The White-Winged Chough

To Adriana Barrueto Etchecoin, and her love of nature

Painting by Daniela Perez M.

Social structure, individual fitness and the effect of climate in an obligate cooperatively breeding bird, the white-winged chough (*Corcorax melanorhamphos*)

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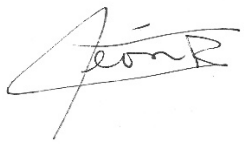
Candidate's Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of the author's knowledge, it contains no material previously published or written by another person, except where due reference is made in the text. All chapters included Rob Heinsohn's input in writing and methodology. Chapter 2 was carried out with Sam Banks' contribution to genetic methods and manuscript input. Chapter 3 had Andrew Cockburn's involvement in parentage analyses methods. Chapter 4 was carried out with Daniela Perez and Iliana Medina's support in data collection and manuscript corrections; while Weliton Menario was involved in personality data analysis. Chapter 5 was made possible by Damien Farines' contributions to social network analyses methods. However, I am the main contributor of each chapter. This research was carried out under approval from the Australian National University Ethics Committee (Protocols A2014/44, A2017/42).



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Abstract

Group-living animals are affected by both the physical and social environment they inhabit, influencing population dynamic processes such as reproductive success, survival and dispersal patterns; all of which ultimately shape the evolution of a species.

During my PhD I examined the social structure of white-winged choughs (*Corcorax melanorhamphos*), a highly social species of Australian bird, which are obligate cooperative breeders, meaning they must breed in groups to successfully produce offspring. Choughs are large birds (350-380g) which reside in Eucalypt woodlands in south-eastern mainland Australia, and can live in groups from three to up to 20 individuals.

Group living in choughs is related to the young individuals' inefficient foraging, with adults providing care to their young for a long time after fledging (Heinsohn, Cockburn et al. 1988, Heinsohn 1991a). Therefore, choughs' reproductive success is highly dependent on food availability for the young, and bigger groups can feed and support more young than smaller groups (Rowley 1978, Heinsohn 1991a). Consequently, choughs' reproductive abilities are highly susceptible to environmental changes that limit food supply. Lack of breeding opportunities is also considered to restrict dispersal in choughs (Beck and Heinsohn 2006). However, when environmental conditions make foraging too hard, mortality increases, rates of dispersal are higher, and birds enter a period of social instability whereby they form more alliances with unrelated individuals and face higher aggression from other choughs (Heinsohn, Dunn et al. 2000).

This thesis is the fifth study of white-winged choughs over a 60 year period but the first that has been conducted with genetic methods after a period of major climatic stability. Two older studies were conducted before molecular methods were available (Rowley 1978, Heinsohn 1992), and two more recent molecular studies were both conducted after periods of extreme drought that had major consequences for population structure and behaviour (Heinsohn, Dunn et al. 2000, Beck and Heinsohn 2006).

After chapter 1, a general introduction, I first explore choughs' social organisation from the population scale, starting with the genetic population structure of choughs and its relation with ecological factors; which is presented in chapters 2 and 3.

More in detail, chapter 2 explores choughs' population structure using population genetics tools. This is the first genetic study on choughs conducted during a period of climatic stability, with no major droughts registered before or during the investigation; and also the first using the new and powerful

genotyping by sequencing method. I calculated within group relatedness, dispersal patterns and genetic differentiation among groups. These results were compared with those obtained from a previous study for a population of choughs that inhabited the same area during a drought period. I also compared genetic patterns of choughs breeding in established suburbs adjacent to reserves versus groups breeding in the woodlands. I found that choughs have female biased dispersal, which contrasts with previous results that showed choughs did not have sex-biased dispersal. I suggest this difference can be due to the different climatic conditions in which studies were conducted. I also found differences in genetic structure between groups breeding in different habitats, with those from the woodlands being more highly related within groups than those from the suburbs. I suggest this is a reflection of higher group instability in urban areas possibly due to higher mortality and dispersal.

The third chapter focuses on the fitness consequences for helpers and breeders under different environmental conditions. I took advantage of demographic data available across five different studies done on these birds under different environmental conditions ranging from 1959 to 2017 to investigate how their populations are affected by different rainfall patterns. Genetic data were available from the most recent three studies, allowing sophisticated comparisons of population genetic structure and inclusive fitness benefits of group members. I found fitness benefits for helpers and breeders differ according to the prevailing climatic conditions and that advantages to breeding in larger groups tend to be minimal under drought conditions. I found that helpers' inclusive fitness augmented with group size, while breeders' did not, and discuss that breeders may obtain other benefits related from bigger groups.

In chapters 4 and 5 I take a closer look at choughs' social dynamics from a behavioural point of view.

On the fourth chapter I focus on white-winged choughs' personality, showing they have consistent individual differences in boldness. I asked if these behaviours differ between and within family groups, and the habitat where they were measured –suburbs versus woodland. I found that flight initiation distance (FID) was an effective method to measure boldness in the wild in this species. I also found that personality is more variable among groups than within groups; and that individuals measured in the suburbs are bolder than those measured in the woodlands. I discuss how group- level similarity in personality may reflect the importance of social learning and cooperation in a social species, and if the nature of these differences could be related to phenotypical plasticity or natural selection. I also did not find that dispersal was affected by FID measurements, and suggest examining other personality traits that have been related to dispersal in other species.

Chapter 5 explores choughs' social structure from a closer perspective, looking at patterns of associations among individuals within the family group during the breeding season, using social network analysis. I also explore if individual cooperative levels –feeding the fledglings and staying close to them- are affected by genetic relatedness -and life history traits (age, sex) - to the group as indication of kin selection. I found that in most groups, unrelated adult choughs of the opposite sex show strong associations, and discuss how this probably reflects the importance of inbreeding avoidance within chough groups. I did not find that relatedness was associated with higher cooperative behaviour within groups, suggesting cooperation would be related to direct benefits, and that indirect fitness benefits are mostly related to high within-group relatedness rather than kin recognition.

The final chapter of the thesis is a synthesis and integration of the main findings of my PhD, including suggestions of future directions based on these results.

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CHAPTER 1

General Introduction

Cooperative breeding is broadly defined as a mating system in which three or more individuals cooperate to raise young (Stacey and Koenig 1990, Cockburn 1998, Boland and Cockburn 2002, Clutton-Brock 2002, Griffin and West 2003, Cockburn 2006, Cockburn 2013, Riehl 2013). It is a very well-studied case of group living in vertebrates, and has been greatly explored in many avian species (Stacey and Koenig 1990, Cockburn 1998, Koenig and Dickinson 2004, Jetz and Rubenstein 2011, Riehl 2013, Koenig and Dickinson 2016). From the total number of bird species in the world, 9% are cooperatively breeding species (Cockburn 2006), and their geographic distribution is highly skewed towards sub-Saharian Africa and Australasia (Jetz and Rubenstein 2011). In Australia 22% of the 258 species of Australian old endemic passerines are cooperative breeders (Cockburn 2006, Heinsohn 2009).

Members of a cooperatively breeding group that take care of offspring that are not their own are known as "helpers" (Brown 1978). Helpers are often grown offspring that have delayed dispersal, but some can be unrelated immigrants (Ekman, Dickinson et al. 2004, Riehl 2010, Riehl 2013). The fitness benefits for helpers of raising the young of others have been largely discussed (Cockburn 1998, Hatchwell and Komdeur 2000), as ever since Darwin formulated his theory of natural selection, (Darwin 1859), cooperative behaviour in animals has been one of the most intriguing subjects in the field of evolutionary biology. Two complementary questions have emerged to understand the evolution of cooperative breeding behaviour. First, why do some birds stay in their parents' territories instead of dispersing and breeding on their own? Second, why do these non-dispersers help to rear the young of others instead of ignoring them or even harming them(Cockburn 1998, Hatchwell and Komdeur 2000)?

Answers to these questions have focused on some of the evolutionary mechanisms outlined in the general study of the occurrence of intra-specific cooperation in populations, such as kin selection, reciprocity, by-product mutualism and group selection (Dugatkin 2002). Other

answers have centred on ecological constraints, life history traits, and the evolutionary history of cooperatively breeding species. Ultimately, all of these factors may have influenced the need for cooperative breeding in different bird species that raise the offspring of others (Koenig and Dickinson 2004).

Mechanisms promoting the evolution of cooperation

To facilitate the understanding of the evolution of cooperation it is essential to identify the costs incurred by the performer of the behaviour, the benefits gained by the receiver, and whether the performer ultimately benefits (Heinsohn and Legge 1999, Croft, Edenbrow et al. 2015). First, for cooperative phenotypes to proliferate, the fitness of those individuals holding the cooperative attribute must be higher than the fitness of the rest of the population. Therefore, if an individual pays a cost to help others, its cooperative trait will only increase in frequency if the benefits gained by helping compensate for the costs paid(Hamilton 1964, Croft, Edenbrow et al. 2015).

One of the evolutionary mechanisms that has traditionally explained the evolution of cooperation in general is kin selection, whereby individuals gain indirect fitness benefits by helping relatives (Hamilton 1964). This evolutionary mechanism is also broadly acknowledged as an important promoter of cooperative breeding (Brown 1987, Dugatkin 1997, Cockburn 1998, Kingma, Hall et al. 2011, Lukas and Clutton-Brock 2012). However, the development of molecular techniques has shown that not all helpers are related to the breeders, and that in some species unrelated members of a group cooperate with the raising tasks as much as related helpers do (Cockburn 1998, Clutton-Brock 2002, Canestrari, Marcos et al. 2005, Riehl 2013). Cooperation among non-relatives has been explained by a range of direct mutual benefits associated with cooperating with others. For instance, group living itself can improve

an individual's direct fitness as larger groups are better able to detect predators and raise their young (Koenig and Dickinson, 2004).

The direct fitness benefits of helping have also received much attention (Clutton-Brock 2002, Kingma, Hall et al. 2011). Reciprocity (Trivers 1971) and by-product mutualism (Clutton-Brock 2009) in particular have been proposed as promoters of intra-specific cooperation. Reciprocity refers to the help that individuals give in exchange for future repayments. For example, Wilkinson (1984) observed that food sharing between vampire bats depended on reciprocity, independent of the relatedness between individuals. Cooperation through by-product mutualism, a term introduced by Brown (1983), occurs when an individual act provides a benefit to others, with by-product benefits being especially important for many cooperatively breeding vertebrate species (Davies, Krebs et al. 2012). This is particularly noticeable in cases where the helper's survival depends on a large group, for example to raise the young, defend resources and keep predators away, and where the costs of helping are not too high (Clutton-Brock, O'Riain et al. 1999).

Group augmentation is another mechanism that would favour the evolution of cooperation. In this case, group augmentation allows the evolution of cooperative breeding if the helpers raise reproductive success, and therefore the benefits of living in bigger groups enhance survival and/or future reproductive success (Kingma, Santema et al. 2014).

All these evolutionary mechanisms are not mutually exclusive, and are likely to interact (West, Griffin et al. 2007), recognising that the forces that promoted the origin of cooperation in an animal society and those that maintain the observed level of cooperation may be different (West, Griffin et al. 2007, Shen, Emlen et al. 2017).

The effect of environmental conditions and life history traits on cooperative breeding

Several different types of environmental conditions have been suggested to favour the evolution of cooperative breeding, though there is little consensus on the matter. In order to understand the role of ecology in cooperatively breeding species, researchers have carried out long-term empirical studies of free-living individual cooperative species (Ligon and Burt 2004, Koenig and Walters 2015). However, these studies have not been as useful as expected to compare or predict the role of environmental conditions in cooperative breeding, though they have been of great value for understanding the ecology of these single species (Cockburn 2013). For example, these studies have identified ecological factors promoting cooperative breeding such as a shortage of suitable breeding territories (Stacey and Ligon 1991), lack of mates (Clarke 1989) and the high costs of leaving a group, such as finding food, and defending and inheriting good quality territories (Ligon and Ligon 1990). Most analyses comparing a wide range of species link the emergence of cooperative breeding to unpredictable and seasonal environments (Du Plessis, Siegfried et al. 1995, Rubenstein and Lovette 2007, Jetz and Rubenstein 2011). However other studies have proposed that non-seasonal and stable environments promote cooperative breeding (Ford, Bell et al. 1988, Gonzalez, Sheldon et al. 2013), arguing that a lack of peak resource periods would not allow birds to obtain the necessary resources to disperse and breed on their own.

A study by Shen, Emlen et al. (2017) proposed that different sources in environmental variation, both spatial and temporal, influence different types of group-living benefits. More specifically, they suggest that spatial variation would lead to delayed dispersal if the natal area offers higher access to food and/or safety, giving cooperative breeders “resource defence benefits”. “Collective action benefits” refer to those obtained when each additional individual increases the group’s efficiency in foraging, feeding and defending the young. These benefits

are expected to be more important in environments that are temporally variable and unpredictable, in which young are more likely to starve; or where challenging conditions are more common, such as when brood parasitism or predation risk are high (Heinsohn 2009, Feeney, Medina et al. 2013, Shen, Emlen et al. 2017). These two sources of environmental variation, spatial and temporal, make it hard to establish a distinct relationship between cooperative breeding and climate, suggesting that shifting the focus to the benefits gained by breeding cooperatively is key to understand the rise of this mating system (Shen, Emlen et al. 2017).

Besides correlations between ecological conditions and cooperatively breeding species, researchers also investigate the association between life-history characteristics and this mating system, such as dispersal and longevity (Arnold and Owens 1998, Hatchwell and Komdeur 2000). For instance, species with slow life histories, where individuals mature late, have high adult survival rates, lower reproductive rates and delayed dispersal, appear to have high rates of cooperative breeding (Fry 1977, Poiani and Jermiin 1994, Arnold and Owens 1998). However, it is difficult to establish if life-history traits are the cause or the effect in the correlation between cooperative breeding and low adult mortality. While some claim that higher survivorship leads to habitat saturation, delayed dispersal and therefore cooperation between individuals (Arnold and Owens 1998), others have suggested the opposite: since living in groups confers predation defence and other advantages, that can be the ultimate cause of decreased mortality (Ligon and Burt 2004).

Cooperative breeding in birds is recognised as an ancestral state in many bird lineages, with several cooperative species belonging to taxonomic groups with other cooperative breeding species (Ligon and Burt 2004). Therefore, some studies have explored the role of life history by analysing the phylogenetic relationships of bird lineages that contain cooperatively breeding

birds (Ligon and Burt 2004, Ekman and Ericson 2006). A study conducted in the Australo-Papuan corvid family found multiple transitions from cooperative to non-cooperative behaviour in that lineage, concluding that different families responded to different selection forces (Ekman and Ericson 2006). It is important to draw attention to the fact that many closely related species inhabit similar environments, which hinders the task of identifying the role of common ancestry from natural selection, and their interaction, in the promotion of cooperative breeding.

Studies linking cooperative breeding to long-lived bird species with slow life histories and small clutch sizes are usually explored within taxonomic families (Cockburn 2003). It has also been noted that there are biases in the selection of the species studied from each family, with most of them living at high latitudes (Cockburn, 2003). Beauchamp (2014) conducted a study in the same geographical region, to control for ecological effects, comparing pairs of phylogenetically related species of cooperative and pair breeders, controlling also for body size. No difference in fecundity between cooperative and pair breeding species was found, however maximum longevity was higher in cooperative breeders. There are still many questions to be answered, such as why helpers' efforts vary within and between species; and which are the specific benefits of helping (Heinsohn, 2004). As proposed by Ligon and Burt (2004), integrating further natural history data into studies of cooperatively breeding species, is an essential step towards the answers of these questions.

The effect of the social environment in cooperatively breeding species

Group living in animals involves the formation of significant and complex relationships with conspecifics. These relationships form an individual's social environment. Alongside the physical environment, the social environment plays a major role in species' evolution, influencing different population dynamic processes such as reproductive success (Schülke,

Bhagavatula et al. 2010), longevity (Williams and Shattuck 2015), dispersal patterns (Wey, Spiegel et al. 2015), gene flow (Wolf and Trillmich 2008), disease transmission (Hamede, Bashford et al. 2009), information flow (Goodale, Beauchamp et al. 2010) and even the maintenance of cooperation (Ohtsuki, Hauert et al. 2006).

More recently, studies of the causes and consequences of sociality have focused on the effects sociality has on individuals, which can interact in many different ways, such as competing, cooperating, mating, grooming, foraging or fighting (Whitehead 2008). These interactions are non-random, and each type can occur between different individuals and between a different number of individuals, and have different fitness consequences for each one involved (Whitehead, Bejder et al. 2005, Sih, Hanser et al. 2009, Hock, Ng et al. 2010).

The synthesis of the patterns of relationships among individuals of the same species that overlap in their ranges can be defined as the social structure of a population (Whitehead 2008). The patterns of interactions among social animals are particularly interesting, since their behaviour is highly dependent on the behaviour of other members of the group (Wey et al., 2008). Consequently, researching the evolution of cooperation in social species is becoming more targeted at investigations of the social structure of animal populations (Madden et al., 2009; Wey et al., 2008).

Traditionally, the methods for determining social complexity among animal behavioural scientists were focused on measures of group size and mating systems. However, these methods do not adequately address relationships between individuals, which are assumed homogeneous (Wey et al., 2008). Social network analysis is a technique used by sociologists since the 1930s to study human relationships, and considers the associations and interactions between individuals using quantitative measures of these relationships. Having quantitative measures permits statistical testing of the relationships among individuals allowing the

detailed description of their social structures (Wey et al., 2008). The construction and analysis of social networks in animal species has advance considerably in the past few years (Hasenjager and Dugatkin 2015, Krause, James et al. 2015, Farine 2017). Nowadays, social network analysis is an important tool among behavioural ecologists used to explore the causes and consequences of complex social and ecological interactions in animal communities (Krause, James et al. 2015).

The intense sociality of cooperatively breeding species makes them ideal models for exploring the influences on social organisation (Griesser, Barnaby et al. 2009, Dey, Reddon et al. 2013). For instance, a study in apostle birds (*Struthidea cinerea*) investigated the social dynamics of these cooperative breeders using social network analysis (Griesser, Barnaby et al. 2009). They focused on the general population structure, exploring the changes in the interactions between social groups during the non-breeding and the breeding season. Interactions between apostle bird groups are more frequent during the non-breeding season, highlighting the overall social importance of winter times for individuals from different social groups to associate with each other. This ultimately facilitates dispersal opportunities and the formation of new groups.

Studying cooperative species using social networks can also help understanding of the evolution of cooperation in social animals (Croft, James et al. 2006, Hill, Walker et al. 2011). Croft, James et al. (2006) studied cooperative interactions of predator inspection in guppies (*Poecilia reticulata*), finding that females preferred performing predator inspections in the lab with other individuals with which they had strong social associations in the wild. Their findings were consistent with theoretical predictions of the evolution of reciprocal cooperation, in which more familiar individuals are more likely to cooperate with each other (Dugatkin 1997).

The role of personality in social species

Animal personalities, also known as temperament, coping styles, and behavioural profiles, refer to the differences in behaviour between individuals that persist through time (Carter, Feeney et al. 2013) and across situations (Réale, Reader et al. 2007). Personality traits are measurable (Groothuis and Carere 2005), and have been studied in many vertebrates and invertebrates (Groothuis and Carere 2005, Bell, Hankison et al. 2009). Réale, Reader et al. (2007) divided measurable behavioural traits that reflect personality into five categories in order to simplify the approach to personality studies: boldness-shyness (an individual's reaction to a risky situation such as fear of predators or humans), exploration-avoidance (an individual´s reaction to a novel situation, such as habitats, objects or food), activity (the level of activity of an individual under non-risky and non-novel environments), aggressiveness (an individual's agonistic reaction towards members of their own species) and sociability (an individual's reaction to the absence or presence of conspecifics, which is not aggression). Traditionally study of phenotypic adaptations to variable environmental conditions has focused on morphological and physiological attributes (Gotthard and Nylin 1995, Schmidt-Nielsen 1997). However, the increasing body of investigation focused on animal personality has increased interest in behaviour as an adaptive trait (Smith and Blumstein 2008, Wolf and Weissing 2012, Carere and Maestripieri 2013).

Personality traits have been shown to influence the social structure of animals by affecting population dynamic processes such as dispersal (Dingemanse, Both et al. 2003, Cote and Clobert 2006, Cote, Clobert et al. 2010, Chapman, Hulthén et al. 2011), habitat use (Wilson and McLaughlin 2007, Boon, Réale et al. 2008) and social foraging (Kurvers, Prins et al. 2009). Further, different personality types vary in frequency and distribution within populations, affecting the interactions between individuals (Pike, Samanta et al. 2008, Aplin, Farine et al.

2013). The study of animal personality in relation to their social organisation, opens new potential opportunities to understand population dynamic processes such as dispersal or competition from a behavioural perspective, delivering a deeper and more complete evolutionary perspective on the social structure of group-living species.

Main objective of thesis

The main aim of this thesis is to explore social structure in a cooperatively breeding bird species, the extremely social white-winged choughs (*Corcorax melanorhamphos,* hereafter often referred to as simply 'choughs'). This is the fifth major study of chough behaviour, with the first study, one of the earliest on any cooperatively breeding bird, carried out by Ian Rowley over 60 years ago (Rowley 1975, Rowley 1978). The second and third studies were conducted by Robert Heinsohn from 1985-1989 and 1993-1997 (Heinsohn 1987, Heinsohn 1988, Heinsohn 1991a, Heinsohn 1991b, Heinsohn 1992, Heinsohn 1995), and the fourth study was conducted by Nadeena Beck from 2003-2006. The studies from the 1990s onwards included molecular methods that were key to understanding the composition of social groups and dispersal patterns. The 1990s and 2000s studies were both conducted during or immediately after major droughts, which had major impacts on the chough population structure and individual behaviour. What we learnt from these studies changed our perspective on what had been considered 'normal' chough behaviour before studying them under drought conditions (Heinsohn, Dunn et al. 2000, Beck and Heinsohn 2006).

The studies presented in this thesis were conducted after a long period of relative climatic stability, with no officially recognised droughts for more than 10 years. In general terms, I explored how choughs' social structure is organised and affected by climatic and

anthropogenic use of the land using genetic, demographic and behavioural data, using comparisons between the different studies to maximum effect.

The study species, white-winged choughs

White-winged choughs, endemic to eastern Australia, are one of few bird species considered to be obligate cooperative breeders, meaning they have to breed in groups to ensure their reproductive success. They live in stable groups that range between three to up to 20 individuals (Rowley 1978, Heinsohn 1992). Choughs in pairs have never been observed to breed successfully, and successful reproduction for trios is also very rare (Heinsohn 1992).

Chough groups consist of a breeding pair and many non-breeding helpers that are usually the offspring from previous years. The breeding season starts in August or September and can continue until March. All group members, both helpers and breeders, contribute to each task related to reproduction, cooperating to build the nest, incubating eggs, and to feeding and defending the young on the nest and after fledgling (Rowley 1978, Boland 1998). Group size in choughs mainly increases via the offspring produced every year (Heinsohn 1992). Kidnapping recently fledged young from neighbouring groups is another, more infrequent, method to enlarge group size in this species (Heinsohn 1991b). White-winged choughs are not strictly territorial, and do not defend foraging or breeding areas. During the breeding season when choughs have nests, they occupy areas of approximately 20 ha, which can be extended to 1000 ha when they are not breeding (Heinsohn 1991a). Conflict and aggression between groups does occur when groups meet by chance (Heinsohn 2009). They do not have a biased sex-ratio like many species of cooperative breeders (Beck, Peakall et al. 2008). The combined evidence suggests that obligate cooperative breeding in choughs is not a consequence of habitat saturation or shortage of mates (Rowley 1978, Heinsohn 1991a).

White-winged choughs can live more than 15 years, taking a long time to reach sexual maturity, and are ready for reproduction when they are four years old (Rowley 1978, Heinsohn, Cockburn et al. 1988). This delayed sexual maturity is likely an adaptation to the length of time required to reach required levels of foraging proficiency (Lack 1954), and it is observed in many bird species that take time to acquire complex foraging abilities, for example predatory birds (Johnston 1982).

Predation of eggs and nestlings, especially by pied currawongs (*Strepera graculina*), is an important cause of nest failure. However, because choughs are large birds, group size does not seem to influence their defence capabilities, with groups of four being able to defend their nests just as effectively as larger groups (Heinsohn 1992). There is evidence of some group members acting as sentinels around the nest and producing alarm calls to alert the rest of the group to the presence of a predator (Boland 1998).

The importance of food resources in chough society is evidenced by reports of larger chough groups harassing the nests of smaller ones, suggesting these attacks are an indirect way of decreasing competition for food resources (Heinsohn 1988). These attacks are even more common during drought periods, when it is harder to dig for arthropods in the hard soil (Heinsohn 2009).

Obligate cooperative breeding in choughs is necessary mostly because of the foraging difficulties faced by young choughs, which need help from the whole group to be fed. Young choughs take 6-10 months to reach foraging independence and are fed by the adults over this period (Heinsohn 1991a). They then continue to improve at foraging slowly throughout their extended period of immaturity (Heinsohn et al., 1988; Heinsohn, 1991a), with the number of group members limiting the groups' reproductive success (Rowley, 1978; Heinsohn, 1991a).

Rowley (1978) found that young choughs from larger groups have higher survival rates than those in small groups, establishing further the multiple benefits of their cooperation. Since then, many studies have explored different aspects regarding the value of group size in chough society. For instance, Heinsohn, Cockburn et al. (1988) observed that individuals from larger groups are more efficient when providing food to nestlings than choughs from smaller groups, carrying larger loads of food to the nestlings, but visiting the nest less often and consuming more food for themselves. The success and stability of chough groups is highly dependent on food availability, which makes them very susceptible to environmental changes that limit food supply, such as droughts. Severe weather disturbances in Australia occur cyclically due to the El Niño Southern Oscillation, generating droughts that have profound consequences for chough society including changes in group composition and in helping behaviour (Heinsohn 2009). In general, when environmental conditions are good for foraging, choughs produce more young, usually do not disperse and have higher levels of within-group relatedness. However, when severe environmental conditions make foraging more difficult, rates of dispersal are higher, and birds form more alliances with unrelated individuals; while more deceptive behaviour is observed when they feed the young (Boland, Heinsohn et al. 1997b). Groups usually fragment when the leading male dies, which also happens more often during droughts, when mortality is higher (Rowley 1978, Heinsohn 1991a, Heinsohn 2009).

Alongside climatic influences, long term research has shown that choughs living in urban environments differ demographically and behaviourally from choughs living in more natural woodland environments (Beck and Heinsohn, 2006). On the one hand, urban areas represent high quality habitats because water and food sources are more stable in comparison to woodlands, especially under drought conditions (Heinsohn, 2009). Conversely, Beck and Heinsohn (2006) also showed that higher fledgling mortality in cities was a result of the

elevated risk of predation. Mortality of older choughs is also likely to be higher in urban areas though evidence for this has been so far lacking.

Thesis outline

Each chapter of the thesis after this introduction, is written as an independent scientific paper in the format intended for publication. Throughout the thesis I use the pronoun 'I' but this will be changed at publication to 'we' to reflect the many collaborations involved in the research.

