). According to this hypothesis, the efforts of helpers to improve either the current reproductive success of breeders or enhance their survival (or likelihood of further breeding attempts), for example through load lightening (Komdeur 1994).

To direct helping behaviour preferentially towards kin, helpers require a mechanism of discrimination between individuals according to their relatedness in complex social groups. Noisy miners possess one of the most complex vocal repertoire systems in birds (Holt et al. 2016), which may enable them to evolve a kin discrimination mechanism and adjust their effort according to the relatedness to breeders. Previous findings in this system provide evidence that noisy miners have the ability to differentiate between different individual conspecifics using only one of their acoustic signals, a recruitment/mobbing call (McDonald 2012). Given this sensitivity, acoustic cues might facilitate preferential care for the relatives, which are also reported in the closely related bell miners (McDonald & Wright 2011). In this species, individuals acoustic similarity might enable miners to identify other individuals with whom they share genes (McDonald and Wright 2011). Consequently, helpers preferentially aid broods to which they are more related, regardless of the spatial proximity of nests (McDonald et al. 2016). Given their complex vocal repertoire systems (Holt et al. 2016), and ability for differentiating between the acoustic signals of individuals (McDonald 2012), noisy miners may also use similar mechanisms and actively select and provision the nest of the relatives.

In addition to acoustic cues, noisy miner helpers might also be able to use other social cues for kin discrimination. For example, the social association between a bird and its parents and siblings, and the parental care after fledging, by both parents and

helpers, might facilitate the identification of relatives. This might happen in societies where offspring stay in the natal territory and consequently there are a high number of social associations between related individuals (Wright et al. 1999; Cornwallis et al. 2009; Hatchwell 2010). The majority of noisy miner helpers are philopatric males that stay in their natal colony. Female noisy miners usually establish a breeding territory and place the nest in the territory for multiple years (Higgins et al. 2001; Barati et al. 2016); therefore, related helpers might have a higher degree of associations with the breeding female. This would provide an opportunity to learn to discriminate between individuals and perhaps recognise kin from familiarity-based relationships.

Group augmentation: a potential direct benefit for unrelated helpers

Despite genetic relatedness being the main determinant of helper provisioning of broods, numerous unrelated birds still provided some help. Provisioning by unrelated helpers cannot be explained by the kin selection hypothesis (Magrath and Whittingham 1997; Clutton-Brock et al. 2000, Wright et al. 2010). Therefore, direct benefits should also be considered to account for help provided by unrelated helpers, and these may also be applicable to related helpers as well.

Direct benefits in this system can be explained in the context of the ‘group augmentation’ hypothesis (Brown 1983; Connor 1986; Kokko et al. 2001, Kingma et al. 2014). This hypothesis predicts that offspring that were assisted (i.e. recipients of helper investment) would be beneficial for helpers and improve their fitness through an increase in the benefits of living in larger groups (Kingma et al. 2014), so this benefit favours the evolution of helping behaviour. As helpers are predominantly philopatric males, it is predicted that they would breed at the same colony and stay at their natal colony for their lifetime (Higgins et al. 2001) and, therefore, can potentially benefit from the increased group size and the presence of male recruits

who may improve the fitness of the helpers.

In addition to any potential benefits that recruits might provide for unrelated helpers through their helping behaviour, increased group size may facilitate more effective colony defence against intruders (Arnold et al. 2005). This is a typical social behaviour of noisy miners, usually referred to as ‘mobbing’ behaviour. Noisy miners actively mob predators in large groups (Arnold 2000), and these groups are comprised of both related and unrelated individuals. Therefore, an increase in the group size or maintaining the group size at a high optimal level would benefit helpers in the future in defending the colony and preserving access to resources within it (Arnold 2000, Arnold et al. 2005). Therefore, while other direct benefit pathways might also be possible, group augmentation appears to be the potential driver of helping behaviour by unrelated helpers in the noisy miner breeding system.

Conclusion

In noisy miners, it has previously been suggested that helpers are usually close relatives (Poldmaa et al. 1995, Higgins et al. 2001), but this is the first study to confirm this using molecular analyses and detailed behavioural observations. This chapter provides an insight into the dynamics of helping behaviour in a common, cooperatively breeding passerine that has surprisingly remained little studied. The results of this chapter demonstrate that helping behaviour in the noisy miner is primarily driven by kin selected investment in relatives and the accrual of indirect fitness benefits, adding to the existing evidence that supports this hypothesis in cooperatively breeding birds (Nam et al. 2010, Wright et al. 2010, Browning et al. 2012, Green et al. 2016). Much like their congener, the bell miner (Wright et al. 2010), numerous unrelated helpers also provided care for young in this complex social system however, with helpers, particularly males, covering a large spectrum of

relatedness. Although unrelated helpers can potentially gain different types of direct fitness benefits, attendance and investment of unrelated helpers and patterns of help relative to sex-biased dispersal most closely fit group augmentation theory. Other, non-mutually exclusive types of direct benefit might also be important in this system however, so further information, including long-term demographic and survivorship data as well as lifetime reproductive success of individual birds would be beneficial in testing these. Finally, this study also demonstrated that first-year and adult helpers provided similar rates of help, an unexpected result for cooperatively breeding species.

References

- Abbott, C. L., Poldmaa, T., Loughheed, S., Clarke, M., Boag, P.T. (2002). Hierarchical analysis of genetic population structure in the noisy miner using DNA microsatellite markers. *Condor*, 104, 652-656.
- Andrews S. P., Reid, N. (2000). The user guide to Newholme: A Dictionary of natural resources information for Newholme field laboratory. University of New England, Armidale.
- Arnold, K. E. (2000). Group mobbing behaviour and nest defence in a cooperatively breeding Australian bird, *Ethology*, 106, 385-393.
- Arnold, K. E., Owens, I. P. F., Goldizen, A. W. (2005). Division of labour within cooperatively breeding groups. *Behaviour*, 142, 1577-1590.
- Barati, A., Etezadifar, F., McDonald P. G. (2016). Fragmentation in eucalypt woodlands promotes nest-tree occupancy by a despotic species, the noisy miner (*Manorina melanocephala*). *Austral Ecology*, 41, 897-905
- Bartoń, K. (2015). MuMIn: Multi-Model Inference. R package version 1.13.4. <http://CRAN.R-project.org/package=MuMIn>.
- Bates, D., Maechler, M., Bolker, B., Walker, S. (2014). lme4: Linear mixed-effects models using Eigen and S4_. R package version 1.1-7, <URL: <http://CRAN.R-project.org/package=lme4>>.
- Breitwisch, R., Merritt, P. G., Whitesides, G. H. (1984). Why do northern mockingbirds feed fruit to their nestlings? *Condor*, 86, 281-287.

- Brown, J. L. (1980). Fitness in complex avian social systems. *In Evolution of social behaviour* (ed. H. Markl), pp: 115-128. Weinheim: Verlag-Chemie
- Brown, J. L. (1983). Cooperation: a biologist's dilemma. *Advances in the Study of Behavior*, 13, 1-37.
- Brown, J. L. (1987). *Helping and communal breeding in birds*. Princeton, NJ: Princeton University Press.
- Brown, J. L., Brown, E. R. (1980). Reciprocal aid-giving in a communal bird. *Z. Tierpsychol*, 53, 313-324.
- Brown, J. L., Brown, E. R., Brown, S. D., Dow, D. D. (1982). Helpers: effects of experimental removal on reproductive success. *Science*, 215, 421-422.
- Browning, L. E., Patrick, S.C., Rollins, L.A., Griffith, S. C., Russell, A. F. (2012). Kin selection, not group augmentation, predicts helping in an obligate cooperatively breeding bird. *Proceedings of the Royal Society B*, 279, 3861-3869.
- Burnham, K. P., Anderson, D. R. (2004). Multimodel inference: understanding AIC and BIC in model selection. *Sociological Methods & Research*, 33, 261-304.
- Canestrari, D., Marcos, J. M., Baglione, V. (2005). Effect of parentage and relatedness on the individual contributions to cooperative chick care in the carrion crow (*Corvus corone corone*). *Behaviora Ecology and Sociobiology*, 57, 422-428.
- Clarke, M. F., Jones, D. A., Ewen, J. G., Robertson, R. J., Griffith, R., Painter, J., Boag, P. T., Crozier, R. (2002). Male-biased sex ratios in broods of the cooperatively breeding bell miner (*Manorina melanophrys*). *Journal of Avian Biology*, 33, 71-76.
- Clutton-Brock, T. H. (1991). *The Evolution of Parental Care*. Princeton, New Jersey: Princeton University Press.
- Clutton-Brock, T. H. (2002). Breeding together: kin selection and mutualism in cooperative vertebrates. *Science*, 296, 69-72.
- Clutton-Brock, T. H. (2009). Cooperation between non-kin in animal societies. *Nature*, 462, 51-57.
- Clutton-Brock, T. H., Brotherton, P. N. M., O'Riain, M. J., Griffin, A. S., Gaynor, D., Sharpe, L., Kansky, R., Manser, M. B., McIlrath, G. M. (2000). Individual contributions to babysitting in a cooperative mongoose (*Suricata suricatta*). *Proceedings of the Royal Society B*, 267, 301-305.

- Cockburn, A. (1998). Evolution of helping behavior in cooperatively breeding birds. *Annual Review of Ecology, Evolution, and Systematics*, 29, 141-177.
- Cockburn, A. (2006). Prevalence of different modes of parental care in birds. *Proceedings of the Royal Society B*, 273, 1375–1383.
- Connor, R. C. (1986). Pseudo-reciprocity: investing in mutualism. *Animal Behaviour*, 34, 1562-1584.
- Cornwallis, C. K., West, S. A., Griffin, A. S. (2009). Routes to indirect fitness in cooperatively breeding vertebrates: kin discrimination and limited dispersal. *Journal of Evolutionary Biology*, 22, 2445–2457.
- Dawson, R.J.G., Gibbs, H.L., Hobson, K.A., Yezerinac, S.M. (1997). Isolation of microsatellite DNA markers from a passerine bird, *Dendroica petechia* (the yellow warbler), and their use in population studies. *Heredity*, 79, 506-514
- Dickinson, J. L. (2004). A test of the importance of direct and indirect fitness benefits for helping decisions in western bluebirds. *Behavioral Ecology*, 15, 233-238.
- Dow, D. D., Whitmore, M. J. (1990). Noisy miners: variations on the theme of communality. In: Stacey, P. B., Koenig, W. D. (eds) *Cooperative breeding in birds*. Cambridge University Press, Cambridge, pp: 559-592.
- Emlen, S. T. (1997). Predicting family dynamics in social vertebrates. In *Behavioural ecology, an evolutionary approach* (eds J. R. Krebs & N. B. Davies), pp. 301–337. Oxford, UK: Blackwell Scientific Publications.
- Frith, C. B., Frith, D. W. (2001). Nesting biology of the spotted catbird, (*Ailuroedus melanotis*), a monogamous bowerbird (Ptilonorhynchidae), in Australian Wet Tropics upland rainforests. *Australian Journal of Zoology*, 49, 279-310.
- Goodnight, K. F., Queller, D. C. (1999). Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Molecular Ecology*, 8, 1231-1234.
- Green, J. P., Freckleton, R.P., Hatchwell B. J. (2016). Variation in helper effort among cooperatively breeding bird species is consistent with Hamilton’s Rule. *Nature Communications* (DOI: 10.1038/ncomms12663).
- Griffin, A. S., West, S. A. (2003). Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. *Science*, 302, 634-636.
- Griffiths, R., Double, M., Orr, K., Dawson, R. (1998). A DNA test to sex most birds. *Molecular Ecology*, 7, 1071-1075.

- Grueber, C. E., Nakagawa, S., Laws, R. J., Jamieson, I.G. (2011). Multimodel inference in ecology and evolution: challenges and solutions. *Journal of Evolutionary Biology*, 24, 699-711.
- Hamilton, W. D. (1964). The genetical evolution of social behaviour. I and II. *Journal of Theoretical Biology*, 7, 1-52.
- Harrell, F. E. (2001). *Regression Modeling Strategies: With Applications to Linear Models, Logistic Regression, and Survival Analysis*. Springer, New York.
- Hatchwell, B. J. (2009). The evolution of cooperative breeding in birds: kinship, dispersal and life history. *Philosophical Transactions of the Royal Society B*, 364, 3217-3227.
- Hatchwell, B. J. (2010). Cryptic kin selection: kin structure in vertebrate populations and opportunities for kin-directed cooperation. *Ethology*, 116, 203-216.
- Heinsohn, R. G., Cockburn, A. (1994). Helping is costly to young birds in cooperatively breeding white-winged choughs. *Proceedings of the Royal Society B*, 256, 293-298.
- Heinsohn, R. G., Cockburn, A., Cunningham, R. B. (1988). Foraging, delayed maturation, and advantages of cooperative breeding in white-winged choughs, (*Corcorax melanorhamphos*). *Ethology*, 77, 177-186.
- Heinsohn, R., A. Cockburn. (1994). Helping is costly to young birds in cooperatively breeding white-winged choughs. *Proceedings of the Royal Society B*, 256:293-298.
- Heinsohn, R., Legge, S. (1999). The cost of helping. *Trends in Ecology and Evolution*, 14, 53-57.
- Higgins, P. J., Peter, J. M., Steele W. K. (2001) *Handbook of Australian, New Zealand and Antarctic Birds*. Tyrant-Flycatchers to Chats. Vol 5. Oxford University Press, Melbourne.
- Hobbs, J. E., Jackson, I. J. (1977). "Climate", in Lea, D. A.M., Pigram, J.J.J., Greenwood, L.M. (Eds), *An Atlas of New England*. Vol. 2. The Commentaries, Department of Geography, University of New England.
- Holt, J., Barati, A., McDonald, P. G. (2016). The complex acoustic repertoire of a highly social species, the noisy miner (*Manorina melanocephala*). *Emu*, in press.

- Jamieson, I. G., Craig, J. L. (1987). Critique of helping behaviour in birds: a departure from functional explanations. In *Perspectives in ethology*, vol. 7 (ed. P. Bateson & P. Klopfer), pp: 79-98. New York: Plenum Press.
- Jetz, W., Rubenstein, D. R. (2011). Environmental uncertainty and the global biogeography of cooperative breeding in birds. *Current Biology*, 21, 72-78.
- Kalinowski, S.T., Taper, M. L., Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, 16, 1099-1106.
- Kingma, S. A., Santema, P., Taborsky, M, Komdeur, J. (2014). Group augmentation and the evolution of cooperation. *Trends in Ecology and Evolution*, 29, 476-484.
- Koenig, W. D., Dickinson, J. L. (2004). *Ecology and evolution of cooperative breeding in birds*. Cambridge, UK: Cambridge University Press.
- Koenig, W. D., Dickinson, J. L. (2016). *Cooperative Breeding in Vertebrates: Studies of Ecology, Evolution, and Behavior*, Cambridge University Press.
- Kokko, H., Johnstone, R. A., Clutton-Brock, T. H. (2001). The evolution of cooperative breeding through group augmentation. *Proceedings of the Royal Society B*, 268, 187-196.
- Komdeur, J. (1994). The effect of kinship on helping in the cooperative breeding seychelles warbler (*Acrocephalus sechellensis*). *Proceedings of the Royal Society B*, 256, 47-52.
- Komdeur, J. (1996). Influence of helping and breeding experience on reproductive performance in the Seychelles warbler: a translocation experiment. *Behavioral Ecology*, 7, 326-333.
- Konovalov, D. A., Manning, C., Henshaw, M. T. (2004). KINGROUP: a program for pedigree relationship reconstruction and kin group assignments using genetic markers. *Molecular Ecology Notes*, 4, 779-782.
- Kopps, A.M, McDonald, P. G., Rollins, L. A. (2013). Isolation and characterisation of polymorphic microsatellite loci for noisy miners (*Manorina melanocephala*), with successful cross-amplification in bell miners (*M. melanophrys*). *Conservation Genetic Resources*, 5, 39-41.
- Langen, T. A. (1996). Skill acquisition and the timing of natal dispersal in the white-throated magpie-jay (*Calocitta formosa*). *Animal Behaviour*, 51, 575-588.
- Magrath, R. D., Whittingham, L. A. (1997). Subordinate males are more likely to help

- if unrelated to the breeding female in cooperatively breeding white-browed scrubwrens. *Behavioral Ecology and Sociobiology*, 41, 185-192.
- Marshall, T. C., Slate J., Kruuk, L. E. B., Pemberton J. M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, 7, 639-655.
- Mazerolle, M. J. (2015). AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 2.0-3. <http://CRAN.R-project.org/package=AICcmodavg>.
- McDonald, P. (2012). Cooperative bird differentiates between the calls of different individuals, even when vocalizations were from completely unfamiliar individuals. *Biology Letters*, 8, 365-368.
- McDonald, P. G., Kazem, A. J. N., Clarke, M. F., Wright, J. (2008a). Helping as a signal: does removal of potential audiences alter helper behavior in the bell miner? *Behavioral Ecology*, 19, 1047-1055.
- McDonald, P. G., Kazem, A. J. N., Wright, J. (2007). A critical analysis of ‘falsefeeding’ behaviour in a cooperatively breeding bird: disturbance effects, satiated nestlings or deception? *Behavioral Ecology and Sociobiology*, 61, 1623-1635.
- McDonald, P. G., te Marvelde, L., Kazem, A. J. N., Wright, J. (2008b). Helping as a signal and the effect of a potential audience during provisioning visits in a cooperative bird. *Animal Behaviour*, 75, 1319-1330.
- McDonald, P. G., Wright, J. (2011). Bell miner provisioning calls are more similar among relatives and are used by helpers at the nest to bias their effort towards kin. *Proceedings of the Royal Society B*, 278, 3403-3411.
- McDonald, P.G., Rollins, L.A., Godfrey, S. (2016). The relative importance of spatial proximity, kin selection and potential ‘greenbeard’ signals on provisioning behaviour among helpers in a cooperative bird. *Behavioral Ecology and Sociobiology*, 70, 133-143
- Nakagawa, S., Cuthill, I. C. (2007). Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Reviews*, 82, 591-605.
- Nakagawa, S., Schielzeth, H. (2013). A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4, 133-142.
- Nam, K. B., Simeoni, M., Sharp, S. P., Hatchwell, B. J. (2010). Kinship affects

- investment by helpers in a cooperatively breeding bird. *Proceedings of the Royal Society B*, 277, 3299-3306.
- Painter, J., Crozier R. H., Crozier Y. C., Clarke M. F. (1997). Characterization of microsatellite loci for a cooperatively breeding honeyeater. *Molecular Ecology* 6, 1103-1105.
- Poldmaa, T., Montgomerie, R., Boag, P. (1995). Mating system of the cooperatively breeding noisy miner (*Manorina melanocephala*), as revealed by DNA profiling. *Behavioral Ecology and Sociobiology*, 37, 137-143.
- Queller, D. C., Goodnight, K. F. (1989). Estimating relatedness using genetic markers. *Evolution* 43, 258-275.
- R Core Team.(2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Reid, N., Yan Z., Fittler, J. (1994). Impact of mistletoes (*Amyema miquelii*) on host (*Eucalyptus blakelyi* and *Eucalyptus melliodora*) survival and growth in temperate Australia. *Forest Ecology and Management*, 70, 55-65.
- Russell, A. F., Hatchwell, B. J. (2001). Experimental evidence for kin-biased helping in a cooperatively breeding vertebrate. *Proceedings of the Royal Society B*, 268, 2169-2174.
- Seddon, N., Tobias, J. A., Butchart, S. H. M. (2003). Group living, breeding behaviour and territoriality in the subdesert mesite (*Monias benschi*). *Ibis*, 145, 277-294.
- Solomon, N. G., French, J. A. (1996). *Cooperative breeding in mammals*. Cambridge: Cambridge University Press.
- Stacey, P. B., Koenig, W. D. (1990). *Cooperative Breeding in Birds: Long Term Studies of Ecology and Behaviour* (Cambridge University Press, 1990).
- Taborsky, M. (1984). Broodcare helpers in the cichlid fish (*Lamprologus brichardi*): their costs and benefits. *Animal Behaviour*, 32, 1236-1252.
- te Marvelde, L., McDonald, P. G., Kazem, A. J. N., Wright, J. (2009). Do helpers really help? Provisioning biomass and prey type effects on nestling growth in the cooperative bell miner. *Animal Behaviour*, 77, 727-735.
- Woxvold, I. A., Mulder, R. A., Magrath, M. J. L. (2006). Contributions to care vary with age, sex, breeding status and group size in the cooperatively breeding apostlebird. *Animal Behaviour*, 72, 63-73.

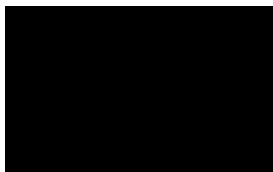
- Wright, J. (2007). Cooperation theory meets cooperative breeding: exposing some ugly truths about social prestige, reciprocity and group augmentation. *Behavioural Processes*, 76, 142-148.
- Wright, J., McDonald, P. G., te Marvelde, L., Kazem, A. J. N., Bishop, C. (2010) Helping effort increases with relatedness in bell miners, but ‘unrelated’ helpers of both sexes still provide substantial care. *Proceedings of the Royal Society B*, 277, 437-445.
- Wright, J., Parker, P.G., Lundy, K. J. (1999). Relatedness and chick feeding effort in the cooperatively breeding arabian babbler. *Animal Behaviour*, 58, 779-785.

Statement of originality

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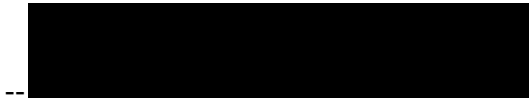
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Statement of Authors' contribution

We, the Research Master/PhD candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

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Does nestling sex influence helper effort in a sexually dimorphic bird with male-biased philopatry?



This chapter will be submitted for publication as a standard paper.

Abstract.

In cooperatively breeding species, the level of investment by both breeders and helpers may be influenced by the sex of offspring in broods that they attend, due to different fitness payoffs associated with investing in male versus female offspring. This might be expected if the sexes differ markedly in either the level of help that they may subsequently provide in later life that could benefit helpers, or the cost of their rearing due to factors such as sexual size dimorphism. Here I investigate how nestling sex influences the provisioning efforts provided by helpers and the subsequent growth and development of offspring in the cooperatively breeding noisy miner (*Manorina melanocephala*). In this species, helpers are predominantly philopatric males, as females disperse to other colonies to find breeding positions. I therefore predicted that helpers might invest more in helpful male-biased broods rather than those with dispersive female offspring, particularly if larger males required more resources than female offspring to successfully fledge. Male miners certainly grew faster in this study, fledging at the same age but with a heavier body mass than female offspring. However, despite this apparent difference in the demand of male-biased broods, and likely differences in the fitness returns of investing in different sexes, brood sex ratio did not influence the amount of food provided at a nest by either the breeding pair or their helper contingent. Therefore, the provisioning strategy of noisy miner helpers does not appear to incorporate differential investment according to offspring sex. This could be due to an inability to recognise nestling sex, or potential future costs that might be imposed by philopatric males that subsequently compete for resources and/or mating opportunities with their past helpers. These costs may outweigh or nullify any benefit accrued by male offspring being retained as part of a subsequent helper contingent.

Keywords. Rearing cost, sexual size dimorphism, cooperatively breeding, noisy miner, *Manorina melanocephala*

Introduction

Parental behaviours such as brood provisioning are costly, particularly if parents are limited in their energetic resources (Clutton-Brock 1991). Therefore, if the cost and benefit of provisioning and/or investment in offspring varies according to their sex (Trivers 1972), attendants are expected to bias their investment towards the sex that maximises their fitness benefits. Sex-biased parental investment can be favoured under a range of conditions (Hardy 2002, Wade et al. 2003). For example, in species with sexual size dimorphism and thus sex-related differences in offspring nutritional needs, parents might direct their efforts preferentially to the larger or more expensive sex (Magrath et al. 2007), because offspring of a larger sex usually require a greater amount of resources to reach independence (Hardy 1997). Facultative sex ratio adjustment and a deviation from equal investment in each sex can be favoured both before egg laying through over production of the beneficial sex (Ewen et al. 2003; Doutrelant et al. 2004) or during the rearing stage through differential resource allocation (Ridley & Huyvaert 2007). However, in spite of numerous investigations that have examined the mechanism and the evolution of investment in different sexes in vertebrates, the majority have focused on sex ratio adjustment, with subsequent sex-related bias in levels of provided care during rearing receiving less research attention.

Cooperative breeding species, where individuals other than parents help in raising young, are excellent systems in which to examine sex-related investment, as philopatric helpers may repay a fraction of the cost of their production by aiding those that once helped them later in life (Emlen et al. 1986). In addition, sex-related differences in fitness benefits and payoffs are more pronounced in cooperatively breeding systems than in non-cooperative species, as sons and daughters usually have different helping patterns in many species. For example, in the long-tailed tit, (*Aegithalos caudatus*), the majority of help is provided by males (ca. 85%; Hatchwell et

al. 2004), whereas in the Seychelles warbler (*Acrocephalus sechellensis*), female helpers contribute more towards rearing young than males (Komdeur 1994). Therefore, from a helper's perspective, these differences in the fitness benefits of each sex are primary due to the patterns of dispersal in each sex and its contribution to helping behaviour (Brown 1987; Hatchwell 2009), which result in both positive and negative social interactions (Brown 1987; Emlen 1997; Kokko et al. 2001; Wright 2007; Hatchwell 2009). As a result, in cooperative breeding species, helpers may have different strategies that lead to differential investment in either female- or male-biased broods according to the different fitness benefits that they may accrue (Emlen 1997). Hypotheses trying to explain why helpers might bias investment according to the sex of recipients fall into three main categories.

First, according to the 'repayment' hypothesis, if male and female helpers contribute to helping behaviour unequally, helpers might preferentially investment in the more helpful sex (Emlen et al. 1986; Griffin et al. 2005). Second, if offspring of one sex remains at the natal territory and competes with its previous helpers for resources, such as mating opportunities and food, then helpers may benefit from investing in the dispersing sex to reduce the costs of sharing resources with the philopatric sex. This is known as the local resource competition hypothesis (Clark 1978). Third, parents and helpers might allocate food according to the energetic requirements of offspring as a result of sexual size dimorphism (Slagsvold 1997). Parents might invest more in the larger sex if it has a higher nutritional need and, as a consequence, helpers are also expected to do likewise if they are investing in offspring as opposed to attending nests as a form of prestige (Zahavi 1975; McDonald et al. 2008a). In addition, helpers might gain other directs benefits, such as increased breeding opportunities (Craig & Jamieson 1988) or access to additional potential alliances during dispersal frays, from preferentially investing in a given sex

(Clutton-Brock et al. 2002; Baglione et al. 2003).