Chapter 2 focuses on the social structure of the study population from a population genetics perspective. I investigated how climate and urbanisation affect the overall population genetic structure and dispersal patterns in choughs. Comparing dispersal patterns of choughs during my study after a long-term period of rainfall stability, I found female biased dispersal in choughs. This contrasts with the findings from a previous study conducted after a severe drought, which suggested a lack of sex-biased dispersal in this species. I also found that groups of choughs breeding in urban areas were less related than from those breeding in adjacent woodlands. I suggest this may be a reflection of higher mortality and dispersal in groups that are exposed to urban habitats, as observed in Beck and Heinsohn (2006) study of white-winged choughs in urban habitats.

In chapter 3 I investigated the profound effect of environmental conditions on chough population structure and the benefits of cooperative breeding. I compared the inclusive fitness benefits obtained by helpers and breeders across three studies using molecular data conducted under different rainfall patterns. I found inclusive fitness benefits varied between breeders and helpers, and also according to environmental conditions, with helpers and breeders receiving fewer benefits during severe drought conditions. The results are discussed

with respect to both the drivers of obligate cooperative breeding and the likely impact of impending climate change.

Chapter 4 explores the influence of individual personalities in the organisation of chough societies, a novel area of research given that the composition of cooperatively breeding groups have rarely been described with respect to personality. I measured flight initiation distance (FID) as a proxy for boldness in a wild population of choughs. I discovered that choughs within the same social groups tend to have similar personalities, whereas personalities differed significantly between groups. Overall, I found that the social structure of choughs is linked with common levels of boldness within groups, which opens the door for investigating the link of heritability and habituation to this behavioural trait.

Chapter 5 investigates the within-group social structure of white-winged choughs using social network analysis, with the aim of understanding the relationships between individuals during the breeding season. I looked at how the associations between individuals are linked to their cooperative behaviour towards the offspring. I found that individuals with higher connections to the rest of the group were more likely to spend more time closer to the fledglings, a form of cooperation. I also found that cooperative effort is not linked to relatedness, implying there are direct benefits associated to cooperation besides kin selection. I showed that focusing on the relationships among individuals within groups of cooperative breeders leads to helpful insights into the proximate causes of the costs and benefits of living in intensely social cooperatively breeding societies.

Finally, chapter 6 summarises the major findings of the thesis and discusses the most fruitful directions for future research.

References

Aplin, L., et al. (2013). "Individual personalities predict social behaviour in wild networks of great tits (*Parus major*)." Ecology letters **16**(11): 1365-1372.

Arnold, K. E. and I. P. Owens (1998). "Cooperative breeding in birds: a comparative test of the life history hypothesis." Proceedings of the Royal Society of London. Series B: Biological Sciences **265**(1398): 739-745.

Beauchamp, G. (2014). "Do avian cooperative breeders live longer?" Proceedings of the Royal Society of London B: Biological Sciences **281**(1787): 20140844.

Beck, N., et al. (2008). "Social constraint and an absence of sex-biased dispersal drive fine-scale genetic structure in white-winged choughs." Molecular ecology **17**(19): 4346-4358.

Beck, N. R. and R. Heinsohn (2006). "Group composition and reproductive success of cooperatively breeding white-winged choughs (*Corcorax melanorhamphos*) in urban and non-urban habitat." Austral ecology **31**(5): 588-596.

Bell, A. M., et al. (2009). "The repeatability of behaviour: a meta-analysis." Animal Behaviour **77**(4): 771-783.

Boland, C. R. J. (1998). "Helpers improve nest defence in cooperatively breeding white-winged choughs." Emu **98**: 320-324.

Boland, C. R. J. and A. Cockburn (2002). "Short sketches from the long history of cooperative breeding in Australian birds." Emu **102**(1): 9-17.

Boland, C. R. J., et al. (1997b). "Deception by helpers in cooperatively breeding white-winged choughs and its experimental manipulation." Behavioral Ecology and Sociobiology **41**(4): 251-256.

Boon, A. K., et al. (2008). "Personality, habitat use, and their consequences for survival in North American red squirrels *Tamiasciurus hudsonicus*." Oikos **117**(9): 1321-1328.

Brown, J. L. (1978). "Avian communal breeding systems." Annual Review of Ecology and Systematics **9**: 123-155.

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: ecology and evolution. Princeton, Princeton University Press.

Canestrari, D., et al. (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**(5): 422-428.

Carere, C. and D. Maestripieri (2013). Animal personalities: behavior, physiology, and evolution, University of Chicago Press.

Carter, A. J., et al. (2013). "Animal personality: what are behavioural ecologists measuring?" Biological Reviews **88**(2): 465-475.

Chapman, B. B., et al. (2011). "To boldly go: individual differences in boldness influence migratory tendency." Ecology letters **14**(9): 871-876.

Clarke, M. F. (1989). "The pattern of helping in the bell miner (*Manorina melanophrys*)." Ethology **80**(1-4): 292-306.

Clutton-Brock, T. (2002). "Breeding together: kin selection and mutualism in cooperative vertebrates." Science **296**(5565): 69-72.

Clutton-Brock, T. (2009). "Cooperation between non-kin in animal societies." Nature **462**(7269): 51-57.

Clutton-Brock, T. H., et al. (1999). "Selfish sentinels in cooperative mammals." Science **284**(5420): 1640-1644.

Cockburn, A. (1998). "Evolution of helping behavior in cooperatively breeding birds." Annual Review of Ecology and Systematics: 141-177.

Cockburn, A. (2003). "Cooperative breeding in oscine passerines: does sociality inhibit speciation?" Proceedings of the Royal Society of London. Series B: Biological Sciences **270**(1530): 2207-2214.

Cockburn, A. (2006). "Prevalence of different modes of parental care in birds." Proceedings of the Royal Society B: Biological Sciences **273**(1592): 1375-1383.

Cockburn, A. (2013). Cooperative breeding in birds: towards a richer conceptual framework. In 'Cooperation and its Evolution.'(Eds K. Sterelny, R. Joyce, B. Calcott and B. Fraser.) pp. 223–245, MIT Press: Cambridge, MA.

Cote, J. and J. Clobert (2006). "Social personalities influence natal dispersal in a lizard." Proceedings of the Royal Society B: Biological Sciences **274**(1608): 383-390.

Cote, J., et al. (2010). "Personality-dependent dispersal: characterization, ontogeny and consequences for spatially structured populations." Philosophical Transactions of the Royal Society B: Biological Sciences **365**(1560): 4065-4076.

Croft, D., et al. (2006). "Social structure and co-operative interactions in a wild population of guppies (*Poecilia reticulata*)." Behavioral Ecology and Sociobiology **59**(5): 644-650.

Croft, D. P., et al. (2015). Assortment in social networks and the evolution of cooperation. Animal Social networks. J. Krause, R. James, D. W. Franks and D. P. Croft. Oxford, UK, Oxford University Press: 13 - 23.

Darwin, C. (1859). "On the origins of species by means of natural selection." London: Murray: 247.

Davies, N. B., et al. (2012). An introduction to behavioural ecology. West Sussex, United Kingdom, John Wiley & Sons.

Dey, C. J., et al. (2013). "Network structure is related to social conflict in a cooperatively breeding fish." Animal Behaviour **85**(2): 395-402.

Dingemanse, N. J., et al. (2003). "Natal dispersal and personalities in great tits (*Parus major*)." Proceedings of the Royal Society of London. Series B: Biological Sciences **270**(1516): 741-747.

Du Plessis, M. A., et al. (1995). "Ecological and life-history correlates of cooperative breeding in South African birds." Oecologia **102**(2): 180-188.

Dugatkin, L. A. (1997). Cooperation among animals, Oxford University Press.

Dugatkin, L. A. (1997). Cooperation among animals: an evolutionary perspective, Oxford University Press on Demand.

Dugatkin, L. A. (2002). "Cooperation in animals: an evolutionary overview." Biology and Philosophy **17**(4): 459-476.

Ekman, J., et al. (2004). Delayed dispersal. Ecology and evolution of cooperative breeding in birds. Cambridge: 35.

Ekman, J. and P. G. Ericson (2006). "Out of Gondwanaland; the evolutionary history of cooperative breeding and social behaviour among crows, magpies, jays and allies." Proceedings of the Royal Society B: Biological Sciences **273**(1590): 1117-1125.

Farine, D. R. (2017). "A guide to null models for animal social network analysis." Methods in ecology and evolution **8**(10): 1309-1320.

Feeney, W. E., et al. (2013). "Brood Parasitism and the Evolution of Cooperative Breeding in Birds." Science **342**(6165): 1506-1508.

Ford, H., et al. (1988). "The relationship between ecology and the incidence of cooperative breeding in Australian birds." Behavioral Ecology and Sociobiology **22**(4): 239-249.

Fry, C. (1977). The evolutionary significance of co-operative breeding in birds. Evolutionary ecology, Springer**:** 127-135.

Gonzalez, J.-C. T., et al. (2013). "Environmental stability and the evolution of cooperative breeding in hornbills." Proceedings of the Royal Society B: Biological Sciences **280**(1768).

Goodale, E., et al. (2010). "Interspecific information transfer influences animal community structure." Trends in ecology & evolution **25**(6): 354-361.

Gotthard, K. and S. Nylin (1995). "Adaptive plasticity and plasticity as an adaptation: a selective review of plasticity in animal morphology and life history." Oikos: 3-17.

Griesser, M., et al. (2009). "Influence of winter ranging behaviour on the social organization of a cooperatively breeding bird species, the apostlebird." Ethology **115**(9): 888-896.

Griffin, A. S. and S. A. West (2003). "Kin discrimination and the benefit of helping in cooperatively breeding vertebrates." Science **302**(5645): 634-636.

Groothuis, T. G. and C. Carere (2005). "Avian personalities: characterization and epigenesis." Neuroscience & Biobehavioral Reviews **29**(1): 137-150.

Hamede, R. K., et al. (2009). "Contact networks in a wild Tasmanian devil (*Sarcophilus harrisii*) population: using social network analysis to reveal seasonal variability in social behaviour and its implications for transmission of devil facial tumour disease." Ecology letters **12**(11): 1147-1157.

Hamilton, W. D. (1964). "The genetical evolution of social behaviour. II." Journal of Theoretical Biology **7**(1): 17-52.

Hasenjager, M. J. and L. A. Dugatkin (2015). "Chapter Three-Social Network Analysis in Behavioral Ecology." Advances in the Study of Behavior **47**: 39-114.

Hatchwell and Komdeur (2000). "Ecological constraints, life history traits and the evolution of cooperative breeding." Animal Behaviour **59**(6): 1079-1086.

Hatchwell, B. and J. Komdeur (2000). "Ecological constraints, life history traits and the evolution of cooperative breeding." Animal Behaviour **59**(6): 1079-1086.

Heinsohn, R., et al. (2000). "Coalitions of relatives and reproductive skew in cooperatively breeding white-winged choughs." Proceedings of the Royal Society of London. Series B: Biological Sciences **267**(1440): 243-249.

Heinsohn, R. and S. Legge (1999). "The cost of helping." Trends in ecology & evolution **14**(2): 53-57.

Heinsohn, R. G. (1987). "Age-dependent vigilance in winter aggregations of cooperatively breeding white-winged choughs (*Corcorax melanorhamphos*)." Behavioral Ecology and Sociobiology **20**(4): 303-306.

Heinsohn, R. G. (1988). "Inter-group ovicide and nest destruction in cooperatively breeding white-winged choughs." Animal Behaviour **36**(6): 1856-1858.

Heinsohn, R. G. (1991a). "Slow learning of foraging skills and extended parental care in cooperatively breeding white-winged choughs." American Naturalist: 864-881.

Heinsohn, R. G. (1991b). "Kidnapping and reciprocity in cooperatively breeding white-winged choughs." Animal Behaviour **41**(6): 1097-1100.

Heinsohn, R. G. (1992). "Cooperative enhancement of reproductive success in white-winged choughs." Evolutionary Ecology **6**(2): 97-114.

Heinsohn, R. G. (1995). "Hatching asynchrony and brood reduction in cooperatively breeding white-winged choughs (*Corcorax melanorhamphos)*." Emu **95**(4): 252-258.

Heinsohn, R. G. (2009). White-winged Coughs: The social consequences of Boom and Boost Boom and Bust: Bird Stories for a Dry Country. Collingwood, CSIRO Publishing**:** 223-239.

Heinsohn, R. G., et al. (1988). "Foraging, Delayed Maturation, and Advantages of Cooperative Breeding in White-winged Choughs, *Corcorax melanorhamphos*." Ethology **77**(3): 177-186.

Hill, K. R., et al. (2011). "Co-residence patterns in hunter-gatherer societies show unique human social structure." Science **331**(6022): 1286-1289.

Hock, K., et al. (2010). "Systems Approach to Studying Animal Sociality: Individual Position versus Group Organization in Dynamic Social Network Models." PLoS ONE **5**(12): e15789.

Jetz, W. and D. R. Rubenstein (2011). "Environmental Uncertainty and the Global Biogeography of Cooperative Breeding in Birds." Current Biology **21**(1): 72-78.

Johnston, T. D. (1982). "Selective costs and benefits in the evolution of learning." Advances in the Study of Behavior **12**: 65.

Kingma, S. A., et al. (2011). "Multiple benefits drive helping behavior in a cooperatively breeding bird: an integrated analysis." The American Naturalist **177**(4): 486-495.

Kingma, S. A., et al. (2014). "Group augmentation and the evolution of cooperation." Trends in ecology & evolution **29**(8): 476-484.

Koenig, W. D. and J. L. Dickinson (2004). Ecology and evolution of cooperative breeding in birds. United Kingdom, Cambridge University Press.

Koenig, W. D. and J. L. Dickinson (2016). Cooperative breeding in vertebrates: studies of ecology, evolution, and behavior, Cambridge University Press.

Koenig, W. D. and E. L. Walters (2015). Temporal variability and cooperative breeding: testing the bet-hedging hypothesis in the acorn woodpecker. Proc. R. Soc. B, The Royal Society.

Krause, J., et al. (2015). Animal Social Networks. New York, USA, Oxford University Press.

Kurvers, R. H., et al. (2009). "The effect of personality on social foraging: shy barnacle geese scrounge more." Proceedings of the Royal Society B: Biological Sciences **277**(1681): 601-608.

Lack, D. (1954). "The natural regulation of animal numbers." The Natural Regulation of Animal Numbers.

Ligon, J. and S. Ligon (1990). "Green woodhoopoes: life history traits and sociality." Cooperative breeding in birds: 33-65.

Ligon, J. D. and D. B. Burt (2004). Evolutionary origins. Ecology and evolution of cooperative breeding in birds. Great Britain, Cambridge University Press.

Lukas, D. and T. Clutton-Brock (2012). "Cooperative breeding and monogamy in mammalian societies." Proceedings of the Royal Society B: Biological Sciences **279**(1736): 2151-2156.

Ohtsuki, H., et al. (2006). "A simple rule for the evolution of cooperation on graphs and social networks." Nature **441**(7092): 502-505.

Pike, T. W., et al. (2008). "Behavioural phenotype affects social interactions in an animal network." Proceedings of the Royal Society B: Biological Sciences **275**(1650): 2515-2520.

Poiani, A. and L. S. Jermiin (1994). "A comparative analysis of some life-history traits between cooperatively and non-cooperatively breeding Australian passerines." Evolutionary Ecology **8**(5): 471-488.

Réale, D., et al. (2007). "Integrating animal temperament within ecology and evolution." Biological Reviews **82**(2): 291-318.

Riehl, C. (2010). "Living with strangers: direct benefits favour non-kin cooperation in a communally nesting bird." Proceedings of the Royal Society B: Biological Sciences **278**(1712): 1728-1735.

Riehl, C. (2013). "Evolutionary routes to non-kin cooperative breeding in birds." Proceedings of the Royal Society of London B: Biological Sciences **280**(1772).

Rowley, I. (1975). Bird life. Sydney, Collins.

Rowley, I. (1978). "Communal activities among White-winged Choughs *Corcorax melanorhamphos*." Ibis **120**: 178-197.

Rubenstein, D. R. and I. J. Lovette (2007). "Temporal environmental variability drives the evolution of cooperative breeding in birds." Current Biology **17**(16): 1414-1419.

Schmidt-Nielsen, K. (1997). Animal physiology: adaptation and environment, Cambridge University Press.

Schülke, O., et al. (2010). "Social bonds enhance reproductive success in male macaques." Current Biology **20**(24): 2207-2210.

Shen, S. F., et al. (2017). "The ecology of cooperative breeding behaviour." Ecology letters **20**(6): 708-720.

Sih, A., et al. (2009). "Social network theory: new insights and issues for behavioral ecologists." Behavioral Ecology and Sociobiology **63**(7): 975-988.

Smith, B. R. and D. T. Blumstein (2008). "Fitness consequences of personality: a meta-analysis." Behavioral Ecology **19**(2): 448-455.

Stacey, P. B. and W. D. Koenig (1990). Cooperative breeding in birds: long term studies of ecology and behaviour. New York, Cambridge University Press.

Stacey, P. B. and J. D. Ligon (1991). "The benefits-of-philopatry hypothesis for the evolution of cooperative breeding: variation in territory quality and group size effects." American Naturalist: 831-846.

Trivers, R. L. (1971). "The evolution of reciprocal altruism." Quarterly review of biology: 35-57.

West, S. A., et al. (2007). "Evolutionary explanations for cooperation." Current Biology **17**(16): R661-R672.

Wey, T. W., et al. (2015). "Natal dispersal in a social landscape: considering individual behavioral phenotypes and social environment in dispersal ecology." Current Zoology **61**(3): 543-556.

Whitehead, H. (2008). Analyzing animal societies: quantitative methods for vertebrate social analysis, University of Chicago Press.

Whitehead, H., et al. (2005). "Testing association patterns: issues arising and extensions." Animal Behaviour **69**(5): e1.

Wilkinson, G. S. (1984). "Reciprocal food sharing in the vampire bat." Nature **308**(5955): 181-184.

Williams, S. A. and M. R. Shattuck (2015). "Ecology, longevity and naked mole-rats: confounding effects of sociality?" Proceedings of the Royal Society B: Biological Sciences **282**(1802).

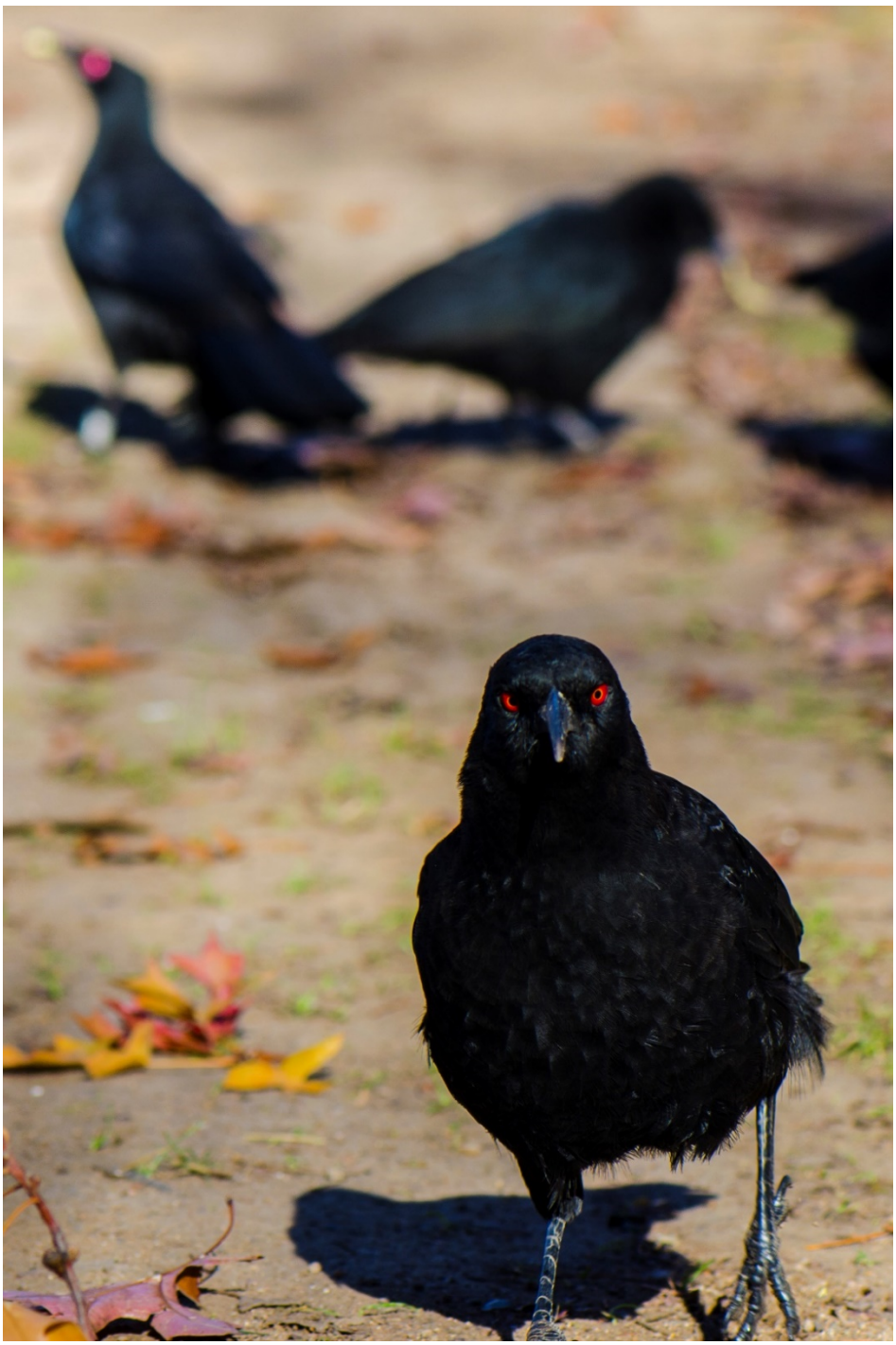
Wilson, A. D. and R. L. McLaughlin (2007). "Behavioural syndromes in brook charr, *Salvelinus fontinalis*: prey-search in the field corresponds with space use in novel laboratory situations." Animal Behaviour **74**(4): 689-698.

Wolf, J. B. and F. Trillmich (2008). "Kin in space: social viscosity in a spatially and genetically substructured network." Proceedings of the Royal Society of London B: Biological Sciences **275**(1647): 2063-2069.

Wolf, M. and F. J. Weissing (2012). "Animal personalities: consequences for ecology and evolution." Trends in ecology & evolution **27**(8): 452-461.

CHAPTER 2

Population genetic structure and sex-biased dispersal reflect costs and benefits of cooperative breeding for a bird living under variable environmental conditions



Abstract

Understanding the effects of environmental conditions in cooperatively breeding species helps reveal the nature of evolutionary forces leading to this type of social system. In particular environmental variability may affect demographic processes such as mortality and dispersal which can ultimately define the fine-scale genetic structure of cooperatively breeding populations, and the direct and indirect fitness opportunities for individuals. We investigated the impact of two types of environmental variability, climate and habitat alteration, on the population genetic structure and dispersal patterns of an obligate cooperatively breeding bird species. We compared within-group relatedness and genetic differentiation among groups of white-winged choughs (*Corcorax melanhoramphos*) sampled over several years of average rainfall, with data from a previous study in the same area conducted during a period of severe drought. Chough groups showed similar significant genetic differentiation among groups during both drought and non-drought periods. However, when examining differences in genetic structure in the present study between groups breeding in highly altered habitat (suburban) areas versus native woodlands, the woodland birds were more highly related within groups. We hypothesise that these differences reflected lower stability of groups in urban areas be due to higher rates of mortality and dispersal. We found that dispersal was female biased which contrasts with previous studies conducted during a drought. Dispersal was female biased in both suburban and woodland habitat but subadult females in suburban habitat were more likely to disperse than adult females. We suggest that higher mortality in chough populations (both during the drought of the previous study, and in suburban habitat in this study) led to more breeding opportunities, resulting in higher dispersal. Our study has important implications for how the social structure of cooperatively breeding species is being impacted under major environmental change including climate and urbanisation.

Introduction

Cooperative breeding is broadly defined as a social and mating system in which more than two individuals work together to rear offspring (Fry 1977, Brown 1978, Cockburn 1998, Hatchwell and Komdeur 2000, Koenig and Dickinson 2016). The motivation of non-reproductive helpers to delay their own reproduction to instead care for young that are not their own makes this social system particularly interesting from an evolutionary perspective. (Cockburn 2006, Hatchwell 2009). If a helper pays a cost to assist others, its cooperative trait will only increase in frequency in the population if the benefits gained by helping compensate for the costs payed (Hamilton 1964, Heinsohn and Legge 1999, Croft, Edenbrow et al. 2015). One important motive for helping is the indirect benefit of kin selection (Hamilton 1964), which is the gain of inclusive fitness obtained by assisting close relatives (Hatchwell and Komdeur 2000, Hatchwell 2009). Many species of cooperative breeders show fine-scale genetic structure, with higher relatedness within their social groups than among groups (Painter, Crozier et al. 2000, Temple, Hoffman et al. 2006, Woxvold, Adcock et al. 2006, Leedale, Sharp et al. 2018), meaning that helpers are indeed aiding the production of close relatives. Helpers may also benefit directly from cooperating, for example, they can increase their own present and future mating opportunities, enhance their probabilities of territory inheritance and learn skills that improve their survival (Cockburn 1998, Riehl 2013).

Understanding the evolution of helping requires identification of the environmental factors that encourage individuals to cooperate rather than disperse and breed as pairs (Arnold and Owens 1998, Hatchwell and Komdeur 2000). These factors have been viewed as ecological constraints that alter food and territory availability, predation rates and other elements, and thereby limit dispersal and independent breeding (Emlen 1982). One manifestation of

ecological constraints is that they may be associated with climatic variability and unpredictability, which can affect the availability of food and suitable territories (Rubenstein and Lovette 2007, Jetz and Rubenstein 2011). Breeding cooperatively in such environments gives individuals the opportunity to maximise fitness by cooperating under challenging environmental conditions, either by helping to raise close relatives or by obtaining direct benefits of group living (Clutton-Brock 2002, Griffin and West 2003, Clutton-Brock 2009, Hatchwell 2009, Riehl 2013).

We studied the impacts of environmental variability, specifically climate (different rainfall patterns) and habitat alteration (urbanisation) on the genetic structure of an obligately cooperatively breeding bird, the white-winged chough (*Corcorax melanorhamphos*). This species must breed in groups to produce offspring, and their reproductive success is highly dependent on food availability for the young, with bigger groups supporting and feeding more young than smaller groups (Rowley 1978, Heinsohn 1991a). Parents and helpers also have to provide food to the young for many months after fledging (Heinsohn 1991a). Long-term studies have shown that the social stability of white-winged choughs is highly susceptible to environmental changes that limit food supply, such as the severe *El Niño* driven droughts that occur in eastern Australia (Chiew, Piechota et al. 1998, Heinsohn 2009), or impose differing constraints such as when the birds live in highly altered urban habitats (Beck and Heinsohn 2006). Droughts have been associated with a decline in the number of helpers, reduction in reproductive output, higher mortality and group disintegration in choughs and other species (Heinsohn, Dunn et al. 2000, van de Pol, Osmond et al. 2012), whereas chough groups in urban environments experience higher fledgling mortality than those breeding in non-urban areas (Beck and Heinsohn 2006). Urbanisation has been a major cause of habitat alteration in recent decades and entails both habitat fragmentation and transformation to landscapes with entirely

different ecological properties. Urbanised areas may be advantageous for bird species by providing stable sources of food and water, or especially challenging due to increased hazards from vehicle traffic, noise and predation (Marzluff 2001, Chiari, Dinetti et al. 2010, Halfwerk, Holleman et al. 2011, Møller, Erritzøe et al. 2011). Studies on the effects of urbanisation on cooperatively breeding birds are scarce but are potentially important in terms of understanding the impact of increased urbanisation on their behaviour and social systems, and for yielding important insights into the costs and benefits of sociality under variable ecological conditions.

This study examines the fine-scale genetic structure of white-winged chough populations using genotyping by sequencing methods (Jaccoud, Peng et al. 2001, Kilian, Wenzl et al. 2012). The aim is to show how opportunities for kin selection and other drivers of cooperative behaviour might change under variable environmental conditions, focusing on climate and habitat alteration. We calculate within group relatedness, dispersal patterns and genetic differentiation among groups, and compare values within the same population between periods of severe drought and near average rainfall (Beck, Peakall et al. 2008). The effects of habitat alteration were explored in the present study by comparing the same parameters mentioned above, between choughs living in natural woodland habitat with those in highly altered suburban habitat. We predicted that within group relatedness should be higher during non-drought periods than during droughts as groups grow from within-group reproduction in more stable conditions (Rowley 1978, Heinsohn, Dunn et al. 2000). We predicted that within group relatedness should be higher in woodland habitat than urban habitat based on higher mortality rates and faster turn-over of group members in the latter.

Methods

Study species

White-winged choughs are large passerine birds (350-380g) endemic to south-eastern Australia. Choughs are one of the few species of obligate cooperatively breeding birds, which means they must breed in groups to produce any young (Rowley 1978). Choughs consume invertebrates found on the ground or by digging up to 10cm beneath the surface. They live in stable groups of 3-20 individuals throughout the year, which typically consist of one breeding pair and many non-breeding helpers who are usually offspring from previous years. Every group member is involved at each reproductive stage by helping with nest building, incubating, feeding nestlings and fledglings and predator defence (Rowley 1978, Boland 1998). Group living in choughs is associated with the long time it requires for juveniles to learn how to forage without help. This means that the parents and helpers have to provide food to the young for many months after fledging, and that, even after independence, juveniles take until they are four years old to reach foraging proficiency and sexual maturity (Rowley 1978, Heinsohn, Cockburn et al. 1988). A difficult foraging niche also means groups have to cooperate to raise nestlings (Heinsohn, Cockburn et al. 1988, Heinsohn 1991a), and that choughs' reproductive success is highly dependent on food availability for the young; with bigger groups being able to support and feed more young than smaller groups (Rowley 1978, Heinsohn 1991a). Pairs and trios of choughs have never been observed to reproduce successfully, and group size is positively correlated to the number of young fledged and their later survival (Heinsohn 1992).

Study population background, field methods and sample collection

We captured and banded 209 choughs from suburbs and woodland areas in northern Canberra, Australian Capital Territory, Australia, between August 2015 and March 2018. (Figure 1). We caught choughs using baited walk-in cage traps. Each captured bird was marked with a standard metal band with number and a large white plastic band with a unique number that was easily visible through binoculars from up to 50 meters away. We used eye colour to determine each individual's age up to four years following Rowley (1975). Birds were classified as "1" (first year, with a completely brown iris); "2" (second year, with a brown iris surrounded by an orange ring); "3" (third year, with an orange iris and a brown inner ring), "4" (fourth year, with an orange iris and yellow inner ring spotted in brown) and "5" (fifth year or older; with a red iris and orange inner ring). Birds were considered adults from age four onwards, when they have reached sexual maturity (Rowley 1978). For genetic analyses, we took a small blood sample (~50µl) from the brachial vein from each banded bird. The sample was stored in 70% ethanol. The Australian National University Animal Ethics Committee approved all field methods (Animal Ethics protocol number: A2014/44, A2017/42).

We genotyped 172 individuals from 27 groups studied in both 2016-2017 and 2017-2018 breeding seasons, 95 females and 77 males. The sex ratio (Male / (Male + Female)) of all genotyped choughs was 0.447, with no significant difference from parity (χ^2 = 1.884, p = 0.1699). The individuals came from 27 group-years with group sizes ranging from four to 16 excluding fledglings (mean = 7.77), and from five to 23 (mean = 9.7) including fledglings (mean number of fledglings per group = 1.48; range = 0 to 6; N = 40).

We banded and took DNA samples from 67-100% of the members of each group. Alongside new additions to the group through reproduction, seven out of eight groups varied in membership of older birds between years.

A total of thirteen choughs disappeared from the study. Choughs have short dispersal distances so (Usually <2km) so these birds were assumed to have died (Beck et al. 2008). In other cases, individuals dispersed to join different groups. We analysed groups separately each year since the number of coalitions and/or identity of the breeding pair, varied between breeding seasons. Group membership of each individual was recorded during the breeding season, between August and January each year, as well as the geographic coordinates of 25 nests. Two groups were found with young fledglings, but we did not find the nest, so they were excluded from the spatial autocorrelation analysis.

We compared results from the present study with a previous study conducted on the same population, during a severe drought, from March 2003 to March 2005 (Beck et al., 2008). In order to compare the genetic structure of white-winged chough population among different climatic conditions, we used genetic data collected during that period of drought, to conduct analyses on chough groups living in the same and surrounding areas of our present study (Figure 1). We examined 83 individuals from 16 groups from the previous study. Sex ratio was 0.51, with 42 males and 41 females, and not significantly different from parity ($\chi^2 = 0.012$, p = 0.913).

Both studies used different molecular techniques. We used SNPs to estimate population genetic parameters, while the previous study conducted during a drought in the mid 2000s used microsatellite markers. Several studies comparing the performance of both types of molecular markers have found that estimates of genetic variation are highly correlated (Coates, Sumerford et al. 2009, Elbers, Clostio et al. 2017), and therefore comparable. Further, the methods we used to compare patterns of spatial genetic structure (F'_{ST} and multilocus spatial autocorrelation) are standardised and therefore are, in principle, comparable among

marker types. Since microsatellites have high diversity, the same populations usually have higher F_{ST} for microsatellites than SNPs (Hedrick 2005, Meirmans and Hedrick 2011).

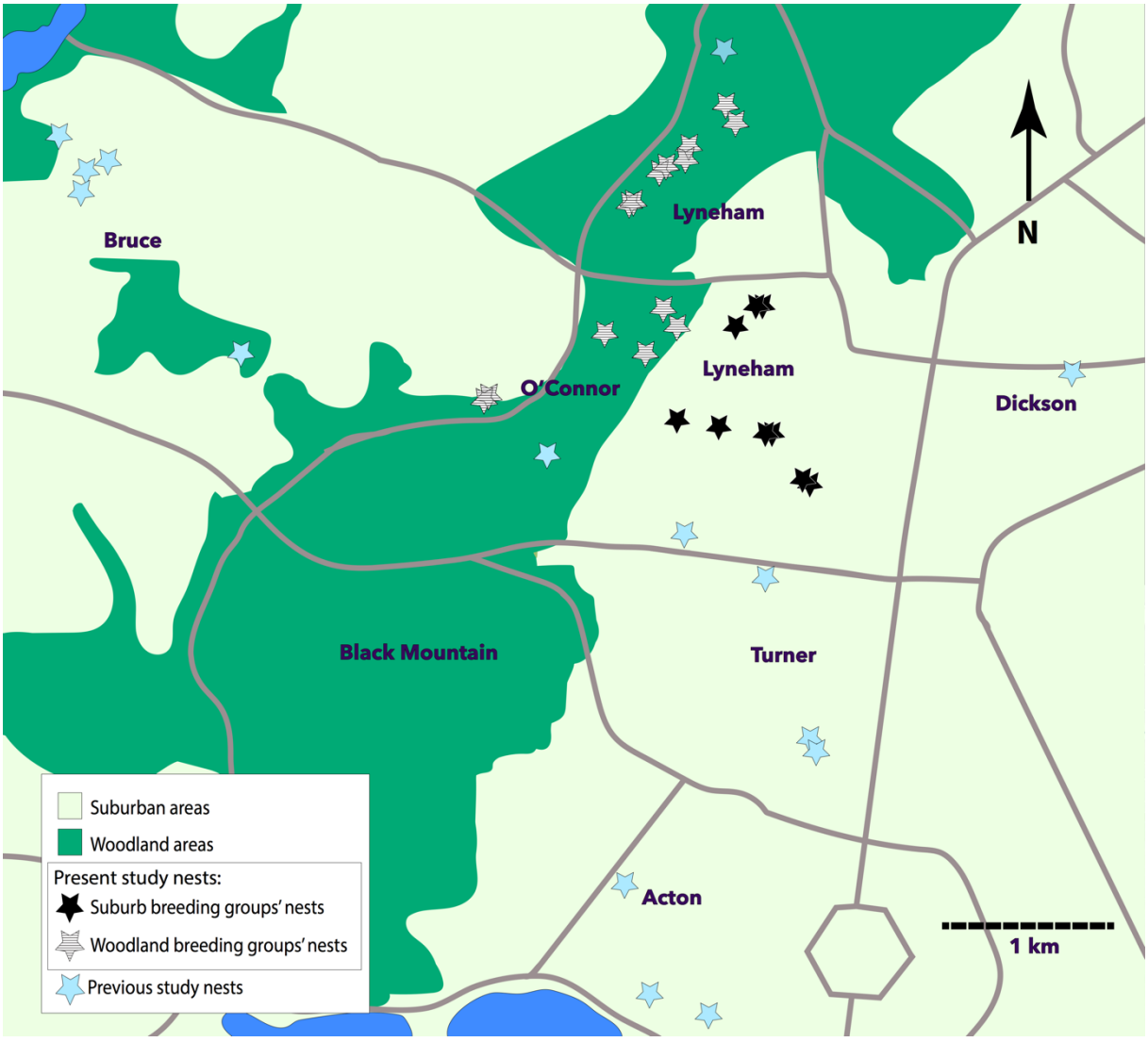


Figure 1. Map of Canberra inner north showing the present and previous study areas with nests of white-winged choughs in suburban (light yellow) and woodland habitat (green).

Genetic methods

DartSeq Genotyping

Codominant SNP genotypes were obtained using a genotyping by sequencing method via the DArTSeq protocol from Diversity Arrays Technology (DArT) Pty Ltd., in Canberra, Australia (Jaccoud, Peng et al. 2001, Kilian, Wenzl et al. 2012). In total, 188 blood samples of ~2µl volume stored in in 70% ethanol, including 172 different birds and 14 individuals genotyped twice as technical replicates, were genotyped. Diversity Arrays P/L conducted the DNA extraction, sequencing and genotyping. The sequencing method is optimised for each organism, selecting the most appropriate complexity reduction method using specific restriction enzymes. Quality control steps of the samples were conducted by Diversity Arrays P/L as described in Melville, Haines et al. (2017). After initial SNP calling by Diversity Arrays P/L, we obtained a genotype matrix with 17,961 genotypes for 172 individuals with an average 10.3% missing data per SNP and average sequencing depth per SNP of 16.77. We then filtered the dataset further on criteria specific to our analyses.

Based on Shaw (2018), we followed filtering steps that optimised fine-scale population genetic analyses. We removed SNP loci that occurred in less than 95% of individuals, with a reproducibility (based on technical replicates of 30% of samples) below 95%. We retained only a single SNP from each clustered sequence. We discarded SNPs with a minor allele frequency (MAF) lower than 0.05, an average read depth of less 10 across both alleles, and an average of the polymorphic information content (PIC) lower than 0.01. We do not expect chough genotypes to adhere to Hardy-Weinberg expectations due to the kinship-based social structure of their populations, therefore we did not filter SNPs following this criterion since it might remove biological signal from the data.

S*ex determination*

Choughs are not sexually dimorphic (Rowley 1978) so we initially determined the sex of a sample of forty individuals using PCR. To do this, we carried out DNA extraction from blood using Qiagen DNeasy Blood and Tissue Kit (QIAGEN, California). We used the method of avian molecular sexing based on the CHD gene (Fridolfsson and Ellegren 1999), using primers P2/P8 to amplify sex chromosome-linked DNA fragments (Griffiths, Double et al. 1998). Knowing the sex of these 40 choughs allowed us to determine the sex of the remaining 132 birds by identifying 63 sex linked SNPs (38 Z-linked and 25 W-linked). Using the SNP report from DArTseq ™, first, we identified W linked SNPs looking for those that are always present in females and not in males. To find W-linked SNPs in the non-identified males, we considered that W-linked SNPs were null in more than 0.8 (allowing for errors) of the already identified males; while they were present in all the identified females. We then looked for Z-linked SNPs which can show heterozygosity in males but should be non-existent in females, with less than 0.05 of identified female birds being heterozygotes for Z-linked SNPs, allowing for errors; while males' heterozygosity for Z-linked SNPs ranged between 0.5 and 0.7.

Genetic diversity

Using GenAlex 6.5 for the overall population we calculated mean observed heterozygosity (H_O), mean expected heterozygosity (H_S), total expected heterozygosity (H_T), inbreeding coefficient within individuals relative to groups (F_{IS}), the group-level fixation index relative to total (F_{ST}) and individual inbreeding coefficient relative to the rest of the population (F_{IT}).

We conducted an analysis of molecular variance (AMOVA) using GenAlex 6.5, calculating group F_{ST} per year, for males and females, for adults (4 years or older) and sub-adults (3years or

younger), and both sexes and life stages combined. For each analysis, we only included individuals from groups that contained two or more members of the same sex or age group. We also conducted this analysis for chough groups from the previous study living in the same and surrounding areas of our study population.

To visualize genetic differences between individuals from different groups, we used the R package DartR to perform principal coordinates analyses for each breeding season (2016-2017 and 2017-2018).

Fine-scale population structure

Using GenAlex 6.5 (Peakall and Smouse 2012) we calculated genetic distances among individuals using Smouse and Peakall (1999) genetic distance metric. We used the pairwise individual genetic distance matrix in a set of multilocus spatial autocorrelation analyses to investigate associations between genetic structure and spatial or social relationships among individuals. The genetic correlation coefficient r takes values between -1 and 1, where zero indicates no genetic correlation. Random permutation (999 times) of the data set generated a null hypothesis of zero genetic autocorrelation among individuals that allowed testing for significance. Within-group relatedness was estimated for all members in all groups in our sample (n=27 groups) with a 95% confidence interval. We compared within-group relatedness for groups with more than one member of each sex (females = 27 groups, males = 24 groups), groups with more than one adult member of the same sex (female adults = 18, male adults = 17 groups), and groups with more than one sub-adult member of the same sex (female sub-adults = 15 groups, male sub-adults = 15 groups). We performed a two tailed t-test, after

testing for equal variances with an F-test (all variances were not significantly different), using R studio version 1.1.453.

We generated geographic and genetic distance matrices separately for each breeding season, for males and females, for adults (four years old or older) and sub-adults (three years old or younger), and for male and female adults and sub-adults. We used the option "Multiple Pops" in GenAlex 6.5 to allow the permutation within all these different categories in order to compare the results between them. The distance classes used for this analysis followed Beck, Peakall et al. (2008) to allow a comparison of the studies. We also analysed data from the previous study including only groups that lived in the surrounding areas of our study (Figure 1). We also took into account the smaller geographic range of the present study population, using only five distance classes both for this study and for Beck, Peakall et al. (2008) (500 m, 1000 m, 2500 m and 3500 m), since after 3500 m genetic correlation between groups is rarely positive (Beck, Peakall et al. 2008). The differences in the geographic scale of both studies might affect the absolute r values, which would differ only due to sampling differences. These might also alter patterns of spatial autocorrelation in the data, but differences among sexes within each study should not be affected.

Finally, we estimated the correlation between group size and multilocus genetic correlation among individuals within each group. For this analysis, we divided chough groups breeding in the suburbs (nests less than 50 m away from a residence), from groups breeding in areas of native eucalypt woodland adjacent to the suburbs (nests more than 200 m away from a residence). We used the R package ggscatter, with the Spearman correlation method, to identify if within genetic correlation and group size were correlated, to compare the result between groups living in the suburbs and those in native woodlands.

Results

SNP filtering

Once filtered, we obtained 1813 SNPs, from the initial 17,961 SNPs of 172 individuals, 95 females and 77 males. (Figure S1). Mean individual H_O increased from 0.191 before filtering to 0.274 after filtering; mean H_T was 0.247 before filtering and 0.311 after filtering; mean MAF was 0.174 unfiltered and 0.22 filtered; while mean F_{IT} was 0.246 before filtering and 0.121 filtered.

Genetic diversity

Mean observed heterozygosity of subpopulations ($H_O = 0.278 \pm 0.003$) was higher than mean subpopulation expected heterozygosity ($H_S = 0.251 \pm 0.003$), and lower than the total expected heterozygosity ($H_T = 0.314 \pm 0.003$). The individual inbreeding coefficient ($F_{IS} = -0.107 \pm 0.005$) was negative and close to zero, while the genetic differentiation between populations is positive ($F_{ST} = 0.2 \pm 0.002$).

Principal Coordinates analysis (PCoA) for breeding seasons 2016-2017 and 2017-2018 (Figure 3) showed that choughs that belong to the same group tended to cluster together and were genetically more similar. However, there were some exceptions where some individuals were closer to others from different groups, showing close relatedness among individuals belonging to different groups. The first PCoA axis from breeding season 2016-2017 explained 8.8% of the variation, axis two 7.6% and axis three 4.9%; and from breeding season 2017-2018, axis one explained 8% of the variation, axis 2 6.2% and axis three 5.5% (Figure S2).

Genetic differences between populations under different rainfall patterns

For the present study, the Analysis of Molecular Variance showed significant genetic differentiation among groups (F_{ST} = 0.148, F'_{ST} = 0.207). F_{ST} values for adult males (F_{ST} = 0.184, F'_{ST} = 0.249), sub-adult males (F_{ST} = 0.181, F'_{ST} = 0.247), and sub-adult females (F_{ST} = 0.171, F'_{ST} = 0.236) were similar, but the value for adult females was lower (F_{ST} = 0.103, F'_{ST} = 0.146). (Table 1). This compares with Beck et al. data, for which we also found genetic differentiation among groups (F_{ST} = 0.165). In the previous study, F_{ST} values for adult males (F_{ST} = 0.171, F'_{ST} = 0.5) and sub-adult males (F_{ST} = 0.143, F'_{ST} = 0.425) were more similar; while sub-adult females (F_{ST} = 0.146, F'_{ST} = 0.444) and adult females (F_{ST} = 0.139, F'_{ST} = 0.411) presented lower F_{ST} values. (Table 1). In the present study, suburban breeding choughs presented a lower F_{ST} value than those breeding in the surrounding woodlands (woodland groups: F_{ST} = 0.185, F'_{ST} = 0.25, suburban groups: F_{ST} = 0.087, F'_{ST} = 0.121) (Table 1).

Table 1. Among group differentiation using analysis of molecular variance (F_{ST}) between all groups (Total), between only males of each group and between only females of each group for both the present and previous studies.

		Among group variation	Value F_{ST}	Value F'_{ST}	df	p
AMOVA results present study	Total	15%	0.148	0.207	26	0.01
	Female adults	10%	0.103	0.146	17	0.01
	Male adults	17%	0.184	0.249	16	0.01
	Female sub-adults	17%	0.171	0.236	14	0.01
	Male sub-adults	18%	0.181	0.247	14	0.01
	Woodland breeding choughs	17%	0.185	0.25	15	0.01
	Suburban breeding choughs	9%	0.087	0.121	8	0.01
AMOVA results previous study	Total	15%	0.163	0.485	14	0.01
	Female adults	12%	0.139	0.411	4	0.01
	Male adults	14%	0.171	0.5	6	0.01
	Female sub-adults	13%	0.146	0.444	5	0.01
	Male sub-adults	13%	0.143	0.425	5	0.01

We compared pairwise relatedness between group members of the same sex for choughs of the present study. We found no significant difference in relatedness when comparing all female members and all male members within groups (female mean = 0.215, SE = 0.003; male

mean= 0.295, SE= 0.006; t = -1.89, df = 49, p-value = 0.064). We also did not find significant differences in relatedness among female and male sub-adults within groups (female mean = 0.272, SE = 0.005; male mean= 0.304, SE= 0.008; t = -0.549, df = 28, p-value = 0.588), however adult females were significantly less related within groups than adult males (female mean = 0.183, SE = 0.007; male mean= 0.319, SE= 0.005; t = -2.5138, df = 33, p-value = 0.017).

The spatial autocorrelation analysis detected significant and positive genetic structure within all groups (0 kilometres distance) (Figure 2). It also showed positive and significant genetic structure between male adults in groups less than 0.5 kilometres apart (Figure 2 d).)The *r*-values between year, sex and age showed no significant differences between individuals within groups (0 kilometres distance). However, when these were calculated for each sex combined to each age class, female adults showed significantly lower values of r within groups (Figure 2 f).). Spatial autocorrelation analysis in Beck et al. (2008) also revealed genetic structure within groups of choughs, and significantly lower r values in adult choughs than subadults at 0 kilometres distance (Figure 2 a).). Beck et al. (2008) found no significant differences per sex or sex and age combined in genetic correlation between groups in any of the distance classes (Figure 2 c). and e).)

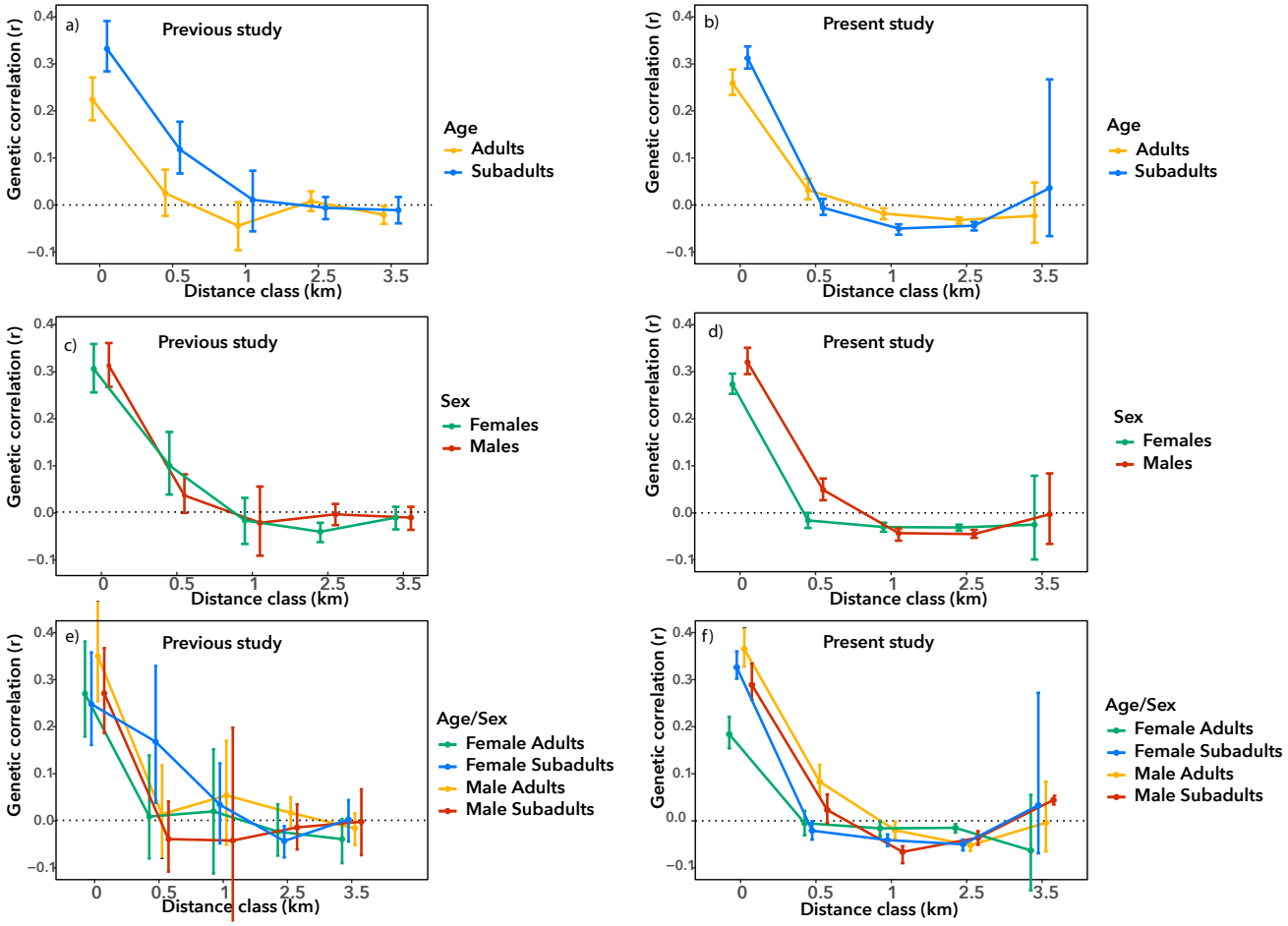


Figure 2. Plots of spatial genetic autocorrelation for individuals comparing a previous study on white-winged choughs (Beck et al., (2008)) to the present study. Each graph shows the genetic correlation coefficient (*r*) as a function of distance (km), grouped by a) previous study per age class, b) present study per age class, c) previous study per sex, d) present study per sex, e) previous study per age class and sex combined, f) present study per age class and sex combined. Upper and lower bounds for the 95% confidence interval around the null hypothesis of no spatial structure (*r*= 0) calculated by bootstrap resampling are presented.

Genetic differences between choughs nesting in the suburbs and in adjacent native woodland

Estimates of genetic autocorrelation (r) within-groups were higher than expected by chance (higher than zero and confidence intervals obtained by permutation) in 20 of the 27 sampled groups (74%, Figure 3). These results were similar to those reported by Beck et al. (2008), where 19 of the 27 (70%) groups they studied were significantly more related within groups than expected by chance. Per year, in 2016 nine out of 13 groups (69%) were more related within than between groups, while in 2017 it was 11 out of 14 groups (79%). From the groups breeding in the suburbs 6 out of 9 (67%) were more related than expected by chance, while from groups breeding in the woodlands 14 of the 16 (88%) were more related than expected by chance.

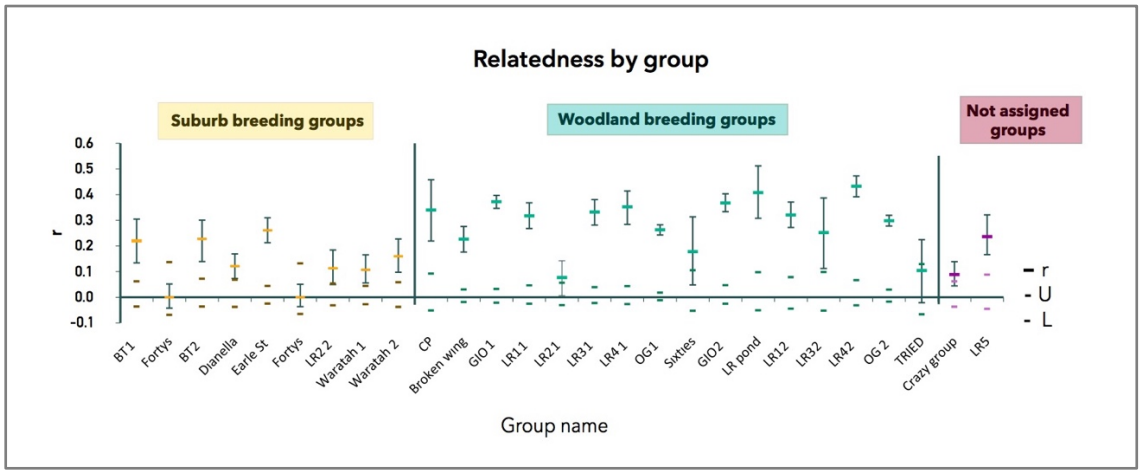


Figure 3. Within-group mean pairwise relatedness values (r) for 27 groups of choughs. Error bars indicate 95% confidence intervals as determined by bootstrap resampling. Thinner lines surrounding x-axis show upper (U) and lower (L) 95% confidence limits for the null hypothesis of "no relatedness" as determined by permutation.