The provisioning strategies of both parents and helpers, according to the sex ratio of provisioned broods has been studied in various bird species (e.g. Ridley & Huyvaert 2007; McDonald et al. 2010; Nam et al. 2011), with results have differed among studies. For example, in the arabian babbler, (*Turdoides squamiceps*) helpers invested more in the opposite sex, probably to increase their future breeding opportunities and decrease the cost of within-sex competition for mating opportunities (Ridley & Huyvaert 2007). Further, sex-biased investment was associated with increasing group size, with male-biased investment occurring in small groups, as increasing the number of males present improved a group's ability to compete in territorial conflicts (Ridley & Huyvaert 2007). In the bell miner (*Manorina melanophrys*), although female offspring usually disperse from the natal colony and thus provide a lower return of benefits than male offspring, helpers did not invest more on male-biased broods (McDonald et al. 2010). Similarly, in the long-tailed tit, despite sexual size dimorphism in nestlings, breeders and helpers did not adjust their effort according to offspring sex (Nam et al. 2011).

In two of these studies, there may actually have been a lack of pronounced differences between male and female offspring in terms of their future fitness benefit to helpers as both male and female helpers provide aid, at least early in life (Ridley & Huyvaert 2007; McDonald et al. 2010, but see Nam et al. 2011). In addition to a helpers' sex, the fitness benefits that helpers gain if they preferentially investment in male or female offspring, may also depend upon their genetic relatedness to the recipients (Wright et al. 2010). For example, unrelated helpers might invest more in the philopatric sex as only the philopatric sex provides direct fitness benefits, such as contributions to future territory and/or colony defence through predator mobbing (Arnold et al. 2005). Thus, simultaneous, non-mutually-exclusive effects of genetic relatedness, helper and

offspring sex and offspring costs might shape helpers' investment patterns, all of which have rarely been assessed in previous studies. To fully understand sex-biased provisioning strategies relative to these potentially influential factors, an ideal system is one in which the nest attendance sex ratio is biased towards the philopatric sex, the cost of rearing offspring differs among the sexes and, finally, both related and unrelated helpers provision the broods. The noisy miner (*Manorina melanocephala*) provides just such an opportunity to explore breeders and helpers' investment strategies. A honeyeater species from the Meliphagidae family, noisy miners are endemic to wooded country in south-eastern Australia, are cooperative breeders and form large colonies with complex internal social structures (Higgins et al. 2001). In this complex society, both kin and non-kin regularly cooperate across a range of social contexts (McDonald 2012). Despite this, kinship is the most important known driver of helping behaviour, with a positive relationship between the level of genetic relatedness and provisioning effort (Chapter 3). However, there are also substantial investments by unrelated helpers, which could be explained in the context of direct fitness benefits that helpers accrue (Chapter 3). Male offspring are the philopatric sex of this species, staying in their natal colony for life whilst female offspring disperse to find new breeding territories (Higgins *et al.* 2001; Chapter 3). Consequently, the majority of helpers in the noisy miner system are male (ca. 93%, Chapter 3). As a result, and consistent with repayment hypothesis, male offspring should have higher value than females in terms of their potential future direct contributions to current helpers. In addition, the species exhibits sexual size dimorphism, with adult males being larger than females (Higgins et al. 2001). As a result, the cost of rearing offspring is likely to vary in relation to sex, with male-biased broods likely requiring greater levels of resources than females. Given this, I predicted that that (1) breeders and helpers should invest more in male-biased broods to gain direct

benefits from their future helping contributions; (2) this male-biased helping effort would be particularly evident in unrelated helpers, as these individuals only accrue direct benefits and do not have access to indirect benefits associated with kin selection.

Methods

Study populations

This study was conducted at three different colonies of noisy miner. Two were situated at the Newholme Field Research Station (30° 25' 24''S; 151° 38' 84 38''E) and Dumaresq Dam Public Reserve (30°30'S, 151°40'E), both approximately 12 km north-west of Armidale, NSW, Australia. Birds were banded at a third colony located 25km east of Armidale at Hillgrove (30°56'S, 151°90'E). All sites consisted of dry, open woodland vegetation with large eucalypt over-storey and sparse under-storey vegetation (Barati et al. 2016).

Fieldwork and behavioural observations

Noisy miners were captured with mist nests or baited walk-in cage traps at these three colonies between August 2012 and November 2015. All birds were weighed, measured and banded using a combination of three colour bands and one metal band issued by the Australian Bird and Bat Banding Scheme. One of the bands was also equipped with a Passive Integrated Transponder (PIT) tag. Before releasing the birds, approximately 70 µL of blood collected from the alar vein via venepuncture, placed in 70% ethanol and then stored at the Avian Behavioural Ecology Laboratory, UNE, at -3°C for future analysis.

To record breeding activities, regular visits were made from the beginning of nest-building and mating in mid-August for three seasons (2013-2015) at Newholme, and between 2014 and 2015 at Dumaresq Dam. Nests were typically high up in trees and I used a ladder and a mirror

attached to an extendable pole to access them or record nest contents (Chapter 3, Barati et al. 2016). Fresh nests were individually marked with a numbered plastic tag attached to a nearby tree and visited daily to record egg-laying dates. From the expected hatching date onwards (around 14 days after the last egg laid was laid in this study), nests were visited daily to record hatch dates. As soon as nestlings were detected, biometric measurements were taken and this continued daily until fledgling. For nestling identification within broods, I marked each nestling on a specific location on their leg with a non-toxic marker until around 7-8 days of age, when I could fit birds with metal leg bands as per adults. During each visit, body mass, tarsus length and wing chord of each nestling was measured. I weighed nestlings on a top-pan balance (Mini-Table-Top One Balance Vaporize, Australia, accuracy 0.01 g). The oldest nestling in each brood was designated as hatch order 1, the second hatch order 2 and the third hatch order 3. Measurements were carried out between approximately 1400 and 1800hrs each day and started within 1 minute of bringing a nestling down from the nest, with nestlings immediately returned once all measurements had been completed. At 11 days post-hatch, nestlings were banded with a combination of three colour bands, of which one colour band was equipped with a PIT tag as per adults, and 70 μ L of blood was collected from the alar vein for use in determining their sex and parentage analysis.

From 13 September 2013 until 30 November 2015, I conducted behavioural observations at 38 nests in order to identify the breeding pair and helpers attending a given nest, whilst also collecting information on brood provisioning behaviour (Chapter 3). Observations were carried out from a hide placed 15-40 m ($28.7 \text{ m} \pm 0.38\text{SE}$, $N=104$) from nests, distances that did not interfere with normal provisioning behaviour (Chapter 3). Observations were made for an average of around 1 hour in duration ($57.88 \text{ minutes} \pm 1.83\text{SE}$). For each nest visit event, I

identified bird identity, prey type, prey size (estimated relative to bill volume) whilst viewing behaviours through either a telescope (Gerber Montana 15-45x) or binoculars (Monarch 7, 10x42, Nikon, Japan; LF). Observational data were dictated to a solid-state audio recorder (Marantz PMD661, Japan). Nest events were also recorded with a camera (2013-2014: analogue Hi8 camcorder, Sony, Japan; 2015: digital Panasonic HC-V270, Korea), placed $5.5 \text{ m} \pm 0.13 \text{ SE}$ ($N=104$) from the nest. Nest provisioning observations were carried out between 0800 and 1700 hours.

Molecular methods and assignment of brood and adult sex ratio

Using blood samples collected from individual birds, DNA was extracted in the Molecular Ecology Laboratory, UNE (details in chapter 3) and all birds were sexed molecularly using sexing polymerase chain reactions (PCRs) involving two primers (P2 and P8) simultaneously in order to amplify homologous parts of the CHD-W and CHD-Z genes (Griffiths et al. 1998). Birds were genotyped at 20 microsatellite loci (Chapter 3) that have been previously isolated and characterised for noisy miners (Painter et al. 1997; Abbott et al. 2002; Kopps et al. 2013). Of the 20 loci amplified, 6 deviated from Hardy-Weinberg equilibrium, so while all 20 loci were used in parentage analyses, only the 14 loci conforming to HWE were used to calculate pairwise genetic relatedness of helpers with breeders (see Chapter 3 for details). All nests included in this analysis had three nestlings, the modal brood size for this species (Higgins et al. 2001). Nests with nestling mortality due to starvation (1 nest) and (predation, 1 nest) were excluded. After the sex of nestlings ($n=82$) and adults ($n=288$) was identified, I calculated brood sex ratio (BSR) for each brood as the proportion of male nestlings. For example, if there were 2 male and one female nestlings, then the BSR at that nest would be $2/3=0.67$. For each focal nestling, I also defined the sibling sex ratio (SSR), which indicated the proportion of male nestmates for a given focal

nestling in a brood. For adult birds, the sex ratio was calculated as the proportion of male birds among those banded at each colony, excluding offspring.

Assignment of the breeding pair at each nest

For each brood, the putative breeding female was identified initially based on strictly maternal behaviours such as nest construction, incubation of clutches and, if individuals were captured, the presence of a brood patch (Higgins et al. 2001; N=8). The sex of putative females was then tested using the above molecular sexing method and the assignment of molecular maternity using Cervus 3.0.7 and data from 20 microsatellite loci (Chapter 3; Marshall et al. 1998; Kalinowski et al. 2007). To determine paternity, all adult males at the colony in the given breeding season were included as potential fathers in analysis. For each offspring, Cervus calculates Trio LOD scores (natural logarithm of the likelihood ratio), giving the likelihood of paternity of that candidate parent relative to a randomly chosen individual in the population, while also considering known maternity. This method is based on simulations using known allele frequencies to calculate differences in LOD scores (Δ LOD), which were then used to rank the two most likely fathers for a given offspring at 95% confidence levels. Candidate fathers suggested by Cervus were then assigned to offspring if Δ LOD scores were $\geq 80\%$ and the number of allele mismatches was not more than one. If there was more than one genetic father indicated for a given brood, and both males provisioned the focal brood (N=2), I defined the breeding male (or social male) as the bird that exhibited a higher provisioning rate at the focal nest, given that breeding males typically provision more than helpers (see Chapter 3).

Genetic relatedness

Pairwise genetic relatedness values (r) were calculated in the program KINGROUP v2 (Konovalov et al. 2004), which estimates the degree of genetic similarity of two individuals based on the proportion of shared alleles between the individuals weighted by the allele frequencies in the whole population (Goodnight & Queller 1999). These pairwise relatedness values range from -1 to +1. If the value is -1, theoretically it means that there are no shared alleles between two individuals, while an r of +1 indicates identical alleles for the two individuals. Therefore values close to zero represent two relatively unrelated individuals, and increasingly positive values between two individuals indicate increasing levels of relatedness (Wang 2002). To test if helpers were significantly related or unrelated to breeders at each nest, I conducted a kinship test in KINGROUP v2 that calculated the significance of genetic relatedness for two individuals. Two individuals could be ‘significantly related’ (primary hypothesis $r=0.5$, null hypothesis $r=0$), or ‘significantly unrelated’ (primary hypothesis $r=0$, null hypothesis of $r=0.5$) based on the ratio required to exclude 95% of 1000 simulated pairwise comparisons (Goodnight & Queller 1999). If neither test reached statistical significance, then r values were assumed to be 0.25. As these individuals were intermediate and not significantly different from unrelated ($r=0$) and related ($r=0.5$) individuals, they were placed in third group: ‘unresolved’. Helpers were therefore placed into three groups with regards to their relatedness status to breeding female or male: 1) birds that were significantly related ($r=0.5$); 2) birds that were significantly unrelated ($r=0$) and 3) birds with ‘unresolved’ status ($r=0.25$). As benefits to helpers could occur via relatedness along either maternal or paternal lineages, or a combination of both (McDonald et al. 2008a-b), I calculated the mean relatedness of helpers to breeders as a proxy for relatedness to the brood. To obtain the mean relatedness to the breeding pair, I

calculated the average value of comparisons to the breeding female and male. For example, if a bird was significantly related to the breeding female ($r=0.5$) but significantly unrelated to the breeding male ($r=0$), then relatedness to the breeding pair was calculated as $(0.5+0)/2=0.25$. This yielded five possible values for relatedness to the breeders for each helper (0, 0.125, 0.25, 0.375, 0.5) (McDonald et al. 2008a-b).

Statistical analyses

To test if the sex ratio of males relative to females differed significantly from the expected 50:50 distribution among both nestlings and adult birds, a goodness of fit test was carried out. Once I had confirmed that nestling's sex ratios did not vary significantly from year to year in each colony, I then combined all colonies and proceeded to test for parity for all years combined following Neuhauser (2004) to account for the non-independence of nestlings within broods.

I conducted an ANOVA test to determine whether hatching order affected the body mass of nestlings at hatching. To test if the body mass of nestlings at hatching was correlated with their body mass at fledgling, I performed a Spearman Spearman's rank correlation test. I also used a t-test to compare body measures of nestling and adult birds to determine if there was evidence of sexual size dimorphism.

Factors influencing growth rate and pre-fledging body mass were modelled by fitting different generalised linear mixed models (GLMMs). This method was employed to account for repeated measures of individuals and broods, and to control for the non-independence of data using the random effects of nest identity and individual nestling incorporated into models. Response parameters used in analyses were average daily body mass gain during the period of approximately linear growth rates between days 2-10 post-hatch (g/day), as a measure of nestling growth, and final body mass prior to fledging (at day 14 post hatch). The aim of this modelling was primarily to assess the effect of nestling sex on these dependent variables; however, the

growth patterns and body mass could also be influenced by the provisioning rate at the nest and the sex of brood mates. Therefore, in the global model, I included provisioning rate per hour and sibling sex ratio (SSR) as fixed effects, as well as focal nestling sex. Provisioning rate increased with nestling age but did not change significantly after 11 days of age (see Chapter 3). Therefore, provisioning rate only included observations collected 11-15 days post hatch at the broods. For each brood, mean provisioning rate during this period (e.g. 11-15 days post hatch) was used as an index of volume of food delivered to the focal brood. In all models, nest identity and hatching order were included as random effects. A total of 6 submodels were then generated from this global model, and these, plus a model containing only the intercept and random terms (Table 4.1), were fitted with the *lmer* function in the R package *lme4* (Bates et al. 2014) in the R environment (R Core Team, 2014) using a Maximum Likelihood approach. After fitting the models, I used Akaike's Information Criteria for small sample size (AICc) to assess model fit, and considered models within ~ 2 AICc of the best fit models as having equivalent levels of support. It has been suggested that if there is not a clear best model (based on model weight > 0.9) then coefficients should be obtained through model-averaging (Grueber et al. 2011). Model averaging was conducted in *MuMIn* package (Bartoń 2015) using the natural average method to obtain model-averaged coefficients (Burnham & Anderson 2004, Grueber et al. 2011).

Table 4.1. List of models fitted to explain the daily growth rate and pre-fledging body mass of nestling noisy miners.

Number	Fixed effects	Random effects
1	Intercept-only	Nest+ Hatch order
2	Sex ¹ + Provisioning rate ² + Sibling Sex Ratio ³	Nest+ Hatch order
3	Provisioning rate + Sibling Sex Ratio	Nest+ Hatch order
4	Sex+ Provisioning rate	Nest+ Hatch order
5	Sex + Sibling Sex Ratio	Nest+ Hatch order
6	Sex	Nest+ Hatch order
7	Provisioning rate	Nest+ Hatch order
8	Sibling Sex Ratio	Nest+ Hatch order

¹ male or female nestling, ² mean number of feeds per hour for broods aged 11-15 days post hatch, ³ percentages of male brood mates for each focal nestling.

To examine the effects of BSR on helper effort, provisioning rate during the periods of stable provisioning was examined (see above and Chapter 3). Previous analyses identified bird status as an important variable that influences provisioning rate (Chapter 3). To examine if BSR influences provisioning rate, I first constructed a GLMM with bird status (7 levels: breeder female, breeder male and 5 levels of helper relatedness) as a fixed effect and then examined if BSR improved model explanatory power. BSR was defined as a categorical variable with two levels based on BSR values (see above). Broods were grouped as male-biased (BSR=0.67, 1) or female-biased (BSR=0, 0.33) to generate approximately equal sample sizes. The modal clutch and brood size was three, therefore broods were either male- or female biased in the majority of nests.

To test if an interaction between status and BSR improved model fit, I compared a model with status and BSR with a model that contained identical fixed effects plus their interaction. Models were compared with a likelihood ratio test and the significance of terms assessed using $\alpha=0.05$. For each term, the effect size and 95% CIs were also reported. In all models, the response variable (provisioning rate) was square-root transformed to normalise and reduce residual

variance. In addition, because female helpers were rare in this system, (~ %7 of helpers, N=7) and were all unrelated to the breeding female at nests that they provisioned, thus I only modelled male helpers' behaviour. In these models, I also included the random effects of nest identity and bird identity, to control for non-independence of data collected from multiple observations of the same individuals and nests. As I conducted multiple observations at each nest, the order of observation was also included as random effect to control for any potential impact of observation sequence on provisioning behaviour. All analyses were performed in the R statistical language and environment (R Core Team, 2014). I used the *lmer* function with a Restricted Maximum Likelihood approach in *lme4* package (Bates et al. 2014) to perform GLMMs.

Results

Nestling and adult sex ratio in different colonies

Overall at the Newholme and Dumaresq Dam colonies, 51% of nestlings were male and nestling sex ratios did not differ significantly from the expected 50:50 distribution across a combined dataset for all colonies and years ($\chi^2_1=0$, $p=1$, N=82, Female: 40, Male: 42). When analysing the reproductive output of each colony separately, neither was significantly different from parity: Dumaresq Dam ($\chi^2_1=0.68$, $p=0.40$, N=19, Female: 6, Male:13), Newholme ($\chi^2_1=0.07$, $p=0.78$, N=63, Female: 34, Male: 29). Similarly, the overall sex ratio of nestlings did not differ from parity in any year (2013: $\chi^2_1=0$, $p=1$, N=22, Female: 12, Male: 10; 2014: $\chi^2_1=0.29$, $p=0.58$, N=37, Female: 18, Male: 25; 2015: $\chi^2_1=0.02$, $p=0.86$, N=17, Female: 10, Male: 7). For nestlings with a known hatch order (N=31), sex ratios did not differ significantly from parity within a given hatch sequence (first-hatched nestlings: $\chi^2_1=0.33$, $p=0.56$, second-hatched nestlings: $\chi^2_1=0.09$, $p=0.73$, third-hatched nestlings: $\chi^2_1=0.50$, $p=0.47$).

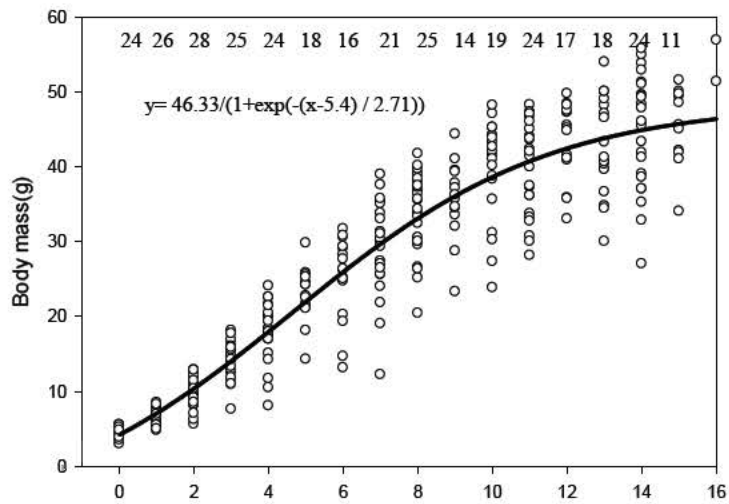
Combined for the three colonies, the overall adult sex ratios of noisy miners banded within each colony were significantly male-biased: 69 % \pm 1 SE (N=288, Female: 89, Male: 199; $Z = -5.34$, $p < 0.001$). Likewise, when each colony was examined separately, a significant male-biased sex ratio was apparent at the Dumaresq Dam ($\chi^2_1 = 4.5$, $p = 0.03$, N=56, Female: 16, Male: 40), Hillgrove ($\chi^2_1 = 5.8$, $p = 0.01$, N=90, Female: 28, Male: 62) and Newholme colonies ($\chi^2_1 = 9.10$, $p = 0.001$, N=142, Female: 45, Male: 97). There was no significant difference in the adult sex ratio among colonies ($Z = 4.65$, $p = 0.90$) or years (2013-2015; $Z = 1.45$, $p = 0.20$).

Nestling body mass at hatching and subsequent development trajectories

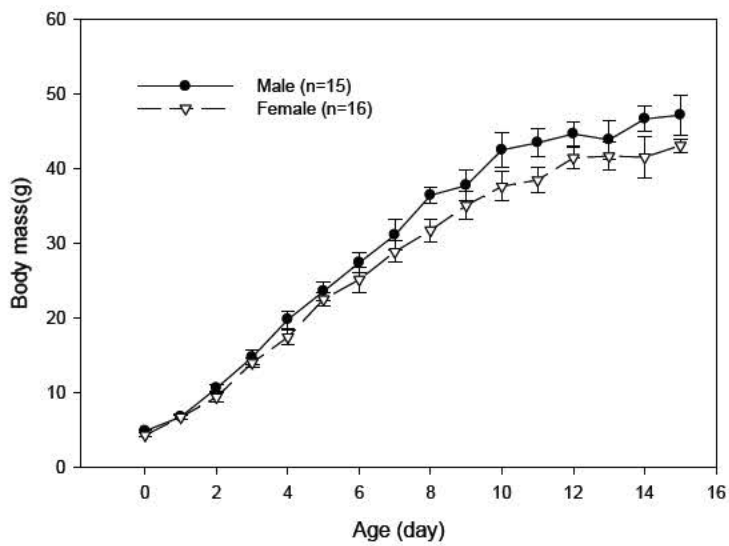
Body mass at hatching did not differ between the sexes of nestlings ($t = -1.80$, $df = 9.57$, $p = 0.10$) although male nestlings were slightly heavier (mean \pm SE: 4.91 g \pm 0.51) than females (4.25 g \pm 0.95). Body mass at hatching was not influenced by hatch order ($F_{2,18} = 2.27$, $p = 0.12$). No significant interaction was detected between sex and hatching order in body mass at hatching ($F_{1,16} = 0.02$, $p = 0.88$), and this variable was also not significantly correlated with pre-fledging body mass ($r^2 = 0.195$, $p = 0.15$). Sigmoid functions provided the best fit for body mass increases between the period 0-15 days of age ($R^2 = 0.89$, $p = 0.0001$) with approximately linear growth between days 2 and 10 (Fig. 4.1a). At most ages, male nestlings had a higher body mass than females (Fig. 4.1b).

I modelled two main growth components: body mass before fledging (i.e. body mass at day 14 post-hatch) and the daily gain in body mass during the linear growth period (days 11-15 post hatch). Variations in the growth rate of body mass were best described with models including nestling sex and provisioning rate, with a model including nestling sex alone receiving the highest support ($\Sigma w_i = 0.59$, Table 4.2). The second best model included both nestling sex and provisioning rate as fixed factors (Table 4.2), and had similar levels of support to the best model

($\Delta AICc = 2.35$; Table 4.2). When these two best-fit models were averaged, the model-averaged coefficients of provisioning rate and their associated 95%CI overlapped with zero (Table 4.3), indicating that provisioning rate index is not a strong explanatory factor for variation in the growth rate of body mass. On most days, male nestlings had a higher body mass than female nestlings (Fig. 4.1b). A model with nestling sex best explained variation in pre-fledging body mass of nestlings ($\Sigma w_i = 0.52$), followed by a model with sibling sex ratio alone ($\Sigma w_i = 0.13$). Close to fledging (day 14), males had around 10% heavier body mass than females of the same age (Fig. 4.1b, Table 4.3). Sibling sex ratio was also supported as explaining pre-fledging body mass as nestlings, with male-biased siblings were lighter prior to fledging than nestlings with only female siblings.



(a)



(b)

Fig. 4.1. Growth patterns of (a) individual nestling noisy miners and (b) mean \pm SE body mass for nestlings of different sexes. Total sample sizes at each age are given in (a), with the number of individuals of each sex measured provided in (b).

Table 4.2. GLMM results for modelling nest growth rate and pre-fledgling body mass of nestling noisy miners. AICc indicates the Akaike's Information Criterion for small samples; Δ AICc the scaled AICc relative to the top model; w_i the Akaike model weight; k the number of parameters and Σw , cumulative model weight. Models that were averaged are shown in bold.

Fixed effects	k	AICc	Δ AICc	w_i	Σw
<i>Linear growth rate</i>					
Sex	6	68.1	0	0.59	0.59
Sex + Provisioning rate	7	70.44	2.35	0.18	0.77
Intercept-only	5	75.17	7.08	0.02	0.98
<i>Pre-fledgling body mass</i>					
Sex	6	196.83	0	0.52	0.52
Sibling Sex Ratio	6	199.55	2.72	0.13	0.66
Intercept-only	5	201.01	4.18	0.06	0.93

Table. 4.3. Effects size, SE and 95% CIs for different factors that influence growth rate and pre-fledgling body mass of noisy miner nestlings (N=31). Estimates of final model terms were calculated using a ML approach.

	Linear growth rate				Pre-fledgling body mass			
	β	SE	95L	95U	β	SE	95L	95U
Fixed-effects								
Intercept	3.40	0.33	2.73	4.08	44.08	3.26	37.57	50.59
Sex (male)	0.71	0.18	0.32	1.11	5.33	1.80	1.60	9.09
Provisioning rate	-0.01	0.01	-0.05	0.01				
Sibling Sex Ratio					- 4.59	0.80	-8.31	-0.86
Random Effect								
	Variance	SD				Variance	SD	
Nest ID	0.20	0.45				12.73	3.56	
Hatching order	0.004	0.06				0.00	0.00	
Residual	0.22	0.47				21.07	4.59	

¹ SSR: sibling sex ratio calculated as proportion of male brood mates

Adult sexual dimorphism

Adult noisy miners showed moderate sexual size dimorphism, with male birds being significantly larger than females in all univariate measures of body size (all $p < 0.001$; Table 4.4). Male noisy miners were 6-10 % heavier than females, and had 2-3.5% larger tarsus size. In addition, males had a 5-7% larger wing length than females (Table 4.4).

Table 4.4. Mean \pm SE of morphometric measurements of female and male adult noisy miners and results of between-sex comparisons.

Traits	Sex	N	Mean \pm SE	<i>t</i>	<i>df</i>	<i>p</i>
Mass (g)	Female	89	69.92 \pm 0.56	-9.49	134.20	< 0.001
	Male	199	76.13 \pm 0.33			
Tarsus (mm)	Female	89	32.20 \pm 0.09	-7.68	161.93	< 0.001
	Male	199	33.10 \pm 0.07			
Wing (mm)	Female	89	134.19 \pm 0.52	-13.47	147.60	< 0.001
	Male	199	142.57 \pm 0.34			

Variations of provisioning rate according to brood sex ratio

The number of birds that were seen attending the nest in addition to breeders tended to be greater in male-biased broods than in female-biased broods (Fig. 4.2), but the difference did not reach statistical significance (GLMM, $\chi^2_1=3.18$, $p=0.07$). Similarly, the number of helpers at the nest did not differ significantly according to BSR (GLMM test, $\chi^2_1=2.8$, $p=0.09$; Fig. 4.2).

Helper composition was strongly male-biased, regardless of whether or not broods attended contained a majority of male ($\chi^2_1= 13.78$, $p < 0.001$) or female nestlings ($\chi^2_1= 7.87$, $p < 0.001$). Overall, 91% and 88% of helpers were male in male- and female-biased broods, respectively.

The proportion of male helpers did not differ significantly according to BSR of broods receiving help ($\chi^2_1 = 0.84$, $p=0.35$). Breeding females and breeding males provisioned the brood at higher rate than helpers (Chapter 3). In both male- and female-biased broods, bird status explained a significant amount of variation in attendant provisioning rate using likelihood ratio tests (male-biased broods: $\chi^2_6 = 81.20$, $p<0.001$, female biased broods: $\chi^2_6 = 33.00$, $p<0.001$). In both male- and female-biased broods the breeding pair provisioned the broods at a higher rate than helpers at the nest (Fig. 4.3).

When breeding pairs were excluded from the analysis and bird status (e.g mean genetic relatedness to breeders) confined to helpers alone, a similar significant influence of status on provisioning rate was found for both male biased ($\chi^2_4 = 29.96$, $p<0.001$) and female biased broods ($\chi^2_4 = 17.56$, $p<0.001$). There was a general pattern of an increase in provisioning rate with the genetic relatedness of a given helper to the breeding pair in all broods (Chapter 3, Fig. 4.3). To examine if BSR influenced provisioning rates in addition to the status of attendants, I further compared models with status as a fixed effect with a model that contained status and BSR as fixed effects. BSR did not improve the model support significantly, either for all birds or when only helpers were included in the models using likelihood ratio tests (all birds: $\chi^2_1 = 2.12$, $p=0.14$; helpers: $\chi^2_1 = 3.00$, $p=0.08$). In addition, interactions between BSR and status were not apparent when all birds ($\chi^2_6 = 10.48$, $p=0.10$) or only helpers were included in the analysis ($\chi^2_4 = 2.35$, $p=0.67$).

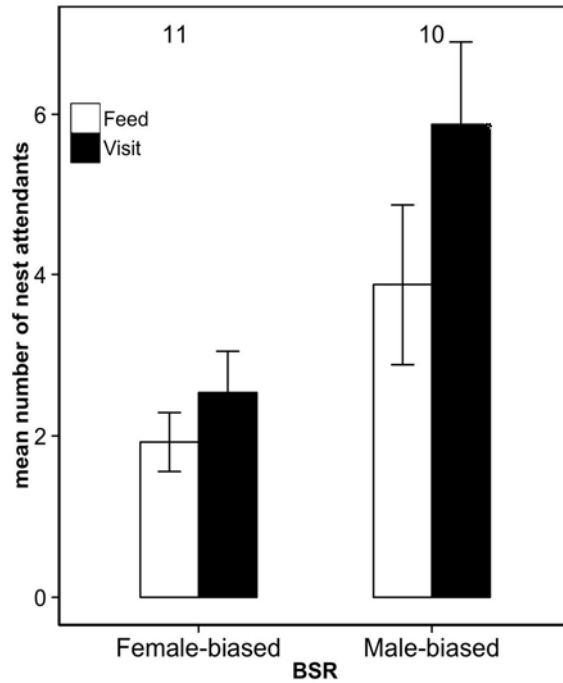


Fig. 4.2. Mean \pm SE of variations in the mean number of nest attendants other than the breeding pair at nests in relation to the nestling brood sex ratio (BSR). Sample sizes for each BSR are presented above the bars.

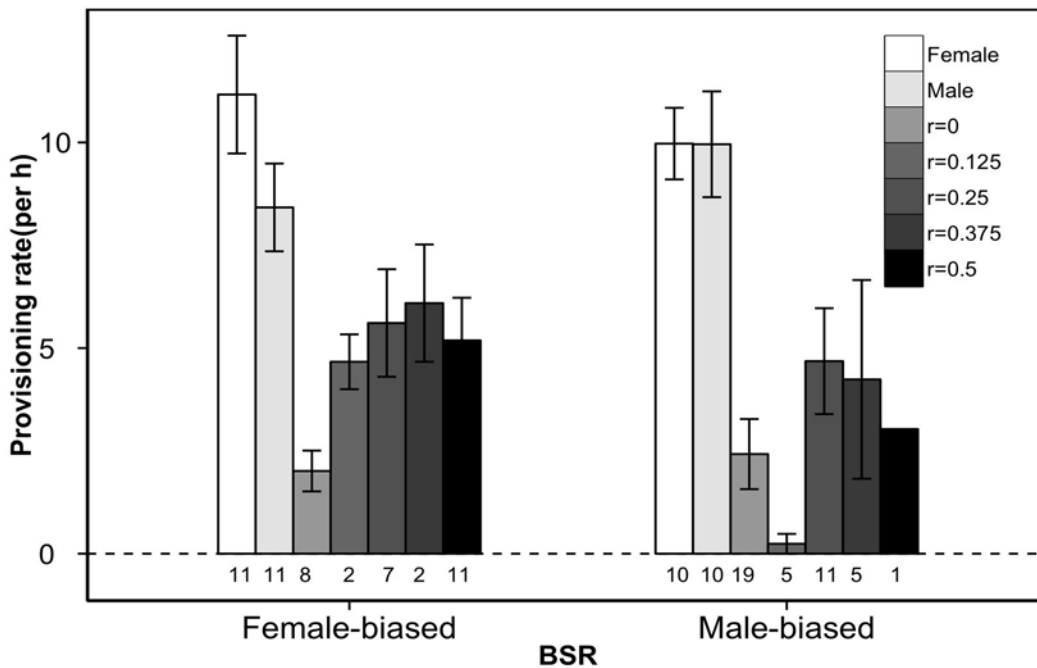


Fig. 4.3. Mean \pm SE of variations in the provisioning rate by breeders and helpers of different genetic relatedness to the breeder pair in relation to brood sex ratio (BSR). Sample sizes for each status class are presented below bars.

Discussion

Offspring sex ratio

The sex ratio of nestlings did not deviate from parity when data from colonies and different breeding seasons were compared as a collated dataset or in isolation. This finding is in line with previous reports for noisy miners (e.g. Arnold et al. 2001). In cooperatively breeding species, it is predicted that breeding females should overproduce the helping sex when that sex is rare in the population (Gowaty & Lennartz 1985; Pen & Weissing 2000). However, this seems unlikely to lead to a male-biased brood in noisy miners, as the overall population sex ratio is clearly biased in favour of the helper sex in colonies (see Results). In addition, the strength of selection on sex ratio adjustment is positively correlated with the benefit that helpers may provide (Griffin et al. 2005). In other words, if helpers have a large effect on the fitness benefit of breeding females, the deviation of sex ratio should be more pronounced towards the helping sex (Griffin & West 2003). In contrast, when the impact of helpers on breeding female fitness is negligible, we may not expect as strong or even any deviation of the sex ratio (Pen & Weissing 2000). Although the effects of helpers on the fitness and survival of breeding females is not known in noisy miners, we did not detect a marked impact of helper provisioning on the growth and body condition of nestlings in this study, potentially explaining why breeding females did not overproduce males as the primary helping sex.

Helpers do not bias investment according to brood sex ratio, regardless of their relatedness to breeders

The level of investment provided by individual helpers did not vary according to the brood sex ratio. Male noisy miners are more philopatric than females, and as such male offspring are

more likely to become helpers at future nests within the colony, potentially repaying the help provided to them by helpers if these were able to secure a breeding position within the colony in later life. In addition, colony members that are predominately male, assist in other types of helping, such as improved territory defence against competitors and predators, a behaviour known as 'mobbing' (Arnold 2000, Arnold et al. 2005). This should be particularly important for unrelated helpers, as they can only gain benefits from philopatric male recruits in the absence of any indirect fitness benefits. From an unrelated helper's perspective, male offspring are therefore seemingly more valuable in terms of their future fitness benefits than females. Despite this, helpers did not adjust their provisioning efforts according to the focal brood sex ratio and there was no support for the repayment hypothesis (Emlen et al. 1986; Griffin et al. 2005).

One potential factor that might explain this deviance from the expected provisioning rate is local competition for resources, such as mating opportunities and food. Given that successfully raised, philopatric male offspring may ultimately compete with their helpers for resources in later life (West et al. 2001), this cost may well outweigh or nullify any benefit of future aid. This trade-off between the short and long term costs and benefits of preferentially investing in male-biased broods may thus be shaping helper provisioning behaviour, and is worthy of further study.

In some cooperatively breeding birds, helpers might preferentially invest in the opposite sex in order to increase their probability of breeding in the future (Craig & Jamieson 1988). In noisy miners, helpers are predominantly males (this study); therefore, to gain this direct benefit of helping, helpers might be expected to invest more in female-biased broods. However, despite this potential direct benefit for helpers, they did not preferentially provision female-biased broods in this study. Two main factors might explain why related and unrelated helpers did not bias their efforts towards female-biased broods in order to gain enhanced breeding opportunities. First, a

substantial proportion of care is provided by related helpers (Chapter 3), and given clear inbreeding avoidance in this system (Chapter 5), it is unlikely that related helpers would form a breeding pair with the females that they care for. Second, female offspring disperse from the natal colony before they start breeding, thus unrelated male helpers also usually do not have the opportunity to mate with the females that they have helped, given females typically disperse to other colonies to breed. So, although a direct benefit of enhanced mating opportunity might explain investment bias towards the opposite sex in some species (Ridley & Huyvaert 2007), it seems unlikely to be relevant in the noisy miner system.

Breeding pairs do not preferentially provision broods according to offspring sex ratio

Similar to helpers, breeders also did not adjust their provisioning efforts according to offspring brood sex ratio. The benefits that breeders and helpers accrue through investment in broods differ. In addition to the prediction that breeders can gain a higher benefit of investment in philopatric and more helpful male offspring (e.g. repayment hypothesis), sex allocation of breeders is also influenced by the difference in the overall breeding success and thus fitness returns of each sex (Koenig et al. 1983). For example, if it is predicted that females would have a higher lifetime reproductive success than their male counterparts, breeders may invest more heavily in female offspring. Although it is predicted that breeders might invest more in philopatric male offspring so that they would benefit in future reproductive activities once nutritionally independent, the strategies of breeders may also depend on external conditions such as habitat quality. In high quality habitats, breeders are expected to invest in the philopatric sex (Hewison & Gaillard 1999; Julliard 2000). In contrast, breeders might invest more on the dispersing sex if local resources such as breeding territories are limited, and retained offspring would increase competition for resources with breeders and their siblings (Clark 1978; Ewen et

al. 2003; Silk & Brown 2008). Therefore, in addition to the overall breeding success of the different offspring sexes, other factors, such as habitat quality, need to be considered for a better understanding of how sex allocation functions in the noisy miner breeding system.

The costly sex and helper provisioning effort

Male nestlings had a greater body mass for much of the time during the nestling phase and fledged at a heavier body mass than their female counterparts after controlling for a hatch order effect. This difference was consistent with adult sexual dimorphism patterns, as adult males had a greater body mass and larger skeletal size measures than their female counterparts. Therefore, male offspring are likely to have higher energetic requirements and be more expensive to raise to fledging and beyond than female offspring. Despite this, helpers did not contribute more to rearing male-biased broods, and an increase in food being delivered to male-biased broods as might have been anticipated (Slagsvold 1997). One explanation could be that the size differences between male and female offspring are relatively small, and did not result in adaptive changes in provisioning behaviour of helpers or parents alike. Male-biased preferential provisioning has typically been documented in species that show greater sexual size dimorphism than found in noisy miners. For example, in the brown songlark (*Cinchorhamphus cruralis*), male nestlings were shown to be on average 49% heavier than female nestlings, and received 42% more food than female nestlings (Magrath et al. 2007).

Moreover, within-nest competition may prevent helpers from directing their provisioning effort to males as result of a stronger hatch order effect on body size. In the noisy miner, hatching occurs asynchronously, with one-day intervals between nestlings being typical (Higgins et al. 2001), and can cause variation in offspring growth at the same nest. Therefore, older nestlings might benefit from having higher body mass than younger brood mates regardless of

their sex. This may result in greater competitive ability in older nestlings within the brood, as they can move more vigorously to a position closest to the attendant when they arrive with food (McRae et al. 1993). However, after fledging, and in the absence of within-nest competition, helpers still might preferentially bias their efforts towards costly males, which requires further examination with a focus on the post-fledging period.

Is there an acoustic cue that might facilitate nestling sex determination by either parents or helpers?

The results herein are consistent with parents and helpers not preferentially biasing their investment according to brood sex ratio in the noisy miner. Regardless of all possible explanations for the lack of biased investment towards beneficial males, it is important to note that any preferential investment in a given sex requires nest attendants being able to discriminate between sexes when provisioning. Breeders and helpers are likely to use one of two different sources of information to separate the nestling sexes. First, they may use acoustic cues of differences in the vocal structure of begging calls of the different sexes. This was not measured during this study, but in the bell miner, a close relative of the noisy miner, no differences were found between the begging characteristics of male and female nestlings (McDonald et al. 2010). Therefore, it is possible that carers do not have sufficient acoustic cues to differentiate nestlings according to their sex.

Second, nest attendants might use visual cues such as a size difference between the sexes of offspring to distinguish and preferentially feed a given nestling sex. In this system, although male nestlings were bigger than females of the same age, the size-differences were unlikely to be readily detectable by provisioners, particularly when a large hatch order effect is also taken into consideration. Moreover, the body condition of nestlings indicated that body mass prior to

fledging was influenced by the sibling sex ratio and offspring raised in a male-biased brood were disadvantaged regardless of the sex of that focal nestling (Tables 4.2-4.3). This suggests that before fledging, the body size of a given nestling is not necessarily an adequate indicator of offspring sex. Further, there is also possibility that nestlings are selected to conceal their sex to prevent helpers preferentially feeding a given sex as shown in the eclectus parrot (*Eclectus roratus*) (Heinsohn et al. 1997). Therefore in this system, it is quite likely that provisioners cannot determine a given nestlings' sex or a brood's sex ratio during provisioning, and this may explain why preferential aid was not detected, even when it may have been advantageous for helpers to do so.

Conclusion

Neither breeder nor the helper noisy miners adjusted their investment according to focal brood sex ratio, despite the apparent difference of fitness benefits resulted from investment in male versus female-biased broods. In cooperatively breeding birds, the fitness consequences of investment according to the recipient's sex are likely to be affected by multiple factors as opposed to a single determinant (Hasselquist & Kempenaers 2002; Lessells 2002). Therefore, the net benefit gained by helpers depends on the relative weight of positive and negative interactions between helpers and offspring (Emlen 1997; West et al. 2005). An alternative explanation that was not able to be ruled out is that provisioners may simply not have been capable of discriminating between nestlings of a different sex. Despite these results, however, breeders and helpers may still invest more in one sex after fledging because of prolonged parental care in this system, so further research targeting this neglected stage of alloparental care would be most informative.

References

- Abbott, C.L., Poldmaa, T., Loughheed, S., Clarke, M., Boag, P.T. (2002). Hierarchical analysis of genetic population structure in the noisy miner using DNA microsatellite markers. *Condor*, 104, 652-656.
- Arnold, K. E. (2000). Group mobbing behaviour and nest defence in a cooperatively breeding Australian bird. *Ethology*, 106, 385-393.
- Arnold, K. E., Owens, I. P. F., Goldizen, A. W. (2005). Division of labour within cooperatively breeding groups. *Behaviour*, 142, 1577-1590.
- Arnold, K.E., Griffith, S.C., Goldizen, A.W. (2001). Sex-biased hatching sequences in the cooperatively breeding noisy miner. *Journal of Avian Biology*, 32, 219-223.
- Baglione, V., Canestrari, D., Marcos, J., Ekman, J. (2003). Kin selection in cooperative alliances of carrion crows. *Science*, 300, 1947-9.
- Bartoń, K. (2015). MuMIn: Multi-Model Inference. R package version 1.13.4. <http://CRAN.R-project.org/package=MuMIn>.
- Neuhauser, M. (2004). Tests for a biased sex ratio when the data are clustered. *Environmnetal and Ecological Statistics*, 11,295–304.
- Barati, A., Etezadifar, F., McDonald P. G. (2016). Fragmentation in eucalypt woodlands promotes nest-tree occupancy by a despotic species, the noisy miner (*Manorina melanocephala*). *Austral Ecology*, 41, 897-905
- Bates, D., Maechler, M., Bolker, B., Walker, S. (2014). lme4: Linear mixed-effects models using Eigen and S4_. R package version 1.1-7, <URL: <http://CRAN.R-project.org/package=lme4>>.
- Brown, J. L. (1987). *Helping and Communal Breeding in Birds*. Princeton, New Jersey: Princeton University Press.
- Burnham, K. P., Anderson, D. R. (2004). Multimodel inference: understanding AIC and BIC in model selection. *Sociological Methods & Research* 33, 261-304.
- Clark, A. B. (1978). Sex ratio and local resource competition in a prosimian primate. *Science*, 201, 163-165.
- Clutton-Brock, T. H. (1991). *The Evolution of Parental Care*. Princeton, New Jersey: Princeton University Press.
- Clutton-Brock, T. H., Russell, A. F., Sharpe, L. L., Young, A. J., Balmforth, Z., McIlrath, C. M.

- (2002). Evolution and development of sex differences in cooperative behavior in meerkats. *Science*, 297, 253-256.
- Craig, J. L., Jamieson, I. G. (1988). Incestuous matings in a communally breeding bird. *American Naturalist*, 131, 58-70.
- Doutrelant C, Covas R, Caizergues A, du Plessis M. (2004). Unexpected sex ratio adjustment in a colonial cooperative bird: pairs with helpers produce more of the helping sex whereas pairs without helpers do not. *Behavioral Ecology and Sociobiology*, 56, 149-154.
- Emlen, S. T. (1997). When mothers prefer daughters over sons. *Trends in Ecology & Evolution*, 12, 291-292.
- Emlen, S. T., Emlen, J. M., Levin, S. A. (1986). Sex-ratio selection in species with helpers-at-the-nest. *American Naturalist*, 127, 1-8.
- Ewen, J. G., Crozier, R. H., Cassey, P., Ward-Smith, T., Painter, J. N., Robertson, R. J., Jones, D. A., Clarke, M. F. (2003). Facultative control of offspring sex in the cooperatively breeding bell miner (*Manorina melanophrys*). *Behavioral Ecology*, 14, 157-164.
- Goodnight, K. F., Queller, D. C. (1999). Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Molecular Ecology*, 8, 1231-1234.
- Gowaty, P. A., Lennartz, M. R. (1985). Sex ratios of nestling and fledgling red-cockaded woodpeckers (*Picoides borealis*) favor males. *American Naturalist*, 126, 347-353.
- Griffin, A. S., Sheldon, B. C., West, S. A. (2005). Cooperative breeders adjust offspring sex ratios to produce helpful helpers. *American Naturalist*, 166, 628-632.
- Griffin, A. S., West, S. A. (2003). Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. *Science*, 302, 634-636.
- Griffiths, R., Double, M., Orr, K., Dawson, R. (1998). A DNA test to sex most birds. *Molecular Ecology*, 7, 1071-1075.
- Grueber, C.E., Nakagawa, S., Laws, R. J., Jamieson, I. G. (2011). Multimodel inference in ecology and evolution: challenges and solutions. *Journal of Evolutionary Biology*, 24, 699-711.
- Heinsohn, R., Legge, S., Barry, S. (1997). Extreme bias in sex allocation in Eclectus parrots. *Proceedings of the Royal Society B*, 264, 1325-1329.
- Hardy, I. C. W. (2002). *Sex ratios: concepts and methods*. Cambridge Univ. Press.
- Hardy, I.C.W. (1997). Possible factors influencing vertebrate sex ratios: an introductory

- overview. *Applied Animal Behaviour Science*, 51, 217-241.
- Hasselquist, D., Kempenaers, B. (2002). Parental care and adaptive brood sex ratio manipulation in birds. *Philosophical Transactions of the Royal Society B*, 357, 363-372.
- Hatchwell, B. J. (2009). The evolution of cooperative breeding in birds: kinship, dispersal and life history. *Philosophical Transactions of the Royal Society B*, 364, 3217-3227.
- Hatchwell, B. J., Russell, A. F., MacColl, A. D. C., Ross, D. J., Fowlie, M. K., McGwan, A. (2004). Helpers increase long-term but not short-term productivity in cooperatively breeding long-tailed tits. *Behavioral Ecology*, 15, 1-10.
- Hewison, A. J. M., Gaillard, J-M. (1999). Successful sons or advantaged daughters? The Trivers–Willard model and sex-biased maternal investment in ungulates. *Trends Ecology and Evolution*, 14, 229-234.
- Higgins, P. J., Peter J. M., Steele, W. K. (2001) *Handbook of Australian, New Zealand and Antarctic Birds*. Tyrant-Flycatchers to Chats. Vol 5. Oxford University Press, Melbourne.
- Julliard, R. (2000). Sex-specific dispersal in spatially varying environments leads to habitat-dependent evolutionarily stable offspring sex ratios. *Behavioural Ecology*, 11, 421-428.
- Kalinowski, S.T., Taper, M. L., Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, 16, 1099-1106.
- Koenig, W. D., Mumme, R. L., Pitelka, F. L. (1983). Female roles in cooperatively breeding acorn woodpeckers. In: *Social Behaviour of Female Vertebrates* (Ed. By S. K. Waser), pp. 235-261. New York: Academic Press.
- Kokko, H., Johnstone, R. A., Clutton-Brock, T. H. (2001). The evolution of cooperative breeding through group augmentation. *Proceedings of the Royal Society B*, 268, 187-196.
- Komdeur, J. (1994). The effect of kinship on helping in the cooperative breeding seychelles warbler (*Acrocephalus sechellensis*). *Proceedings of the Royal Society B*, 256, 47-52.
- Konovalov, D. A., Manning, C. Henshaw, M. T. (2004). KINGROUP: a program for pedigree relationship reconstruction and kin group assignments using genetic markers. *Molecular Ecology Notes*, 4, 779-782.
- Kopps, A. M., McDonald, P.G., Rollins L.A. (2013). Isolation and characterisation of polymorphic microsatellite loci for noisy miners (*Manorina melanocephala*), with successful cross-amplification in bell miners (*M. melanophrys*). *Conservation Genetic*

- Resources*, 5, 39-41.
- Lessells, C. M. (2002). Parentally biased favouritism: why should parents specialize in caring for different offspring? *Philosophical Transactions of the Royal Society B*, 357, 381-403.
- Magrath, M. J. L., Van Lieshout, E., Pen, I., Visser G. H., Komdeur, J. (2007). Estimating expenditure on male and female offspring in a sexually size-dimorphic bird: a comparison of different methods. *Journal of Animal Ecology*, 76, 1169-1180.
- Marshall, T. C., Slate, J., Kruuk, L. E. B., Pemberton, J. M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, 7, 639-655.
- McDonald, P. (2012). Cooperative bird differentiates between the calls of different individuals, even when vocalizations were from completely unfamiliar individuals. *Biology Letters*, 8, 365-368.
- McDonald, P. G., Ewen, J. G., Wright, J. (2010). Brood sex ratio does not affect helper effort in a cooperative bird, despite extreme sex-biased dispersal. *Animal Behaviour*, 79, 243-250.
- McDonald, P. G., Kazem, A. J. N., Clarke, M. F., Wright, J. (2008a). Helping as a signal: does removal of potential audiences alter helper behavior in the bell miner? *Behavioral Ecology*, 19, 1047-1055.
- McDonald, P. G., te Marvelde, L., Kazem, A. J. N., Wright, J. (2008b). Helping as a signal and the effect of a potential audience during provisioning visits in a cooperative bird. *Animal Behaviour*, 75, 1319-1330.
- McRae, S. B., Weatherhaed, P. J., Montgomerie, R. (1993). American robin nestlings compete by jockeying for position. *Behavioral Ecology and Sociobiology*, 33, 101-106.
- Nam, K-B., Meade, J., Hatchwell, B. J. (2011). Do parents and helpers adjust their provisioning effort in relation to nestling sex in a cooperatively breeding bird? *Animal Behaviour*, 82, 303-309.
- Painter, J., Crozier, R. H., Crozier, Y. C., Clarke, M. F. (1997). Characterization of microsatellite loci for a cooperatively breeding honeyeater. *Molecular Ecology* 6, 1103-1105.
- Pen, I., Weissing, F. J. (2000). Sex ratio optimization with helpers at the nest. *Proceedings of the Royal Society of London B*, 267, 539-544.

- R Core Team. (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Ridley, A. R., Huyvaert, K. P. (2007). Sex-biased preferential care in the cooperatively breeding arabian babbler. *Journal of Evolutionary Biology*, 20, 1271-1276.
- Silk, J.B., Brown, G.R. (2008). Local resource competition and local resource enhancement shape primate birth sex ratios. *Proceedings of the Royal Society of London B*, 275:1761-1765.
- Slagsvold, T. (1997). Brood division in birds in relation to offspring size: sibling rivalry and parental control. *Animal Behaviour*, 54, 1357-1368.
- Trivers, R. L. (1972). *Parental investment and sexual selection*. In: *Sexual Selection and the Descent of Man* (Ed. by B. Campbell), pp. 136-179. London: Heinemann.
- Wade, M. J., Shuster, S. M., Demuth, J. P. (2003). Sexual selection favors female-biased sex ratios: the balance between opposing forces of sex-ratio selection and sexual selection. *American Naturalist*, 162: 403-414.
- Wang, J. (2002). An estimator for pairwise relatedness using molecular markers. *Genetics*, 160, 1203-1215.
- West, S. A., Murray, M. G., Machado, C. A., Griffin, A. S., Herre, E. A. (2001). Testing Hamilton's rule with competition between relatives. *Nature*, 409, 510-513.
- West, S. A., Shuker, D. M., Sheldon, B. C. (2005). Sex-ratio adjustment when relatives interact: a test of constraints on adaptation. *Evolution*, 59, 1211-1228.
- Wright, J. (2007). Cooperation theory meets cooperative breeding: exposing some ugly truths about social prestige, reciprocity and group augmentation. *Behavioural Processes*, 76, 142-148.
- Wright, J., McDonald, P. G., te Marvelde, L., Kazem, A. J. N., Bishop, C. (2010). Helping effort increases with relatedness in bell miners, but 'unrelated' helpers of both sexes still provide substantial care. *Proceedings of the Royal Society of London B*, 277, 437-445.
- Zahavi A. (1975). Mate selection—A selection for a handicap. *Journal of Theoretical Biology*, 53, 205-214.

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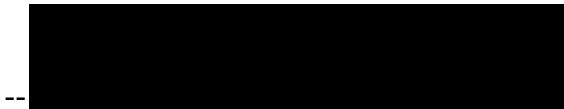
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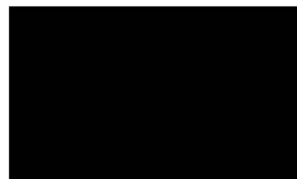
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Extra-pair paternity and inbreeding avoidance in a cooperatively breeding bird, the noisy miner (*Manorina melanocephala*)



This chapter will be submitted for publication as a standard paper.

Abstract.