Group size and relatedness were not significantly correlated overall (R = 0.36, p = 0.076) or when we separated groups according to whether they bred in the suburbs (n = 9) woodland (n = 16) (suburban groups R = 0.64, p = 0.066; woodland groups R = 0.14, p = 0.59). However,

groups breeding in native woodland had significantly higher within-group genetic autocorrelation (mean = 0.290 ± 0.003 s.e.) than those breeding in the suburbs (mean = 0.135 ± 0.003 s.e.) (t = -3.7675, df = 23, p-value < 0.001). There was no significant difference in group size between groups breeding in native woodland and those breeding in the suburbs (woodland groups mean = 8.375 ± 3.496 s.e.; suburb groups mean= 6.778 ± 0.981 s.e.; Welch two sample t-test, t = -1.4574, df = 22.437, p-value = 0.159).

Spatial autocorrelation categorised by breeding habitat showed that both the woodlands and suburbs choughs had significant positive spatial structure within-groups. However, woodland groups were significantly more spatially genetically structured than suburb groups in the 0 kilometres category. Neighbour groups in the woodland category also showed higher genetic structure at the 0.5 km distance class. In woodland groups adult females showed significantly lower genetic correlation than the rest of the categories, while in urban habitats female subadults had significantly lower values than the other categories (Figure 4).

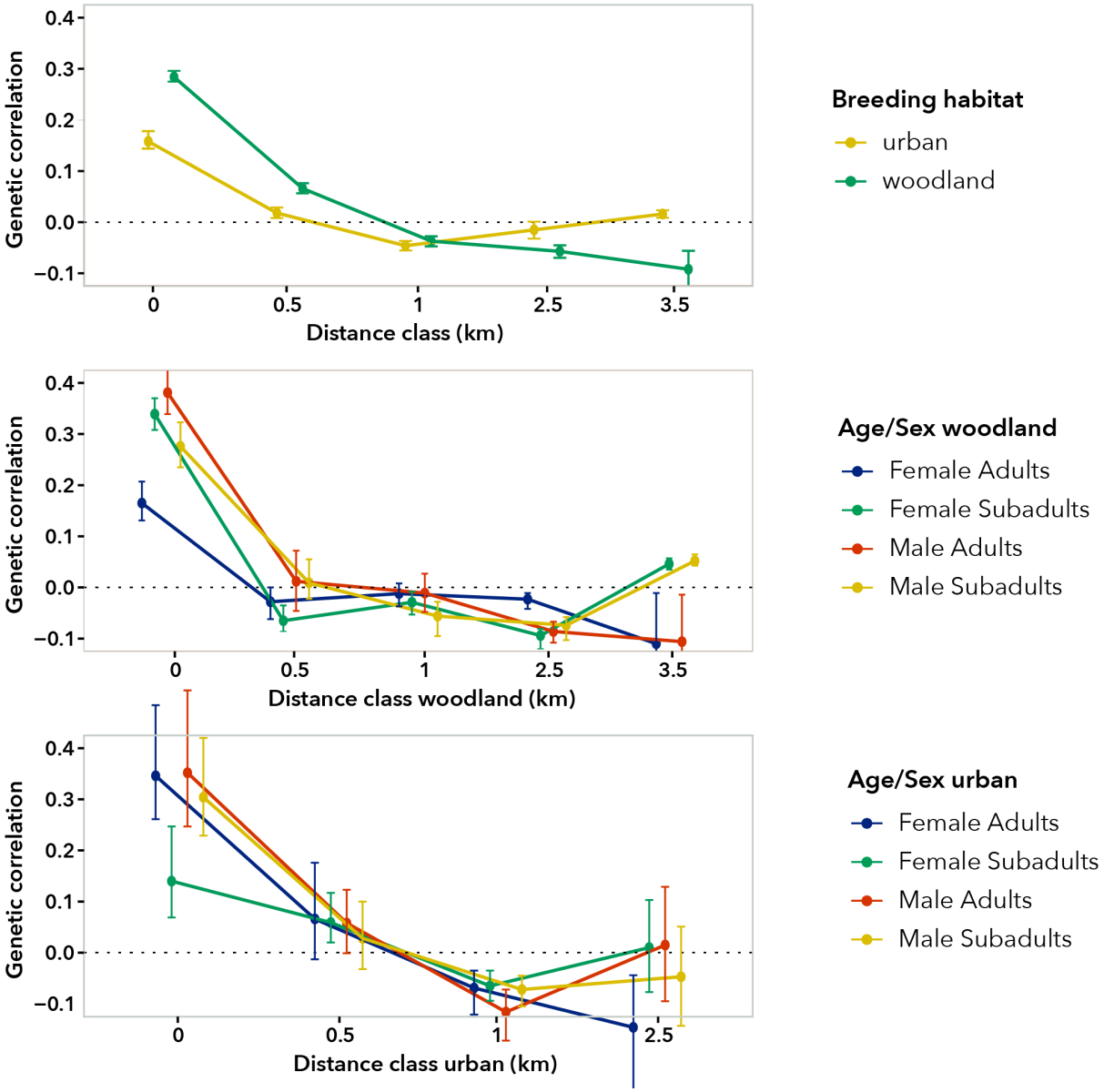


Figure 4. Plots of spatial genetic autocorrelation for individuals breeding in different habitats, showing the genetic correlation coefficient (*r*) as a function of distance, separated by a) breeding habitats: suburbs and native woodland b) individuals breeding in the woodlands classified by age class and sex, c) individuals breeding in the suburbs classified by age class and sex. Upper and lower bounds for the 95% confidence interval around the null hypothesis of no spatial structure (*r*= 0) calculated by bootstrap resampling are presented.

Discussion

Ecological constraints have long been hypothesised as central factors determining variation in animal social systems (Emlen 1982, Brown 1987, Stacey and Koenig 1990, Lott 1991). This study of white-winged choughs compares the structure of social groups and likely inclusive fitness rewards to individuals under two main scenarios of variable environmental conditions, one pertaining to climate and the other to major habitat alteration. Our results showing that dispersal was female biased during a period of rainfall stability contrast with a previous study during extreme drought that showed an absence of sex-biased dispersal (Beck et al., 2008), and suggest a major role for climatic factors in social organisation. Further, the difference in within-group relatedness between chough groups breeding in different habitats provides an important demonstration that both social organisation and the costs and benefits of cooperative breeding can change with anthropogenic habitat alteration.

Variable rainfall patterns

First, we consider the effect of climatic variation, specifically rainfall patterns. The search for climatic correlates of cooperative breeding has been carried out for several decades and continues to be a topic of high interest in evolutionary biology. While some studies have linked non-seasonal environments to the promotion of cooperative breeding (Ford, Bell et al. 1988, Gonzalez, Sheldon et al. 2013), others have found associations with high seasonality and inter-annual rainfall variability (Du Plessis, Siegfried et al. 1995, Rubenstein and Lovette 2007, Jetz and Rubenstein 2011). Nevertheless, cooperatively breeding birds inhabit ecologically and climatically diverse environments, and also show great diversity within this type of social and mating system (Cockburn and Russell 2011, Russell 2016).

We predicted that differences in climatic conditions would lead to different demographic outcomes within a cooperatively breeding species. The hypothesis was not supported by mean

within-group relatedness but was supported by differences in sex-specific dispersal. Mean within group relatedness was higher than expected by chance in 74% of the sampled groups from woodland habitat. This was similar to the proportion found by Beck, Peakall et al. (2008), who carried out their study after a severe multi-year drought across eastern Australia. Thus, both studies showed that chough groups are usually comprised of close relatives. This result was supported by analysis of molecular variance, showing that both the chough populations in this study and that of Beck et al. (2008) were highly structured.

The most remarkable difference was that our study during non-drought weather conditions found evidence of adult female biased dispersal whereas Beck et al (2008) conducted during drought conditions did not. Most bird species have female-biased dispersal (Greenwood 1980, Harrison, York et al. 2014) but white-winged choughs have been considered an exception (Beck, Peakall et al. 2008), like the cooperatively breeding grey-crowned babblers (*Pomatostomus temporalis*) (Blackmore, Peakall et al. 2011). Local genetic structure between individuals in a population is expected to be higher when dispersal is infrequent, and relatedness between individuals to decrease with growing geographic distances (Beck et al. (2008). From our study, the spatial autocorrelation analysis and the F_{ST} and F'_{ST} estimates showed that adult females disperse further than adult males. Sub-adult females and sub-adult males had higher F_{ST} and F'_{ST} values, confirming philopatry of younger birds, supporting observations from previous studies on this species (Rowley 1978, Heinsohn 1992).

The spatial autocorrelation analyses did not show a significant difference in genetic correlation between sexes by age in chough groups during the drought, confirming Beck et al. (2008) finding of an absence of sex-biased dispersal . However, F_{ST} values were the lowest for female adults, which could be due to an actual decline in the female adult population. For instance, Heinsohn et al. (2000) found that group fragmentation was only observed when a severe

drought occurred in 1994 after many years of group stability, which also matched an unusual increase in mortality throughout the whole population, especially of female breeders. During this drought, Heinsohn et al. (2000) observed the formation of an aggressive group composed only of males, suggesting male biased dispersal during this period. In previous studies, conducted under normal rainfall patterns and in woodland populations, white-winged chough dispersal was considered very rare (Rowley 1978, Heinsohn et al. 1992). In summary, white-winged chough dispersal patterns between groups might be strongly determined by environmental conditions, suggesting that the degree of sex-bias in dispersal is a dynamic attribute of this species.

2. Habitat alteration.

We considered the impact of anthropogenic alteration of habitat, specifically urbanisation, on chough social organisation. Although this form of habitat change has been documented as a force changing the distribution and behaviour of species worldwide (Marzluff 2001, La Sorte and Thompson 2007, Meffert and Dziock 2013) few studies have considered its impact on cooperatively breeding birds. When cooperative species persist in altered landscapes, examination of the changes to the social system and individual rewards may offer further opportunities for understanding both the drivers of cooperative breeding and the consequences of environmental change. We showed that there are important differences in within group relatedness between groups breeding in the suburbs and those breeding in the woodlands, indicating the influence that changes in the landscape have in their kinship structure.

Beck et al. (2008) indicated that suburban breeding groups were slightly more related than woodland breeding groups. In contrast, we found that individuals in suburban groups were significantly less related to each other than individuals within woodland groups. Chough groups

are mostly stable, and usually increase group size by adding offspring from previous years (Heinsohn, 1992). Thus, the result of this study could reflect higher mortality in groups breeding in the suburbs as well as higher dispersal among suburban groups, and suggests greater instability of groups breeding in areas with higher human disturbance. Choughs living under stressful environmental conditions such as droughts form new groups composed of unrelated individuals (Heinsohn, Dunn et al. 2000, Beck, Peakall et al. 2008), which would account for the smaller difference in relatedness among groups from different habitats during a drought. This can also be the case for groups breeding under the stress of an urban environment, where bird mortality is higher due to predation, traffic, and poison, among others (personal observation, (Erritzoe, Mazgajski et al. 2003, Beck and Heinsohn 2006, Reijnen and Foppen 2006, Baker, Molony et al. 2008). In contrast, individuals in groups breeding in less disturbed areas are more closely related to each other since they are most likely comprised of a breeding pair and their offspring from previous years (Heinsohn, Dunn et al. 2000, Beck and Heinsohn 2006). One of the two groups breeding in the woodlands with lower within group relatedness (group 'TRIED', Figure 3) was a new group that tried to breed with no success and was only together for one breeding season. The second woodland group with low within-group relatedness ('LR21', Figure 3) was the only group that has been observed to move from the woodlands to the suburbs.

The suburban groups in our investigation had lower F_{ST} values than woodland groups, probably resulting from more group disintegration and reestablishment with unrelated choughs, which has been only common during periods of high environmental stress like droughts (Heinsohn, Dunn et al. 2000). This result again underlines the stress that suburban environments can impose on bird species, even though they can be a relief during drought periods by supplying food and water (Beck and Heinsohn 2006). Finally, we found that sub-adult females in

suburban nesting groups were significantly less related within their groups than the rest of group members. Suburban female sub-adults were also more related to groups with nests that were 500 meters apart, reflecting higher rates of dispersal of younger female helpers to neighbouring groups than occurs among woodland breeding groups. Woodland groups instead showed adult male dispersal to neighbouring groups, and female adult dispersal from more distant groups.

Reduced habitat quality can affect dispersal in cooperative breeders. Examples include red-cockaded woodpeckers (*Picoides borealis*), that need pine tree cavities for breeding (Walters, Copeyon et al. 1992a) and superb fairy wrens (*Malurus cyaneus*) which depend on extensive vegetation patches (Parsons, French et al. 2009). However, little is known about the effects of urbanisation on populations of cooperatively breeding species (Beck and Heinsohn 2006). Canberra, often referred to as the "bush capital" has many areas of parkland and reserves compared to other cities (Ignatieva, Stewart et al. 2011), and these areas may even offer a refuge for choughs during drought conditions that affect the surrounding countryside (Beck et al. 2006). However our study suggests that urbanisation may present a major challenge for cooperatively breeding species, with isolation due to patchy habitats not only affecting dispersal events, but potentially affecting their population genetic structure. For instance, a non-cooperative species, the house sparrow (*Passer domesticus*), showed increased relatedness among individuals that live in close proximity in highly urbanized environments (Vangestel, Mergeay et al. 2011), and lower genetic variability and high genetic differentiation among isolated populations. Such trends have been observed in a variety of bird and vertebrate species in urbanised areas (Delaney, Riley et al. 2010, Unfried, Hauser et al. 2013, Gil and Brumm 2014).

References

Arnold, Kathryn E, and Ian PF Owens. 1998. 'Cooperative breeding in birds: a comparative test of the life history hypothesis', *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265: 739-45.

Baker, Philip J, Susie E Molony, Emma Stone, Innes C Cuthill, and Stephen Harris. 2008. 'Cats about town: is predation by free-ranging pet cats Felis catus likely to affect urban bird populations?', *Ibis*, 150: 86-99.

Beck, R Peakall, and R Heinsohn. 2008. 'Social constraint and an absence of sex-biased dispersal drive fine-scale genetic structure in white-winged choughs', *Molecular ecology*, 17: 4346-58.

Beck, Nadeena R, and Robert Heinsohn. 2006. 'Group composition and reproductive success of cooperatively breeding white-winged choughs (Corcorax melanorhamphos) in urban and non-urban habitat', *Austral ecology*, 31: 588-96.

Blackmore, Caroline J, Rod Peakall, and Robert Heinsohn. 2011. 'The absence of sex-biased dispersal in the cooperatively breeding grey-crowned babbler', *Journal of Animal Ecology*, 80: 69-78.

Boland, Christopher R J. 1998. 'Helpers improve nest defence in cooperatively breeding white-winged choughs', *Emu*, 98: 320-24.

Brown, Jerram L. 1978. 'Avian communal breeding systems', *Annual Review of Ecology and Systematics*, 9: 123-55.

Brown, Jerram L. 1987. *Helping and communal breeding in birds: ecology and evolution* (Princeton University Press: Princeton).

Chiari, Claudia, Marco Dinetti, Cinzia Licciardello, Gaetano Licitra, and Marco Pautasso. 2010. 'Urbanization and the more-individuals hypothesis', *Journal of Animal Ecology*, 79: 366-71.

Chiew, Francis HS, Thomas C Piechota, John A Dracup, and Thomas A McMahon. 1998. 'El Nino/Southern Oscillation and Australian rainfall, streamflow and drought: Links and potential for forecasting', *Journal of Hydrology*, 204: 138-49.

Clutton-Brock, Tim. 2002. 'Breeding together: kin selection and mutualism in cooperative vertebrates', *Science*, 296: 69-72.

Clutton-Brock, Tim. 2009. 'Cooperation between non-kin in animal societies', *Nature*, 462: 51-57.

Coates, Brad S, Douglas V Sumerford, Nicholas J Miller, Kyung S Kim, Thomas W Sappington, Blair D Siegfried, and Leslie C Lewis. 2009. 'Comparative performance of single nucleotide polymorphism and microsatellite markers for population genetic analysis', *Journal of Heredity*, 100: 556-64.

Cockburn, Andrew. 1998. 'Evolution of helping behavior in cooperatively breeding birds', *Annual Review of Ecology and Systematics*: 141-77.

Cockburn, A. 2006. 'Prevalence of different modes of parental care in birds', *Proceedings of the Royal Society B: Biological Sciences*, 273: 1375-83.

Cockburn, Andrew, and Andrew F Russell. 2011. 'Cooperative breeding: a question of climate?', *Current Biology*, 21: R195-R97.

Croft, Darren P, Mathew Edenbrow, and Safi K Darden. 2015. 'Assortment in social networks and the evolution of cooperation.' in Jens Krause, Richard James, Daniel W Franks and Darren P Croft (eds.), *Animal Social networks* (Oxford University Press: Oxford, UK).

Delaney, Kathleen Semple, Seth PD Riley, and Robert N Fisher. 2010. 'A rapid, strong, and convergent genetic response to urban habitat fragmentation in four divergent and widespread vertebrates', *PLoS ONE*, 5: e12767.

Du Plessis, Morné A, W Roy Siegfried, and Adrian J Armstrong. 1995. 'Ecological and life-history correlates of cooperative breeding in South African birds', *Oecologia*, 102: 180-88.

Elbers, Jean P, Rachel W Clostio, and Sabrina S Taylor. 2017. 'Population genetic inferences using immune gene SNPs mirror patterns inferred by microsatellites', *Molecular ecology resources*, 17: 481-91.

Emlen, Stephen T. 1982. 'The evolution of helping. I. An ecological constraints model', *American Naturalist*: 29-39.

Erritzoe, Johannes, Tomasz D Mazgajski, and Łukasz Rejt. 2003. 'Bird casualties on European roads—a review', *Acta Ornithologica*, 38: 77-93.

Ford, HughA, Harry Bell, Ray Nias, and Richard Noske. 1988. 'The relationship between ecology and the incidence of cooperative breeding in Australian birds', *Behavioral Ecology and Sociobiology*, 22: 239-49.

Fridolfsson, Anna-Karin, and Hans Ellegren. 1999. 'A simple and universal method for molecular sexing of non-ratite birds', *Journal of avian biology*: 116-21.

Fry, CH. 1977. 'The evolutionary significance of co-operative breeding in birds.' in, *Evolutionary ecology* (Springer).

Gil, Diego, and Henrik Brumm. 2014. *Avian urban ecology* (Oxford University Press).

Gonzalez, Juan-Carlos T, Ben C Sheldon, and Joseph A Tobias. 2013. 'Environmental stability and the evolution of cooperative breeding in hornbills', *Proceedings of the Royal Society B: Biological Sciences*, 280.

Greenwood, Paul J. 1980. 'Mating systems, philopatry and dispersal in birds and mammals', *Animal behaviour*, 28: 1140-62.

Griffin, Ashleigh S, and Stuart A West. 2003. 'Kin discrimination and the benefit of helping in cooperatively breeding vertebrates', *Science*, 302: 634-36.

Griffiths, Richard, Mike C Double, Kate Orr, and Robert JG Dawson. 1998. 'A DNA test to sex most birds', *Molecular ecology*, 7: 1071-75.

Halfwerk, Wouter, Leonard JM Holleman, C M Lessells, and Hans Slabbekoorn. 2011. 'Negative impact of traffic noise on avian reproductive success', *Journal of applied Ecology*, 48: 210-19.

Hamilton, William D. 1964. 'The genetical evolution of social behaviour. II', *Journal of theoretical biology*, 7: 17-52.

Harrison, Xavier A, Jennifer E York, and Andrew J Young. 2014. 'Population genetic structure and direct observations reveal sex-reversed patterns of dispersal in a cooperative bird', *Molecular ecology*, 23: 5740-55.

Hatchwell, and Komdeur. 2000. 'Ecological constraints, life history traits and the evolution of cooperative breeding', *Animal Behaviour*, 59: 1079-86.

Hatchwell, Ben J. 2009. 'The evolution of cooperative breeding in birds: kinship, dispersal and life history', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364: 3217-27.

Hedrick, Philip W. 2005. 'A standardized genetic differentiation measure', *Evolution*, 59: 1633-38.

Heinsohn, Robert, Peter Dunn, Sarah Legge, and Michael Double. 2000. 'Coalitions of relatives and reproductive skew in cooperatively breeding white-winged choughs', *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 267: 243-49.

Heinsohn, Robert G. 1991a. 'Slow learning of foraging skills and extended parental care in cooperatively breeding white-winged choughs', *American Naturalist*: 864-81.

Heinsohn, Robert G. 1992. 'Cooperative enhancement of reproductive success in white-winged choughs', *Evolutionary Ecology*, 6: 97-114.

Heinsohn, Robert G. 2009. 'White-winged Coughs: The social consequences of Boom and Boost ' in, *Boom and Bust: Bird Stories for a Dry Country* (CSIRO Publishing: Collingwood).

Heinsohn, Robert G, Andrew Cockburn, and RB Cunningham. 1988. 'Foraging, Delayed Maturation, and Advantages of Cooperative Breeding in White-winged Choughs, Corcorax melanorhamphos', *Ethology*, 77: 177-86.

Heinsohn, Robert, and Sarah Legge. 1999. 'The cost of helping', *Trends in Ecology & Evolution*, 14: 53-57.

Ignatieva, Maria, Glenn H Stewart, and Colin Meurk. 2011. 'Planning and design of ecological networks in urban areas', *Landscape and ecological engineering*, 7: 17-25.

Jaccoud, Damian, Kaiman Peng, David Feinstein, and Andrzej Kilian. 2001. 'Diversity arrays: a solid state technology for sequence information independent genotyping', *Nucleic acids research*, 29: e25-e25.

Jetz, Walter, and Dustin R. Rubenstein. 2011. 'Environmental Uncertainty and the Global Biogeography of Cooperative Breeding in Birds', *Current Biology*, 21: 72-78.

Kilian, Andrzej, Peter Wenzl, Eric Huttner, Jason Carling, Ling Xia, Hélène Blois, Vanessa Caig, Katarzyna Heller-Uszynska, Damian Jaccoud, and Colleen Hopper. 2012. 'Diversity arrays technology: a generic genome profiling technology on open platforms.' in, *Data production and analysis in population genomics* (Springer).

Koenig, Walter D, and Janis L Dickinson. 2016. *Cooperative breeding in vertebrates: studies of ecology, evolution, and behavior* (Cambridge University Press).

La Sorte, Frank A, and Frank R Thompson. 2007. 'Poleward shifts in winter ranges of North American birds', *Ecology*, 88: 1803-12.

Leedale, Amy E, Stuart P Sharp, Michelle Simeoni, Elva JH Robinson, and Ben J Hatchwell. 2018. 'Fine-scale genetic structure and helping decisions in a cooperatively breeding bird', *Molecular ecology*, 27: 1714-26.

Lott, Dale F. 1991. *Intraspecific variation in the social systems of wild vertebrates* (Cambridge University Press).

Marzluff, John M. 2001. 'Worldwide urbanization and its effects on birds.' in, *Avian ecology and conservation in an urbanizing world* (Springer).

Meffert, Peter J, and Frank Dziock. 2013. 'The influence of urbanisation on diversity and trait composition of birds', *Landscape Ecology*, 28: 943-57.

Meirmans, Patrick G, and Philip W Hedrick. 2011. 'Assessing population structure: FST and related measures', *Molecular ecology resources*, 11: 5-18.

Melville, Jane, Margaret L Haines, Katja Boysen, Luke Hodkinson, Andrzej Kilian, Katie L Smith Date, Dominique A Potvin, and Kirsten M Parris. 2017. 'Identifying hybridization and admixture using SNPs: application of the DArTseq platform in phylogeographic research on vertebrates', *Royal Society open science*, 4: 161061.

Møller, Anders Pape, Helga Erritzøe, and Johannes Erritzøe. 2011. 'A behavioral ecology approach to traffic accidents: interspecific variation in causes of traffic casualties among birds', *Zoological research*, 32: 115-27.

Painter, J. N., R. H. Crozier, A. Poiani, R. J. Robertson, and M. F. Clarke. 2000. 'Complex social organization reflects genetic structure and relatedness in the cooperatively breeding bell miner, Manorina melanophrys', *Molecular ecology*, 9: 1339-47.

Parsons, Holly, Kristine French, and Richard E Major. 2009. 'The vegetation requirements of Superb Fairy-wrens (Malurus cyaneus) in non-urban edge and urbanised habitats', *Emu*, 108: 283-91.

Peakall, R, and P E Smouse. 2012. 'GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update.', *Bioinformatics*: 2537-39.

Reijnen, Rien, and RUUD Foppen. 2006. 'Impact of road traffic on breeding bird populations.' in, *The ecology of transportation: managing mobility for the environment* (Springer).

Riehl, Christina. 2013. 'Evolutionary routes to non-kin cooperative breeding in birds', *Proceedings of the Royal Society of London B: Biological Sciences*, 280.

Rowley, Ian. 1975. *Bird life* (Collins: Sydney).

Rowley, Ian. 1978. 'Communal activities among White-winged Choughs *Corcorax melanorhamphus*', *Ibis*, 120: 178-97.

Rubenstein, Dustin R, and Irby J Lovette. 2007. 'Temporal environmental variability drives the evolution of cooperative breeding in birds', *Current Biology*, 17: 1414-19.

Russell, Andrew F. 2016. 'Chestnut-crowned babblers: dealing with climatic adversity and uncertainty in the Australian arid zone', *Cooperative Breeding in Vertebrates: Studies in Ecology, Evolution and Behavior*: 150-64.

Shaw, Robyn. 2018. 'The genetic consequences of demography and disturbance in small mammal populations', The Australian National University.

Smouse, Peter E, and Rod Peakall. 1999. 'Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure', *Heredity*, 82: 561-73.

Stacey, Peter B, and Walter D Koenig. 1990. *Cooperative breeding in birds: long term studies of ecology and behaviour* (Cambridge University Press: New York).

Temple, HJ, JI Hoffman, and W Amos. 2006. 'Dispersal, philopatry and intergroup relatedness: fine-scale genetic structure in the white-breasted thrasher, *Ramphocinclus brachyurus*', *Molecular ecology*, 15: 3449-58.

Unfried, Thomas M, Lorenz Hauser, and John M Marzluff. 2013. 'Effects of urbanization on Song Sparrow (*Melospiza melodia*) population connectivity', *Conservation Genetics*, 14: 41-53.

van de Pol, Martijn, Helen L Osmond, and Andrew Cockburn. 2012. 'Fluctuations in population composition dampen the impact of phenotypic plasticity on trait dynamics in superb fairy-wrens', *Journal of Animal Ecology*, 81: 411-22.