In many socially monogamous bird species, both sexes regularly engage in mating outside their social pair bond. While the benefits of extra-pair (EP) mating behaviour are clear and well established for males, such as an increase in the number of sired offspring, the benefits of EP mating behaviour to females are less clear. A dominant theory for the incident of EP mating predicts that socially monogamous females can improve the genetic quality of their offspring and avoid the costs of inbreeding through EP mating. However, in cooperatively breeding species, where breeders benefit from helper contributions in raising their offspring, females may also benefit from engaging in EP mating through the ‘parental care’ hypothesis that predicts females obtain additional help for their offspring through reproductive skew. Conversely, one might also expect decreased care to be provided by cuckolded breeding pair males, and any helpers that are related to these individuals, in broods with EP if differences in paternity and/or relatedness are detectable. However, empirical evidence in support of the ‘parental care’ hypothesis is currently lacking. Here, I examined evidence for both the inbreeding avoidance and parental care hypotheses in the cooperatively breeding noisy miner (*Manorina melanocephala*) by comparing microsatellite-based analysis of extra-pair paternity with provisioning efforts of putative breeding males and their helper contingent. In this species, the putative, social breeding male provides substantial care to offspring, as do both related and unrelated, predominately male, helpers attending nests. Overall, EP mating occurred in 27% of broods with 14% of offspring sired by males other than the identified social male at a given nest. There was a strong tendency to avoid copulation with genetically related individuals, with 86% of social breeding pairs being significantly less related to each other than the general population based on likelihood ratio tests. While EP mating occurred independent of the degree of relatedness between the social breeding

pair at a given nest, nests at which EP mating had occurred had an increased brood provisioning rate than those where the breeding male obtained paternity of the entire brood. However, contrary to *a priori* predictions, this increased brood provisioning rate was not restricted to unrelated male helpers that may have obtained copulations consistent with the ‘parental care’ hypothesis, but was rather observed by breeders, related and unrelated helpers alike. These results demonstrate that in this cooperatively breeding system, there is no support that EP mating might function as a mechanism to reduce the costs of inbreeding depression or to gain benefits of extra helpers.

Keywords. Inbreeding avoidance, extra-pair paternity, noisy miner, cooperative breeding, *Manorina melanocephala*, provisioning effort.

Introduction

Recent developments in the analysis of parentage have confirmed a higher complexity and diversity of mating systems in birds than predicted by social bonds alone, demonstrating that in most species females do not mate exclusively with their social partners, but rather also frequently copulate with males outside their social bond (Birkhead 1998). This phenomenon, which is referred to as extra-pair (EP) mating behaviour or polyandry, is widespread among bird species. For example, only 10% of socially monogamous songbirds are found to be genetically monogamous (reviewed in Griffith et al. 2002). EP mating has broad implications and might influence mate choice as well as sexual conflicts and speciation (Bretman & Tregenza 2005). While the mechanism and evolution of EP mating in birds has been a central focus of many recent studies, how EP mating evolves and what benefits might be accrued still remain a topic of considerable debate (reviewed in Griffith et al. 2002; Westneat & Stewart 2003; Akçay & Roughgarden 2007).

While EP mating enhances the reproductive success or opportunities for males, the benefit/s that females receive are less clear given that EP mating typically does not enhance fecundity (Møller & Birkhead 1994). Further, mating with multiple males can impose significant costs to breeding females (Cornell & Tregenza 2007), such as aggression (Valera et al. 2003) or decreased parental care from her social male (Arnqvist & Kirkpatrick 2005), as well as increased exposure to parasites and pathogens (Martinez-Padilla et al. 2012). Despite these costs, the near ubiquity of EP matings in the majority of taxa suggests that females should receive indirect and/or direct compensatory benefits to support this behaviour. As a result, many hypotheses have been developed to explain what benefits females might gain for polyandrous mating behaviour (Griffith et al. 2002).

One of these hypotheses suggests that females use EP mating as a mechanism to avoid inbreeding and to gain an indirect benefit of improved offspring fitness (Griffith et al. 2002; Westneat & Stewart 2003; Akçay & Roughgarden 2007). This is because inbreeding can cause negative effects on offspring's traits such as survival or immunity (Reid et al. 2011; Gohli et al. 2013; Keller & Waller 2002; Hemmings et al. 2012) or even egg hatchability (Kingma et al. 2013). Therefore, EP mating with genetically dissimilar males might improve the genetic diversity of offspring (Kempnaers 2007) and, consequently, offspring fitness (Cohas et al. 2009; Harrison et al. 2011). If EP mating evolves as a mechanism to mitigate any negative consequences of inbreeding depression, a higher genetic relatedness between breeding females and their social mate would be expected in broods where females engage in EP mating compared to those where the social mate gained paternity of the entire brood. For example in the red-backed fairy-wren (*Malurus melanocephalus*), females are more likely to engage in EP mating the greater their genetic similarity with their social mate (Varian-Ramos & Webster 2012).

In addition to indirect genetic benefits, females might gain direct benefits from EP mating as a result of copulation with multiple males, including reduced harassment, access to additional resources, or securing a future partner (reviewed in Forstmeier et al. 2014). However, the main form of direct benefit that females might gain is increased parental care by additional males that access copulations (Kempnaers 1993; Ihara 2002). This direct benefit, known as the 'prenatal care' hypothesis, implies that females recruit extra males to help them in rearing the young, assuming that extra males provide care according to their certainty of paternity (e.g. Burke et al. 1989).

Although females can potentially gain the benefits provided by extra pair males, they may also risk the costly response of the cuckolded social mate. Female multiple copulations would

decrease genetic relatedness of this cuckolded male to the brood and, as such, males are predicted to facultatively adjust their parental care in relation to their decreased paternity share in the brood (Sheldon 2002). These costs might constrain and influence the evolution of EP mating behaviour.

Cooperatively breeding species are an excellent system to examine female EP mating as the costs associated with EP mating may be mitigated as a result of helpers' efforts (Rubenstein 2007). In other words, if social males decrease their 'parental care' in response to female unfaithfulness (Albrecht et al. 2006), helpers might compensate for any deficits in parental care associated with less active social males. Despite numerous investigations examining how female polyandry may influence a cuckolded male's investment in broods, results to date have not been consistent across species. Some studies suggest that cuckolded males reduce parental investment in broods with EP mating, whereas others report no change or even an increase in parental care despite female unfaithfulness (reviewed in Du et al. 2015). Moreover, in cooperatively breeding species, copulation outside the pair bond would alter the mean genetic relatedness of helpers to the resultant brood, particularly in species where helpers are related to the breeding male. Thus, according to kin selection theory (Hamilton 1964), related helpers are expected to reduce their efforts as a result of decreased relatedness, whereas unrelated helpers would be anticipated to not change their helping effort. Despite this, how helpers in cooperative systems respond to EP mating and how this is mitigated by their genetic relatedness to the subsequent brood is poorly understood. Given this, ideally the responses of cuckolded males and helpers to EP mating would be examined in cooperatively breeding species where social mates provide a high level of paternal care and both related and unrelated helpers actively provision broods.

In this chapter, I first examine if EP mating functions as a mechanism for inbreeding avoidance and whether or not females gain ‘parental care’ benefits in the cooperatively breeding noisy miner (*Manorina melanocephala*). Second, I test how cuckolded putative breeding males and male helpers of various relatedness levels respond to female EP mating behaviour. The noisy miner is a honeyeater species from the Meliphagidae family endemic to wooded country in south-eastern Australia. Noisy miners are a highly social species that lives year-round in large colonies characterised by a highly complex social structure. Most importantly for this study, noisy miners are cooperative breeders (Higgins *et al.* 2001), during which both related and unrelated helpers contribute to the care of broods (Chapter 3). Noisy miners are a particularly useful system to examine the influences of EP mating on the subsequent provisioning behaviour of a cuckolded social male mate for two reasons. First, helpers vary in the level of their genetic relatedness to both members of the social breeding pair, with the breeding male being the male that provisions broods at the highest rate at nests (Chapter 3). Second, helpers are predominantly male and female helpers are generally rare, so there is minimal confounding of the effect of helper’s sex on the outcome (Chapter 3). Given this, I predict that 1) EP mating will be positively associated with the genetic similarity of social mates (e.g. putative breeding pair); 2) If females seek extra parental care through EP mating, cuckolded males will reduce their provisioning as a result of a decreased share in the paternity of a brood; 3) EP mating would result in reduction in the care provide by related but not unrelated helpers as a consequence of their decreased genetic relatedness to the broods.

Methods

Study populations and general fieldwork

This study was conducted at two noisy miner colonies situated at Newholme Field Research Station (30° 25' 24''S; 151° 38' 84 38''E), a working rural property owned by the University of New England (UNE) and Dumaresq Dam Public Reserve, a public recreational site (30°30'S, 151°40'E). Both areas are located approximately 12 km north-west of Armidale, NSW, Australia. Between September 2013 and December 2015, noisy miners were captured and banded (see chapter 3 for details). Briefly, adult birds were caught with the aid of mist nets or baited walk-in cage traps before being fitted with a unique combination of three plastic colour leg bands and one uniquely numbered metal band issued by the Australian Bird and Bat Banding Scheme. Birds were measured, aged as either less than or greater than one year of age (see Chapter 3 for details) and approximately 70 µL of blood collected from the alar vein via venepuncture and placed in 70% ethanol and then stored at the Avian Behavioural Ecology Laboratory, UNE, at -3°C for future analysis. Nestlings were banded in the same manner at around 10-14 days post-hatch. Nests were searched for every 2-3 days from mid-August each year and, once found, marked with a plastic numbered tag and visited at least every other day to examine their contents using a mirror attached to an extendable pole. Nest visits were made daily when nestlings were 13 days post-hatch and close to fledging so that a precise fledge date could be noted for each brood.

Observations of provisioning behaviour

From 13 September 2013 until 30 November 2015, I conducted behavioural observations at 29 nests in order to identify the putative breeding pair and helpers attending a given nest, whilst

also collecting information on brood provisioning behaviour (see Chapter 3 for details). Observations were carried out from a hide placed 15-40 m ($28.66 \text{ m} \pm 0.38\text{SE}$, $N=104$) from nests, a distance that did not impact provisioning behaviour (see Chapter 3). Observations were for an average of around 1 hour in duration ($57.88 \text{ minutes} \pm 1.83\text{SE}$). For each nest visit event, I dictated bird identity, prey type, prey size (estimated relative to bill volume) as well as observation time, date and location onto a digital recorder (Marantz PMD661, Japan) whilst viewing behaviours through either a telescope (Gerber Montana 15-45x) or binoculars (Monarch 7, 10x42, Nikon, Japan). Nest events were also recorded with a camera (2013-2014: analogue Hi8 camcorder, Sony, Japan; 2015: digital Panasonic HC-V270, Korea), placed $5.5 \text{ m} \pm 0.13 \text{ SE}$ ($N=104$) from the nest. Observations were carried out between 0800 and 1700 hours.

Molecular methods and genetic relatedness

DNA was extracted in the Molecular Ecology Laboratory, UNE (details in chapter 3). Individuals were sexed molecularly using sexing polymerase chain reactions (PCRs) involving two primers (P2 and P8) simultaneously in order to amplify homologous parts of the CHD-W and CHD-Z genes (Griffiths et al. 1998). Birds were genotyped at 20 microsatellite loci (chapter 3) previously isolated and characterised from noisy miners (Kopps *et al.* 2013, Abbott *et al.* 2002, Painter *et al.* 1997). For these 20 loci, allele range per loci varied from 3 to 15 ($7.55 \pm 0.64 \text{ SE}$). Of the 20 loci amplified, 6 deviated from Hardy-Weinberg equilibrium, so while all 20 loci were used in parentage analyses, only the 14 loci conforming to HWE were used to calculate pairwise genetic relatedness of female breeders with the putative breeding male and their contingent of helpers.

I calculated the pairwise genetic relatedness value (r) in the program KINGROUP v2 (Konovalov et al. 2004). This relatedness coefficient estimates the degree of genetic similarity of

two individuals based on the proportion of shared alleles between the individuals weighted by the allele frequencies in the whole population (Goodnight & Queller 1999). These pairwise relatedness values range from -1 to +1. If the value is -1, theoretically it means that there are no shared alleles between two individuals, while an r of +1 indicates identical alleles for the two individuals. Therefore values close to zero represent two relatively unrelated individuals, and increasingly positive values between two individuals indicate increasing levels of relatedness (Wang 2002). To test if putative male breeders and helpers were significantly related or unrelated to breeding females at each nest, I performed a kinship test in KINGROUP v2, which calculated the significance of genetic relatedness for two individuals. Two individuals could be ‘significantly related’ (primary hypothesis $r = 0.5$, null hypothesis $r = 0$), or ‘significantly unrelated’ (primary hypothesis $r = 0$, null hypothesis of $r = 0.5$) based on the ratio required to exclude 95% of 1000 simulated pairwise comparisons (Goodnight & Queller 1999). If neither test reached statistical significance, then r values were assumed to be approximately 0.25. As these individuals were intermediate and not significantly different from unrelated ($r = 0$) and related ($r = 0.5$) individuals, they were placed into a third group: ‘unresolved’. Helpers and putative male breeders were therefore placed into three groups with regards to their relatedness status to breeding female: 1) birds that were significantly related ($r = 0.5$); 2) birds that were significantly unrelated ($r = 0$) and 3) birds with ‘unresolved’ status ($r = 0.25$) (McDonald et al. 2008a-b).

Parentage analysis and the identification of extra-pair (EP) mating

For each brood, the putative breeding female was identified based on the presence of strictly maternal behaviours such as nest construction, incubation of clutches and, if captured, the presence of a brood patch (Higgins et al. 2001; $N = 8$). The sex of putative females was then

tested using the above molecular sexing method and assignment of molecularly maternity using Cervus 3.0.7 and data from 20 microsatellite loci (Chapter 3; Marshall et al. 1998; Kalinowski et al. 2007). To determine paternity, all males that could have sired offspring were considered as potential fathers. Therefore all adult males at the colony in the given breeding season were included as potential fathers in these analyses. For each offspring, Cervus calculates Trio LOD scores (natural logarithm of the likelihood ratio), giving the likelihood of paternity of that candidate parent relative to a randomly chosen individual in the population, while also considering known maternity. This was done based on simulations on allele frequency that were used to calculate differences in LOD scores (Δ LOD), which were then used to rank the two most likely fathers for a given offspring at 95% confidence levels. Candidate fathers suggested by Cervus were then assigned to offspring if Δ LOD scores were $\geq 80\%$ and allele mismatch was not more than one mismatch. If there was more than one genetic father indicated for a given brood, and both males provisioned the focal brood (N=2), I defined the putative male breeder (or social male) as the bird that exhibited a higher provisioning rate at the focal nest, given that breeding males typically provision more than helpers (Chapter 3). Therefore for each brood, the breeding male was identified as the male that had paternity in the brood and provisioned at highest rate among other males attending the nest. EP mating in a brood was assumed if 1) there was more than one known matched father at the brood level (N=3 broods) and 2) when one or two members of a brood did not match with the best father suggested for their brood mate(s) (N=4 broods). To quantify relatedness between siblings in a brood in the absence of known parents, the software COLONY 2.0 (Jones & Wang 2010) was used. By determining if individuals from the same brood were either full or half siblings, the absence or occurrence of EP mating could be detected. To test the validity of this method, I included both nestlings with known parents

(N=57) using Cervus as outlined above and those broods with un-sampled fathers.

Reconstructions of sibling relationships agreed with the Cervus results and were supported by probabilities of at least 90%. Therefore, when analysing broods with unknown fathers, either half or full-sibling relationships were assumed to be an accurate assignment if the probability produced by Colony software for that match was at least 0.9. There is possibility that a whole brood was sired by an extra pair male, but the combination of behavioural observations and molecular methods used herein matched the putative breeding male in all nests without mixed paternity. Putative male breeders were then categorised as significantly related or unrelated to the focal breeding female at a given nest using the same methodology as outlined above.

Statistical analysis

To determine if breeding males were more often unrelated to breeding females at their focal nest than expected by chance, a goodness of fit test was carried out. The frequency distribution of different relatedness groups of helpers (sex and age), in relation to EP mating, was assessed with 2×2 contingency tables.

To examine if genetic relatedness values between breeders and helpers (using the continuous r value calculated using the Queller and Goodnight formula (Queller & Goodnight, 1989), mean number of helpers and total provisioning rate differed between broods with and without EP mating, I constructed Generalized Linear Mixed Models (GLMMs) with the term of interest as a single fixed effect using the *lmer* function in the *lme4* package (Bates *et al.* 2014) in R environment (R Core Team, 2014). The significance of each term was determined by comparing the fit of the model to that of the intercept-only model using likelihood ratio tests with an $\alpha=0.05$. Provisioning rate was the main index of parental behaviour and helper effort in this breeding system, increasing with nestling age but not differing significantly after 11 days of age (see

Chapter 3). Therefore, analyses examining provisioning rate only including observations collected 11-15 days post hatch.

Previous analysis suggested that bird status was an important variable that influenced provisioning rate (Chapter 3). The percentages of EP nestlings and EP broods was calculated as the proportion of EP nestlings or EP, relative to all known nestlings and broods, respectively. Further, the 95% CIs around these estimations were calculated using a binomial distribution (Brown et al. 2001). To examine if the presence of EP mating influenced provisioning rate beyond this, I first constructed a GLMM model with bird reproductive status (4 levels: breeder female, breeder male, related, unrelated) as a fixed effect and then examined if adding EP mating (two levels with binary distribution) improved model explanatory power. Similarly, to test if an interaction between status and EP mating improved model fit, I compared a model with status and EP mating with a model that had same fixed effect plus their interactions. Models were compared with likelihood ratio tests and the significance of terms was confirmed at $\alpha=0.05$. For each term the effect size and 95% CIs are also reported. In all models, the response variable (provisioning rate) was square-root transformed to normalise and reduce residual variance. In addition, because female helpers were rare in this system, (~ %6 of helpers, N=7) and were all unrelated to the breeding female at the nests provisioned, I only modelled male helper behaviour to avoid the issue of rank deficiency. Similarly, given that for broods with EP mating there were no helpers categorised as ‘unsolved’, I excluded this group of helpers from the analysis with regards to the effects of EP mating on provisioning rate. In the models, I also included nest identity, bird identity and observation order as random effects to control for non-independence of data collected from multiple observations of the same individuals and same nests. All analyses were performed in the R statistical language and environment (R Core Team, 2014). I used *lmer*

function with REML approach in *lme4* package (Bates *et al.* 2014) to perform GLMM modelling.

Results

Mate choice and genetic relatedness of breeders and male helpers

In 86% of broods, female and putative male breeders were significantly unrelated genetically, with this proportion significantly higher than predicted by random mating ($\chi^2_1= 7.1$, $p<0.01$). There was a significant difference in the level of genetic relatedness values (r) between breeding females and putative breeding males with male helpers at the nest. Mean relatedness between breeding females and the putative male breeder was significantly lower than the mean relatedness of breeding females with helper males provisioning at the same nest ($\chi^2_1= 4.07$, $p=0.04$, $\beta=0.09$, 95% CI= 0.002, 0.19; Fig. 5.1).

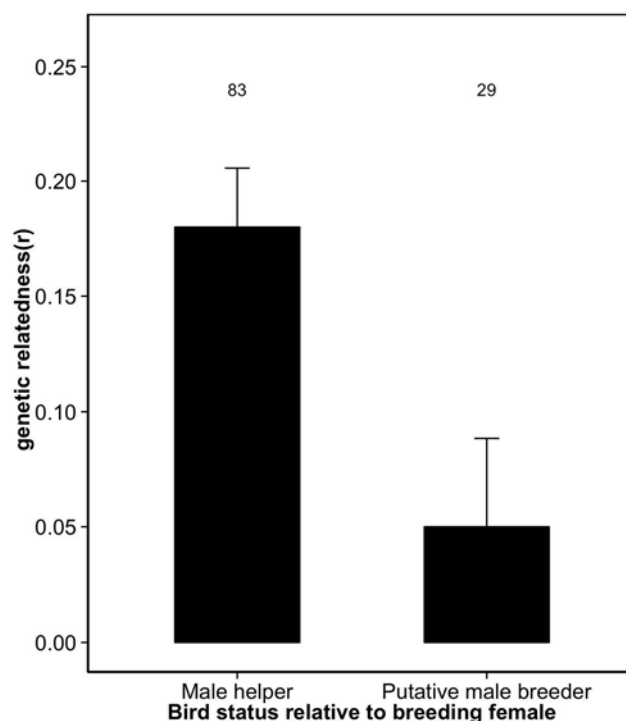


Fig. 5. 1. Mean \pm SE genetic relatedness of breeding female to putative male breeder (social male) and other males that provisioned her brood (e.g. helpers). Sample size for each group is presented above bars.

Genetic relatedness of breeders and helpers in relation to extra-pair mating

Of the 75 offspring genotyped (N=29 broods), we determined candidate fathers for 57 nestlings (N=24 broods), with only 3 of these offspring (N=3 broods) sired by a sampled male other than the putative breeding male (two with 0, and one with 1 allele mismatch). Seven of the 18 nestlings without a known sire occurred in broods (N=7 broods) where we could identify fathers for some of the brood. Allele mismatches between these offspring and the most likely sampled sire ranged from 3 to 7 (3 mismatch: N=2; 4 mismatch: N=3; 6 mismatch: N=1 and 7 mismatch: N=1; mean \pm SE: 4.42 \pm 0.57). In some broods (N=4) no known sire was determined for any of the nestlings. Subsequent analysis in COLONY showed that one nest contained a half-

sibling to the other nestlings, suggesting an additional occurrence of EP mating. Therefore we counted 11 nestlings (14% of all nestlings, 95% CIs: 6-22%) across 8 broods (27% of all broods, 95% CIs: 12-47%) that were sired by extra pair males in these populations. There were either one (N=5 broods) or two EP nestlings (N=3 broods) per brood (mean±SE: 1.37 ± 0.20), with all of these EP nests having broods of 3 offspring in total. The relatedness of the breeding pair did not differ between broods with and without EP mating ($\chi^2_1 = 0.03$, $p=0.81$, $\beta = -0.01$, 95% CI= -0.16, 0.12; Fig. 2). The mean genetic relatedness of breeding females to male helpers did not vary in broods with and without EP mating ($\chi^2_1 = 1.16$, $p=0.27$, $\beta = -0.06$, 95% CI= -0.15, 0.03).

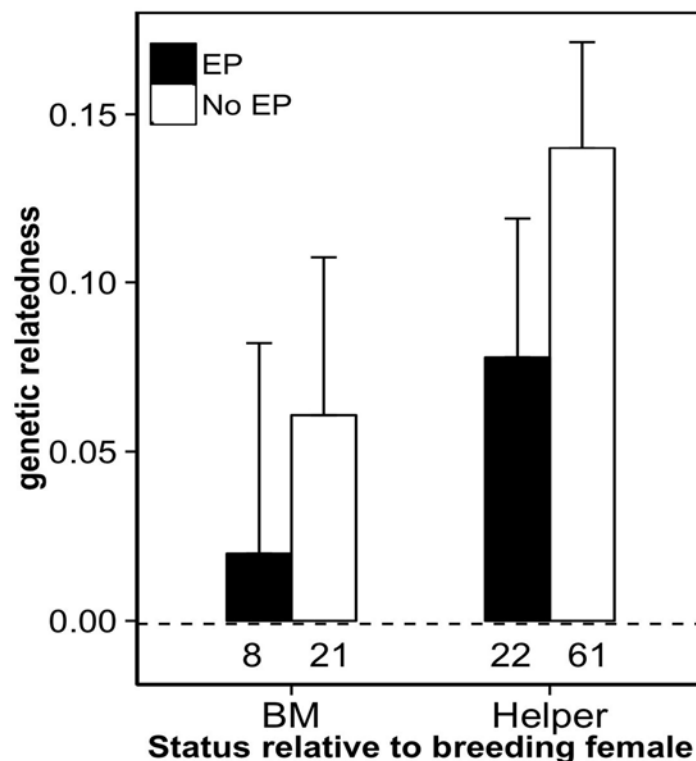


Fig. 5. 2. Mean ± SE genetic relatedness of breeding female to the putative male breeder (BM) and to male helpers (Helper) at the nest in broods with EP (solid bars) and without EP (open bars). Sample sizes (number of individuals in the given group) are presented below each corresponding bar.

Helper attendance and age/sex structure according to the presence of extra-pair mating

Overall, I recorded 1759 provisioning events in 21 broods without EP and 752 provisioning events in 8 broods with EP during a total of 112.28 hours of nest observation across the 29 broods. During these, a total of 130 individuals provisioned focal broods. The mean number of helpers tended to be slightly greater in EP broods than in broods without EP, however, the differences were not significant ($\chi^2_1 = 1.7$, $p = 0.19$, $\beta = 1.4$, 95% CI = -0.7, 3.71; Fig. 5.3). Although, percentage of adult helpers (age ≥ 2) were significantly higher in both broods with EP mating (74%, $\chi^2_1 = 3.2$, $p < 0.05$) and without EP mating (76%, $\chi^2_1 = 9.03$, $p < 0.001$), no difference was apparent between broods with EP and without EP mating (contingency table; $\chi^2_1 = 0.004$, $p = 0.94$). The sex ratio of helper contingents was extremely male-biased in both broods with EP and broods without EP (88% and 94% respectively), and the frequency of male helpers also did not differ in relation to the EP mating status of a given nest (contingency table, $\chi^2_1 = 0.27$ $p = 0.59$). In the majority of broods in which EP mating detected (87% of broods, $N = 7$), EP males did not provision the brood at all, and the proportion of broods that EP male did not provide care was significantly higher than those that EP male was seen provisioning ($\chi^2_1 = 10.11$, $p = 0.001$).

Provisioning response to extra-pair mating according to nest attendant status

Overall provisioning rate was higher in broods in which EP mating was detected compared to those where it was not (Fig. 5.3). As expected, a model including reproductive status (e.g. breeding female, putative male breeder, related and unrelated helpers) had significantly higher support in explaining variation in provisioning rate of individuals when compared to an intercept-only model ($\chi^2_3 = 43.83$, $p < 0.001$). Overall, breeding females provisioned broods at the highest rate followed by putative male breeders, related and then unrelated male helpers (Fig.

5.4). To examine if the presence of EP matings at a nest also explained variation in provisioning rate in addition to the relatedness status of attendants, I further tested for changes in the explanatory power of the model when EP mating and status were included in the model as fixed effects. A model with EP mating and bird status had significantly more support than a model with only bird status ($\chi^2_1= 4.11$, $p=0.04$). However, adding the interaction of status and EP mating did not result in a significant difference in the variance explained ($\chi^2_3= 0.44$, $p=0.93$), indicating that there was not a significant difference among birds of different status for their response to EP mating presence (Fig. 5.4).