Vangestel, Carl, Joachim Mergeay, Deborah A Dawson, Viki Vandomme, and Luc Lens. 2011. 'Spatial heterogeneity in genetic relatedness among house sparrows along an urban–rural gradient as revealed by individual-based analysis', *Molecular ecology*, 20: 4643-53.

Walters, Jeffrey R, Carole K Copeyon, and JH Carter III. 1992a. 'Test of the ecological basis of cooperative breeding in red-cockaded woodpeckers', *The Auk*: 90-97.

Woxvold, Iain A, Greg J Adcock, and Raoul A Mulder. 2006. 'Fine-scale genetic structure and dispersal in cooperatively breeding apostlebirds', *Molecular ecology*, 15: 3139-46.

Supplemental information

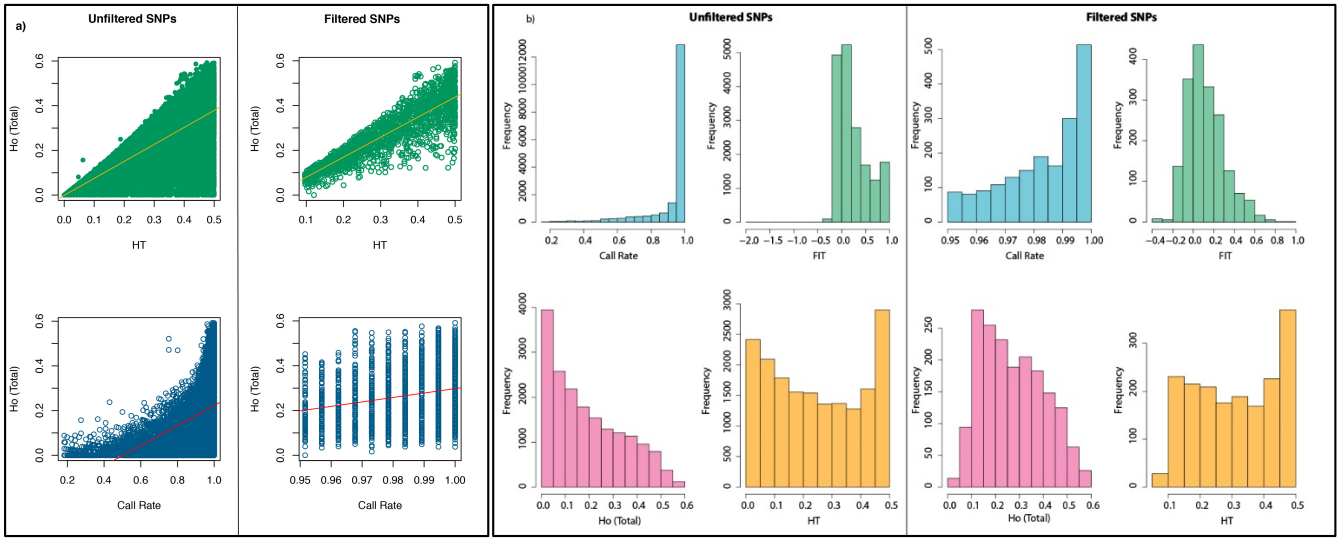


Figure S1. a) Expected heterozygosity (H_T) versus observed heterozygosity (Ho) for unfiltered and filtered SNPs and H_O vs Call Rate, for unfiltered and filtered SNPs; b) Histograms of call rate, reference allele frequency , individual inbreeding coefficient (F_{IT}), observed heterozygosity (Ho) and expected heterozygosity (HT); before and after filtering.

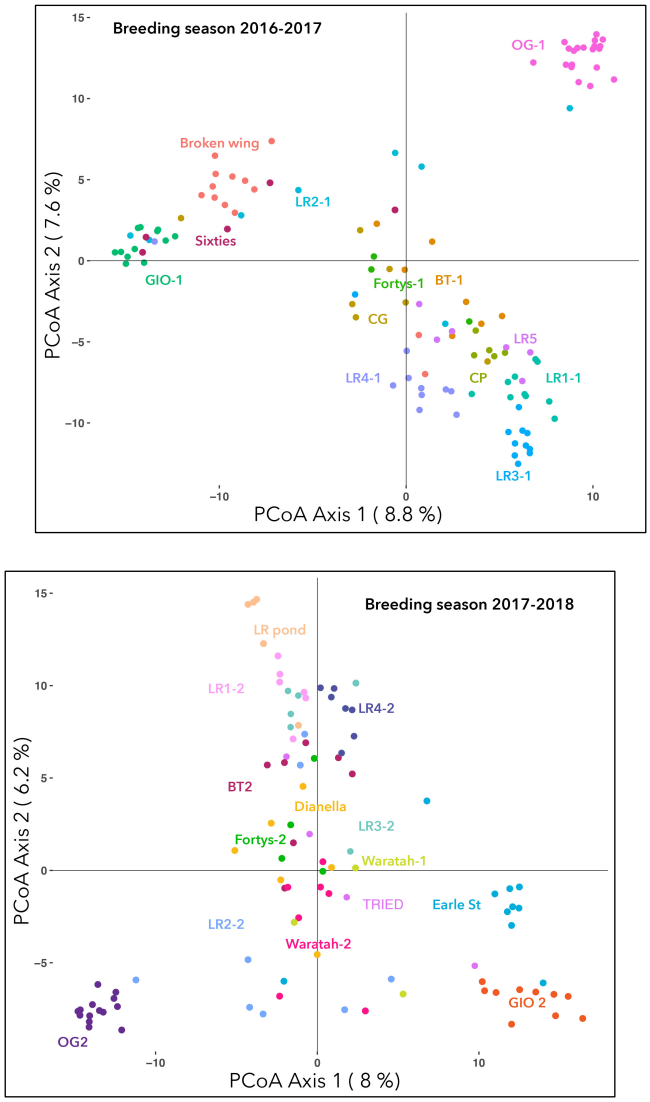


Figure S2. Principal coordinates analysis of white-winged chough populations during the 2016-2017 and 2017-2018 breeding seasons (left and right respectively). Each point represents an individual chough, and each colour represents group membership. Proximity indicates genetic similarity between the individuals.

CHAPTER 3

Long term studies reveal major climate related fitness variation for breeders and helpers in a cooperatively breeding bird

Abstract

The origin and maintenance of cooperative breeding, a social and mating system in which more than two individuals cooperate to raise a single brood, are of great interest in evolutionary biology, as helpers appear to forgo their own reproductive opportunities to raise the offspring of others. Some of the most widely established explanations are linked to ecological constraints that limit independent breeding and encourage individuals to wait for breeding opportunities and instead gain inclusive fitness benefits at least in the short term. I took advantage of demographic data available across five different studies of an obligate cooperatively breeding bird species, the white-winged chough (*Corcorax melanorhamphos*). The studies were conducted in the same geographic region under different environmental conditions from 1959 to 2017. Over all five studies the number of fledglings produced was dependent on group size in years with high rainfall but there was no such effect in periods of drought. Genetic information including relatedness between group members was available for the most recent three studies, which I used to compare population traits and the inclusive fitness benefits acquired by group members. I found that fitness benefits differed for helpers and breeders, and also varied according to the prevailing climatic conditions. Offspring equivalents for breeders did not increase with group size in two studies and decreased significantly with group size in one study. Offspring equivalents for helpers increased with group size in two studies confirming they received a direct inclusive fitness benefit in some years. However, belonging to larger groups led to a decrease in fitness for helpers in one period of drought. This study is important because it shows that inclusive fitness benefits via increased production of young, even in an obligately cooperative bird species, may not be available under all environmental conditions. Rather than increased reproduction the benefits of group living during drought years may simply include increased survival while waiting for conditions to improve.

Considering Australia is a continent with a highly variable environment in terms of rainfall and temperature, and that this variability is predicted to become more extreme due to climate change, understanding the effects of climate at the population level of a social species, is relevant from an evolutionary and a conservational point of view.

Introduction

Cooperative breeding occurs when more than two individuals work together to produce offspring (Emlen 1982, Brown 1987, Cockburn 1998). The non-parental individuals, known as helpers, may help the breeders to produce more young, or instead lighten the load on breeders (Cockburn 1998, Hatchwell 1999). The motivation behind the cooperative behaviour of helpers is not easy to explain from an evolutionary point of view, as natural selection should favour independent breeding. Besides direct benefits related to helping, such as territory inheritance or gaining future allies (Cockburn 1998, Hatchwell and Komdeur 2000, Clutton-Brock 2002); one of the classic hypotheses accounting for the evolution of cooperative breeding is kin selection, an indirect reproductive strategy that consists of actions that enhance the breeding success and survival of those that are closely related (Hamilton 1964, Eberhard 1975). Whether cooperation towards relatives is a direct and active choice that involves kin recognition Considering that cooperative breeding often occurs in vertebrates that live in highly related social groups, indirect fitness benefits are certainly important for helpers, which have more opportunities to help kin when cooperating (Hatchwell 2010). Examples in which cooperation is greater towards relatives have been found in many cooperatively breeding birds, such as long-tailed tits (*Aegithalos caudatus*) (Russell and Hatchwell 2001, Nam, Simeoni et al. 2010), wild turkeys (*Meleagris gallopavo*) (Krakauer 2005) and chestnut-crowned babblers (*Pomatostomus ruficeps*) (Browning, Patrick et al. 2012).

Another factor considered important for explaining the evolution of cooperative breeding is the ecological conditions that may have facilitated its emergence. For example, one classic view of cooperative breeding was that it resulted from habitat saturation and higher natal philopatry due to the lack of territories for young birds to disperse to (Komdeur 1992, Hatchwell and Komdeur 2000). A related hypothesis is that cooperative breeding is associated with particular

climates and weather patterns that affect the costs and benefits of helping versus breeding (Brown 1978, Emlen 1982). Studies on the effect of climatic conditions on individual species have enabled meta-analyses associating species with varied manifestations of cooperative breeding to specific climatic traits, with most of them reaching the conclusion that environmental unpredictability and variability promotes the emergence of this mating system (Ford, Bell et al. 1988, Arnold and Owens 1999, Jetz and Rubenstein 2011). A comparative analysis by Jetz and Rubenstein (2011), suggested that cooperative breeding in passerines is correlated with highly variable climates, especially with high inter-annual rainfall variability, while in non-passerines it is related to temperature variation. This was consistent with the findings of a previous comparative study on 45 species of African starlings by Rubenstein and Lovette (2007) which also found a link between cooperatively breeding species, highly seasonal environments and temporal variability in rainfall. If so, cooperative breeding would present an opportunity to maximise fitness under unpredictable and variable breeding conditions (Koenig, Pitelka et al. 1992, Du Plessis, Siegfried et al. 1995, Jetz and Rubenstein 2011). More specifically, if independent breeding is limited by any ecological constraint, such as lack of suitable breeding territories, high predation pressure, or food limitations, individuals would benefit by breeding cooperatively if they obtain indirect fitness by helping to rear close relatives, as well as direct fitness benefits of group-living such as defence from predators (Wright, Berg et al. 2001) and conspecifics (Heinsohn 1988).

In order to disentangle the influence of the environment on the evolution of cooperative breeding from other factors, like phylogenetic relatedness or life history traits (Cockburn and Russell 2011), it is potentially revealing to investigate how different climatic conditions affect individual fitness within the same species of cooperatively breeding bird. This study examines the inclusive fitness benefits to both breeders and helpers in an obligate cooperatively

breeding bird species endemic to Australia, the white-winged chough (*Corcorax melanorhamphos*), and the effect dramatic changes in climatic conditions can have on its society. Choughs are obligate cooperative breeders which means they are always found in groups and that breeding pairs cannot reproduce successfully without help. The members of these groups are usually highly related to each other, and are composed mainly of a monogamous pair and their helper offspring (Heinsohn, Dunn et al. 2000, Beck and Heinsohn 2006). Their reproductive success, both of fledglings produced per nest and offspring surviving to the next breeding season (Heinsohn 1992, Heinsohn 1995), is highly dependent on food availability for the young. Bigger groups are able to support and feed more young than smaller groups (Rowley 1978, Heinsohn 1991a). Dispersal in choughs is considered rare under normal climatic conditions (Rowley 1978, Heinsohn 1991a), and is probably restricted by a lack of breeding opportunities (Beck and Heinsohn 2006). However social stability among choughs appears susceptible to environmental conditions that make foraging difficult. Drought in particular appears to make foraging more difficult for choughs, since they feed on invertebrates obtained by digging and sifting through the leaf litter, which are not readily available in hard dehydrated soils (Heinsohn 2009). Therefore during droughts, mortality increases and this leads to increased dispersal and alliance formation between unrelated individuals (Heinsohn, Dunn et al. 2000, Beck and Heinsohn 2006).

In this chapter I examine the inclusive benefits attained by individual choughs, and how fluctuating environmental factors affect these benefits. I took advantage of demographic data available across five different studies done on these birds under different environmental conditions between 1959 and 2017. I investigated how individuals and populations are affected by different rainfall patterns, and given the likely increase in the number and severity of droughts and high temperatures in Australia due to climate change (Hennessy, Fawcett et

al. 2008), ask if environmental factors such as rainfall variability are likely to affect the costs and benefits of cooperation in this species in the long term.

Methods

White-winged chough data over five studies in the Canberra region

Populations of white-winged choughs have been studied in the Canberra region over five different periods, totalling 27 years, since 1959. I refer to these as the 1960s, the 1980s, the 1990s, 2000s and 2010s studies. The 1960s study was carried out in Eucalypt woodland approximately 20km from Canberra (Rowley 1978) whereas the remaining four studies were carried out in the woodland nature reserves, with very similar floristic characteristics, adjacent to Canberra (Rowley, 1978; Heinsohn, 1992). These study periods differed in the climatic conditions in which they were carried out, and in the technology available to examine population structure. Genetic data for individuals and populations were available for the last three studies (1990s, 2000s and 2010s) only. Mean annual rainfall was higher in the earlier two studies and lower in the later three studies. The 1990s and 2000s studies both included severe drought years. For annual rainfall across the five studies see Figure 1.

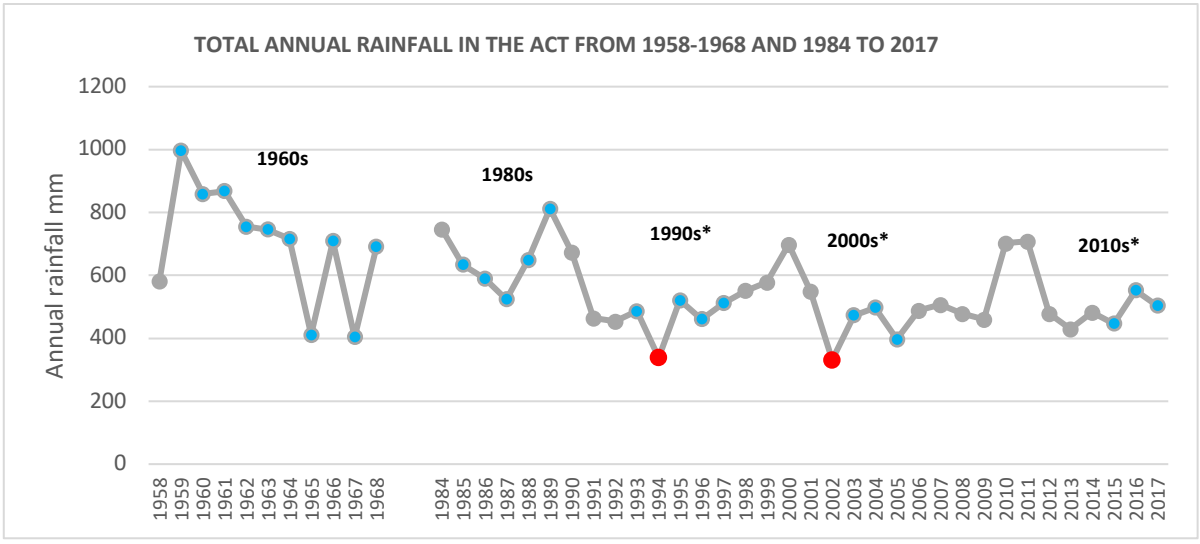


Figure 1. Total annual rainfall (mm) in the Australian Capital Territory between the years 1958 to 1968 and 1984 to 2017 (Bureau of Meteorology, 2108, Available at: http://www.bom.gov.au/climate/data/). The years in which the five different studies have been undertaken are highlighted with blue dots: Rowley (1978) is the 1960s study, carried out between the years 1959 and 1968. Heinsohn (1992) is the 1980s, conducted between 1985 and 1989. Heinsohn et al. (2000) corresponds to the 1990s studied, conducted between 1993 and 1997. Beck et al. (2008) is the 2000s study, conducted between 2002 and 2005. The present study or the 2010s study was conducted between 2015 and 2017. The two most severe drought years are represented by red dots. The last three studies indicated with * include molecular data. The long-term average rainfall for Canberra is 617.4 mm

(Bureau of Meteorology, 2109, Available at: http://www.bom.gov.au/climate/current/annual/act/summary.shtml).

Choughs were studied for the first time by Ian Rowley (1960s study) at Geary's Gap, about 20 kilometres away from Canberra, from 1959 to 1968. This area has streams and stock-dams that provided permanent water sources to choughs (Rowley, 1978). He studied group formation, maintenance and composition including age and sex (determined mainly by laparotomy) of the individuals.

A second period of study on choughs in Canberra took place from 1985 to 1989 (1980s study), a period with no droughts. Group membership was stable over these years with groups mostly growing via retention of offspring from previous years (Heinsohn 1992).

The first molecular study (third study overall) on white-winged choughs' social system was conducted in the early 1990s by Heinsohn et al. (2000). They studied a population of choughs between 1993 and 1997 using DNA fingerprinting techniques. They never observed group fragmentation during 1993, but after a severe drought during 1994 breeding season, preceded by the coldest winter in Canberra in the last 150 years, chough society experienced high mortality -especially of adult females- and breakup of groups that lost their breeding female. Groups became largely composed of multiple small factions of relatives that joined forces to form larger groups, and parentage was often shared by three or more breeders.

A second molecular study (2000s study, fourth study overall) was conducted between 2002 and 2005, and used microsatellite data to analyse within-group relatedness, spatial autocorrelation and genetic structure of the population (Beck and Heinsohn 2006, Beck, Peakall et al. 2008). This study began during a drought that extended from March 2002 to February 2003, when temperatures were also above average. The next two years of the study presented higher temperatures than average, but rainfall was near average after the drought.

In summary, both earlier molecular studies were carried out during periods of high mortality and great social disruption (Heinsohn et al. 2000, Beck et al. 2008).

The study presented here (2010s study) is the third with molecular data and the fifth overall. I studied a population of choughs in Canberra inner-north, from 2015 to 2017, following a long period of environmental stability, with no severe droughts recorded since 2003. I banded 209 choughs mainly from nature reserves, woodland areas and neighbouring suburbs in northern Canberra, Australian Capital Territory, Australia, between August 2015 and March 2018.

In this study, I analysed the genetic relationships of 104 banded choughs from 16 groups. I banded 67-100% of the group members in each case. Three groups were studied in both the 2016-2017 and 2017-2018 breeding seasons but varied in members between years, without taking into account the offspring. Choughs were assumed to have died if they were never seen again since they do not disperse very far (Beck 2006).

Group membership of each individual was recorded during the breeding season, between August and January each year. Individual dispersal during the breeding season was only observed by one individual during the present study.

To keep the results directly comparable to those of previous studies I only included groups of choughs breeding in the woodland reserves adjacent to Canberra, and not those living in the suburbs as previous studies have shown suburban choughs to have different rates of reproduction and mortality (Beck and Heinsohn, 2006; also see Chapter 2).

Further field methods are described in Chapter 2. The Australian National University Animal Ethics Committee approved all field methods.

Genetic methods

Genetic data for this study

To obtain genetic data I used a genotyping by sequencing method via the commercial company Diversity Arrays Technology (DArT Seq) (Jaccoud, Peng et al. 2001). For this study I genotyped 104 individuals, 58 females and 46 males. The individuals came from 16 groups with group size ranging from four to 16 without fledglings (mean = 8.06), and from five to 23 including fledglings (mean = 10.06). See chapter 2 for details of genotyping methods.

Determination of relatedness and parentage

In order to determine relatedness among individuals I used the R package SNPRelate, using Maximum Likelihood Estimation (MLE) (Zheng, Levine et al. 2012). The value of parentage given by MLE was multiplied by two in order to match traditional relatedness coefficients. These results were supported with parentage analysis, so I could determine parents, siblings and half sibling relationships. I repeated DartSeq analyses on 13 individuals that I used as a reference for "self-relatedness". For parentage analysis I used a homozygote opposite test developed by Huisman (2017), which identifies offspring and potential parents that are homozygotes for the same locus but present the opposite allele. Each time a potential parent offspring pair are opposite homozygotes for a locus one point is assigned to the potential parent, which results in selecting the potential parents with the lowest score as the actual parents. With a selection of potential parents with the lowest scores, I performed an additional test that focuses on the heterozygosity of the offspring, and look for a potential mother and father that are not identical homozygotes for that same locus. This test is known as the HIPHOP criterion, since in the case of "Homozygous Identical Parents, Heterozygous Offspring are Precluded", and was developed by Cockburn et al. (unpublished) (Figure 2).

In order to be able to compare relatedness relationships with previous studies, I used the information from the analyses described above to classify the relatedness among individuals as first order or second order relatedness. I classified them as "0.5" if they were first order

relatives (full-siblings, parent-offspring relationships), and as “0.25” if they were second-order relatives (half-siblings, aunt/uncle-niece/nephew, grandparent-grandchild relationships). All other relationships were marked as “0”. Even though kinship was obtained using microsatellite markers in the 1990s and 2000s studies, while we used single nucleotide polymorphisms (SNPs); from previous studies we know that both methods are both sufficient and comparable to estimate kinship (Hauser, Baird et al. 2011).

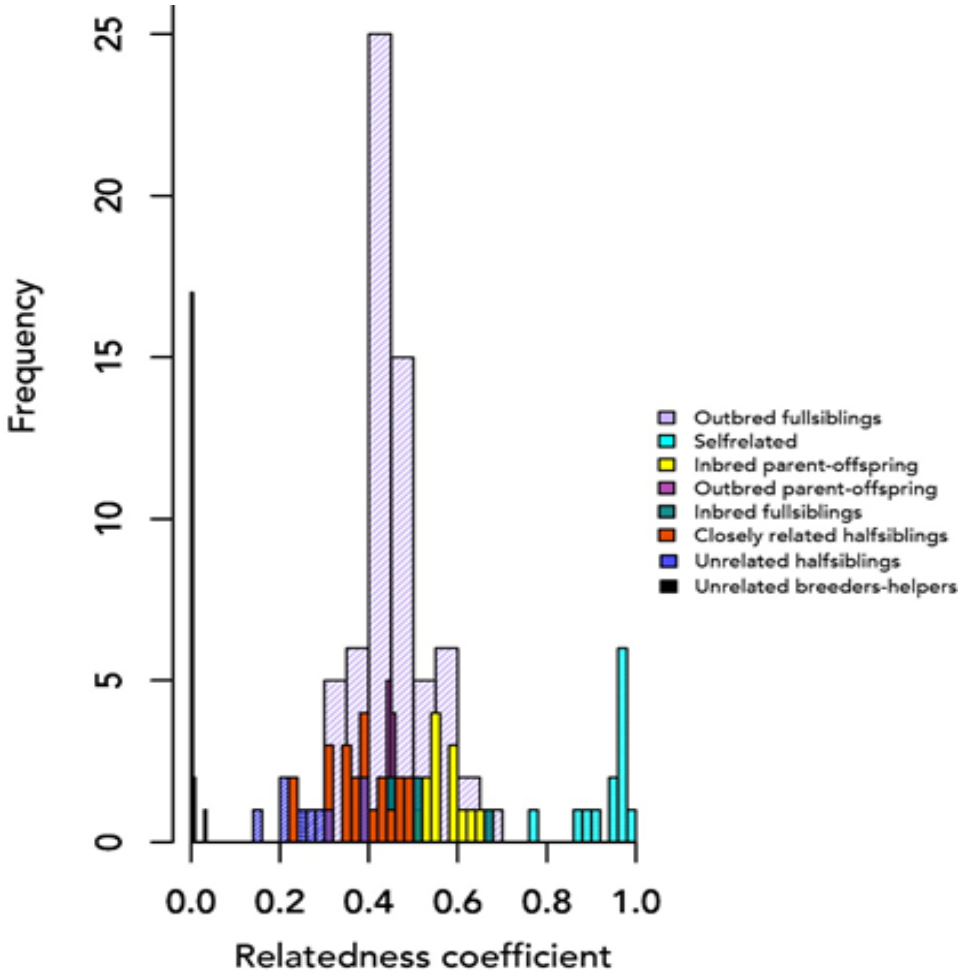


Figure 2. Relatedness coefficients calculated using the Maximum Likelihood Estimation multiplied by 2, and assigned to pedigree relationships among individuals estimated by the HIPHOP test. The histogram shows halfsiblings, siblings and parent-offspring coefficients as a continuum, however they can be distinguished from completely unrelated individuals and self-related individuals. First order relatives mostly share a higher

relatedness coefficients than lower degree relationships. A sample of each relationship category was used to make this figure.

Data collection from previous studies and fitness calculations

Offspring equivalents can be defined as the unit of inclusive fitness (Eberhard 1975). The offspring of diploid organisms share on average half of their genetic material with their parents. This is also true between siblings, which means the value of offspring equivalents of having one sibling is the same as having one offspring, while nieces and nephews share 0.25 of identical genetic material with their aunts and uncles.

In this study, for each group that I had genetic information, I identified all first order relative relationships, such as parent-offspring and full siblings, which were assigned with a 0.5 relatedness coefficient. For second order relatives I assigned a 0.25 relatedness coefficient; and group members related less than 0.25 were assigned with a "0" relatedness coefficient. I used the information from Table 1 from Heinsohn, Dunn et al. (2000) and from Table 2, Chapter 5 in Beck (2006), plus data from the current study, to estimate relatedness coefficients among individuals within their social groups, group sizes, number of fledglings produced per nesting attempt, and sex ratios of these studies. I used data from Rowley (1978) and Heinsohn (1992) to obtain data on fledgling production with respect to group sizes in the studies on choughs carried out in the 1960s and 1980s respectively.

I calculated fitness benefits obtained by breeders in each group per nesting attempt, only for the three last studies that had genetic data (1990s, 2000s, and 2010s) by calculating offspring equivalents per breeder and helper within each group (Hamilton 1964). The first two studies carried out in the 1960s and 1980s did not have any genetic data and were left out of the fitness benefits calculations.

Offspring equivalents per breeder in the genetic studies that only had one breeding pair, corresponded to the total offspring produced by the group. For those groups that had more than two breeders, I estimated the relatedness of each parent to each offspring independently, as I knew the relatedness among parents. I calculated mean relatedness and multiplied it by two to obtain a mean offspring equivalent per breeder value.

To calculate mean offspring equivalents per helper per group I used the relationship of each helper to the parents and the offspring, to establish if each helper was first-order relative to the offspring (siblings), a second-order relative to the offspring (grandparent or aunt/uncle) or non-related to the offspring. I added the relatedness values, and divided them by the number of genotyped helpers in that group to obtain a mean relatedness value per helper, which was multiplied by two in order to obtain the number of offspring equivalents per helper per group.

Statistical analyses

I compared the mean group size between the five studies using an ANOVA and Tukey post hoc tests, being group size the response variable. I also compared mean number of breeders per group between the three later studies that include genetic information, with an ANOVA, with number of breeders per group as the response variable.