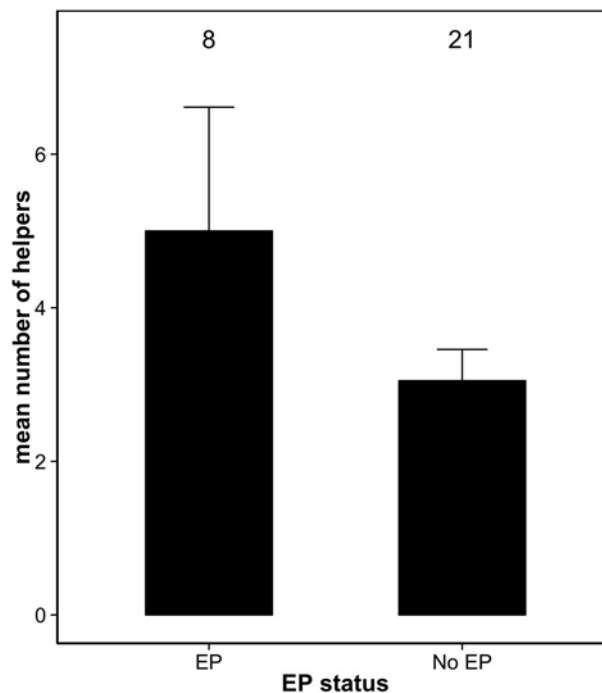


Fig. 5. 3. Mean \pm SE number of helpers at nests with and without EP. Sample sizes for each group of nests are given above corresponding bar.

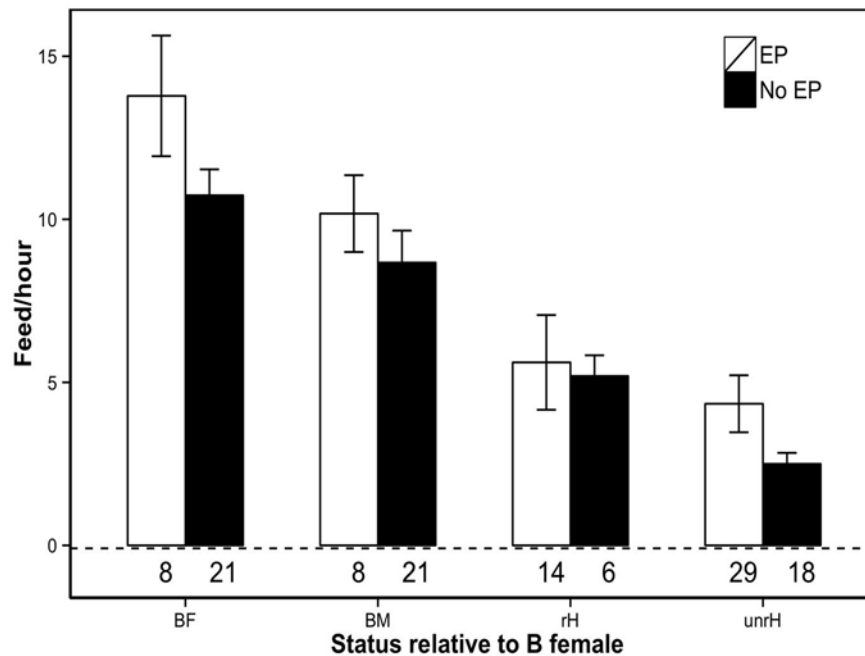


Fig. 5. 4. Mean \pm SE provisioning arte per hour by birds of different social class at the nest with EP and without EP. Sample sizes for each group of broods are given below each bar.

Discussion

Frequency of extra pair offspring in the noisy miner system

Extra pair males sired 14% of nestlings, while 27% of broods contained at least one EP nestling. These results are informative, as different mating systems have been previously suggested for noisy miners, with initial assumptions being that noisy miners were highly promiscuous with females observed mating with multiple males (Dow 1978). The first molecular-based study found that EP mating was rare and only 3.5 % of nestlings being sired by EP males (Poldmaa et al. 1995). The rate of EP mating detected in noisy miner broods in this study was relatively low compared to other species typically considered promiscuous. For example, 85-90% of broods are reported to contain EP offspring in the superb fairy-wren (*Malurus cyaneus*) (Mulder & Magrath 1994; Double & Cockburn 2000) and splendid fairy-

wren (*Malurus splendens*) (Brooker et al. 1990), while up to 80% of broods in the Australian magpie (*Gymnorhina tibicen*) are the result of EP mating (Durrant & Hughes 2005). The values to date for noisy miners are moderate in comparison.

Moreover, in passerine bird species that show a level of EP mating, on average approximately 11% of offspring are found to be the result of EP paternity (Griffith et al. 2002). Therefore the range of 6-22% EP offspring found in this study is close to the average EP mating rate for passerine bird species, seemingly contrasting the high level of promiscuous mating suggested by Dow (1978) prior to the advent of molecular techniques. The results herein do differ from the level reported in the one previous study (Poldmaa et al. 1995). The sample sizes used in the current study and Poldmaa et al. (1995) are similar (31 vs 29 broods, respectively), so it is unlikely that the variation of the rate of EP mating found in this study and that of Poldmaa et al. (1995) stems from methodological differences. These variations could be due to other factors such as colony structure and group composition of the focal study populations, and demonstrate flexibility in the mating system of noisy miners to some extent, and suggest an intermediate mating strategy to that proposed by either Poldmaa et al (1995) or Dow (1978).

Poldmaa et al. (1995) suggested that the monopolisation of paternity by one male was a consequence of a high degree of genetic relatedness within that focal group. However, the results herein do not match this expectation and rather demonstrate that helper contingents in noisy miners are not limited to related individuals alone and, in the focal colonies reported herein at least, did not appear to drive EP patterns. Nonetheless, the prevalence of EP mating in this system and its variation between populations could be associated with different social environments on a brood by brood basis such as the composition of a given helper contingent. For example, a high number of unrelated helpers surrounding a breeding female, and thus a high

number of potential EP males, could influence the variability of EP mating rates between populations of the same species as previously noted for social birds (Stacey 1982). Further, variations in the rate of EP mating among populations of the same species could also be influenced by population-specific dispersal patterns. Low rates of dispersal could lead to increased numbers of relatives in the group and therefore an enhanced chance of inbreeding, thus breeding females in this scenario may be more likely to engage in EP mating to avoid inbreeding. For example, in the Australian magpie, the rate of EP mating has been shown to be associated with genetic structure of group members that is influenced by dispersal patterns; 44% of EP mating recorded in groups where juveniles dispersed, but this rate increases to 80% in groups where juveniles are retained (Durrant & Hughes 2005). These suggest that the rate of EP mating might vary between populations as a result of different ecological and demographic conditions, an area worthy of additional consideration when examining EP mating behaviour.

Inbreeding avoidance in noisy miners

In the majority of broods, female and putative male breeders were not closely genetically related (see Results). This suggests that despite presence of both related and unrelated males in the helping group, females mated selectively with unrelated males more often than expected by chance, showing a clear inbreeding avoidance. In most bird species, there is a general rule of avoiding inbreeding due to the fitness costs associated with mating with kin as a consequence of inbreeding depression (Frankham et al. 2002; Kokko & Ots 2006). For example in purple-crowned fairy-wrens (*Malurus coronatus*), incestuous mating causes 30% hatching failure (Kingma et al. 2013). Therefore various mechanisms have evolved for inbreeding avoidance in avian species. There are two possible mechanisms that seem most likely to allow female noisy miners to avoid mating with closely related individuals. First, although the sex ratio of offspring

is not biased, there is a consistent male-biased adult sex ratio across populations as a consequence of female-biased dispersal and subsequent mortality (Chapter 4). This sex-biased dispersal naturally acts as a mechanism to reduce inbreeding, separating opposite-sex kin in space and therefore preventing matings between kin (Hazlitt et al. 2004, 2006; Guillaume & Perrin 2009; Liebgold et al. 2011). Dispersal acts as an important means of fostering inbreeding avoidance in other species as well, including the great tit (*Parus major*), where the level of inbreeding negatively correlated with dispersal distance from the natal breeding area (Szulkin & Sheldon 2008).

Second, noisy miners might also use a form of kin recognition mechanism, which occur in other cooperatively breeding birds (Jamieson et al. 2009) to avoid incestuous mating. This is in particular important for breeding females to avoid mating with the philopatric males that stay in the same territories and natal colony in this species. Females and males might use their complex acoustic repertoire systems (Holt et al. 2016) to be able to differentiate between kin and non-kin when selecting mates. Noisy miners have shown the ability to differentiate between different individuals using acoustic cues previously (McDonald 2012), and the closely related bell miner (*Manorina melanophrys*) uses acoustic cues to favour aiding kin (McDonald & Wright 2011), suggesting that a similar mechanism for inbreeding avoidance might be operating. Whether any discrimination occurs based on familiarity, an innate preference or learnt template of a form of signal is currently unknown, but given noisy miners also adjust helping effort towards relatives (Chapter 3) then some form of kin recognition and thus avoidance during mating by breeding females seems highly likely.

Although the genetic similarity of the putative breeding mate is assumed to be a driver of EP mating behaviour, current evidence from avian systems is contradictory. While genetic similarity

between mates has shown to influence EP mating decisions by females (Kleven et al 2005; Tarvin et al. 2005; Freeman-Gallant et al. 2006), in other studies a lack of relationship between mate genetic similarity and EP copulations has been reported (Kleven & Lifjeld 2005; Bouwman et al. 2006; Edly-Wright et al. 2007). One explanation is that the importance of EP mating for inbreeding avoidance is masked by other determinants such as dispersal patterns. EP mating is more likely to act as a mechanism for inbreeding avoidance in species that show a lack of dispersal. For example, incestuous mating in purple-crowned fairy-wrens (*Malurus coronatus*) occurs when sex-biased dispersal is limited (Kingma et al. 2009) and as discussed above the lack of juvenile dispersal can almost double the EP mating rate in Australian magpies (Durrant & Hughes 2005). The female-biased dispersal in noisy miners probably shapes inbreeding avoidance as discussed above, and differences in a reliance upon dispersal patterns may well be the common factor in at least some of the contradictory results reported above.

It is important, however, to note that although, genetic similarity to the putative breeding male does not appear to be a determinant of EP mating behaviour in the noisy miner, females might still seek extra male copulations in order to benefit from their genetic quality, which is usually defined in the context of the ‘genetic quality’ hypothesis (Griffith et al. 2002). According to the ‘genetic quality’ hypothesis, EP mating might be based on the higher heterozygosity of extra pair males compared to their putative social partner (Harrison et. al. 2013) and needs further examinations in noisy miners.

No evidence for polyandry as a means by which females enhance help provided to broods

Despite EP offspring being present in some broods, this did not result in additional care being provided by EP males. This is in contrast to the ‘communal polyandry’ mating system for noisy miners suggested by Dow (1978) and further does not support the suggestion that female noisy

miners engage in EP mating as a mechanism to recruit EP males as helpers (Dow 1978; Dow and Whitmore 1990). At the very least, if females are engaging in extra-pair matings, these are not resulting in high numbers of EP offspring. Further, in some cooperatively breeding species, the number of helpers was found to be positively associated with EP mating. For example in the superb starling (*Lamprotornis superbus*), the number of helpers within the group predicted the probability of EP mating behaviour by breeding females. Females with a lower number of helpers were more likely to copulate with an EP male to gain the direct benefits of additional helpers (Rubenstein 2007). However, in the current study EP mating did not result in either a higher number of helpers or an increased rate of provisioning from successful EP males, indicating that the function of EP mating in noisy miners is unlikely to be related to the recruitment of extra helpers. As a result, cooperative breeding in noisy miners does not support ‘communal polyandry’ or reproductive skew, but instead is more consistent with kin selection being one of the main drivers of helping behaviour in this species, although unrelated helpers do provide substantial aid (see details in Chapter 3).

Response of cuckolded males to female EP mating

EP mating resulted in overall increased brood provisioning rate in EP broods compared to the broods where the putative breeding male obtained paternity of the entire brood. Despite this, contrary to predictions, cuckolded males did not reduce their provisioning rate towards broods where they had lost some share of paternity, a result that was not consistent with the ‘parental care’ hypothesis.

Generally, the responses of cuckolded males to reduced paternity are thought to be influenced by two main determinants. First, a cuckolded male's behaviour towards a brood in which he has

lost partial paternity should be a trade-off of the costs and benefits of continuing to care for that brood, and second the ability of male breeder to assess their share of parentage and the predictability of cuckoldry is also important in influencing their behaviour. It is also important to consider that even when males have lost some paternity in the noisy miner system, they still typically have the greatest share in paternity of the brood, with the majority of EP broods (62% of 29 broods) still having more than half offspring sired by the putative male breeder. This pattern has also been found in other species, such as the mexican jay (*Aphelocoma ultramarina*), where cuckolded putative male breeders still provided the highest level of care to broods (Li & Brown, 2002). One possibility is that polyandrous females limit the parentage share of extra pair males within broods to avoid cuckolded males reducing their level of care (Du et al. 2015). This has some support in that a recent meta-analysis using 48 species of fish, insects, birds, and mammals, also demonstrated that the response of cuckolded males to female polyandrous mating behaviour depends on the strength of cuckoldry and the cost of parental care for future reproductive success of cuckolded male (Griffin et al. 2013). Cuckolded males may therefore be flexible and relatively tolerant to female EP mating behaviour if the parental care being provided does not negatively influence lifetime reproductive success (Grafen 1980).

When examining the response of cuckolded male to a decreased share of paternity, it is important to note that a breeding male's response is likely influenced by their ability to assess parentage share in the brood, which is typically thought to be difficult (Kempnaers & Sheldon 1997). Although cuckolded males can maximise their fitness by reducing parental care for unrelated offspring, low certainty about paternity would result in the risk of putative male breeders abandoning their own offspring (Maynard-Smith 1977; Wolf et al., 1988). Currently, the mechanisms of parentage detection are not well known in birds, and experimental manipulations of parentage have yielded inconsistent responses of cuckolded males (Kempnaers

et al. 1998), suggesting that parentage assessment could be difficult and highly variable among species. In some species, the accessibility of females in their fertile period is assumed to act as a cue for males to assess their parentages (Davies et al. 1992; Komdeur 2001), however this would be difficult to ascertain in a highly social species like the noisy miner.

One possibility that cannot be ruled out in this study, given provisioning rates were only able to be measured at the brood rather than individual nestling level, is that cuckolded males only provisioned their own offspring when attending nests, ignoring any extra pair nestlings. This would be possible if male breeders had some cues to identify extra pair nestlings, however preferential provision seems unlikely given the overall patterns in nest attendance, as the lower number of sired offspring in a cuckolded nest would suggest that breeding males would have a lower provisioning rate when attending mixed paternity broods, not a greater one as found here. Nonetheless this is an area worth examining with cross-fostering experiments that enable experimental manipulation of the paternity levels of breeding males.

Conclusion

In this chapter, I have shown that EP males sired 14% of offspring in the focal populations of noisy miner. Considering the mean level of EP mating rate in passerine birds, this level of EP mating lies around the average rate for passerine birds, and is moderate in comparison to many other highly promiscuous species. This result therefore suggests that mating system in noisy miner is unlikely to be highly promiscuous as proposed previously (Dow 1978; Brown 1987) but rather confirm to a more typical level seen in passerines that is flexible from colony to colony.

Two main hypotheses to explain female EP mating in birds suggest that this behaviour might be beneficial in the form of avoiding inbreeding and through gaining extra parental care provided by EP males (Kempnaers & Dhondt 1993, Griffith et al., 2002). However, in the noisy miner

cooperatively breeding system, there was no evidence that EP mating might function as a mechanism to reduce the costs of inbreeding depression. I argue that other factors such as female-biased dispersal might function as mechanism to avoid inbreeding, thus the relative importance of EP mating could be dependent on other ecological conditions. Further, EP mating did not either lead to additional help provided by EP males, nor a reduction in the care provided by cuckolded males. Therefore, no evidence was found to support parental care hypothesis as a drive of EP mating in the noisy miner. Despite this, I cannot rule out that EP mating behaviour may have other functions such as increasing 'genetic quality' (Griffith et al. 2002) and offspring heterozygosity and consequently their fitness or 'insurance' in case the breeding male is infertile.

References

- Abbott, C.L., Poldmaa, T., Loughheed, S., Clarke, M., Boag, P.T. (2002). Hierarchical analysis of genetic population structure in the noisy miner using DNA microsatellite markers. *Condor*, 104, 652-656.
- Akçay, E., Roughgarden, J. (2007). Extra-pair paternity in birds: review of the genetic benefits. *Evolutionary Ecology Research*, 9, 855-868.
- Albrecht, T, Kreisinger, J., Pialek, J. (2006). The strength of direct selection against female promiscuity is associated with rates of extrapair fertilizations in socially monogamous songbirds. *American Naturalist*, 167, 739-744.
- Arnqvist, G., Kirkpatrick, M. (2005). The evolution of infidelity in socially monogamous passerines: the strength of direct and indirect selection on extrapair copulation behavior. *American Naturalist*, 165, S26-S37.

- Bates, D., Maechler, M., Bolker, B., Walker, S. (2014). lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7, <URL: <http://CRAN.R-project.org/package=lme4>>.
- Birkhead, T. (1998). *Sperm competition in birds: mechanisms and function*. In Sperm Competition and Sexual Selection (eds Birkhead, T. R., Møller, A. P.), pp; 579-622. Academic Press, London.
- Bouwman, K. M., Burke, T., Komdeur, J. (2006). How female reed buntings benefit from extra-pair mating behaviour: testing hypotheses through patterns of paternity in sequential broods. *Molecular Ecology*, 15, 2589-2600.
- Brown, L.D., Cai, T.T., DasGupta, A. (2001). "Interval Estimation for a Binomial Proportion". *Statistical Science*, 16, 101–133
- Bretman, A., Tregenza, T. (2005). Measuring polyandry in wild populations: a case study using promiscuous crickets. *Molecular Ecology*, 14, 2169-2179.
- Brooker, M.G., Rowley, I., Adams, M., Baverstock, P. R. (1990). Promiscuity: an inbreeding avoidance mechanism in a socially monogamous species? *Behavioral Ecology and Sociobiology*, 26, 191-199.
- Brown, J. L. (1987). *Helping and communal breeding in birds*. Princeton University Press, Princeton.
- Burke, T., Davies, N. B., Bruford, M. W., Hatchwell, B. J. (1989). Parental care and mating behaviour of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting. *Nature*, 338, 249-251.
- Cohas, A., Bonenfant, C., Kempnaers, B., Allainé, D. (2009). Age-specific effect of heterozygosity on survival in alpine marmots (*Marmota marmota*). *Molecular Ecology*, 18, 1491-1503.

- Cornell, S. J., Tregenza, T. (2007). A new theory for the evolution of polyandry as a means of inbreeding avoidance. *Proceedings of the Royal Society B*, 274, 2873-2879.
- Davies, N. B., Hatchwell, B., Robson, T., Burke, T. (1992). Paternity and parental effort in dunnocks *Prunella modularis*: how good are male chick-feeding rules? *Animal Behaviour*, 43,729-746.
- Double, M., Cockburn, A. (2000). Pre-dawn infidelity: females control extra-pair mating in superb fairy-wrens. *Proceedings of the Royal Society B*, 267, 465-470.
- Dow, D. (1978). Reproductive behavior of the noisy miner, a communally breeding honeyeater. *Living Bird*, 16,163-185.
- Dow, D. D., Whitmore M. J. (1990). Noisy miners: variations on the theme of communality. In: Stacey, P. B., Koenig, W. D. (eds) *Cooperative breeding in birds*. Cambridge University Press, Cambridge, pp: 559-592.
- Du, B., Guan, M., Ren, Q., Chen, G. (2015). Cuckolded male ground tits increase parental care for the brood. *Animal Behaviour*, 110, 61-67.
- Durrant, K. L., Hughes, J. M. (2005). Differing rates of extra-group paternity between two populations of the Australian magpie (*Gymnorhina tibicen*). *Behavioral Ecology and Sociobiology*, 57, 536-545.
- Edly-Wright, C, Schwagmeyer, P. L., Parker, P. G. & Mock, D. W. (2007). Genetic similarity of mates, offspring health and extrapair fertilization in house sparrows. *Animal Behaviour*, 73, 367-378.
- Forstmeier, W., Nakagawa, S., Griffith, S.C., Kempenaers, B. (2014). Female extra-pair mating: adaptation or genetic constraint? *Trends in Ecology and Evolution*, 29, 456-464.
- Frankham, R., Ballou J. D., Briscoe D. A. (2002). *Introduction to conservation genetics*.

Cambridge University Press, Cambridge.

Freeman-Gallant, C. R., Wheelright, N. T, Meiklejohn, K. E. & Sollecito, S. V. (2006). Genetic similarity, extrapair paternity, and offspring quality in Savanna sparrows (*Passerculus sandwichensis*). *Behavioral Ecology*, 17, 952-958.

Gohli, J., Anmarkrud, J.A., Johnsen, A., Kleven, O, Borge, T., Lifjeld, J.T. (2013). Female promiscuity is positively associated with neutral and selected genetic diversity in passerine birds. *Evolution*, 67, 1406-1419.

Goodnight, K. F., Queller, D. C. (1999). Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Molecular Ecology*, 8, 1231-1234.

Grafen, A. (1980). Opportunity cost, benefit and degree of relatedness. *Animal Behaviour*, 28, 967-968.

Griffin, A. S., Alonzo, S. H., Cornwallis, C. K. (2013). Why do cuckolded males provide paternal care? *PLoS Biology*, 11, e1001520.

Griffith, S. C., Owens, I. P. F., Thuman, K. A. (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology*, 11, 2195-2212.

Griffiths, R., Double, M., Orr, K., Dawson, R. (1998). A DNA test to sex most birds. *Molecular Ecology*, 7, 1071-1075.

Guillaume, F., Perrin, N. (2009). Inbreeding load, bet hedging, and the evolution of sex-biased dispersal. *American Naturalist*, 173, 536-541.

Hamilton, W. D. (1964). The genetical evolution of social behaviour. I and II. *Journal of Theoretical Biology*, 7,1-52.

Harrison, X.A., Bearhop, S., Inger, R. et al. (2011). Heterozygosity–fitness correlations in a migratory bird: an analysis of inbreeding and single-locus effects. *Molecular Ecology*, 20,

4786-4795.

- Harrison, X.A., York J.E., Cram, D.L., Young A.J. (2013). Extra-group mating increases inbreeding risk in a cooperatively breeding bird. *Molecular Ecology*, 22, 5700-5715.
- Hazlitt, S., Eldridge, M., Goldizen, A. (2004). Fine-scale spatial genetic correlation analyses reveal strong female philopatry within a brushtailed rock-wallaby colony in southeast Queensland. *Molecular Ecology*, 13, 3621–3632
- Hazlitt, S., Sigg, D., Eldridge, M., Goldizen, A. (2006). Restricted mating dispersal and strong breeding group structure in a mid-sized marsupial mammal (*Petrogale penicillata*). *Molecular Ecology*, 15, 2997-3007.
- Hemmings, N. L., Slate, J., Birkhead, T. R. (2012). Inbreeding causes early death in a passerine bird. *Nature Communication*. 3, 863.
- Higgins, P. J., Peter, J. M., Steele, W.K. (2001). *Handbook of Australian, New Zealand and Antarctic Birds*. Tyrant-Flycatchers to Chats. Vol 5. Oxford University Press, Melbourne.
- Holt, J., Barati, A., McDonald, P. G. (2016). The complex acoustic repertoire of a highly social species, the noisy miner, *Manorina melanocephala*. *Emu*, in press.
- Ihara, Y. (2002). A model for evolution of male parental care and female multiple mating. *American naturalist*, 160, 235-244.
- Jamieson, I.G., Taylor, S.S., Tracy, L.N., Kokko, H., Armstrong D.P. (2009). Why some species of birds do not avoid inbreeding: insights from New Zealand robins and saddlebacks. *Behavioral Ecology*, 20, 575-584.
- Jones, O., Wang, J. (2010). COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources*, 10, 551-555.
- Kalinowski, S. T., Taper, M. L., Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment.

- Molecular Ecology*, 16, 1099-1106.
- Keller, L. F., Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in Ecology and Evolution*, 17,230-241.
- Kempnaers, B. (1993). A case of polyandry in the blue-tit—female extra-pair behavior results in extra male help. *Ornis Scandinavica*. 24, 246-249.
- Kempnaers, B. (2007). Mate choice and genetic quality: a review of the heterozygosity theory. *Advances in the Study of Behavior*, 37, 189-278.
- Kempnaers, B., Lanctot, R. B., Robertson, R. J. (1998). Certainty of paternity and paternal investment in eastern bluebirds and tree swallows. *Animal Behaviour*, 55, 845-860.
- Kempnaers, B., Sheldon, B. C. (1997). Studying paternity and paternal care: pitfalls and problems. *Animal Behaviour*, 53, 423-427.
- Kempnaers, B., Dhondt, A.A. (1993). Why do females engage in extra-pair copulations? A review of hypotheses and their predictions. *Belgian Journal of Zoology*, 123, 93-103.
- Kingma, S. A., Hall, M.L., Peters, A. (2013). Breeding synchronization facilitates extrapair mating for inbreeding avoidance. *Behavioral Ecology*, 24,1390-1397.
- Kingma, S.A., Hall, M. L., Segelbacher, G., Peters, A. (2009). Radical loss of an extreme extra-pair mating system. *BMC Ecology*, 9,15.
- Kleven, O., Jacobsen, F., Robertson, R. J., Lifjeld, J. T. (2005). Extrapair mating between relatives in the barn swallow: a role for kin selection? *Biology Letters*, 1, 389-392.
- Kleven, O., Lifjeld, J. T. (2005). No evidence for increased offspring heterozygosity from extrapair mating in the reed bunting (*Emeriza schoeniclus*). *Behavioral Ecology*, 16, 561-565.
- Kokko, H., Ots, I. (2006). When not to avoid inbreeding. *Evolution*, 60, 467-475.

- Komdeur, J. (2001). Mate guarding in the seychelles warbler is energetically costly and adjusted to paternity risk. *Proceedings of the Royal Society B*, 268, 2103-11.
- Konovalov, D. A., Manning, C., Henshaw, M. T. (2004). KINGROUP: a program for pedigree relationship reconstruction and kin group assignments using genetic markers. *Molecular Ecology Notes*, 4, 779-782.
- Kopps, A. M., McDonald, P.G., Rollins, L.A. (2013). Isolation and characterisation of polymorphic microsatellite loci for noisy miners (*Manorina melanocephala*), with successful cross-amplification in bell miners (*M. melanophrys*). *Conservation Genetic Resources*, 5, 39-41.
- Li, S-H., Brown, J. L. (2002). Reduction of maternal care: a new benefit of multiple matings. *Behavioral Ecology*, 13, 87-93.
- Liebgold, E. B., Brodie E. D., Cabe, P. R. (2011). Female philopatry and malebiased dispersal in a direct-developing salamander, *Plethodon cinereus*. *Molecular Ecology*, 20, 249-257
- Marshall, T. C., Slate, J., Kruuk, L., Pemberton, J. M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, 7, 639-655.
- Martinez-Padilla, J., Vergara, P., Mougeot, F., Redpath, S. M. (2012). Parasitized mates increase infection risk for partners. *American Naturalist*, 179, 811-820
- Maynard-Smith, J. (1977). Parental investment: a prospective analysis. *Animal Behaviour*, 25, 1-9.
- McDonald, P. (2012). Cooperative bird differentiates between the calls of different individuals, even when vocalizations were from completely unfamiliar individuals. *Biology Letters*, 8, 365-368.