I calculated sex ratio as number of adult males divided by the sum of total number of adults on each of the three studies that had information on birds' sex: 1990s, 2000s and 2010s. I performed a Chi-square goodness of fit test to test for significance during each study.

I also looked at the relationship between each group mean relatedness and group size between the three studies with genetic information (1990s, 2000s and 2010s) using three LMs, one per study. The response variable was mean group relatedness and the fixed effect was group size.

In order to examine the relationship between fitness and group size (without offspring) in all five studies I performed a linear model of the relationship between number of offspring per nest –as a proxy of fitness-, selecting group size, study and their interaction as fixed effects. Residuals were examined for linearity and homoscedasticity for all models and no obvious deviations from model assumptions were found. I checked that the interaction between group size and study was significant by performing a likelihood ratio test between the interaction model and the model without the interaction. I also built separate linear models of the relationship between number of fledglings –as a proxy of fitness- and group size, for each of the five studies.

I performed a Linear Model (LM) on the three studies with genetic information (1990s, 2000s and 2010s studies) of the relationship between offspring equivalents per breeder and adult group size; with offspring equivalent per breeder as the response variable, and adult group size, study and their interaction as fixed effects. I performed the same analysis on offspring equivalents per helper for different group sizes.

I used RStudio (Version 1.1.453) (RStudio 2018) for all analyses.

Results

Group size and production of young across five studies

Sex ratio of the 1990s study was 0.671 and significantly male biased ($\chi^2 = 9.89$, $p < 0.01$). Sex ratio of the 2000s and 2010s studies was not significantly biased (2000s: sex ratio = 0.521, $\chi^2 = 0.123$, $p = 0.726$; 2010s: sex ratio= 0.456, $\chi^2 = 0.563$, $p = 0.453$).

There were significant differences in mean group size over the five studies ($F= 11.7$, $df= 4$, $p <0.01$). Pairwise comparisons showed that mean group size from the 1980s, 1990s and 2010s studies was significantly larger than mean group size from the 1960s study. Mean group size in the 1990s study was also larger than mean group sizes in the 2000s study (Tukey test: $p < 0.05$) (Figure 3).

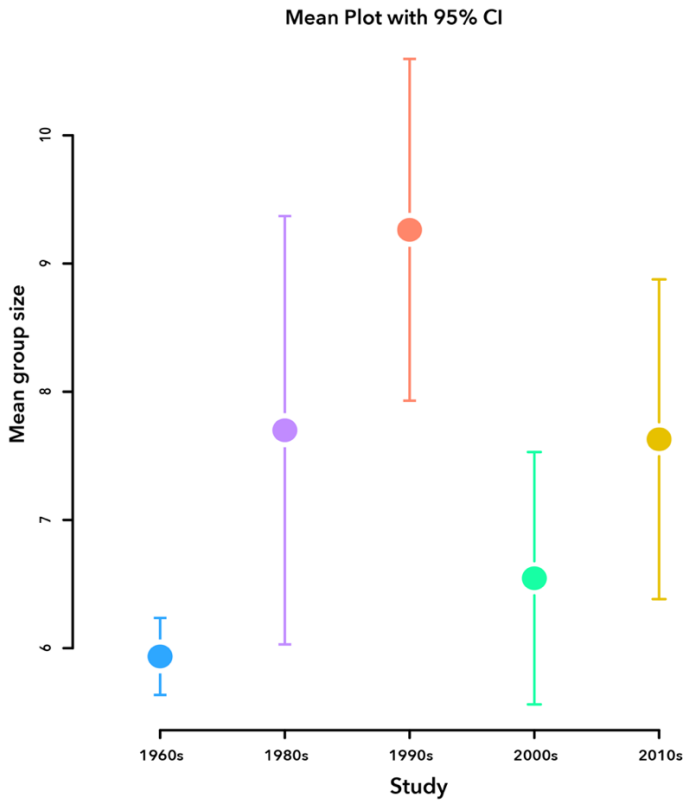


Figure 3. Boxplots showing mean group size and 95% confidence interval per study (1960s (n=186), 1980s (n=20), 1990s (n=19), 2000s (n=22) and 2010s (n=27)). Mean group size was significantly lower for the 1960s study when compared to 1980s, 1990s and 2010s study. Mean group size of the 1990s study was significantly higher than 2000s and 1960s study.

I fitted a linear model of number of fledglings as a function of group size, study and their interaction, which was significant ($F = 2.528$, d.f. = 4 , $p < 0.05$). This model showed a significant positive association between group size and number of offspring when data across all five studies were pooled ($t = 2.414$, p-value < 0.05) (Figure 4).

When the five studies were analysed separately, number of offspring was significantly positively dependent on group size for the three studies conducted during the non-drought periods of the 1960s ($F(1,10)=68.75$, $p<0.01$), the 1980s ($F(1,18)=24.43$, $p<0.01$) and 2010s ($F(1,11)=6.281$, $p<0.05$). Linear models indicated that the number of offspring was significantly but negatively dependent on group size in the first study during drought (1990s, $F(1,12)=20.96$, $p<0.01$); this relationship was not significant for the second study during drought (2000s, $F(1,14)= 3.54e-31$, $p= 1$) (Figure 5).

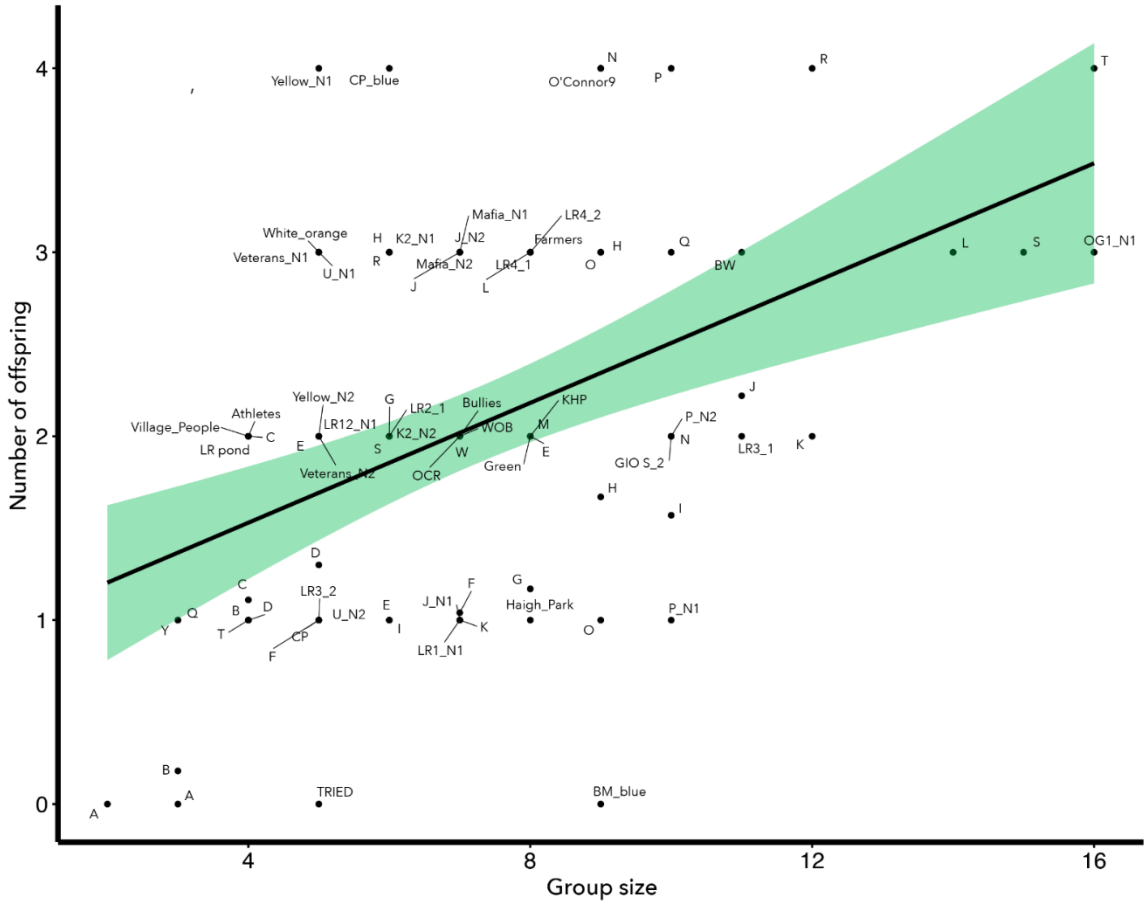


Figure 4. Regression line (and confidence interval) of the relationship between number of offspring produced per group and group size over all five studies (1960s, 1980s, 1990s, 2000s and 2010s). Number of offspring per group was significantly and positively dependent on group size when all groups of all studies were combined.

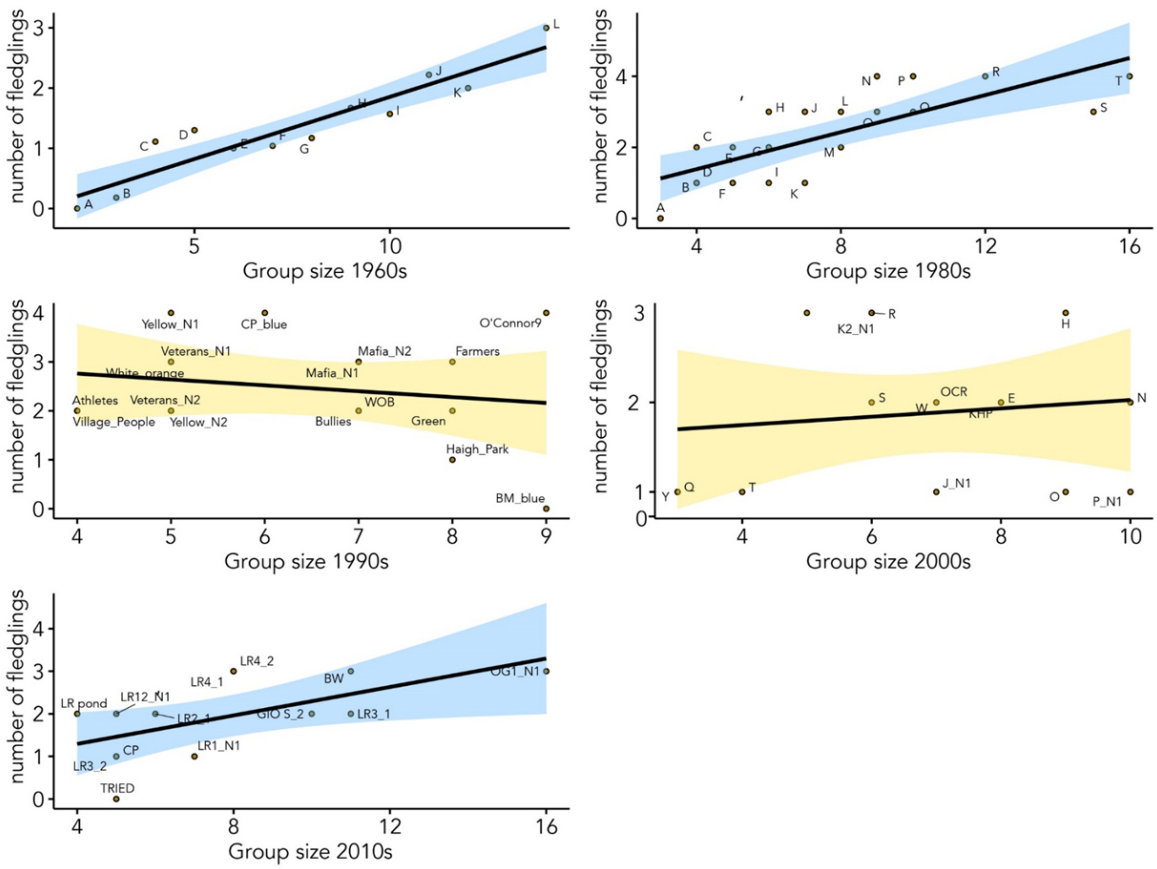


Figure 5. Number of fledglings versus group size for each study. The relationship was significant and positive for the 1960s, 1980s and 2010s studies. It was significant and negative for the 1990s study, and not significant for the 2000s study.

Group composition and inclusive fitness benefits

Linear models showed that mean group relatedness increased significantly with group size in the 2000s study ($F(1,14)= 18.11$, $p < 0.01$) , and was close to significant for the 2010s study ($F(1,11)= 4.602$, $p= 0.055$). The 1990s study did not show a significant association between relatedness and group size ($F(1,12)= 1.197$, $p= 0.295$).

I found no difference in the mean number of breeders per group between the three later studies (Mean for 1990s = 2.53, SD = 0.915; mean for 2000s = 2.44, SD= 0.814; mean for 2010s = 2.08, SD = 0.862). I did find a positive and significant association between number of breeders and group size for the 2010s study ($F(1,11)= 5.232$, $p= 0.043$), but not for the 1990s ($F(1,13)= 0164$, $p= 0.69$) and 2000s ($F(1,13)= 0.201$, $p= 0.66$) studies (Figure 6.).

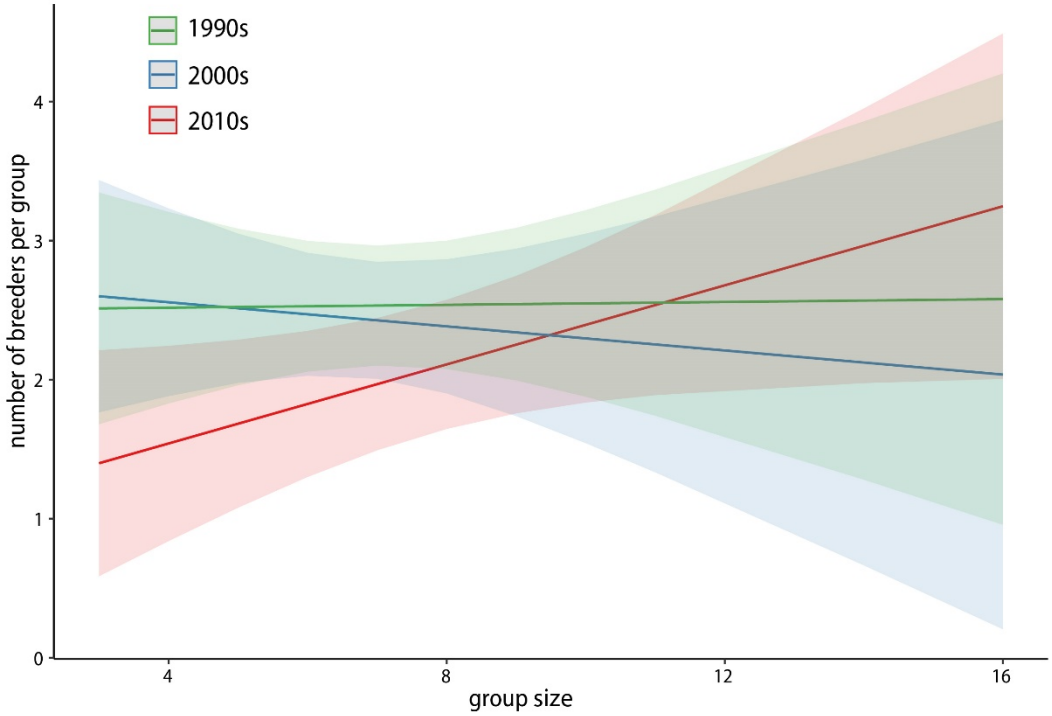


Figure 6. Regression lines (and confidence intervals) depicting relationship between number of breeders per group and group size in the 1990s, 2000s and 2010s studies. Number of breeders per group was significantly and positively dependent on group size in the 2010s, but not in the 1990s and 2000s studies.

The number of offspring equivalents per breeder did not increase with group size when the three studies with molecular data were analysed together ($F(1, 52) = 0.1342$, $p = 0.72$). However there was a significant interaction between study and group size ($F = 4.89$, d.f. = 2, $p < 0.05$) which revealed that the number of offspring equivalents per breeder in the 1990s study fell significantly as group size increased ($t = -2.989$, $p < 0.01$). Group size did not have a significant effect on offspring equivalents per breeder in the 2000s ($t = -0.019$, $p = 0.985$) and 2010s ($t = 0.996$, $p = 0.324$) studies (Figure 7).

The interaction between study and group size for offspring equivalents per helper was significant ($F = 6.78$, d.f. = 2, $p < 0.01$), and revealed different trends across the studies.

Offspring equivalents per helper were significantly and positively dependent on group size in the 2000s study (t = 4.341, p-value < 0.01) and the 2010s (t = 4.133, p-value < 0.01). Each additional helper received 0.21 offspring equivalents in the 2010s study and 0.23 offspring equivalents in the 2000s study. However, offspring equivalents per helper were negatively dependent on group size in the 1990s study, with each additional helper decreasing the offspring equivalents by 0.1 (t = -3.424 , p-value < 0.01) (Figure 8).

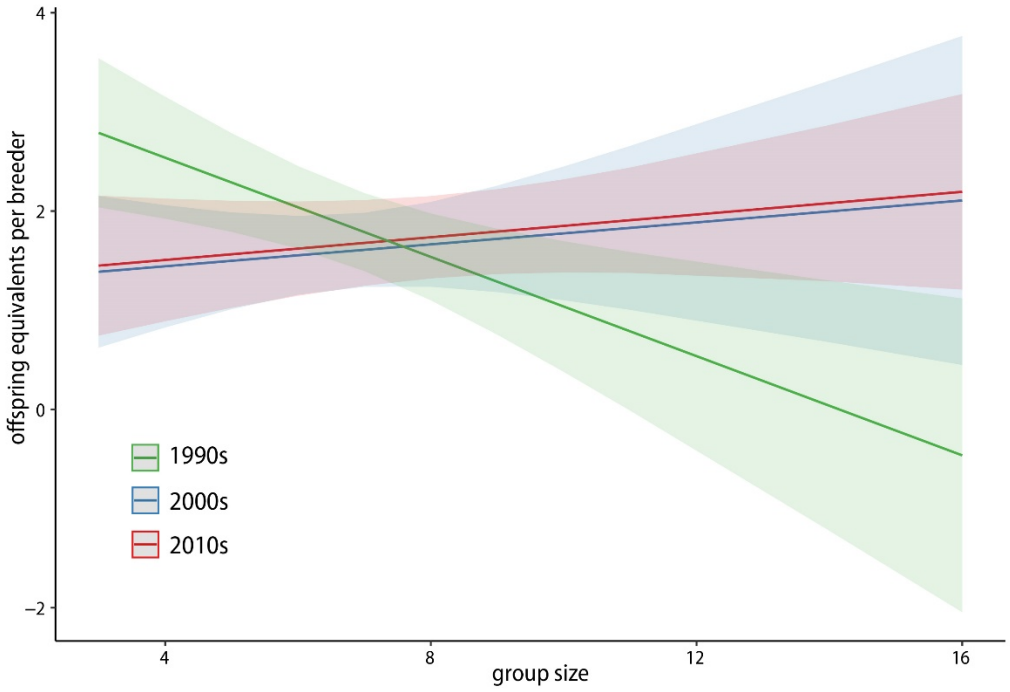


Figure 7. Regression lines (and confidence intervals) depicting relationships between offspring equivalents per breeder and group size for each molecular study. The relationship was significant and negative for the 1990s study; and non-significant for the 2000s and 2010s study.

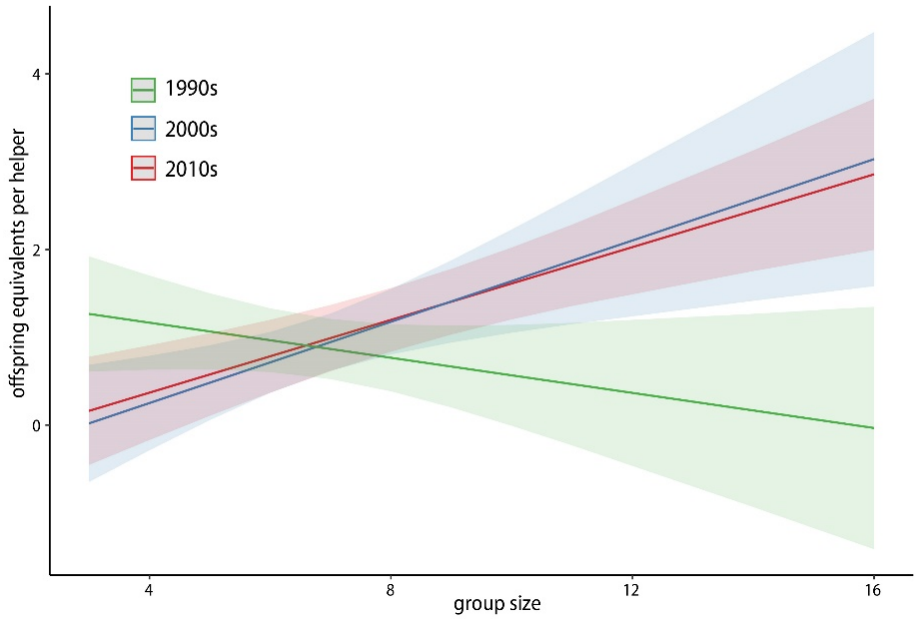


Figure 8. Regression lines (and confidence intervals) depicting relationships between offspring equivalents per helper and group size for each study with genetic data. The relationship was significant and positive for the 2000s and 2010s study, but significant and negative in the 1990s study.

Discussion

Ecological constraints that limit breeding opportunities, together with the inclusive fitness benefits of helping kin to breed, have become a major paradigm for explaining the evolution of cooperative breeding (Stacey and Koenig 1990, Clutton-Brock 2002, Koenig and Dickinson 2004, Hatchwell 2007, Hatchwell 2009). Cooperative breeding has been linked to specific environmental conditions such as highly variable climates; including high inter-annual rainfall variability in passerine birds and high inter-annual temperature variation in non-passerines (Jetz and Rubenstein 2011). Accordingly, environmental variability and unpredictability are suggested to limit survival and reproductive opportunities, leaving group living and the indirect benefits of kin selection as the best route to fitness for many individuals (Koenig, Pitelka et al. 1992, Arnold and Owens 1999, Jetz and Rubenstein 2011). Comparative analyses have been useful for furthering our understanding of the ecological patterns that explain the rise and maintenance of this particular social system (Rubenstein and Lovette 2007, Jetz and Rubenstein 2011, Feeney, Medina et al. 2013) but few studies have attempted to elucidate the impact of fluctuating environmental conditions on the costs and benefits of cooperative breeding in single species. Here I used data from studies of the same species conducted in the same region but over dramatically different environmental conditions over 60 years to elucidate the specific role played by ecological conditions in shaping the costs and benefits of cooperative breeding. By elucidating the fitness drivers under variable climatic conditions it was possible to estimate how inclusive benefits of this behaviour are likely to shift when climate changes in the future.

Habitat saturation in the form of high population density and shortage of breeding vacancies was proposed as an ecological constraint that encourages individuals to become helpers

instead of breeders or floaters (Komdeur 1992). High longevity of breeders may be linked to habitat saturation and is also likely to encourage cooperative breeding (Hatchwell and Komdeur 2000). In choughs, the long time they take to learn how to forage proficiently, limited breeding opportunities, and their need for help when they do breed, may all discourage dispersal (Heinsohn 1991a, Beck, Peakall et al. 2008). Conversely they can gain inclusive fitness by staying with their natal group and helping to raise related young. These pressures to delay dispersal explain the highly genetically structured populations of choughs, with immature and mature birds remaining in their parents' territory for long periods (Heinsohn 1991a, Beck, Peakall et al. 2008).

During the first two studies in the 1960s and 1980s, conducted during periods of near average rainfall, chough dispersal was considered uncommon (Rowley 1978, Heinsohn 1992). However, after drought periods in later studies, an increase in adult mortality opened breeding opportunities, which led to dispersal, group fragmentation and the formation of new groups (Heinsohn, Dunn et al. 2000, Heinsohn 2009). Interestingly, I found that drought periods affected the 1990s and 2000s populations differently. The drought that preceded the 1990s study caused high mortality among female breeders in the population (Heinsohn, Dunn et al. 2000). This led to a significantly male biased sex ratio in that study only, in contrast with non-sex biased populations of the 2000s and 2010s studies. The 1990s study witnessed the formation of an all-male group of choughs (the Mafia) that destroyed the nests of other groups (Heinsohn 2009). Mean group size during this time was also the largest, and was likely to be related to the greater need for safety in numbers as aggression between chough groups increased (Heinsohn, Dunn et al. 2000, Heinsohn 2009). Within-group relatedness was not significantly associated with group size in the 1990s study, most likely due to group disintegration and formation of new groups promoted by the higher number of female

breeding vacancies (Heinsohn, Dunn et al. 2000). In spite of the drought preceding the 2000s study that also led to the formation of new groups, both the 2000s and 2010s studies showed that relatedness among group members went up with group size. In the case of the 2010s population, larger groups were mostly formed by retention of offspring from previous years, as was observed in the non-genetic studies of the 1960s and 1980s (Rowley 1978, Heinsohn 1991a). In the case of the 2000s population, the new groups tended to be formed by related factions of birds, while some other family groups remained intact (Beck 2006).

The biggest similarity I observed between the two chough populations studied during drought (1990s and 2000s) was that the number of fledglings produced did not increase with group size as it did in the other three studies conducted during non-drought periods. The reasons for this are not clear, however, it might be linked to the opening of breeding opportunities during drought periods, which allowed more individuals to breed in the newly formed, but also smaller, groups of choughs. However this result is also likely to be due to higher food availability in wetter conditions such that each chough can make a more substantial contribution to feeding nestlings. This hypothesis is supported by an experimental study that showed that helper contributions at the nest and production of fledglings increased when additional food was supplied in the field (Boland et al 1997). My results suggest that the investment strategy of these cooperative breeding birds is additive in wet conditions, with each extra individual contributing to produce and maintain more offspring; but may be compensatory in dry conditions whereby breeders and possibly other group members reduce their contributions so that a similar number of young are produced at all group sizes (Hatchwell 1999). Another possibility could be related to bigger groups depleting food resources faster during years of droughts, when the amount of invertebrates declines considerably; decreasing the amount of food available for the chicks .

I did not find a significant difference in the mean number of breeders per group between the three molecular studies, and only the 2010s study showed that the number of breeders increased with group size. However this result was probably disproportionately driven by the two largest groups in that study. The largest group had three breeders: one male, father to all the group's offspring, and two first-order relative females, resulting in unusually highly related offspring. Different members from the same social group obtaining different benefits associated with group-living is not a new concept (Baglione, Marcos et al. 2002), and encourages a closer look at the dynamics within groups of cooperative breeding animals. Ultimately, the effect that droughts may have in populations of cooperative breeders may vary depending on which individuals face higher mortality. For instance, if mortality affects helpers and breeders unevenly it would influence the availability of breeding positions (Walters, Copeyon et al. 1992a, Hatchwell and Komdeur 2000), or if it affects males and females differently, repercussions on the populations' sex ratio and dispersal patterns might be different (see chapter 2).