- McDonald, P. G., Kazem, A. J. N., Clarke, M. F., Wright, J. (2008a). Helping as a signal: does removal of potential audiences alter helper behavior in the bell miner? *Behavioral Ecology*, 19, 1047-055.
- McDonald, P. G., te Marvelde, L., Kazem, A. J. N., Wright, J. (2008b). Helping as a signal and the effect of a potential audience during provisioning visits in a cooperative bird. *Animal Behaviour*, 75, 1319-1330.
- McDonald, P. G., Wright, J. (2011). Bell miner provisioning calls are more similar among relatives and are used by helpers at the nest to bias their effort towards kin. *Proceedings of the Royal Society B*, 278, 3403-3411.
- Møller, A. P., Birkhead, T. R. (1994). The evolution of plumage brightness in birds is related to extrapair paternity. *Evolution*, 48, 1089-1100.
- Mulder, R. A., Magrath, M. J. L. (1994). Timing of prenuptial molt as a sexually selected indicator of male quality in superb fairy-wrens (*Malurus cyaneus*). *Behavioral Ecology*, 5, 393-400.
- Painter, J., Crozier R. H., Crozier, Y. C., Clarke M. F. (1997). Characterization of microsatellite loci for a cooperatively breeding honeyeater. *Molecular Ecology* 6,1103-1105.
- Poldmaa, T., Montgomerie, R., Boag, P. (1995). Mating system of the cooperatively breeding noisy miner (*Manorina melanocephala*), as revealed by DNA profiling. *Behavioral Ecology and Sociobiology*, 37, 137-143.
- Queller, D. C., Goodnight, K. F. (1989). Estimating relatedness using genetic markers. *Evolution* 43, 258-275.
- R Core Team.(2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Reid, J. M., Arcese, P., Sardell, R. J., Keller, L. F. (2011). Additive genetic variance, heritability,

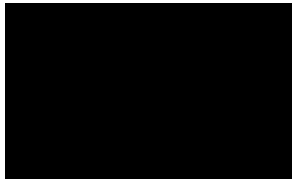
- and inbreeding depression in male extra-pair reproductive success. *American Naturalist*, 177, 177-187.
- Rubenstein, D. R. (2007). Female extrapair mate choice in a cooperative breeder: trading sex for help and increasing offspring heterozygosity. *Proceedings of the Royal Society B*, 274, 1895-1903.
- Sheldon, B. C. (2002). Relating paternity to paternal care. *Proceedings of the Royal Society B*, 357, 341-350.
- Stacey, P. B. (1982). Female promiscuity and male reproductive success in social birds and mammals. *American Naturalist*, 120, 51-64
- Szulkin, M., Sheldon B.C. (2008). Dispersal as a means of inbreeding avoidance in a wild bird population. *Proceedings of the Royal Society B*, 275, 703-711.
- Tarvin, K. A., Webster, M. S., Tuttle, E. M., Pruett-Jones, S. (2005). Genetic similarity of social mates predicts levels of extrapair paternity in splendid fairy-wrens. *Animal Behaviour*, 70, 945-955.
- Valera, F., Hoi, H., Krištín, A. (2003). Male shrikes punish unfaithful females. *Behavioral Ecology*, 14, 403-408.
- Varian-Ramos, C.W., Webster, M. S. (2012). Extrapair copulations reduce inbreeding for female red-backed fairy-wrens, (*Malurus melanocephalus*). *Animal Behaviour*, 83, 857-864.
- Wang, J. (2002). An estimator for pairwise relatedness using molecular markers. *Genetics*, 160, 1203-1215.
- Westneat, D. F., Stewart, I. R. (2003). Extra-pair paternity in birds: causes, correlates, and conflict. *Annual Review of Ecology, Evolution, and Systematics*, 34, 365-396.
- Wolf, L., Ketterson, E. D., Nolan, V. (1988). Paternal influence on growth and survival of dark-eyed junco young: do parental males benefit? *Animal Behaviour*, 36, 1601-1618.

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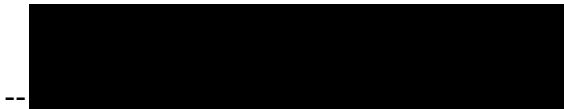
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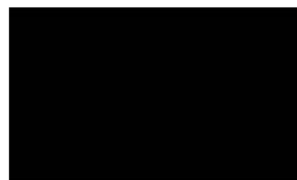
	Author's Name (please print clearly)	% of contribution
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Date

Nestlings reduce their predation risk by attending to predator-information encoded within conspecific alarm



This chapter will be submitted for publication as a standard paper.

Abstract.

Predation is one of the main threats to nestlings of altricial species, with predators often locating nests via eavesdropping acoustic begging signals of nestlings. Nestlings still need to signal nutritional needs to attendants by begging however, but may be able to adjust their begging behaviour based on the specific current level of risk by monitoring both intra- and interspecific alarm calls near the nest. This is especially true if alarm calls encode additional relevant information such as predator type, although these fine scale differences remain poorly elucidated. I show that noisy miner (*Manorina melanocephala*) nestlings can differentiate between terrestrial and aerial alarm calls of their own species, as they suppressed begging behaviour for longer in response to terrestrial rather than aerial alarm calls broadcast near the nest. This differential response is potentially due to greater danger associated with terrestrial calls and thus predators, as these are given when raptors perch near to the nest for example, as opposed to aerial alarms that signify a flying predator likely to leave the nest area quickly. In contrast, nestlings ignored alarm calls of the sympatric grey butcherbird (*Cracticus torquatus*) and continued to beg, indicating that miner nestlings had not learnt to associate butcherbird alarm calls with a potential predation threat. Surprisingly, nestlings did attend to the non-alarm calls of a sympatric parrot species, the eastern rosella (*Platycercus eximius*), by reducing their begging intensity. Whilst not an alarm call, parrot calls were found to be more structurally similar to noisy miner alarm calls than those of the butcherbird, suggesting nestlings were likely responding based upon similarity to a known signal as opposed to expressing a learnt behaviour. Together these results show that while nestlings respond adaptively to two different intraspecific alarm signals, they have not learnt to respond to the alarm calls of sympatric species with a similar predator guild.

Keywords. Parent-offspring communication, predation, alarm call, nestling vocalisation, *Manoriana melanocephala*, noisy miner

Introduction

In many animal taxa, offspring produce begging signals to solicit food from parents (Bell 2008). In birds, solicitation displays usually incorporate begging calls, particularly in altricial nestlings where offspring are totally dependent on parents for their nutrition and needs (Wright & Leonard 2002). These begging calls act as proximate cues of need, with parents and even nest attendants in cooperative systems adjusting their feeding effort to begging intensity and its acoustic components (Wright & Leonard 2002; McDonald et al. 2009a). Although increased begging calls can provoke higher provisioning rates by parents, begging calls are also costly to nestlings. Two main costs of begging for nestlings are an increased metabolic rate (Chappell & Bachman 2002) and an increased risk of predation (McDonald et al. 2009b). Whilst metabolic costs associated with the escalation of begging seem insufficient to prevent their increase (Schleich & Busch 2004), enhanced predation risk with greater levels of signal appears to be the main cost of begging through increased attractions of predators to the nest (McDonald et al. 2009a; Ibáñez-Álamo et al. 2012).

One way by which the predation risk associated with begging signals could be mitigated by nestlings is through responding appropriately to parental alarm signals (Davies et al. 2004; Platzen & Magrath 2004; Madden et al. 2005; Magrath et al. 2006). In many taxa, parents use alarm calls to warn offspring of the presence of danger (Templeton et al. 2005; Griesser 2008; Suzuki 2011). This parent-offspring communication is particularly important for altricial nestlings, which cannot physically escape predators and typically lack the ability to recognize them for much of their development (Kullberg & Lind 2002).

In addition to warning nestlings generally, parental alarm calls can encode

information on the type and urgency of predators (Fichtel & Kappeller 2000; Platzen & Magrath 2005), which can allow nestlings to respond in the most adaptive manner (Platzen & Magrath 2005). ‘Mobbing’ calls are given by many species to defend nests against stationary predators posing an immediate threat to young, while in some species a different alarm call is given to signal potential danger such as a flying raptor (Klump & Shalter 1984; Evans et al. 1993; Bradbury & Vehrencamp 1998, Farrow et al. 2017). The way young animals respond to these different alarm calls is thought to be adaptive and is associated with differences in the type and level of predation risk (Hanson & Coss 2001; Platzen & Magrath 2005). For example, in California ground squirrels (*Spermophilus beecheyi*), juveniles show stronger responses to alarm calls for ground predators than they do to those targeting aerial predators, most likely as a consequence of differential predation risk (Hanson & Coss 2001). In Japanese great tits (*Parus major*), different parental alarm signals elicit specific responses to predators in young. Nestlings either leave the nest hollow in response to alarm calls that are given for snakes, or crouch down into the nest in response to alarm calls given to corvids (Suzuki 2011).

In these previous studies, research was conducted at a time when the offspring were able to respond with movement. However, for altricial birds, movement away from the nest is not possible for much of their development. Given this, an appropriate adjustment of begging following alarm calls of that species may be particularly important to enhance nestling survival (Mateo 1996). Despite increasing number of studies on parent-offspring communication in the presence of dangers such as an increased predation risk (e.g. Suzuki 2011), the acoustic responses of nestlings to different intraspecific alarm call signals remain relatively poorly understood.

Further, nestlings typically live in environments where they could potentially eavesdrop on the alarm calls of other species to further mitigate their predation risk. This eavesdropping on the alarm cues or signals of other species may well provide an important opportunity to acquire additional predator information, either through innate mechanisms or learnt responses (Haff & Magrath 2012). Innate responses to interspecific alarm calls are most likely if calls are similar between taxa (Haff & Magrath 2013), while conversely learnt responses most easily arise through personal experience or social learning from other nestlings (Magrath et al. 2015).

The aim of this current study was to understand the mechanism by which nestling noisy miners (*Manoriana melanocephala*), an altricial species, respond to acoustically different, functionally referential intraspecific alarm calls in comparison to familiar interspecific alarm calls. Noisy miners are an Australian honeyeater with altricial nestlings and open cup nest that is often predated by range of predators (Higgins et al. 2001; Barati et al. 2016). Noisy miners further possess a complex acoustic repertoire (Holt et al. in press) and produce two functionally referential alarm calls for different predatory types (Farrow et al. 2017): 1) Terrestrial alarm call (hereafter chur call), a vocalisation with a broad frequency and multiple harmonics (Fig. 6.1b) that is given to ground-based or perched threats and attracts other miners to the area (Dow 1977; Kennedy et al. 2009; Holt et al. in press). 2) Aerial alarm calls, which include a series of high-pitched, up-slurred whistles (Fig. 6.1a), are given in response to aerial predators, primarily brown goshawks (*Accipiter fasciatus*), and pied currawongs (*Strepera graculina*), in the study area (Farrow et al. 2017, Holt et al. in press). These two types of alarm call are functionally referential and elicit different anti-predator behaviour responses in adult birds tested in both the field and under controlled laboratory conditions (Farrow et al. 2017). Both types of predators (e.g. terrestrial and

aerial) pose a threat to nestlings, and may potentially use the conspicuous vocalisations nestlings produce to locate them. Ceasing vocalisation production when these predators are near would therefore be beneficial for nestlings and may help mitigate their predation risk.

In addition to intraspecific alarm calls, nestling noisy miners are frequently exposed to two different interspecific calls at the focal study site. First, the grey butcherbird (*Cracticus torquatus*), is common in noisy miner colonies and produces a distinct alarm call in response to potential threats to the nest (Higgins et al. 2001). Given that butcherbirds are closely associated with noisy miners and also give alarm calls when exposed to similar predators as miners (Low 1994; Higgins et al. 20001), nestling noisy miners would also benefit from ceasing vocalisation production in response to butcherbirds alarm calls. Second, the chatter calls of the eastern rosella (*Platycercus eximius*) are also commonly heard in the study area, providing a non-alarm control vocalisation. This system therefore provided an opportunity to compare nestling responses to two intraspecific and two interspecific calls. I predicted that: 1) nestlings would beg less in response to hearing intraspecific alarm calls in order to reduce their risk of being overheard by potential predators; 2) different intraspecific alarm calls would elicit different responses in nestlings because they are referential signals; 3) nestlings may have learnt to respond to the pertinent alarm calls of the butcherbird, but not the irrelevant rosella vocalisations.

Methods

Study population and general methods

The focal populations of this study were two colonies of noisy miners located at Newholme Field Research Station of the University of New England (30° 25' 24''S;

151° 38' 84 38"E) and Dumaresq Dam (30°30'S, 151°40"E), located 10 and 12 km, respectively, north-west of Armidale, NSW, Australia. The most common canopy species in these areas is New England peppermint (*Eucalyptus nova-anglica*) (Andrews & Reid 2000), with sparse understorey vegetation dominated by introduced pasture grasses, consistent with the typical habitat of noisy miners (Higgins *et al.* 2001). Fieldwork was conducted in the 2015 breeding season from mid-September to late October. To find nests, I surveyed study sites every 2-3 days for signs of nesting activity from mid-August when nesting activities normally commence at these colonies (Barati *et al.* 2016). Once nest sites were located, they were marked with small numbered cattle ear tags (Allflex, Australia) attached to a nearby tree. I checked the contents with direct observation (using a ladder) or by a mirror attached to the end of a 10-metre pole to determine hatching dates. I noted the hatching date for each nest, and monitored the brood at least every other day. To assess nestling status (e.g. survived, predated) whilst minimising disturbance, I used the same methods as outlined above, but once nestlings' vocalisations were audible from the ground (approximately 5 days post-hatch), I used this as a cue to their status. Broods were monitored until experiments were conducted at the age of 14 days.

Stimuli recording and preparation

Noisy miners' chur and aerial alarm calls were recorded between September 2013 and October 2015. Chur calls are given to approaching humans (N=17 individuals), while adult miners were provoked to give aerial alarm calls by throwing a hat in the air nearby (N=10 individuals). Vocalisations emitted by individuals were recorded at 44.1kHz using a portable Professional Solid State Recorder (Marantz PMD661, Japan) with 16-bit accuracy in uncompressed wav format using a Sennheiser shotgun microphone (ME67, USA), protected by a fur windshield (Rycote Softie, UK). The

identity of the focal bird was determined using the unique leg colour bands fitted to each adult in these colonies (see Chapter 3 for details) using binoculars (10x42, Nikon, Japan) or a telescope (Gerber Montana 15-45x). The amplitude of five exemplars of each vocalisation type was determined in the field using a Sound Level Meter (Digitech QM-1589) at a distance of *ca.* 15 m from the bird. The original amplitude of vocalisation SPL (sound pressure level) at 1m was then calculated using the formula of a 6dB reduction for each doubling of distance between emission and reception (Naguib & Wiley, 2001). The alarm calls of butcherbirds were also recorded by approaching active nests (n=7) on foot. Butcherbirds' nests were placed in average distance of 14.5 ± 1.5 m (mean \pm SE, n=7) from active noisy miners' nests. Butcherbirds produce a distinctive alarm call in response to approaching humans (Barati, personal obs), and it is this vocalisation that was recorded. Finally, eastern rosella chatter calls were recorded from calling individuals that were perched on trees (n=5). Butcherbird and rosella calls were recorded using the same equipment and settings as those used for noisy miners (see above). Recorded calls were first grouped based on their type (chur, aerial, butcherbird or rosella) and the caller's identity. For each individual/call type, I constructed a 2-minute playback sample using Raven Pro (v1.4; Cornell Laboratory of Ornithology). Each playback track consisted of 1 minute silence, followed by 20 seconds playback of the relevant stimulus, 20 seconds silence then finally another 20 seconds of playback. Only calls with a high signal to noise ratio were used.

Playback experiment design

I assessed how noisy miner nestlings responded to broadcast stimuli from 10 September to 30 November 2015. Fourteen different broods were exposed to playback, all when nestlings were 14 days post hatch. This age is just prior to

fledging, so maximised the probability that nestlings were familiar with the different alarm calls and may have learnt to modify their behaviour accordingly. Prior to playbacks commencing, I installed a small tie-clip microphone under the nest cup (ECM-44B Sony, Japan) run by cables to a hide ~ 30 m away from the nest and connected to a solid-state audio recorder (Marantz PMD661, Japan), allowing recording of nestling vocalisations at high quality. Microphones were attached under the nest cup at least 1 hour before playbacks commenced to avoid any potential disturbance effects on nestling or attendant behaviour. No birds alarm called towards or interfered with the microphone over the experiment. I also placed a speaker (GG0191, JBL, USA) at a distance of ~ 5 m from the nest. It was connected to an audio player (Apple iPod) in the hide to allow playback of uncompressed wav files prepared above at a distance of ~ 30 m. Playback amplitudes were similar to the natural amplitude of the focal calls recorded for different calls (chur call: 83 dB, aerial alarm call: 89 dB) and remained consistent during all trials.

Playback experiments were conducted between 0700 to 1200 h and 1400 to 1700. At the beginning of each trial, one or two observers entered the hide, although playbacks did not commence for a 10-minute period to allow broods to resume begging normally following any potential disturbance. After this period, playbacks were initiated when no adult birds were present within approximately 10 m of the nest and nestlings were vocalising. Nestlings normally resumed begging regardless of stimuli if an adult bird physically visited the nest with food, so if this occurred during playbacks that given trial was aborted (n=10 trials). Trials were also aborted if I detected an additional call being given by a free-living bird (e.g., a chur or aerial call) during the playback period (n=5). Begging data were recorded and measured only when broods were unattended for the duration of the trial. In each trial one of four

stimuli were present in the stimulus broadcast: (1) intraspecific chur calls, (2) intraspecific aerial alarm calls (3) butcherbird alarm calls or (4) rosella chatter calls (non-alarm). In all trials, nests were subject to the broadcast of four different calls. Calls were selected randomly from multiple available recordings for each call type. The order of call types was rotated within (for nests receiving more than 1 playback; N=10) and between nests. If more than one playback was broadcast, there was an interval of at least 30 minutes between trials to allow begging activity to return to normal. I commenced recording of all vocalisations at the nest before playback and continued until 5 minutes after playbacks had ceased in uncompressed WAV format (44.1 kHz, 16 bits) for later analysis.

Acoustic analysis of brood begging in response to stimulus presentation

Recorded begging calls were analysed in Raven Pro 1.4 (Cornell Laboratory of Ornithology). All spectrograms were constructed with a 256-point, 172 Hz grid spacing, Hanning window function with overlap set at 75% and a 3 dB Filter Bandwidth of 248 Hz. Calls were then filtered (highpass 500 Hz) to remove background noise at frequencies lower than the focal vocalisations. By examining spectrograms of recordings of each trial in Raven, I first identified the exact time playback started, then counted the number of begging bouts that occurred 10 seconds prior to playback commencing, and also during the first 10 seconds during playback (Fig. 6.1). After the 2 minutes playback was completed, I then identified the time between the end of playback and the time nestling resumed begging as suppression time. Therefore, suppression time was defined as the interval between the end of playback and the start of nestling begging. For each playback trial, this time was identified on corresponding spectrogram in Raven software. I again counted the number of begging bouts per 10 seconds starting from the first begging call for this

period. Each begging bout consisted of a single syllable that was given repeatedly as shown in Fig. 6.1a. I measured acoustic properties of 5 immediate bouts of begging before playback (n=376 begging bouts) and 5 begging bouts after playback (n=376 begging bouts). However, if any of these five begging bouts were not clear enough for measurement, such as there were additional signals occurring at the same time, I measured the next available begging bout. The measurements were: (1) call amplitude using a root-mean-square algorithm (2) maximum frequency (kHz) or the frequency at maximum amplitude or frequency of the begging bout when it was loudest, and (3) length of each begging bout as the time differences between the starts and the end of a bout as shown in Fig. 6.2.

Spectrographic cross-correlation to determine stimuli similarity

I used spectrographic cross-correlation (SPCC) to quantify the similarity of stimuli tracks, both within and between call types. I first used Sample Manager 3.2.0 (AudioPhile Engineering, USA) to convert stereo audio files to a mono audio file. Calls were then bandpass-filtered at 1000–22050 Hz (Holt et al. 2016) and normalised before spectrograms were constructed with a 256-point, 172 Hz grid spacing, Hanning window function and overlap set at 75%, with 3 dB Filter Bandwidth of 248 Hz. I then compared spectrograms using the batch correlator tool in Raven Pro 1.4 (Charif et al 2004). I obtained the peak correlation score for each pair of calls, which vary between 0, (for orthogonal signals) to 1 (identical signals) (Charif et al 2004).

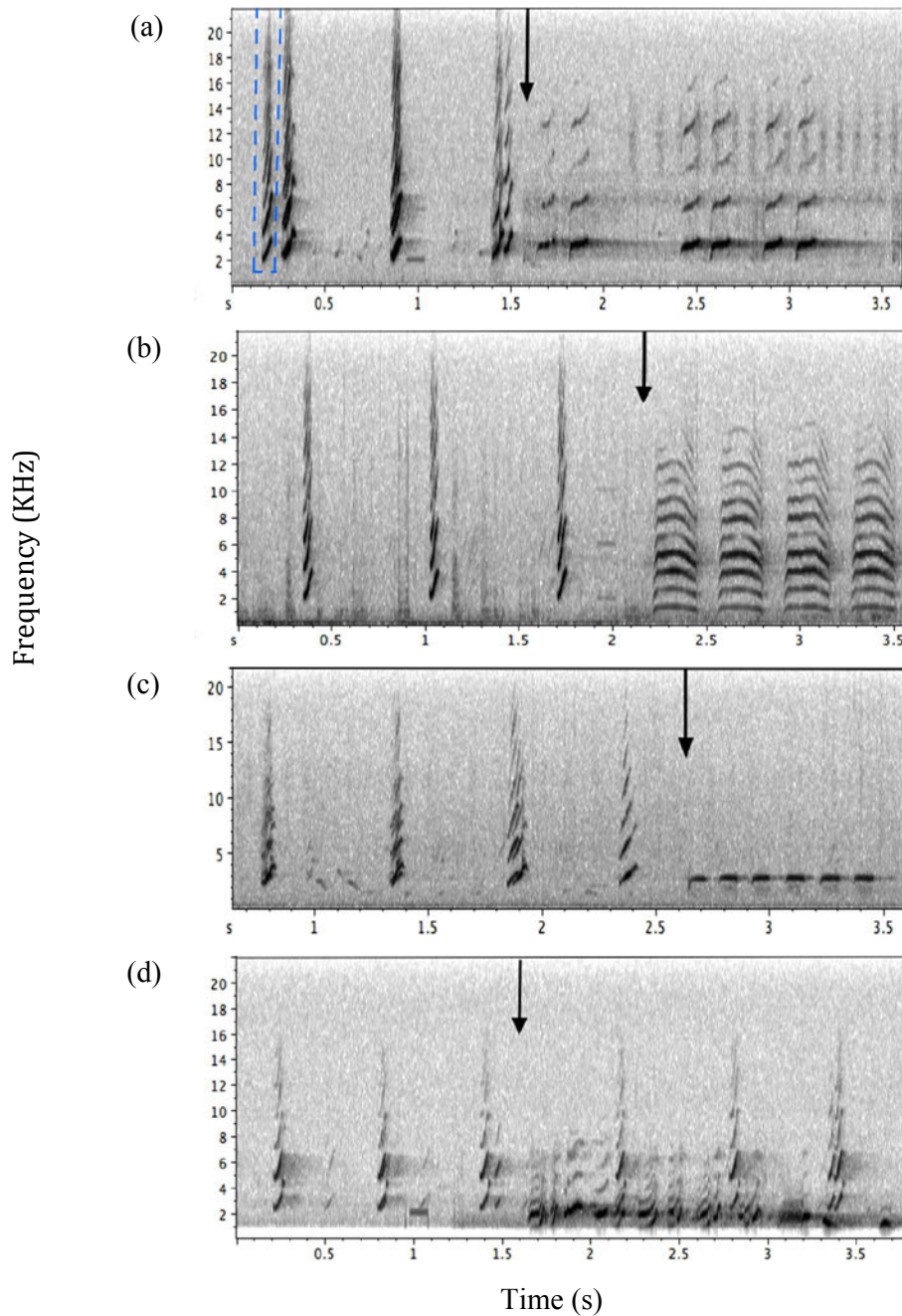


Fig. 6.1. Spectrograms of exemplars of nestling noisy miners responding to different playback stimuli. Nestlings suppressed vocalisations when they heard intraspecific aerial (a) or chur alarm calls (b), sympatric rosella chatter calls (c) but kept begging after playback of playback of the sympatric butcherbird alarm call (d). On each spectrogram the time that playback commenced is indicated with arrows. An example begging syllable produced by the nestlings is highlighted by the dashed line (a).

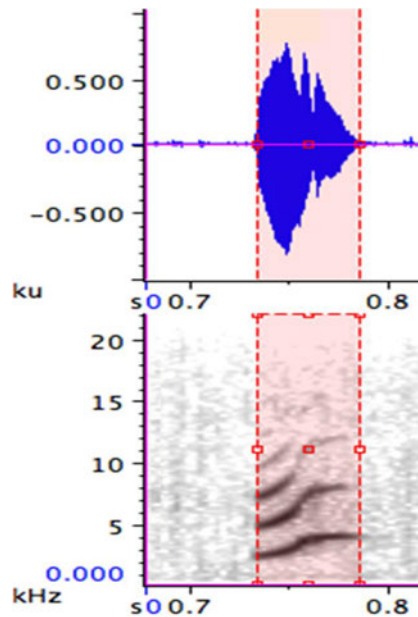


Fig. 6.2. Sample spectrogram showing an example begging bout and how it was measured in Raven software.

Statistical analyses

To test if the begging rate during test periods was similar to begging prior to playbacks commencing, I fitted a generalized linear mixed effects model (GLMM) with a poisson distribution including begging rate (e.g. number of begging bouts per 10 seconds) as the response variable and call type (chur, aerial, butcherbird or rosella) as main effect. Similar GLMMs were fitted to test if begging rate would change in response to each stimulus, whether stimulus type predicted the strength of response, the probability of begging being suppressed, the length of suppression time and the begging rate after suppression, I fitted different GLMMs with the term of interest as a fixed effect and begging rate, probability of suppression and the time of suppression as response variables with either poisson (begging rate and suppression time) or binominal (probability of suppression) distributions. In all models playback order and nest identity were included as random effects. The significance of the fixed terms

fitted were determined by comparing the model with a model that had only the intercept term and random effects using likelihood ratio tests (LRTs). If there were significant, I then performed *post hoc* Tukey tests to investigate within subject differences.

In order to analyse the changes in begging structure with regards to playback types, I first averaged measured characteristics of 5 begging calls before and after playback for each playback trial. I then calculated the changes in these begging properties for each trial (e.g. after playback minus control). To reduce the dimensions of begging properties, I conducted a principal component analysis (PCA). To test if the begging structure varies in relation to stimulus type, I fitted a generalized linear model (GLM) with a gaussian distribution with call type as a fixed effect and the first extracted component of the PCA as the response variable. To examine the SPCC similarities between and within call types, I fitted additional GLMMs. In the models the response variables were pairwise similarity between different calls, including the terms of interest (e.g. call types) as fixed effects and playback order and nest identity as random effects. To test the significance of the term of interest, GLMMs were compared with a reduced model that contained only the intercept term and random effects using likelihood ratio tests (LRTs). To examine within subject differences *post hoc* Tukey tests were used. All statistical analysis tests were performed in the R statistical language and environment (R Core Team, 2015) using the *lme4* package (Bates et al. 2014).