Since the number of breeders was higher in larger groups of choughs during the 2010s study, offspring equivalents per breeder did not increase with group size. I suggest that during years of high rainfall, larger groups of choughs are able to maintain the offspring of more members of their group, enabling some helpers to become breeders without dispersing. During these wet periods, when fledgling starvation is less probable, breeders may also benefit more from other direct benefits from belonging to larger groups such as predator defence (Riehl 2013). In the case of drought years, larger groups were probably unable to produce or maintain more offspring due to a lack of resources, preventing breeders from obtaining extra fitness benefits when belonging to larger groups. However, they still produce offspring during these periods and may benefit from compensatory help and energetic benefits at this time (Hatchwell, 1999).

In other cooperative breeding species that breed with kin, dispersal events are key to avoid inbreeding depression (Daniels and Walters 2000, Nelson-Flower, Hockey et al. 2012). Breeding during droughts, choughs could benefit from additional genetic diversity as new unrelated members join the groups.

Within group relatedness and offspring equivalents for helpers increased with group size in the 2000s and 2010s studies meaning that each additional helper obtained inclusive fitness via increasing production of young. However, inclusive fitness benefits for helpers decreased during the 1990s study, because larger groups were comprised of unrelated individuals. Variable inclusive fitness benefits according to environmental conditions is an important consideration for understanding the costs and benefits of cooperative breeding for white-winged choughs. Negative impacts on reproduction and inclusive fitness benefits during drought suggest that the advantages of cooperative breeding during these periods may be comprised of other direct benefits such as increased survival and genetic diversity.

The interaction of evolutionary mechanisms promoting cooperative breeding is intricate, not only affecting different species in different ways, but also varying in their influence within the same species under different environmental conditions. From this viewpoint, an important consideration is related to the environmental variability that characterises Australia, which is regularly affected by floods and droughts induced by ENSO events (Heinsohn 2009). Given a predicted increase in extreme climatic events in the world and the Australian continent, such as an increase in the severity of droughts and floods (Trenberth 2007, Møller, Fiedler et al. 2010), it is relevant to contemplate the effects they will have in cooperatively breeding birds, as this mating system is highly determined and influenced by environmental conditions. This is especially relevant in Australia, where 22% of old endemic passerines are cooperative breeders, compared to nine per cent for the rest of the world (Cockburn 2006, Heinsohn 2009).

Future long-term studies of species varying in the manifestation of cooperative breeding under different environmental conditions would greatly benefit our understanding of the evolution of this mating system, and its future directions.

References

Arnold, Kathryn E, and Ian PF Owens. 1999. 'Cooperative breeding in birds: the role of ecology', *Behavioral Ecology*, 10: 465-71.

Baglione, Vittorio, José M Marcos, Daniela Canestrari, and Jan Ekman. 2002. 'Direct fitness benefits of group living in a complex cooperative society of carrion crows, Corvus corone corone', *Animal Behaviour*, 64: 887-93.

Beck, R Peakall, and R Heinsohn. 2008. 'Social constraint and an absence of sex-biased dispersal drive fine-scale genetic structure in white-winged choughs', *Molecular ecology*, 17: 4346-58.

Beck, Nadeena. 2006a. 'Causes and consequences of dispersal in an obligate cooperative breeder, the white-winged chough (*Corcorax melanorhamphos*)', The Australian National University.

Beck, N. R., & Heinsohn, R. 2006b. 'Causes and consequences of dispersal in an obligate cooperative breeder, the white-winged chough (*Corcorax melanorhamphos*)', The Australian National University.

Beck, Nadeena R, and Robert Heinsohn. 2006. 'Group composition and reproductive success of cooperatively breeding white-winged choughs (Corcorax melanorhamphos) in urban and non-urban habitat', *Austral ecology*, 31: 588-96.

Brown, Jerram L. 1978. 'Avian communal breeding systems', *Annual Review of Ecology and Systematics*, 9: 123-55.

Brown, Jerram L. 1987. *Helping and communal breeding in birds: ecology and evolution* (Princeton University Press: Princeton).

Browning, LE, SC Patrick, LA Rollins, SC Griffith, and AF Russell. 2012. 'Kin selection, not group augmentation, predicts helping in an obligate cooperatively breeding bird', *Proceedings of the Royal Society of London B: Biological Sciences*, 279: 3861-69.

Clutton-Brock, Tim. 2002. 'Breeding together: kin selection and mutualism in cooperative vertebrates', *Science*, 296: 69-72.

Cockburn, Andrew. 1998. 'Evolution of helping behavior in cooperatively breeding birds', *Annual Review of Ecology and Systematics*: 141-77.

Cockburn, Andrew. 2006. 'Prevalence of different modes of parental care in birds', *Proceedings of the Royal Society B: Biological Sciences*, 273: 1375-83.

Cockburn, Andrew, and Andrew F Russell. 2011. 'Cooperative breeding: a question of climate?', *Current Biology*, 21: R195-R97.

Daniels, Susan J, and Jeffrey R Walters. 2000. 'Between-year breeding dispersal in red-cockaded woodpeckers: multiple causes and estimated cost', *Ecology*, 81: 2473-84.

Du Plessis, Morné A, W Roy Siegfried, and Adrian J Armstrong. 1995. 'Ecological and life-history correlates of cooperative breeding in South African birds', *Oecologia*, 102: 180-88.

Eberhard, Mary Jane West. 1975. 'The evolution of social behavior by kin selection', *The Quarterly Review of Biology*, 50: 1-33.

Emlen, Stephen T. 1982. 'The evolution of helping. I. An ecological constraints model', *American Naturalist*: 29-39.

Feeney, William E, I Medina, M Somveille, R Heinsohn, ML Hall, RA Mulder, JA Stein, RM Kilner, and Naomi E Langmore. 2013. 'Brood Parasitism and the Evolution of Cooperative Breeding in Birds', *Science*, 342: 1506-08.

Ford, HughA, Harry Bell, Ray Nias, and Richard Noske. 1988. 'The relationship between ecology and the incidence of cooperative breeding in Australian birds', *Behavioral Ecology and Sociobiology*, 22: 239-49.

Hamilton, William D. 1964. 'The genetical evolution of social behaviour. II', *Journal of theoretical biology*, 7: 17-52.

Hatchwell, and Komdeur. 2000a. 'Ecological constraints, life history traits and the evolution of cooperative breeding', *Animal Behaviour*, 59: 1079-86.

Hatchwell, B. J., and J. Komdeur. 2000b. 'Ecological constraints, life history traits and the evolution of cooperative breeding', *Animal Behaviour*, 59: 1079-86.

Hatchwell, Ben J. 2007. 'Avian reproduction: role of ecology in the evolution of cooperative breeding', *Current Biology*, 17: R845-R47.

Hatchwell, Ben J. 2009. 'The evolution of cooperative breeding in birds: kinship, dispersal and life history', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364: 3217-27.

Hatchwell, Ben J. 2010. 'Cryptic Kin Selection: Kin Structure in Vertebrate Populations and Opportunities for Kin-Directed Cooperation', *Ethology*, 116: 203-16.

Hatchwell, BJ. 1999. 'Investment strategies of breeders in avian cooperative breeding systems', *The American Naturalist*, 154: 205-19.

Hauser, Lorenz, Melissa Baird, RAY Hilborn, Lisa W Seeb, and James E Seeb. 2011. 'An empirical comparison of SNPs and microsatellites for parentage and kinship assignment in a wild sockeye salmon (Oncorhynchus nerka) population', *Molecular ecology resources*, 11: 150-61.

Heinsohn, Robert, Peter Dunn, Sarah Legge, and Michael Double. 2000. 'Coalitions of relatives and reproductive skew in cooperatively breeding white-winged choughs', *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 267: 243-49.

Heinsohn, Robert G. 1988. 'Inter-group ovicide and nest destruction in cooperatively breeding white-winged choughs', *Animal behaviour*, 36: 1856-58.

Heinsohn, Robert G. 1991a. 'Slow learning of foraging skills and extended parental care in cooperatively breeding white-winged choughs', *American Naturalist*: 864-81.

Heinsohn, Robert G. 1992. 'Cooperative enhancement of reproductive success in white-winged choughs', *Evolutionary Ecology*, 6: 97-114.

Heinsohn, Robert G. 1995. 'Hatching asynchrony and brood reduction in cooperatively breeding white-winged choughs Corcorax melanorhamphos', *Emu*, 95: 252-58.

Heinsohn, Robert G. 2009. 'White-winged Coughs: The social consequences of Boom and Boost ' in, *Boom and Bust: Bird Stories for a Dry Country* (CSIRO Publishing: Collingwood).

Hennessy, Kevin, R Fawcett, D Kirono, F Mpelasoka, D Jones, J Bathols, P Whetton, M Stafford Smith, M Howden, and C Mitchell. 2008. 'An assessment of the impact of climate change on the nature and frequency of exceptional climatic events', *Australian Government Bureau of Meteorology: Melbourne*.

Huisman, Jisca. 2017. 'Pedigree reconstruction from SNP data: parentage assignment, sibship clustering and beyond', *Molecular ecology resources*, 17: 1009-24.

Jaccoud, Damian, Kaiman Peng, David Feinstein, and Andrzej Kilian. 2001. 'Diversity arrays: a solid state technology for sequence information independent genotyping', *Nucleic acids research*, 29: e25-e25.

Jetz, Walter, and Dustin R. Rubenstein. 2011. 'Environmental Uncertainty and the Global Biogeography of Cooperative Breeding in Birds', *Current Biology*, 21: 72-78.

Koenig, Walter D, and Janis L Dickinson. 2004. *Ecology and evolution of cooperative breeding in birds* (Cambridge University Press: United Kingdom).

Koenig, Walter D, Frank A Pitelka, William J Carmen, Ronald L Mumme, and Mark T Stanback. 1992. 'The evolution of delayed dispersal in cooperative breeders', *Quarterly Review of Biology*: 111-50.

Komdeur, Jan. 1992. 'Importance of habitat saturation and territory quality for evolution of cooperative breeding in the Seychelles warbler', *Nature*, 358: 493.

Krakauer, Alan H. 2005. 'Kin selection and cooperative courtship in wild turkeys', *Nature*, 434: 69-72.

Møller, Anders Pape, Wolfgang Fiedler, and Peter Berthold. 2010. *Effects of climate change on birds* (Oxford University Press: New York).

Nam, Ki-Baek, Michelle Simeoni, Stuart P Sharp, and Ben J Hatchwell. 2010. 'Kinship affects investment by helpers in a cooperatively breeding bird', *Proceedings of the Royal Society B: Biological Sciences*, 277: 3299-306.

Nelson-Flower, Martha J, Philip AR Hockey, Colleen O'Ryan, and Amanda R Ridley. 2012. 'Inbreeding avoidance mechanisms: dispersal dynamics in cooperatively breeding southern pied babblers', *Journal of Animal Ecology*, 81: 876-83.

Riehl, Christina. 2013. 'Evolutionary routes to non-kin cooperative breeding in birds', *Proceedings of the Royal Society of London B: Biological Sciences*, 280.

Rowley, Ian. 1978. 'Communal activities among White-winged Choughs Corcorax melanorhamphus', *Ibis*, 120: 178-97.

RStudio, Team. 2018. "RStudio: integrated development for R.(RStudio, Inc., Boston, MA, USA)." In.

Rubenstein, Dustin R, and Irby J Lovette. 2007. 'Temporal environmental variability drives the evolution of cooperative breeding in birds', *Current Biology*, 17: 1414-19.

Russell, Andrew F, and Ben J Hatchwell. 2001. 'Experimental evidence for kin-biased helping in a cooperatively breeding vertebrate', *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268: 2169-74.

Stacey, Peter B, and Walter D Koenig. 1990. *Cooperative breeding in birds: long term studies of ecology and behaviour* (Cambridge University Press: New York).

Trenberth, Kevin E. 2007. 'Warmer oceans, stronger hurricanes', *Scientific American*, 297: 44-51.

Walters, Jeffrey R, Carole K Copeyon, and JH Carter III. 1992a. 'Test of the ecological basis of cooperative breeding in red-cockaded woodpeckers', *The Auk*: 90-97.

Wright, Jonathan, Elena Berg, Selvino R De Kort, Vladimir Khazin, and Alexei A Maklakov. 2001. 'Cooperative sentinel behaviour in the Arabian babbler', *Animal Behaviour*, 62: 973-79.

Zheng, Xiuwen, David Levine, Jess Shen, Stephanie M. Gogarten, Cathy Laurie, and Bruce S. Weir. 2012. 'A high-performance computing toolset for relatedness and principal component analysis of SNP data', *Bioinformatics*, 28: 3326-28.

CHAPTER 4

Personality measures in an obligate cooperatively breeding bird reveal within-group similarities in boldness

Abstract

In the last two decades the consistency of individual differences in behaviour, or animal personalities, have been explored from an evolutionary perspective as phenotypic traits shaped by natural selection. However, studies of the importance of animal personality in group-living species is only in its initial stages. Considering that some studies have found that the interaction between individuals can influence individuals' personalities and ultimately affect their adaptive capacity, it is especially important to take into account the influence of individual personalities, and how they interact, in highly social species. In this study, I explored whether individual differences in behaviour are linked to the social structure of a cooperatively breeding bird species the white-winged chough (*Corcorax melanorhamphos*). Choughs were tested for consistent individual differences in boldness by recording their flight initiation distance (FID), a metric widely used as a proxy for boldness as a personality trait. FID was not affected by individual traits including age, sex and breeding status, and nor was FID a predictor of individual dispersal behaviour. FID was significantly affected by extrinsic factors including the number of birds around the focal individual, the number of tests and the interval between them, all of which led to decreases in FID. Choughs became habituated to the tests and bolder throughout the study, but there were no significant differences between individuals in this measure. Contrary to predictions that boldness would vary according to individual traits within groups, I found no variation within social groups. Habitat type affected FID, with choughs in natural woodlands displaying significantly higher FID values than those living near areas occupied by humans. These results suggest there is either phenotypical plasticity in boldness with those living near humans becoming more habituated to their presence, or that boldness in choughs is selected for when they live near humans. This study shows that groups of white-winged choughs are composed of individuals displaying similar

personality traits rather than a combination of different personality traits, though the reasons for individuals converging on consistently similar behaviour remain unclear.

Introduction

Consistent individual differences in behaviour, "behavioural phenotypes" or "animal personality", refer to the differences in behaviour between individuals that persist through time and across situations (Sih, Bell et al. 2004, Réale, Reader et al. 2007, Székely, Moore et al. 2010, Carter, Feeney et al. 2013); and have been studied in a wide array of taxa, including both vertebrates and invertebrates (Groothuis and Carere 2005, Bell, Hankison et al. 2009). Accordingly, behaviours that reflect personality are consistent, meaning these are less variable within-individuals than they are among individuals. In this way, even though behavioural traits may change as individuals age or use different environments, the differences between individuals will be maintained (Réale, Reader et al. 2007). The adaptive nature and variability of personality traits within and between individuals defines their ecological and evolutionary importance, as they add to the capacity of populations to persist in changing environments (Dall, Houston et al. 2004, Carrete and Tella 2009).

The first step towards establishing a particular behaviour as a personality trait is to verify that the behaviour is repeatable, meaning that the results of the test are consistent when performed at different times (Bell, Hankison et al. 2009, Carter, Feeney et al. 2013). A meta-analysis on the repeatability of behaviour conducted by Bell, Hankison et al. (2009) found that around 35% of behavioural phenotypic variation within populations was explained by individual differences. Yet, when measuring personality in the field there are many factors that can influence its repeatability that should be accounted for, such as difference in environmental conditions and habituation to the implemented test (Martin and Réale 2008).

Social context can be a crucial environmental influence to consider when studying animal personality (Laskowski and Pruitt 2014, Koski and Burkart 2015, Edwards, Burke et al. 2017). This is because the interaction between individuals can influence their personalities and

ultimately affect their adaptive capacity. Furthermore, the social environment can influence the behavioural responses of individuals (Webster and Ward 2011) by making them behave more similarly, which has been observed in social animal species such as great tits (*Parus major*) (van Oers, Klunder et al. 2005) and threespine sticklebacks (*Gasterosteus aculeatus*) (Webster, Ward et al. 2007). On the other hand, social niche specialisation states that individuals with different personalities are likely to adopt specific roles in their populations, favouring differences between individuals caused by social conflict (Bergmuller and Taborsky 2010). Social conflict would force individuals to have different roles, such as different dispersal (Cote and Clobert 2006) or foraging strategies (Michelena, Sibbald et al. 2008). Here I assessed whether individual personalities of an obligate cooperatively breeding bird species can be measured in the wild, which factors influence this trait, and how it relates to the overall population structure. I studied white-winged choughs (*Corcorax melanorhamphos*), an obligate cooperatively species of bird, which lives year round in permanent stable groups ranging from three to 20 individuals (Rowley 1978) .

I first verified whether individuals in a wild population of choughs had consistent individual differences in a widely studied personality trait, boldness. To obtain that trait, I measured flight initiation distance (FID), which is the distance at which a focal individual flees from a potential approaching predator (Blumstein 2006). FID is widely used to assess individual boldness, and has been proven to be highly repeatable in many bird species; such as burrowing owls (*Athene cunicularia*) (Carrete and Tella 2009), dunnocks (*Prunella modularis*) (Holtmann, Santos et al. 2017) and barn swallows (*Hirundo rustica*) (Møller 2014).

I next examined whether boldness varied among social group members and if members of social groups behaved similarly, to evaluate the role of the social environment in white-winged choughs. Specifically, I was interested in exploring if: (1) birds belonging to the same group

display similar personalities as it has been observed in common marmosets, a species of highly sociable mammals that also lives in stable groups (Koski and Burkart 2015), or (2) on the contrary; groups are formed by individuals that differ in personality traits. From studies in social niche specialisation, it has been observed that individuals that display different personality traits may adopt different social roles within their populations. For example, a study in common lizard (*Lacerta vivipara*) showed that individuals that dispersed from high-density populations, showed less social tolerance, a consistent behavioural trait across time (Cote and Clobert 2006).

Lastly, I asked whether boldness affects individual dispersal in white-winged chough populations. Dispersal in animal societies, which can be defined as the movement of individuals form their natal social or breeding site/group to another site, has profound implications in population genetic structure and dynamics, and is usually linked to life-history traits such as sex and age (Clobert, Baguette et al. 2012). The link between behavioural traits, including boldness, and dispersal patterns is currently a much explored topic (F. Fraser, F. Gilliam et al. 2001, Spiegel, Leu et al. 2017). Differences in personality among dispersing individuals can tell us if a specific personality trait is associated with a specific social role (Bergmuller and Taborsky 2010). In addition, they can also reveal if animals with certain personality traits are the ones moving within the population, ultimately explaining how individuals are organised from a personality perspective within the population.

Methods

Study species and site

I studied an individually banded population of wild white-winged choughs (*Corcorax melanorhamphos*) in nature reserves and on the edges of suburbs in Canberra, A.C.T., Australia (see chapter 2). Choughs live in stable social groups that range from three to 20 birds comprised of a breeding pair and many helpers that are usually their immature (1-4 year old) offspring. During the breeding season that can extend from mid-July to March, the members of a group cooperate in every task related to rearing the young of the breeding birds. During the cold months (from April to the beginning of July) chough groups gather in big foraging flocks of up to 70 birds without losing the identity of their social group (Heinsohn 1987). I collected data for this study during the winter of 2017 and 2018 before breeding had commenced.

Habitat and dispersal

I classified choughs as occupying either suburban or woodland habitat when measured. Most individuals were found in the suburbs and immediate adjacent woodland, from where they move interchangeably during the day. I classified these choughs as "suburban" (n = 85 individuals in 11 groups). The remaining social groups of choughs were found on Lyneham ridge, a woodland area further away where they can mostly be found in winter and during the breeding season. I classified these choughs as "woodland" (n = 20 individuals in three groups) (Figure 1). I also identified individual dispersal by noting whether a bird had ever been observed to move from a group to another.

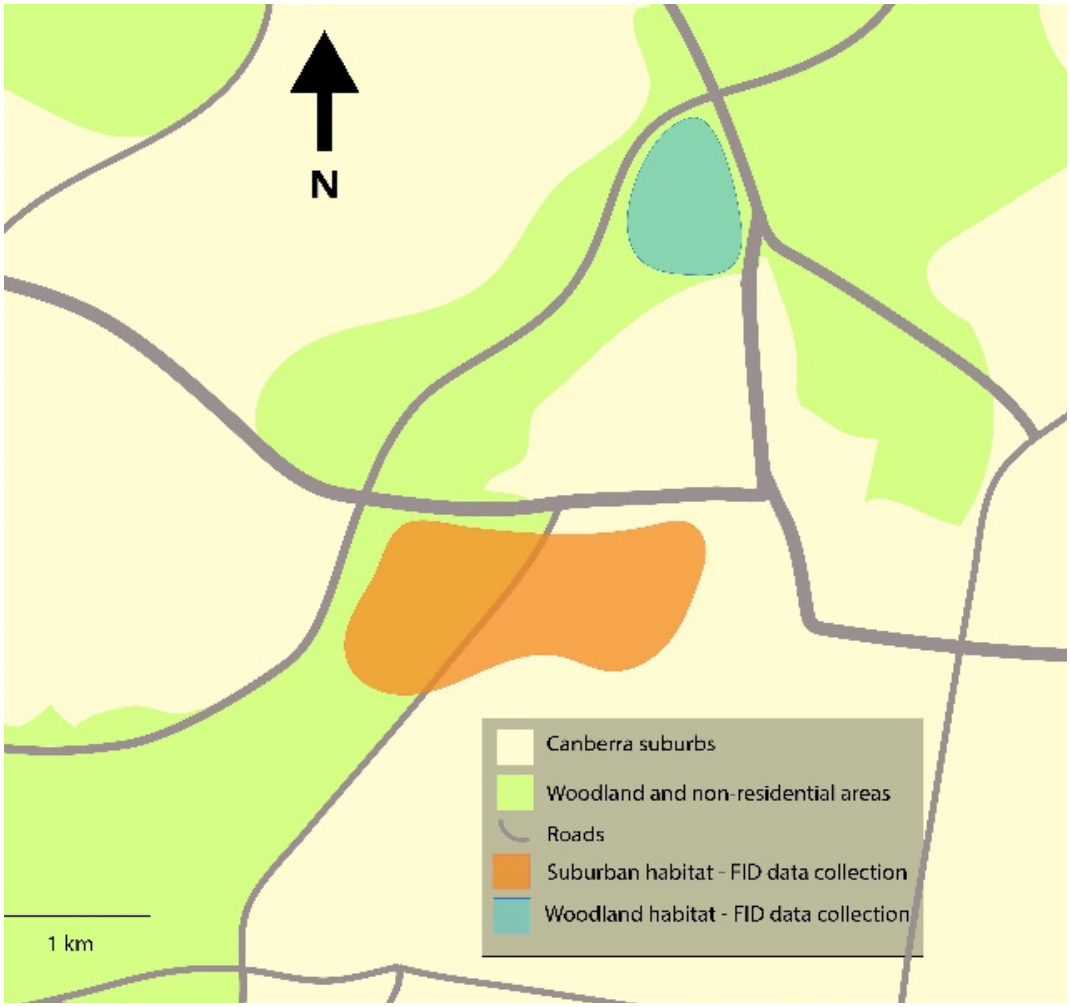


Figure 1. Map showing the different areas where measurements of flight initiation distance (FID) of white-winged choughs were recorded during the autumn and winter of 2017 and 2018. The orange area indicates the "suburban habitat, where choughs move interchangeably during the day. The blue area indicates the "woodland habitat", surrounded mostly by native woodland and grassy non-residential areas.

Age and Breeding status

Each chough's age was determined by the colour of its iris, ranging from one to five years (See Chapter 2 methods, Rowley, 1978). Choughs reach sexual maturity in their fourth year, and are therefore considered as sub-adults if they are three years or younger, and as adults if they are four years or older. Sex and breeding status, if they have ever been breeders, or are helpers within their groups, were determined using molecular techniques (see chapter 2 methods). Chough breeding status was determined using genetic methods (see chapter 2). I classified them as breeders if they were identified as ever having produced offspring. All other individuals measured were classified as helpers.

Flight initiation distance of individuals (FID) and data sampling

I measured flight initiation distance (FID) of 105 individual choughs of which 57 were measured both in 2017 and 2018. FID is a standard method used to measure boldness (Réale, Reader et al. 2007, Weston, McLeod et al. 2012) and entails measuring the distance at which a bird flees in response to the approach of an investigator.

To measure FID, I identified a banded chough (the focal individual) that was not obstructed by other birds or objects and with direct line of sight to an approaching investigator. The focal individual was always the one closest and directly in front to the approaching investigator to minimise the chance of fleeing due to other individuals' cues. The focal chough was always approached by the same investigator (CL) wearing the same clothes, at a constant speed of approximately 1.3 meters/seconds. The approaching speed was measured by following the rhythm of the song "Seven Nation Army" by the White Stripes (practiced multiple times before starting the official data collection), by an investigator with drumming experience. The starting approaching point was set at 15 to 20 m of distance, to reduce the "intruder starting distance" effect (Blumstein 2003). The approaching investigator instantly stopped walking once the focal individual moved away by walking or flying. A second investigator, standing more than 20 m away from the focal individual recorded the following data: date; group and focal individual identity; number of birds that were apart by two chough body lengths or less from the focal individual prior to approach. Lastly, both investigators measured the distance, using a measuring tape, between the starting position of the approaching investigator and the exact position at which the focal individual moved away (FID).

Statistical analyses

All the analyses stem from a global linear mixed effects model built in RStudio version 1.2.1335 (RStudio 2018) using the package *lmerTest* (Kuznetsova, Brockhoff et al. 2017). FID was the response variable and the model included the following fixed effects: individual traits, age (adults or subadult), sex (male or female), breeding status (helper or breeder), and the extrinsic factors, test number (first, second test etc.), days between tests, number of birds around when FID was measured, and year (2017 or 2018). I included the random effects group membership (Group ID), individual ID and date.

I calculated FID repeatability for individual choughs using the interclass correlation coefficient. The coefficient is calculated by dividing the variance among individuals by the total variance within individuals among measurements plus the variance among individuals, and indicates the total phenotypic variance of a trait that can be attributed to differences between individuals (Hayes and Jenkins 1997, Bell, Hankison et al. 2009, Roche, Careau et al. 2016).

Does FID vary between and within groups?

To determine whether FIDs differed between individuals I used a likelihood ratio test between the full model including the random factor individual ID, against the model without the random effect in question. To establish whether groups differed in FID measures I used a likelihood ratio test of the full model including the random factor group ID, against the model without the group ID.

To determine whether individual FIDs differed within groups, I also fitted a model adding a nested random effect of individual ID within each group (Individual ID within Group ID). I performed a likelihood ratio test of the model including the nested random effect, against the model without the nested random effect.

Since FID was shown to become progressively shorter as an individual was repeatedly tested, I used a linear mixed-effects model to test whether habituation differed significantly between individuals. To do this I included an interaction between animal ID and test number as a random factor in the main model, which allows each individual to have an intercept of FID and a slope variance for test number. Finally, I used a likelihood ratio test of the full model, against the model without the interaction between animal ID and test number.

Differences in FID according to habitat data were analysed in a separate model, since the difference in number of birds between both habitats was large (n = 20 birds measured in woodland habitat versus n = 85 measured in the suburbs) and confounded the other terms of the original model. I also performed a linear mixed effects model with the same fixed effects to which I added habitat, recorded as a fixed effect (woodland or suburb). Random effects were only individual ID and date, since group ID is correlated to habitat.