Results

Changes in begging rate in response to different playback stimuli

The rate of begging call production (call syllables per 10 seconds) in control periods prior to stimulus playback did not differ between broods exposed to different playback types (GLMM: $\chi^2_3=2.28$, $p=0.51$). There was a significant decrease in begging rates in response to aerial alarm calls (GLMM, $\chi^2_1=87.14$, $p<0.001$), chur alarm calls (GLMM, $\chi^2_1=66.88$, $p<0.001$) and rosella chatter calls (GLMM, $\chi^2_1=24.86$, $p<0.001$) (Fig. 6.3). In contrast, butcherbirds calls did not provoke a significant decline in the begging rate of nestlings (GLMM, $\chi^2_1=10.82$, $p=0.07$, Fig. 6.3). When comparing the comparative strength of changes in begging rate during the playback period across different call types, there was a significant difference (GLMM, $\chi^2_3=4.825$, $p<0.001$). When call types were compared using a *post hoc* test, a greater reduction in the begging rate in response to aerial alarm call (*post hoc* GLMM, $\beta=-12.8\pm 1.96$, $p=0.0001$), chur alarm call (*post hoc* GLMM, $\beta=-14.35\pm 2.07$, $p=0.0001$) and rosella chatter call stimuli (*post hoc* GLMM, $\beta=-0.47\pm 2.06$, $p=0.0001$) was apparent relative to butcherbird calls (Fig. 6.3). Similarly, the probability that nestlings' suppressed begging was significantly different in relation to call type (GLMM, $\chi^2_3=70.21$, $p<0.001$) with significantly lower probability in response to butcherbird calls than to aerial alarm (*post hoc* GLMM, $\beta=0.87\pm 0.10$, $p<0.001$) and chur alarm calls ($\beta=0.84\pm 0.10$, $p<0.001$). While there was significantly higher probability that nestlings suppressed begging vocalisations in response to intraspecific alarm calls than to interspecific calls (GLMM, $\chi^2_1=66.26$, $p<0.001$; Figs 6.1-6.4), the proportion of times that nestlings suppress begging was not different for two types of intraspecific alarm calls (*post hoc* GLMM, $\beta=0.03\pm 0.10$, $p<0.9$; Fig. 6.4) and two interspecific calls (*post hoc* GLMM, $\beta=-0.21\pm 0.10$, $p=0.20$; Fig. 6.4). Further,

suppression time was different in relation to call type (GLMM, $\chi^2_3 = 2112.5$, $p < 0.0001$). All pairwise comparisons of suppression time differed significantly (Fig 6.5; Table 6.1). Nestlings suppressed begging vocalisations for the longest time in response to chur alarm calls, followed by aerial alarms and then rosella chatter calls. Suppression time in response to butcherbirds was the shortest among all call types (Fig. 6.5, Table 6.1). When nestlings resumed begging again, begging rate remained independent of the stimulus type that they were exposed to during playbacks (GLMM, $\chi^2_3 = 1.49$, $p = 0.68$).

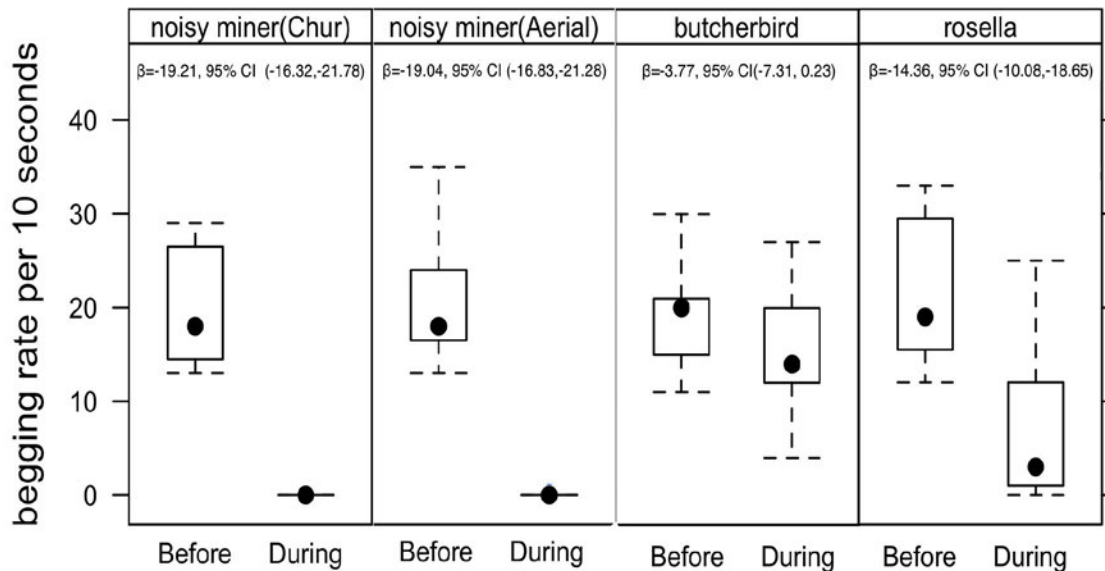


Fig. 6. 3. Begging rates of nestling noisy miners before playbacks (control) and in response to changes in the begging rate in response to different playback stimuli. For each call type, effect size and 95% CIs are given in the corresponding panel. In both before and after playback, begging rate is the number of begging bouts in a 10-second time frame.

Table 6.1. Pair-wise comparisons of suppression time of begging by nestling noisy miners in response to playback stimuli (call type A-call type B).

Call Type A	Call Type B	β	SE	z	p
butcherbird	Aerial	-4.15	0.33	-12.41	< 0.001
chur	Aerial	0.91	0.04	19.52	< 0.001
rosella	Aerial	-0.69	0.06	-10.09	< 0.001
chur	butcherbird	5.07	0.33	15.18	< 0.001
rosella	butcherbird	3.46	0.33	10.25	< 0.001
rosella	Chur	-1.60	0.06	-25.36	< 0.001

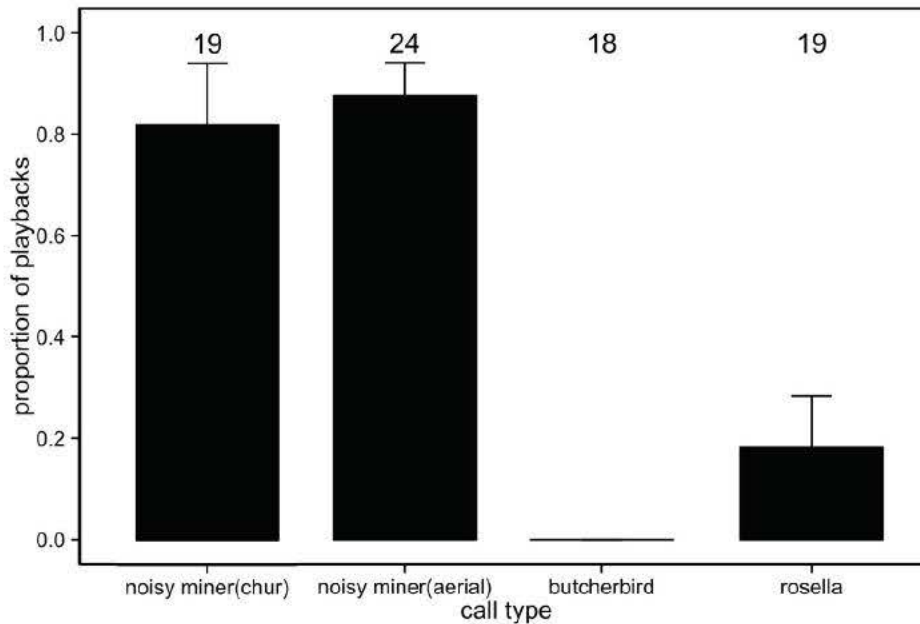


Fig. 6. 4. Mean \pm se of proportion of playbacks that nestling noisy miners suppressed vocalisation in response to different playback stimuli. Numbers above bars represent sample sizes for each playback type.

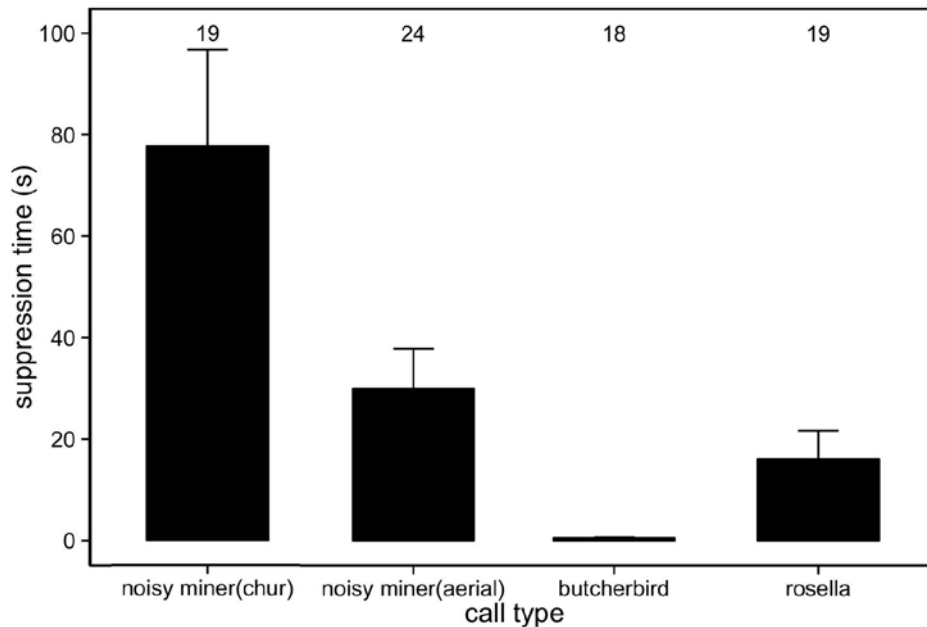


Fig. 6. 5. Mean \pm se suppression time of nestling noisy miners to different playback stimuli. Numbers above bars represent sample sizes for each playback type.

Changes in the spectral structure of begging vocalisations during playbacks

I first calculated the degree of change (the values 10 seconds after playback minus the values 10 seconds before playback) of three begging characteristics (e.g. amplitude, maximum frequency and duration) and then extracted principal components with eigenvalues larger than 1 through PCA. Only the first component (hereafter: PC1) had an eigenvalue that was greater than 1. PC1 was positively correlated with changes in begging call amplitude (Pearson correlation= 0.69, df=78, $p < 0.0001$), maximum frequency (Pearson correlation= 0.73, df=78, $p < 0.001$) and begging duration (Pearson correlation=0.87, df=78, $p < 0.001$). Therefore, larger PC1 values reflect increasing call amplitude, maximum frequency and duration. PC1 was significantly different in relation to stimulus types (GLMM, $\chi^2_3 = 15.5$, $p < 0.001$). Nestlings begged with lower amplitude and maximum frequency, and shorter duration

after the playback of both aerial and chur calls (*post hoc* GLMM, aerial: $\beta=1.06\pm 0.32$, $p<0.001$; chur: $\beta=1.09\pm 0.33$, $p<0.001$; Fig. 6.6), however, no differences was apparent between these two types of intraspecific calls (*post hoc* GLMM, $\beta=0.02\pm 0.31$, $p=0.99$). Further, variations of begging structure did not significantly differ in response to two interspecific calls (*post hoc* GLMM, $\beta=0.37\pm 0.33$, $p=0.68$).

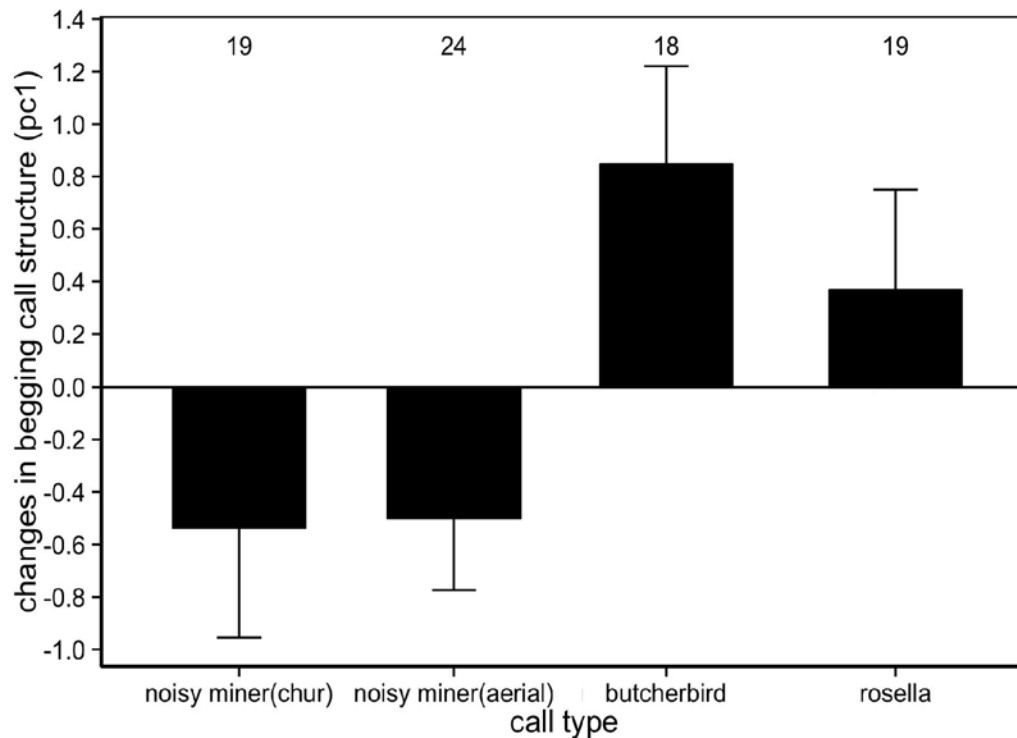


Fig. 6.6. Mean \pm SE of variations in the acoustic structure (represented by PC1) of nestlings begging after playback of different stimuli. Numbers above bars represent sample sizes for each playback type.

Similarity of stimulus signals across different vocalisation types

I compared the similarity both between and within the different call types used as stimuli during these playbacks using spectrographic cross correlation (SPCC). As expected, calls from different birds of the same call type (e.g. within-stimulus) were significantly more similar than between-stimulus (GLM, $\chi^2_1=6.46$, $p<0.001$). The

similarity of calls varied in relation to call type (GLMM, $\chi^2_5=0.29$, $p<0.001$). When between-stimulus calls were compared, both chur and aerial alarm calls were significantly more similar to rosella chatter calls than to butcherbird calls (*post hoc*, GLMM, aerial: $\beta=0.014\pm 0.002$, $p<0.001$; chur: $\beta=0.09\pm 0.002$, $p<0.01$). However, two different intraspecific calls were not significantly different in their similarity to rosella chatter calls (*post hoc*, GLMM, $\beta=-0.001\pm 0.003$, $p=0.99$) or butcherbird calls (*post hoc*, GLMM, $\beta=0.003\pm 0.001$, $p=0.23$).

Discussion

This study provides experimental evidence that nestling noisy miners attended to alarm signals given by conspecifics and suppressed their begging on hearing those signals alone, broadening the taxa in which this result has been documented (Davies et al. 2004, Platzen & Magrath 2004). This study was further able to demonstrate that noisy miner nestlings responded more strongly to intraspecific rather than interspecific alarm calls, with the strength of response in terms of suppression time varying significantly for the two different intraspecific alarm calls broadcast. This suggests that nestlings can differentiate between these functionally referential intraspecific alarm calls. Further, among the two interspecific stimuli played back to nestlings, nestlings responded only to the call that was more similar to intraspecific, despite this not being a vocalisation specific to the presence of predators and thus being associated with danger.

Changes in the begging rate and suppression time in response to playback

Nestling noisy miners showed a strong response to intraspecific alarm calls, and in over 80% of trials, broods suppressed vocalisation upon hearing these alarm calls regardless of their type. These results suggest that nestling noisy miners could

effectively respond to intraspecific alarm calls nearby and reduce their predation risk, adding to very rare examples of this behaviour in other avian taxa (e.g. white-browed scrubwren, *Sericornis frontalis*, Platzen & Magrath 2004).

Although nestlings showed similar, suppressive responses to chur and aerial alarm calls, nestlings ceased begging for a longer period in response to chur calls rather than aerial alarm calls of adult noisy miners. In this system, chur alarm calls warn of ground predators, often in response to approaching potential threats or when a disturbance is detected within the species' colony (Farrow et al. 2017). This call attracts colony members to the area and elicits mobbing behaviour (Kennedy et al. 2009, Holt et al. 2016). Previous studies have found that this call is given in response to foxes (*Vulpes vulpes*), feral cats (*Felis catus*) and perched predatory birds in the study area (Farrow et al. 2017; Holt et al. 2016). Therefore, chur calls usually signal a more urgent threat to nestlings, such as a raptor that has perched nearby, than aerial alarm calls do where the flying predator is quickly gone from the immediate area (Kennedy et al. 2009; Holt et al. 2016; Farrow et al. 2017).

Given that the response of nestlings in terms of the probability of suppression and the reduction in begging rate did not differ when exposed to two intraspecific alarm calls, nestling response is likely an immediate and innate reaction to these calls. However, the functions of chur and aerial alarm calls suggest that aerial alarm calls encode a less urgent threat compared to chur alarm calls, thus it is likely that nestlings have learnt to associate chur calls with immediate and greater danger and fine-tune their response based on the information that they have gained during nestling period. This might explain why the suppression time is longer in response to chur calls. Differentiating between alarm calls and showing adaptive adjustments in responses to these has rarely been reported in nestling birds. To date, only white-browed

scrubwren nestlings have been shown to respond to ground alarm calls more intensely than to aerial alarm calls, with ground predators posing a greater threat to the nestlings (Platzen & Magrath 2005).

Changes in begging acoustic structure in response to broadcast of different call types

Nestlings modified their begging acoustic structure following exposure to either aerial or chur alarm call stimuli from conspecifics. They begged at a lower amplitude, lower maximum frequency and for a shorter time after they resumed begging following exposure to these alarm calls. Generally, nestling vocalisations can attract predators to the nest area (McDonald et al. 2009, Magrath et al. 2010) and there is evidence that the acoustic properties of begging are associated with the degree of predation risk. For example, species with a lower amplitude of begging call experience lower predation risk (Briskie 1999) because lower amplitude reduces the locatability of broods by predators. Therefore, it may be possible that altered acoustic properties provides a reduction in the risk of broods being detected by predators, acting as a passive defence that makes begging calls more difficult for predators to locate (Briskie et al. 1999). However, if the begging calls are honest signals of need, these changes can also influence the amount of food that nestlings would receive from nest attendants (Wright 1998; Hinde & Kilner 2007; Horn & Leonard 2008), so such changes would likely be transient and short-term in nature. This implies that acoustically adjustment of begging calls to intraspecific alarm calls represents a trade-off between avoiding danger and receiving sufficient food, and the intensity of this response perhaps depends on the nestlings' hunger level.

Responses of nestlings to different interspecific calls

Nestling noisy miners responded differently to the two different calls used in this experiment. They largely ignored the alarm calls of the sympatric grey butcherbird. Similar to noisy miners, butcherbirds also mob potential nest predators, even in mixed mobbing groups with noisy miners (Higgins et al. 2001; Low 1994). Further, butcherbirds are closely associated with noisy miners in the studied colonies and usually built their nest at a close distance to those of noisy miners (see methods). Therefore, nestling noisy miners have the opportunity to learn the link between Butcherbird alarm calls and a potential threat during their developmental period before fledging, however the results show that this did not occur. Despite this, it cannot be ruled out that nestlings will learn to respond to butcherbird alarms when they are older. Thus, for understanding the response of noisy miners to interspecific alarm calls, it would be useful to examine the response of adults and fledglings to butcherbird alarm calls.

Further, it is worth noting that learning to respond to an interspecific alarm signal can be influenced by the geographical variation in the density of interspecific species (Magrath & Bennett 2012). For example, adult superb fairy-wrens (*Malurus cyaneus*) respond to the aerial alarm calls of noisy miners only in areas with high density of noisy miners, but they ignore this call if their territories do not overlap with a noisy miner population (Magrath & Bennett, 2012). This suggests that learning to respond to an interspecific alarm call could be closely related to the degree to which focal broods are exposed to these signals on an individual nestling basis. Finally, butcherbird alarm calls recorded herein may also serve more than one role, and thus be given in contexts outside of predation risk. If this were the case, then the link between predator presence and the production of this signal would be weakened, and

perhaps lead to noisy miner broods not being selected to associate this signal with a pertinent cue of an impending threat. Detailed analysis of the butcherbird vocalisation repertoire and alarm call production is needed to quantify the importance of these relationships.

In contrast to their response to butcherbirds, nestlings showed a partial response to sympatric rosella chatter calls, which are not used as alarm calls but rather function to attract flying groups to perch near the caller (Higgins et al. 2001). Our analysis of call similarity based on spectrographic cross-correlation suggested that this response is most likely due to recognition error by broods, given that rosella chatter calls are somewhat similar to noisy miner alarm calls. If animals rely on one or a few key acoustic features to recognise familiar vocalisations, and if these features are similar between intraspecific and interspecific vocalisations, then individuals will respond to interspecific calls (Fallow et al. 2011, 2013, Magrath et al. 2009). This mechanism could be beneficial to nestling birds if calls encode information about likely threats, but can also lead to inappropriate responses if interspecific calls are neutral relative to the probability of a predator being present. The latter appears the most likely explanation here.

The cost of inappropriate responses to non-alarm calls is probably minor compared with the cost of not responding to honest and accurate alarm call signals. There is evidence that various sensory pathways develop with age in nestlings of other species, enabling individuals to acoustically discriminate between familiar and unfamiliar individuals (Sharp et al. 2005) and focus their attention on sound features of their own species' song (Soha & Marler 2000), including adult noisy miners (McDonald 2012). These abilities can then be used to improve the learning of their own species' signals from the many interspecific calls and signals in the surrounding acoustic environment

(Davis et al. 2004). Given this, it is important to consider development process of nestlings when assessing their responses to conspecific alarm calls. Contrary to nestling responses, adult noisy miners usually ignore rosella calls (Barati, personal obs.), therefore it is likely that miners do eventually learn to ignore these irrelevant stimuli as they mature. This has been confirmed in some species. For example, offspring of the white-browed scrubwren adaptively change their response to alarm calls as they grow and show stronger responses to aerial alarm calls after they have fledged (Magrath et. al. 2006).

Interestingly, the changes in begging acoustic properties occurred only in response to intraspecific alarm calls, not interspecific signals. Broods did change their begging rate following exposure to rosella chatter calls, but they did not modify the acoustic structure of their begging calls, suggesting that the response to rosella calls is probably an immediate short-term response, most likely due to recognition confusion and a failure to completely discriminate interspecific calls from intraspecific alarm calls. Nestlings may learn rapidly to discriminate intraspecific alarm calls from similar calls of other species, but this seems unlikely given the broods were close to fledging at the time of this experiment. How noisy miners distinguish between different alarm calls and the role of learning in this process remains unclear, and further research is required using nestlings that have been isolated from exposure to some calls to tease these factors apart.

Conclusion

In conclusion, I show that nestling noisy miner broods suppressed begging vocalisations in response to intraspecific alarm calls in a manner consistent with adaptively reducing their predation risk. Our results confirm previous findings that nestlings can lower the risk of predation and other threats using likely innate

responses (Haff & Magrath 2010; Magrath et al. 2010). Moreover, I demonstrate herein that nestlings can, either innately or through learnt responses, discriminate between two different conspecific alarm calls that signal different relative threats. The study thus provides experimental evidence that the response of offspring to a particular intraspecific alarm call in this system is linked to the degree of danger that particular signal is likely to indicate. I also provide as far as I am aware the first empirical evidence that nestlings adjust the structure of their begging calls when they are exposed to changes in predation risk, again likely a response that reduces the risk of detection by a predator.

References

- Andrews, S. P., Reid, N. (2000). The user guide to Newholme: A Dictionary of natural resources information for Newholme field laboratory, University of New England, Armidale.
- Barati A., Etezadifar F., McDonald P.G. (2016). Fragmentation in eucalypt woodlands promotes nest-tree occupancy by a despotic species, the noisy miner (*Manorina melanocephala*). *Austral Ecology*, 41, 897-905.
- Bates, D., Maechler, M., Bolker, B., Walker, S. (2014). lme4: Linear mixed-effects models using Eigen and S4-. R package version 1.1-7, <URL: <http://CRAN.R-project.org/package=lme4>>.
- Bell, M. B. (2008). Strategic adjustment of begging effort by banded mongoose pups. *Proceedings of the Royal Society B*, 275, 1313-1319.
- Bradbury, J. W., Vehrencamp, S. L. (1998). *Principles of animal communication*. Sunderland (MA): Sinauer.
- Briskie, J. V., Martin, P. R., Martin, T. E. (1999). Nest predation and the evolution of nestling begging calls. *Proceedings of the Royal Society B: Biological Sciences*, 266, 2153-2159.
- Chappell, M. A., Bachman, G. C. (2002). Energetic costs of begging behaviour. In Wright, J., Leonard, M. L. (Eds.), *The evolution of*

- begging, competition, cooperation and communication (pp: 143-162).
Dordrecht, Germany: Kluwer Academic Publishers.
- Charif, R. A., Clark, C. W., Frisrup, K. M. (2004). *Raven 1.2 User's Manual*. Ithaca, New York: Cornell Laboratory of Ornithology.
- Davies, N. B., Madden, J. R., Butchart, S. H. M. (2004). Learning fine-tunes a specific response of nestlings to the parental alarm calls of their own species. *Proceedings of the Royal Society B*, 271, 2297-2304.
- Dow, D. D. Indiscriminate interspecific aggression leading to almost sole occupancy of space by a single species of bird. *Emu*. 77, 115-21 (1977).
- Evans, C. S., Evans, L., Marler, P. (1993). On the meaning of alarm calls: Functional reference in an avian vocal system. *Animal Behaviour*, 46, 23-38.
- Fallow, P. M., Gardner, J. L., Magrath, R. D. (2011). Sound familiar? Acoustic similarity provokes responses to unfamiliar heterospecific alarm calls. *Behavioral Ecology*, 22, 401-410.
- Fallow, P. M., Pitcher, B. J., Magrath, R. D. (2013). Alarming features: birds use specific acoustic properties to identify heterospecific alarm calls. *Proceedings of the Royal Society B*, 280, 20122539.
- Farrow L., Doohan, S., McDonald, P. G. (2017). Alarm calls are referential and elicit context-specific anti-predator behaviour among receivers in a highly cooperative bird. *Behavioral Ecology*, 28, 724–731.
- Fichtel, C., Kappeler, P. M. (2002). Anti-predator behavior of group-living Malagasy primates: mixed evidence for a referential alarm call system. *Behavioural Ecology and Sociobiology*, 51, 262-275.
- Griesser, M. (2008). Referential calls signal predator behavior in a group-living bird species. *Current Biology*, 18, 69-73.
- Haff, T. M., Magrath, R. D. (2010). Vulnerable but not helpless: nestlings are fine tuned to cues of approaching danger. *Animal Behaviour*, 79, 487-496.
- Haff, T. M., Magrath, R. D. (2013). Eavesdropping on the neighbours: Fledglings learn to respond to heterospecific alarm calls. *Animal Behaviour*, 85, 411-418.
- Haff, T.M., Magrath, R. D. (2012). Learning to listen? Nestling response to heterospecific alarm calls. *Animal Behaviour*, 84, 1401-1410.

- Hanson, M. T., Coss, R. G. (2001). Age differences in the response of California ground squirrels (*Spermophilus beecheyi*) to conspecific alarm calls. *Ethology*, 107, 259-275.
- Higgins, P. J., Peter, J. M., Steele, W. K. (eds). (2001). *Handbook of Australian, New Zealand and Antarctic birds*, Oxford Univ. Press, Melbourne.
- Hinde, C. A., Kilner, R. M. (2007). Negotiations within the family over the supply of parental care. *Proceedings of the Royal Society B*, 274, 53-60.
- Holt, J., Barati, A. & McDonald, P. G. The complex acoustic repertoire of a highly social species, the noisy miner, *Manorina melanocephala*. *Emu*. in press (2017).
- Horn, A. G., Leonard, M. L. (2008). Acoustic interactions in broods of nestling birds. *Journal of Comparative Psychology*, 122, 28-304.
- Ibáñez-Álamo, J.D., Arco, L., Soler, M. (2012). Experimental evidence for a predation cost of begging using active nests and real chicks. *Journal of Ornithology*, 153, 801-807.
- Kennedy, R., Evans, C., McDonald, P. (2009). Individual distinctiveness in the mobbing call of a cooperative bird the noisy miner (*Manorina melanocephala*). *Journal of Avian Biology*, 40, 481-490.
- Klump, G. M., Shalter M. D. (1984). Acoustic behaviour of birds and mammals in the predator context I. Factors affecting the structure of alarm calls II. The functional significance and evolution of alarm signals. *Z Tierpsychol.* 66, 189-226.
- Kullberg, C., Lind, J. (2002). An experimental study of predator recognition in great tit fledglings. *Ethology*, 108, 429-441.
- Low, T. (1994). Invasion of the savage honeyeaters. *Australian Natural History*, 24, 26-33
- Madden, J. R., Kilner, R. M., Davies, N. B. (2005). Nestling responses to adult food and alarm calls: 1. Species specific responses in two cowbird hosts. *Animal Behaviour*, 70, 619-627.
- Magrath R. D., Platzen, D., Kondo, J. (2006). From nestling calls to fledgling silence: adaptive timing of change in response to aerial alarm calls. *Proceedings of the Royal Society B*, 273, 2335-2341.

- Magrath, R. D., Bennett, T. (2012). A micro-geography of fear: learning to eavesdrop on alarm calls of neighbouring heterospecifics. *Proceedings of the Royal Society B*, 279, 902-909.
- Magrath, R. D., Haff, T. M., Horn, A. G., Leonard, M. L. (2010). Calling in the face of danger: how predation risk affects acoustic communication by parent birds and their offspring. *Advances in the Study of Behavior*, 41, 187-253.
- Magrath, R. D., Pitcher, B. J., Gardner, J. L. (2009). Recognition of other species' aerial alarm calls: speaking the same language or learning another? *Proceedings of the Royal Society B*, 276, 769-774.
- Magrath, R. D., Haff, T. M., Fallow, P. M., Radford, A. N. (2015). Eavesdropping on heterospecific alarm calls: From mechanisms to consequences. *Biological Reviews*, 90, 560-586.
- Mateo, J. M. (1996). The development of alarm call response behaviour in free living juvenile Belding's ground squirrels. *Animal Behaviour*, 52, 489-505.
- McDonald, P. G., Wilson, D. R., Evans, C. S. (2009). Nestling begging increases predation risk, regardless of spectral characteristics or avian mobbing. *Behavioral Ecology*, 20, 821-829.
- McDonald, P.G. (2012). Cooperative bird differentiates between the calls of different individuals, even when vocalisations were from completely unfamiliar individuals. *Biology Letters*, 8, 365-368.
- Naguib, M., Wiley, R. H. (2001). Estimating the distance of a source of sound: mechanisms and adaptations for long-range communication. *Animal Behaviour*, 62, 825-837.
- Platzen, D., Magrath R. D. (2005). Adaptive differences in Response to two types of parental alarm call in altricial nestlings. *Proceedings of the Royal Society B*, 272, 1101-1106.
- Platzen, D., Magrath R. D. (2004). Parental alarm calls suppress nestling vocalisation. *Proceedings of the Royal Society B*, 271, 1271-1276.
- R Core Team. (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

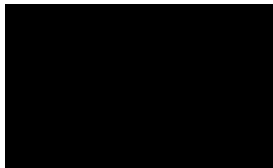
- Schleich, C. E., Busch, C. (2004). Energetic expenditure during vocalisation in pups of the subterranean rodent (*Ctenomys talarum*). *Naturwissenschaften*, 91, 548-551.
- Sharp, S. P., McGowan, A., Wood, M. J., Hatchwell, B. J. (2005). Learned kin recognition cues in a social bird. *Nature*, 434, 1127-1130.
- Soha, J. A., Marler, P. (2000). A species-specific acoustic cue for selective song learning in the white-crowned sparrow. *Animal Behaviour*, 60, 297-306.
- Suzuki, T. N. (2011). Parental alarm calls warn nestlings about different predatory threats. *Current Biology*, 21, 15-16.
- Templeton, C. N., Greene, E., Davis, K. (2005). Allometry of alarm calls: black-capped chickadees encode information about predator size. *Science*, 308, 1934-1937.
- Wright, J. (1998). Helpers-at-the-nest have the same provisioning rule as parents: experimental evidence from play-backs of chick begging. *Behavioural Ecology and Sociobiology*, 42, 423-429.
- Wright, J., Leonard, M. L., editors. (2002). *The evolution of begging: competition, cooperation and communication*. Dordrecht (the Netherlands): Kluwer Academic Press.

Statement of originality

We, the Research PhD candidate and the candidate's Principal Supervisor, certify that the work displayed here including the text, figures and diagrams are the candidate's original work.

Name of candidate: Ahmad Barati

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Date



Principal supervisor

11/12/2016

Date

Statement of Authors' contribution

We, the Research Master/PhD candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

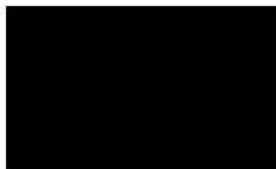
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General Discussion



Overview and main findings

Although noisy miners have been focus of several intensive studies in the last two decades, the majority of these have focused on the negative biodiversity impact of noisy miner aggression on other avian species. By contrast, information on the noisy miner cooperative breeding social system is relatively scarce. Therefore, this study examined some important aspects of cooperative breeding in this system using a combination of detailed observations and experiments manipulating marked, genotyped individuals over a three year period.

Overall, this study examined five distinct aspects. First, I investigated patterns of nest occupancy (Chapter 2). Across the next three chapters I further tested different hypothesis regarding the evolution of cooperative breeding, including the importance of direct and indirect benefits of helping (Chapter 3), and the effects of brood sex ratio (Chapter 4) and extra pair copulation on helper effort (Chapter 5). Finally, Chapter 6 addresses the possibility of a novel form of alloparental care for young that helpers could potentially provide, looking at the mechanism of acoustic communication between nestlings and conspecifics concerning predation risk. Below I summarise and discuss the main findings of the thesis, draw a general conclusion and provide further suggestions to provide a better understanding of the cooperative breeding system in this species.

Nest-site selection may help facilitate cooperative breeding

Selection of a nest site might have important implications for cooperative breeding. In addition to other factors that might influence a breeding female's nest site selection, such as protection from predators and extreme climate (Martin 1995), for cooperatively breeding birds, an ideal nest location is in areas that facilitates efficient

nest accessibility for helpers. In this study, breeding female noisy miners tended to chose the same particular sites for nesting and renesting over multiple breeding seasons. At a colony scale, female noisy miners had a tendency to choose nest areas close to open patches, and also selected eucalypt trees with a lower diameter and canopy height than the available trees in the surrounding area. In addition, noisy miner nests had low levels of concealment (Chapter 2). This selection of a nest site close to open areas and low concealment might maximise the probability that helpers will find and attend the nest area more effectively, as helpers forage in these regions (Dow 1979; Dow & Whitmore 1990). This could be particularly important for female noisy miners as they use a ‘headup’ posture when approaching or leaving the nest area (Dow 1978a), a conspicuous display that may act as a tool to signal the location of the nest site to potential helpers. Thus, choosing to nest near an open area and with a lower tree density may increase the probability of being seen by potential helpers and facilitate the early recruitment of helpers to the nest.

Further, in open areas brooding or incubating females would more easily locate approaching intruders or potential predators, and this early detection may further improve the effectiveness of a group mobbing strategy in this species and thus nest defence (Arnold 2000; Taylor et al. 2008). In addition, noisy miners usually spend substantial time foraging on the ground (Maron 2009), so proximity to these opens areas could improve provisioning efficiency by breeders and helpers and facilitate shorter feeding trips between foraging areas and the brood.

Although nest site secretion can potentially be very important and likely influences the patterns of helping observed in cooperatively breeding species, this area has received little research attention. Nest site location might further be influenced by the spatial distribution of breeders relative to potential helpers throughout the colony. For

example, in group-living species, females might select nest sites that overlap with a larger potential contingent of helpers to facilitate helper recruitment for her brood.

Multiple benefits of helping drive noisy miner cooperative breeding

The evolution of the seemingly altruistic behaviour helpers in cooperatively breeding systems has raised the question as to why and how this costly behaviour could have evolved and be favoured by natural selection. Amongst different potential explanations for helping behaviour (Box 1. Chapter 1), kin selection (Hamilton 1964; Maynard-Smith 1964; Cockburn 1998; Green et al. 2016) appears to be the dominant driver for the evolution of most cooperatively breeding systems. This study was able to demonstrate a clear positive relationship between the provisioning effort of helpers and their genetic relatedness to breeders, (Chapter 3) suggesting that helping behaviour in the noisy miner is primarily kin-based, with helpers accruing the indirect fitness benefits from investment in relatives. Further evidence to support this comes from the fact that although closely related helpers were lower in number than unrelated helpers in this system, related helpers provided more feeds to broods than their unrelated counterparts. This study therefore adds further empirical support to the suggestion that at the broad-scale, kinship controls variation in helper effort in cooperatively breeding birds (Green et al. 2016).

Kin selection has been shown to have a central role in the evolution of the cooperative breeding in birds and explains variation both within (e.g. Nam et al 2010; Wright et al. 2010) and between-species (Cockburn 1998; Green et al. 2016), with most cooperatively breeding groups containing related non-breeding helpers that are typically retained offspring. Despite this, kin selection did not appear to be the only explanation for helping behaviour in this system, as unrelated helpers still provisioned broods, albeit at a lower rate. Helping by unrelated helpers is not a unique

phenomenon to miners, and has been observed in around 44% of cooperative breeding species (Cockburn 1998; Riehl 2013). Further, factors such as promiscuous mating and incorporation of unrelated immigrants may erode genetic relatedness in ‘family’ groups and thus indirect fitness benefits become less important or negligible (Riehl 2013). Overall, these suggest that direct fitness benefit of cooperative breeding (i.e. increased group size), are sufficient to maintain cooperation, even if the helper-breeder relatedness is low (Kingma et al. 2014). Considering the social structure of noisy miners and their group-mobbing behaviour (Arnold 2000), the most plausible direct benefit in this system could be accrued through group augmentation (Kokko et al. 2001; Kingma et al. 2014). Group augmentation in this system could be manifested as actively providing help at the nest or involvement in predator-mobbing behaviour. Under the expectations of active group augmentation, increased group size can benefit both related and unrelated helpers, as larger groups might facilitate more effective mobbing of predators and other intruders into the colony. In addition, passive group augmentation might also be beneficial to helpers, as increased group size can dilute the predation pressure for a given individual (Kingma et al. 2014). It is important to note that other types of benefits of helping behaviour may have remained concealed because of the relatively short period of this study. For example, unrelated helpers might increase the probability of their gaining a future breeding position after some years of helping at the nest of a female if she subsequently re-pairs. Therefore, further additional study might be needed to completely uncover longer-term benefits and costs associated with the helping behaviour of noisy miners.

Helping irrespective of brood sex ratio, despite male offspring likely yielding greater benefits to helpers

Cooperatively breeding species are particularly important for testing investment

according to the sex of offspring, because different fitness payoffs are associated with investing in males versus females in many of these systems (Emlen 1986). Helpers in the noisy miner colonies studied herein were mainly philopatric males, with non-breeding females rarely providing help. Philopatric male offspring are anticipated to not only repay their costs of production to breeders and helpers when they become helpers, but also provide direct benefits in the forms of active (e.g. mobbing predators) or passive (e.g. dilution of predation risk) group augmentation. Given this, for both breeders and helpers male recruits are more beneficial to produce and care for. According to the helper-repayment hypothesis, breeding female noisy miners would be expected to overproduce males as the sex that provides most help to a breeder's subsequent broods (Gowaty & Lennartz 1985). Moreover, helpers were anticipated to preferentially provision male-biased broods regardless of their genetic relatedness to breeders. Yet, no evidence was found to support adjustment of sex ratio by breeding females or preferential provisioning by helpers according to the sex ratio of broods (Chapter 4). A given helper's genetic relatedness to breeders at a focal nest also did not influence their provisioning strategy according to the sex ratio of provisioned broods.

Despite philopatric males being potentially able to repay their rearing cost for breeders and helpers as described above, most studies have not found any effects of brood sex ratio on both parents and helpers provisioning behaviour (e.g. McDonald et al. 2010; Nam et al. 2011), and evidence for such adjustment is rare (e.g. Ridley & Huyvaert 2007). Therefore the results of this study support a general view that sex ratio adjustment and investment are not universally applicable in bird species (Komdeur & Pen 2002, West et al. 2005). These patterns raise the possibility that perhaps long-term differences between sexes are not pronounced enough for sex-

allocation to act at different stages of breeding. For example, although philopatric males are apparently more beneficial to invest in, they may also be costly for both related and unrelated helpers due to increased competition for resources such as food and mating. This highlights the importance of assessing the long-term costs and benefits associated with investing in each sex when examining the effect of broods sex ratio on subsequent investment by both parents and helpers.

No evidence for an adaptive function of extra pair copulation

Results of this study demonstrated that extra pair copulation did occur in the noisy miner breeding system and confirmed results in previous studies (Dow 1978b; Poldmaa et al. 1995). However, I found no evidence to support the suggestion that noisy miners are promiscuous (Dow 1978b), as the rate of extra pair mating (i.e. 14% of offspring) was relatively low and is inconsistent with a promiscuous mating system. Further examination did not provide evidence that extra pair copulations might function as a mechanism to avoid inbreeding or allow breeding females to benefit from the parental care of extra pair males. Instead, inbreeding avoidance in this system appeared to be shaped by other factors, such as female-biased dispersal. Occurrence of extra pair copulation was also expected to influence the parental care provided by the cuckolded breeding male as a result of reduced share in paternity. However, males that obtained extra pair paternity did not provide help at the nest, and cuckolded males did not reduce their parental care.

Although the theory of kin selection (Hamilton 1964; Maynard-Smith 1964), predicts that if a male is cuckolded, he should avoid wasting resources on the offspring of his rivals, despite numerous studies investigating the response of cuckolded males in cooperatively breeding birds (reviewed in Du et al. 2015), their pattern of paternal care does not clearly follow this expectation. The cuckolded breeding male's level of

parental care has largely been independent of female extra pair mating behaviour, with cuckolded males rarely abandoning offspring or reducing care (e.g. Westneat et al. 1995; Peterson et al. 2001; Du et al. 2015). I have therefore highlighted the importance of considering details of mating behaviour of females and males when examining the adaptive benefits of extra pair mating, and also considered a males' ability to assess his paternity of broods. Low certainty of parentage for both cuckolded and extra pair males can potentially influence their decisions on how to respond to extra pair mating, assuming that females control whether or not extra pair copulations occur, which seems likely in most bird species given forced copulation is rare (Maynard-Smith 1977).

Evidence for a new pathway for helpers to provide alloparental care?

Although helping behaviour in cooperatively breeding birds is known to occur across a range of modalities such as antiparasite behaviour (Pacheco et al. 2008), mobbing of potential predators (Griesser & Ekman 2005), incubation (Heinsohn & Cockburn 1994) and sentinel behaviour (Hailman et al. 1994), the main and most dominant form of helping focused on by researchers has been alloparental feeding (Emlen 1986; Cockburn 1998). This extreme bias impairs our ability to fully understand the dynamics of cooperative breeding systems, as it likely neglects many benefits that helpers can provide. Therefore, I dedicated Chapter 6 of this thesis to examining the effectiveness and possibility of a new form of help in which helpers could produce functionally discrete alarm calls and warn nestlings about different types of danger.

I showed that noisy miners could communicate effectively with nestlings concerning imminent danger, with nestlings effectively responding to various types of alarm calls produced by breeders and helpers (Chapter 6). This type of help is relevant

in noisy miners because they effectively use group-mobbing tactics for nest defence (Arnold 2000) and nest defence in this system is an important form of helping behaviour (Arnold et al. 2005). Noisy miners give mobbing calls to terrestrial predators but produce aerial alarm calls in response to danger posed by flying predators (Farrow et al. 2017). Chapter 6 of the thesis suggest possibility and mechanisms of using alarm signals as a form of help provided to breeders. Results show that not only are nestling noisy miners able to show adaptive responses to different miner alarm calls, but also the possibility that helpers in this system provide a new form of helping that has been reported in other cooperatively breeding species to date.

Closing remarks and further directions

In this thesis, I have addressed some of the main questions about the evolution and dynamics of cooperative breeding in a common but relatively rarely studied bird. Briefly, I show that kin selection is the main driver of the evolution of cooperative breeding in noisy miners, and that helpers primarily gain indirect benefits associated with helping their relatives. However, the factors underpinning cooperative breeding in this species are not limited to kin selection alone, with support for other benefits such as active or passive group augmentation being accrued through helping behaviour. Further, no support was found that brood sex ratio influences investment by helpers, as helper investment remained independent of brood sex ratio, regardless of the relatedness of helpers in attendance. I further detected a strong inbreeding avoidance system among breeders, however despite this extra pair fertilisation still occurred in 27% of broods, although I could find no evidence for an adaptive explanation for extra pair copulations. This study also demonstrated the importance of acoustic communication as a tool to improve the fitness and survival of offspring in

complex cooperatively breeding systems.

This study therefore provides insights into factors that underpin the evolution and maintenance of the complex cooperative breeding system seen in noisy miners that has relevance to many other societies. Further research that could build on these results would benefit from further experimental manipulation of the breeding system and perhaps focusing on longer-term information on offspring recruitment into the helper and/or breeding population are needed for improved understanding of this complex system. Manipulations of brood sex ratio through cross-fostering combined with cross-generational analyses of the costs and benefits of raising each sex would also be useful for examining sex-biased investment by breeders and helpers. In addition, the different rate of extra-pair mating detected in this study and in other populations of this species (e.g. Poldmaa et al. 1995), highlights the importance of examining factors that might shape the rate of extra-pair mating between populations of the same species. Experimental reduction in the costs of helping behaviour along with increased broods demands (e.g. intensified begging behaviour) would challenge helpers and could be useful to assess the importance of genetic relatedness on helper effort. Finally, despite group augmentation being thought to provide direct fitness benefits to both related and unrelated helpers in this as well as many other cooperatively breeding systems, these types of benefits have rarely been quantified and need further consideration.

References

- Arnold, K. E. (2000). Group mobbing behaviour and nest defence in a cooperatively breeding Australian bird. *Ethology*, 106, 385-393.
- Arnold, K. E., Owens, I. P. F., Goldizen, A. W. (2005). Division of labour within cooperatively breeding groups. *Behaviour*, 142, 1577-1590.
- Cockburn, A. (1998). Evolution of helping behavior in cooperatively breeding birds.

- Annual Review of Ecology and Systematics*, 29, 141-177.
- Dow, D. (1978a). Reproductive behavior of the noisy miner, a communally breeding honeyeater. *Living Bird*, 16, 163-185.
- Dow, D. D. (1978b). Breeding biology and development of the young of *Manorina melanocephala*, a communally breeding honeyeater. *Emu*, 78, 207-22.
- Dow, D. D. (1979). The influence of nests on the social behaviour of males in *Manorina melanocephala*, a communally breeding honeyeater. *Emu*, 79, 71-83.
- Dow, D. D., Whitmore M. J. (1990) Noisy Miners: variations on the theme of communality. In: Cooperative Breeding in Birds: Long-Term Studies of Ecology and Behavior (ed Stacey & Koenig) pp: 561-592. Cambridge University Press, Cambridge.
- Du, B., Guan, M., Ren, Q., Chen, G. (2015). Cuckolded male ground tits increase parental care for the brood. *Animal Behaviour*, 110, 61-67.
- Emlen, S. (1986). *Cooperative breeding in birds and mammals*. In: Krebs JR, Davies NB (eds) Behavioural ecology, 2nd edn. Blackwell, Oxford, pp: 301-337.
- Farrow, L., Doohan S., McDonald P.G. (2017). Alarm calls are referential and elicit context-specific anti-predator behaviour among receivers in a highly cooperative bird. *Behavioral Ecology*, 28, 724–731.
- Gowaty, P. A., Lennartz, M. R. (1985). Sex ratios of nestling and fledgling red-cockaded woodpeckers (*Picoides borealis*) favor males. *American Naturalist*, 16, 347-353.
- Green, J. P., Freckleton, R.P., Hatchwell B. J. (2016). Variation in helper effort among cooperatively breeding bird species is consistent with Hamilton's Rule. *Nature Communications* (DOI: 10.1038/ncomms12663).
- Griesser, M., Ekman, J. (2005). Nepotistic mobbing behavior in the siberian jay (*Perisoreus infaustus*). *Animal Behaviour*, 69, 345-352.
- Hailman, J. P., McGowan, K. J., Woolfenden, G. E. (1994). Role of helpers in the sentinel behaviour of the florida scrub jay (*Aphelocoma c. coerulescens*). *Ethology*, 97,119-140.
- Hamilton, W. D. (1964). The genetical evolution of social behavior. *Journal of Theoretical Biology*, 7, 1-52.
- Heinsohn, R., Cockburn, A. (1994). Helping is costly to young birds in cooperatively

- breeding White-winged Choughs. *Proceedings of the Royal Society B*, 256, 293-298.
- Kingma, S. A., Santema, P., Taborsky, M., Komdeur, J. (2014). Group augmentation and the evolution of cooperation. *Trends in Ecology and Evolution*, 29, 476-484.
- Kokko, H., Johnstone, R. A., Clutton-Brock, T. H. (2001). The evolution of cooperative breeding through group augmentation. *Proceedings of the Royal Society B*, 268, 187-196.
- Komdeur, J., Pen, I. (2002). Adaptive sex allocation in birds. *Philosophical Transactions of the Royal Society B*, 357, 373-386.
- Maron, M. (2009). Nesting, foraging and aggression of Noisy Miners relative to road edges in an extensive Queensland forest. *Emu*, 109,75-81.
- Martin, T. E. (1995). Avian life history evolution in relation to nest sites, nest predation, and food. *Ecological Monographs*, 65,101-127.
- Maynard-Smith, J. (1964). Group selection and kin selection. *Nature*, 201, 145-147.
- Maynard-Smith, J. (1977). Parental investment: a prospective analysis. *Animal Behaviour*, 25,1-9.
- McDonald, P. G., Ewen, J. G., Wright, J. (2010). Brood sex ratio does not affect helper effort in a cooperative bird, despite extreme sex-biased dispersal. *Animal Behaviour*, 79, 243-250.
- Nam, K-B., Meade, J., Hatchwell, B. J. (2011). Do parents and helpers adjust their provisioning effort in relation to nestling sex in a cooperatively breeding bird? *Animal Behaviour*, 82, 303-309.
- Nam, K. B., Simeoni, M., Sharp, S. P., Hatchwell, B. J. (2010). Kinship affects investment by helpers in a cooperatively breeding bird. *Proceedings of the Royal Society B*, 277, 3299-3306.
- Pacheco, M. L., McDonald, P. G., Wright, J., Kazem, A. J. N., Clarke, M. F. (2008). Helper contributions to antiparasite behavior in the cooperatively breeding bell miner. *Behavioral Ecology*, 19, 558-566.
- Peterson, K. A., Thusius, K. J., Whittingham, L. A., Dunn, P. O. (2001). Allocation of male parental care in relation to paternity within and among broods of the common yellowthroat (*Geothlypis trichas*). *Ethology*, 107, 573-586.
- Poldmaa, T., Montgomerie, R., Boag, P. (1995). Mating system of the cooperatively breeding noisy miner (*Manorina melanocephala*), as revealed by DNA

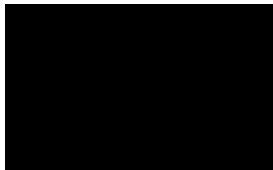
- profiling. *Behavioral Ecology and Sociobiology*, 37, 137-143.
- Ridley, A. R., Huyvaert, K. P. (2007). Sex-biased preferential care in the cooperatively breeding arabian babbler. *Journal of Evolutionary Biology*, 20, 1271-1276.
- Riehl, C. (2013). Evolutionary routes to non-kin cooperative breeding in birds. *Proceedings of the Royal Society B*, 280, 20132245.
- Taylor, R. S., Oldland J. M., Clarke, M. F. (2008). Edge geometry influences patch-level habitat use by an edge specialist in south-eastern Australia. *Landscape Ecology*, 23, 377-89.
- West, S. A., Shuker, D. M., Sheldon, B. C. (2005). Sex-ratio adjustment when relatives interact: a test of constraints on adaptation. *Evolution*, 59, 1211-1228.
- Westneat, D. F. (1990). Genetic parentage in the indigo bunting: a study using DNA fingerprinting. *Behavioral Ecology and Sociobiology*, 27, 67-76.
- Wright, J., McDonald, P. G., te Marvelde, L., Kazem, A. J. N., Bishop, C. (2010) Helping effort increases with relatedness in bell miners, but ‘unrelated’ helpers of both sexes still provide substantial care. *Proceedings of the Royal Society B*, 277, 437-445.

Statement of originality

We, the Research PhD candidate and the candidate's Principal Supervisor, certify that the work displayed here including the text, figures and diagrams are the candidate's original work.

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Date

Statement of Authors' contribution

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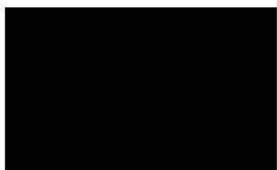
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