Lastly, to test if dispersal was affected by FID, I built a general linear model with binomial distribution. Dispersal was defined as having occurred if an individual was ever observed to move from one group to another. The selected fixed effects were sex, breeding status and individual FID. Group membership was included as a random effect as well as social group size. I obtained a P-value from performing a likelihood ratio test between the full model including group membership, against the model without the effect in question. I did the same with social group size. I checked for residuals normality and homoscedasticity for all models.

Results

Do choughs have consistent flight initiation distances?

The FID of the 105 white-winged choughs measured during the winter of 2017 and 2018, ranged from 39 cm to 1114 cm, with a mean of 403 ± 87 S.D. cm. Focal individuals were on average tested 7.5 times (range = 2-21 times) over a mean interval of 99 days (range = 1 to 342 days). Prior to approach, focal individuals were accompanied by a mean of 2.4 choughs (range = 0 to 15 birds).

FID was significantly different among individuals (X^2 = 121.9, d.f. = 1, p-value < 0.01), confirming the existence of consistent behavioural differences, or personality, among individuals. Using the interclass correlation coefficient (see methods), FID repeatability or individual consistency of the behaviour, was 27%.

Differences among groups were also significant (χ^2 =10.1, d.f. = 1, p-value < 0.01). However FID did not differ among individuals within their social groups (χ^2 =1.97, d.f. = 1, p-value = 0.16) (Table 1, Figure 2).

Is FID affected by individual traits or extrinsic factors?

FID was not significantly affected by the age category, sex or breeding status of the individual. It was also not affected by the year they were measured (Table 1). FID decreased significantly when the number of birds around the focal individual increased (t = -2.58, d.f. = 736.9, p-value < 0.05). Interval of days between tests also had a significant effect on FID, with FID increasing as the interval in days increased (t = 2.06, d.f. = 492.5, p-value < 0.05). Finally, individual FID also significantly decreased with test number (t = -2.38, d.f. =250.9, p-value < 0.05), meaning researchers could get increasingly closer to birds after each time they were tested. (Table 1).

There were no significant differences between slope variances in the number of times FID was tested among individuals (χ^2 = 4.059, d.f. = 2, p-value = 0.1314) meaning that choughs do not show significant differences among individuals in habituation to the test.

Table 1. Effect on flight initiation distance (FID) of age category, sex, breeding status, test number, interval of days between tests were conducted, the number of birds around the focal individual, and the year data were collected. Significance is showed in bold and by "*" if p-value < 0.05.

		FID		
	Trait	t	df	p-value
Fixed effects	**Age category**	-0.67	219.7	0.505
	Sex	-0.28	90.57	0.781
	Breeding status	0.63	108.5	0.53
	Test number	-2.38	250.9	**0.018***
	Interval of days between tests	2.06	492.5	**0.04***
	Number of birds around focal individuals	-2.58	736.9	**0.01***
	Year	-0.12	59.3	0.903
Random effects		**χ2**	**df**	**p-value**
	Individual ID	121.9	1	**< 0.01***
	Group ID	10.1	1	**< 0.01***
	Individual ID : Group ID	1.97	1	0.1603

FID was significantly different between birds measured in woodland and suburbs (t = 3.214, d.f. =133.13, p-value < 0.01), with woodland birds showing higher FID values than suburban ones (suburb mean FID = 382.53 ± S.E. = 6; woodland mean FID = 494.34 ± S.E. = 16.35) (Figure 2).

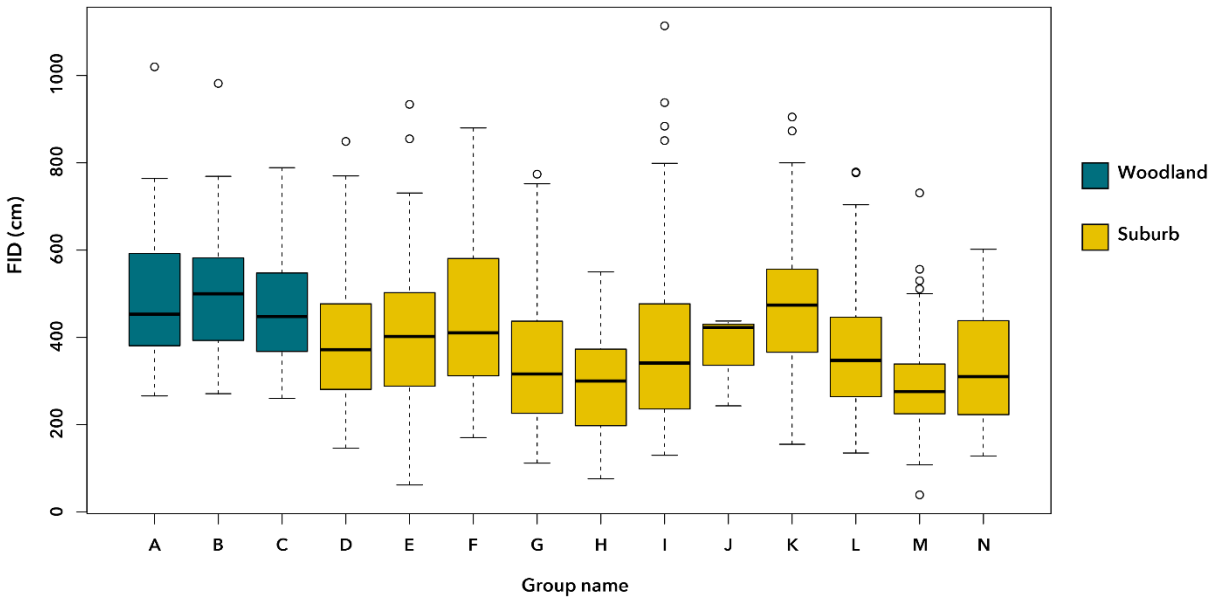


Figure 2. Boxplots of Flight Initiation Distances (FID) measurements by social group. Measurements recorded in woodland habitat are presented in green, while measurements recorded in the suburbs and woodland patch close to it are presented in orange.

Does FID predict individual dispersal?

Individual dispersal to other groups was not significantly affected by individual FID. Dispersal was affected by sex, with males dispersing significantly less than females ($z = -2.167$, $p < 0.05$). Breeding status did not show a significant effect on dispersal, ($z = -0.689$, $p = 0.491$) (Table 2).

Table 2. Effect on dispersal of sex, breeding status and mean FID. All dispersers were adult birds, so this category was not included in the model. P-values lower than 0.05 are indicated by "*"

	Dispersal	
Trait	z value	p-value
Sex	-2.167	**0.0302***
Breeding status	-0.689	0.491
FID	0.093	0.9259

Discussion

In this study, I explore for the first time which factors reflect personality traits in the framework of an obligate cooperatively breeding species of bird. Using FID measurements as a proxy of boldness, I found that white-winged choughs' behavioural phenotypes are consistently different among individuals (Blumstein 2006, Seltmann, Öst et al. 2012) and confirmed that FID is an effective proxy for one dimension of personality in this species. Individuals within groups tended to have similar personalities and habitat also influences boldness, as individuals living further from human habitation tended to be less bold. Choughs showed a degree of progressive habituation to the personality test, but this did not differ significantly among individuals. Dispersing individuals did not differ from non-dispersers in their FID values. Below I discuss how the dynamic behavioural ecology of a group-living species, the white-winged chough, has proven fruitful for research into animal personalities in the context of intense sociality.

I used previous studies as a framework where personality traits assessed in captivity correlate with those measured in the wild (Duckworth 2006, Herborn, Macleod et al. 2010, Cole and Quinn 2011) to identify a method that reflects a highly repeatable behavioural trait in choughs in their natural habitat. It remains important to find a method to measure boldness in isolation from other individuals in white-winged choughs in order to be able to compare between social and non-social contexts (Webster, Ward et al. 2007, Koski and Burkart 2015). This is something I could not have done in a captive setting since choughs react strongly to being handled and kept apart from their group. FID repeatability was 27%, a metric that quantifies the individual consistency of the phenotypic trait (Dingemanse, Both et al. 2002), indicating repeatable inter-individual behavioural differences (Roche, Careau et al. 2016).

Neither sex nor age had an impact on boldness in choughs. However individuals behaved differently, reducing the FID distance, when other conspecifics were in close proximity. This suggests that social environment plays a large role in the choughs' response to predatory threats, and fits with previous observations that individuals become less vigilant when surrounded by more birds (Heinsohn 1987, Beauchamp 2001). Conversely, other studies in birds have found opposite trends, with FIDs increasing when birds were in larger flocks because they flee as soon as the most alert individual becomes alarmed (Fernández-Juricic, Jimenez et al. 2002). Studies on optimal escape theory have established that an individual decides when to escape from a potential predator depending on the costs and benefits associated with it (Cooper Jr and Frederick 2007). The presence of conspecifics has been observed to shorten the FID of individuals, possibly because of the loss of social opportunities, making fleeing more costly when others are around (Cooper 2009). Looking in detail at the life-history traits associated with individuals that are more likely to decrease their FID when others are around, and relating them to their social position within their groups using social network analysis, could reveal the social role of different personality traits (Krause, James et al. 2010).

FID became significantly shorter each time the test was repeated, making choughs less responsive to an approaching investigator. This change in behaviour over time or plasticity, implies habituation to the test (Dingemanse, Kazem et al. 2010). Habituation happened regardless of how many times the test was performed, which, from a practical point of view, means we can re-evaluate the number of necessary tests to measure repeatability and maybe reduce it in future studies (Adolph and Hardin 2007). I also found that the magnitude of habituation decreased when the interval between successive tests was larger. This was also the case in a study of habituation performed in great tits (*Parus major*) showing that exploration behaviour increased with consecutives tests, but declined as the interval between

tests increased, suggesting that memory may play a role in habituation (Dingemanse, Bouwman et al. 2012).

The propensity to habituate to the test can be considered a personality trait itself , reflecting the capacity or range of individual phenotypical plasticity (Dingemanse, Kazem et al. 2010). However, test habituation did not vary among individual choughs, although the low p-value suggests that further study may be warranted. Differences in plasticity related to boldness could potentially expose a meaningful aspect of choughs' capacity to adapt to challenging environments (Dingemanse, Kazem et al. 2010). Individual differences in habituation can provide an opportunity to determine the heritability of boldness in this species, by combining heritability estimates with pedigree information, and establish the evolutionary potential of this behavioural phenotype (Dingemanse, Both et al. 2002, Réale, Reader et al. 2007, Edwards, Burke et al. 2017). Additionally, measuring differences in habituation among choughs could also reveal variation in individuals' learning abilities (Dingemanse, Bouwman et al. 2012).

FID did not differ between breeders and helpers which suggests that boldness is not a personality trait that determines which individual will gain a breeding position in chough social groups. Nevertheless, since some helpers are still too young to become breeders, measuring boldness of helpers close to breeding age, and then comparing those that eventually become breeders with those that do not, would be a more accurate evaluation of the influence of personality in gaining breeding positions.

Interestingly, I did not find significant differences in boldness within social groups. When animals living in social contexts show similar behaviours, they may be facing social processes that encourage conformity, which is the tendency of individuals to adopt the behaviour of most of the group (Efferson, Lalive et al. 2008). Another social process that may influence individuals is facilitation, which occurs when individuals are affected by the presence of other group

members by changing the rate of a behaviour or even doing behaviours they would not do on their own (Zajonc 1965). Group-level similarity in personality traits has also been observed in primates such as the common marmoset (*Callithrix jacchus*) (Koski and Burkart 2015), and chimpanzees (*Pan troglodytes*) (Koski 2011). Koski and Burkart (2015) suggest that since cooperating requires high coordination and consideration of the activities of other group members, this means individuals may ultimately adjust their individual behavioural phenotypes to those of the rest of the group. The marmoset study also proposes that social learning may play a big part in the similarity of personalities within social groups, since learning from others not only requires social proximity but also copying and paying attention to the other group members (Koski and Burkart 2015). From social niche specialisation we might have expected groups to be composed of individuals that differ in personality traits, by adopting different roles within their social groups (Bergmuller and Taborsky 2010). In this way, all social niches would be occupied by individuals with different personalities, driven by social conflict or the availability of different social niches. However, even though boldness did not differ within groups of choughs, it was significantly different among groups. Therefore, it may be the case that the population is comprised of different personality types, however these are not distributed between groups evenly. In this way, cooperation can be favoured within groups, influenced by conformity and facilitation, but each group being comprised of different levels of boldness keeps variability in this personality trait. Differences among social groups also suggest there may be a genetic component of boldness, especially considering groups of choughs are highly related (Beck, Peakall et al. (2008); also see chapters 2 and 3). There may also be environmental effects to which the entire group is exposed. In view of the high plasticity of boldness in choughs, their social environment may play a role in the differences

in boldness among groups, with individuals modifying their behaviour to match their social environment (Koski and Burkart 2015).

The need to account for differences in personality within social groups highlights once again the importance of finding a method to measure boldness, or other personality traits in a non-social context. However, considering that choughs live constantly in the presence of conspecifics, this may be the most informative measure of boldness if we are interested in how they behave in their natural environment. The lack of significant differences in boldness within groups of choughs does not mean this is the case for other personality traits. Diversity in personality traits within populations of social animals has been found in other species, and other traits (Bergmuller and Taborsky 2010).

Another environmental factor that affects differences in FID among chough groups is habitat differences. Choughs measured in suburban areas had lower FIDs than those measured in the woodlands. In some animal species from high predation risk habitats, individuals have been shown to be consistently less bold (Dingemanse, Kazem et al. 2010). This is a general trend in birds, where individuals measured in urban areas have lower FID responses compared to conspecifics in rural areas (Cooke 1980, Møller, Tryjanowski et al. 2015). The effect of urbanisation in bird species is a well-developed field in conservation biology, and changes in anti-predator behaviour in bird species have been well documented (Carrete and Tella 2011, Møller and Ibáñez-Álamo 2012). The underlying causes of differences in FID between urban and woodland individuals may be due to plasticity or habituation to the presence of humans. Combining data on genetic information, urbanisation elements and behavioural phenotypes, could help us investigate the heritability of personality traits. It could also help examining how much FID differences are caused by local adaptation due to natural selection, as it has been

observed in European bird species (Møller, Tryjanowski et al. 2015), versus behavioural plasticity (Sprau and Dingemanse 2017).

Individual FID measures or breeding status were not significant drivers of dispersal. However sex was a significant predictor of dispersal, with adult females (all dispersers were adults) being more likely to disperse from a group in which they were previously observed. Bird dispersal is more often female biased (Dale 2001), and this finding in choughs is consistent with the findings from chapter 2, which found female biased dispersal based on genetic data. Dispersal of helpers is also expected as an inbreeding avoidance mechanism when looking for breeding opportunities outside the natal group (Koenig, Haydock et al. 1998). From a personality perspective, I propose investigating other personality traits that have been observed to be related to dispersal such as exploratory behaviour (great tits *Parus major*) (Quinn et al., 2011; Dingemanse et al., 2003), sociality (common lizard *Lacerta vivipara*) (Cote and Clobert 2006) and aggression (western bluebirds *Sialia mexicana*) (Aguillon and Duckworth 2015). A more comprehensive method would be to focus on behavioural syndromes, which refers to an array of correlated behavioural traits (Sih, Bell et al. 2004), instead of single personality traits to obtain a deeper view of individual choughs' personalities according to the context of dispersal (Sih, Bell et al. 2004). At an individual level, dispersal decisions would not be based only on phenotypical traits, but also on environmental signals (Wey, Spiegel et al. 2015). Therefore accounting for differences between the environments the individuals are moving from would help facilitate the identification of personality traits related to dispersal (Cote and Clobert 2006).

Wey, Spiegel et al. (2015) recommended focusing on the social environment when studying dispersal patterns in relation to personality, given its variability and how it may affect different personality types. In the case of highly social white-winged choughs it seems essential that

future studies account for personality measures related to sociality, such as sociability and aggression (Blumstein, Wey et al. 2009), by applying tests that measure these behavioural traits in the field.

The role of personality within groups of choughs remains a question to be further explored. It is especially important to investigate the degree of specialisation in boldness and how this relates to the breeders' and helpers' positions within their social groups, as this could help explain the traits that increase the chances of individuals becoming breeders. The next step to understand better the adaptive value of personality traits is to test the heritability of this behavioural trait by collecting personality and pedigree data over a larger set of generations. Future studies should focus on the evolutionary implications of personality differences among choughs, especially why individual differences exist, remain constant through time, and how much they change when ecological conditions vary.

References

Adolph, Stephen C, and Johanna S Hardin. 2007. 'Estimating phenotypic correlations: correcting for bias due to intraindividual variability', *Functional Ecology*, 21: 178-84.

Aguillon, Stepfanie M, and Renée A Duckworth. 2015. 'Kin aggression and resource availability influence phenotype-dependent dispersal in a passerine bird', *Behavioral ecology and sociobiology*, 69: 625-33.

Beauchamp, Guy. 2001. 'Should vigilance always decrease with group size?', *Behavioral Ecology and Sociobiology*, 51: 47-52.

Beck, R Peakall, and R Heinsohn. 2008. 'Social constraint and an absence of sex-biased dispersal drive fine-scale genetic structure in white-winged choughs', *Molecular ecology*, 17: 4346-58.

Bell, Alison M, Shala J Hankison, and Kate L Laskowski. 2009. 'The repeatability of behaviour: a meta-analysis', *Animal Behaviour*, 77: 771-83.

Bergmuller, R., and M. Taborsky. 2010. 'Animal personality due to social niche specialisation', *Trends Ecol Evol*, 25: 504-11.

Blumstein, Daniel T. 2003. 'Flight-initiation distance in birds is dependent on intruder starting distance', *The Journal of Wildlife Management*: 852-57.

Blumstein, Daniel T. 2006. 'Developing an evolutionary ecology of fear: how life history and natural history traits affect disturbance tolerance in birds', *Animal Behaviour*, 71: 389-99.

Blumstein, Daniel T, Tina W Wey, and Karisa Tang. 2009. 'A test of the social cohesion hypothesis: interactive female marmots remain at home', *Proceedings of the Royal Society B: Biological Sciences*, 276: 3007-12.

Carrete, Martina, and José L Tella. 2009. 'Individual consistency in flight initiation distances in burrowing owls: a new hypothesis on disturbance-induced habitat selection', *Biology letters*, 6: 167-70.

Carrete, Martina, and José L. Tella. 2011. 'Inter-individual variability in fear of humans and relative brain size of the species are related to contemporary urban invasion in birds', *PloS one*, 6: e18859.

Carter, Alecia J, William E Feeney, Harry H Marshall, Guy Cowlishaw, and Robert Heinsohn. 2013. 'Animal personality: what are behavioural ecologists measuring?', *Biological Reviews*, 88: 465-75.

Clobert, Jean, Michel Baguette, Tim G Benton, and James M Bullock. 2012. *Dispersal ecology and evolution* (Oxford University Press).

Cole, Ella F, and John L Quinn. 2011. 'Personality and problem-solving performance explain competitive ability in the wild', *Proceedings of the Royal Society B: Biological Sciences*, 279: 1168-75.

Cooke, AS. 1980. 'Observations on how close certain passerine species will tolerate an approaching human in rural and suburban areas', *Biological Conservation*, 18: 85-88.

Cooper Jr, William E, and William G Frederick. 2007. 'Optimal flight initiation distance', *Journal of theoretical biology*, 244: 59-67.

Cooper, William E. 2009. 'Flight initiation distance decreases during social activity in lizards (Sceloporus virgatus)', *Behavioral Ecology and Sociobiology*, 63: 1765-71.

Cote, Julien, and J Clobert. 2006. 'Social personalities influence natal dispersal in a lizard', *Proceedings of the Royal Society B: Biological Sciences*, 274: 383-90.

Dale, Svein. 2001. 'Female-biased dispersal, low female recruitment, unpaired males, and the extinction of small and isolated bird populations', *Oikos*, 92: 344-56.

Dall, Sasha RX, Alasdair I Houston, and John M McNamara. 2004. 'The behavioural ecology of personality: consistent individual differences from an adaptive perspective', *Ecology letters*, 7: 734-39.

Dingemanse, Niels J, Christiaan Both, Piet J Drent, Kees Van Oers, and Arie J Van Noordwijk. 2002. 'Repeatability and heritability of exploratory behaviour in great tits from the wild', *Animal behaviour*, 64: 929-38.

Dingemanse, Niels J, Karen M Bouwman, Martijn Van De Pol, Thijs van Overveld, Samantha C Patrick, Erik Matthysen, and John L Quinn. 2012. 'Variation in personality and behavioural plasticity across four populations of the great tit Parus major', *Journal of Animal Ecology*, 81: 116-26.

Dingemanse, Niels J, Anahita JN Kazem, Denis Réale, and Jonathan Wright. 2010. 'Behavioural reaction norms: animal personality meets individual plasticity', *Trends in ecology & evolution*, 25: 81-89.

Duckworth, Renée A. 2006. 'Behavioral correlations across breeding contexts provide a mechanism for a cost of aggression', *Behavioral Ecology*, 17: 1011-19.

Edwards, Hannah A, Terry Burke, and Hannah L Dugdale. 2017. 'Repeatable and heritable behavioural variation in a wild cooperative breeder', *Behavioral Ecology*, 28: 668-76.

Efferson, Charles, Rafael Lalive, Peter J Richerson, Richard McElreath, and Mark Lubell. 2008. 'Conformists and mavericks: the empirics of frequency-dependent cultural transmission', *Evolution and Human Behavior*, 29: 56-64.

F. Fraser, Douglas, James F. Gilliam, Michael J. Daley, An N. Le, and Garrick T. Skalski. 2001. 'Explaining Leptokurtic Movement Distributions: Intrapopulation Variation in Boldness and Exploration', *The American Naturalist*, 158: 124-35.

Fernández-Juricic, E, MD Jimenez, and E Lucas. 2002. 'Factors affecting intra-and inter-specific variations in the difference between alert distances and flight distances for birds in forested habitats', *Canadian Journal of Zoology*, 80: 1212-20.

Groothuis, Ton GG, and Claudio Carere. 2005. 'Avian personalities: characterization and epigenesis', *Neuroscience & Biobehavioral Reviews*, 29: 137-50.

Hayes, Jack P, and Stephen H Jenkins. 1997. 'Individual variation in mammals', *Journal of Mammalogy*, 78: 274-93.

Heinsohn, Robert G. 1987. 'Age-dependent vigilance in winter aggregations of cooperatively breeding white-winged choughs (Corcorax melanorhamphos)', *Behavioral Ecology and Sociobiology*, 20: 303-06.

Herborn, Katherine A, Ross Macleod, Will TS Miles, Anneka NB Schofield, Lucille Alexander, and Kathryn E Arnold. 2010. 'Personality in captivity reflects personality in the wild', *Animal Behaviour*, 79: 835-43.

Holtmann, Benedikt, Eduardo SA Santos, Carlos E Lara, and Shinichi Nakagawa. 2017. 'Personality-matching habitat choice, rather than behavioural plasticity, is a likely driver of a phenotype–environment covariance', *Proceedings of the Royal Society B: Biological Sciences*, 284: 20170943.

Koenig, Walter D, Joseph Haydock, and Mark T Stanback. 1998. 'Reproductive roles in the cooperatively breeding acorn woodpecker: incest avoidance versus reproductive competition', *The American Naturalist*, 151: 243-55.

Koski, and Burkart. 2015. 'Common marmosets show social plasticity and group-level similarity in personality', *Scientific reports*, 5: 8878.

Koski, Sonja E. 2011. 'Social personality traits in chimpanzees: temporal stability and structure of behaviourally assessed personality traits in three captive populations', *Behavioral Ecology and Sociobiology*, 65: 2161-74.

Krause, J, Richard James, and DP Croft. 2010. 'Personality in the context of social networks', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365: 4099-106.

Kuznetsova, Alexandra, Per B Brockhoff, and Rune Haubo Bojesen Christensen. 2017. 'lmerTest package: tests in linear mixed effects models', *Journal of Statistical Software*, 82.

Laskowski, Kate L, and Jonathan N Pruitt. 2014. 'Evidence of social niche construction: persistent and repeated social interactions generate stronger personalities in a social spider', *Proceedings of the Royal Society B: Biological Sciences*, 281: 20133166.

Martin, Julien GA, and Denis Réale. 2008. 'Temperament, risk assessment and habituation to novelty in eastern chipmunks, Tamias striatus', *Animal Behaviour*, 75: 309-18.

Michelena, Pablo, Angela M Sibbald, Hans W Erhard, and James E McLeod. 2008. 'Effects of group size and personality on social foraging: the distribution of sheep across patches', *Behavioral Ecology*, 20: 145-52.

Møller, Anders Pape, Piotr Tryjanowski, Mario Díaz, Zbigniew Kwieciński, Piotr Indykiewicz, Cezary Mitrus, Artur Goławski, and Michał Polakowski. 2015. 'Urban habitats and feeders both contribute to flight initiation distance reduction in birds', *Behavioral ecology*, 26: 861-65.

Møller, AP. 2014. 'Life history, predation and flight initiation distance in a migratory bird', *Journal of evolutionary biology*, 27: 1105-13.

Møller, AP, and Juan D Ibáñez-Álamo. 2012. 'Escape behaviour of birds provides evidence of predation being involved in urbanization', *Animal Behaviour*, 84: 341-48.

Réale, Denis, Simon M Reader, Daniel Sol, Peter T McDougall, and Niels J Dingemanse. 2007. 'Integrating animal temperament within ecology and evolution', *Biological reviews*, 82: 291-318.

Roche, Dominique G, Vincent Careau, and Sandra A Binning. 2016. 'Demystifying animal 'personality'(or not): why individual variation matters to experimental biologists', *Journal of Experimental Biology*, 219: 3832-43.

Rowley, Ian. 1978. 'Communal activities among White-winged Choughs Corcorax melanorhamphus', *Ibis*, 120: 178-97.

RStudio, Team. 2018. "RStudio: integrated development for R.(RStudio, Inc., Boston, MA, USA)." In.

Seltmann, Martin W, Markus Öst, Kim Jaatinen, Shannon Atkinson, Kendall Mashburn, and Tuula Hollmén. 2012. 'Stress responsiveness, age and body condition interactively affect flight initiation distance in breeding female eiders', *Animal Behaviour*, 84: 889-96.

Sih, Andrew, Alison Bell, and J. Chadwick Johnson. 2004. 'Behavioral syndromes: an ecological and evolutionary overview', *Trends in Ecology & Evolution*, 19: 372-78.

Spiegel, Orr, Stephan T Leu, C Michael Bull, and Andrew Sih. 2017. 'What's your move? Movement as a link between personality and spatial dynamics in animal populations', *Ecology Letters*, 20: 3-18.

Sprau, Philipp, and Niels J Dingemanse. 2017. 'An approach to distinguish between plasticity and non-random distributions of behavioral types along urban gradients in a wild passerine bird', *Frontiers in Ecology and Evolution*, 5: 92.

Székely, Tamás, Allen J Moore, and Jan Komdeur. 2010. *Social behaviour: genes, ecology and evolution* (Cambridge University Press).

van Oers, Kees, Margreet Klunder, and Piet J Drent. 2005. 'Context dependence of personalities: risk-taking behavior in a social and a nonsocial situation', *Behavioral Ecology*, 16: 716-23.

Webster, Mike M, and Ashley JW Ward. 2011. 'Personality and social context', *Biological Reviews*, 86: 759-73.

Webster, MM, AJW Ward, and PJB Hart. 2007. 'Boldness is influenced by social context in threespine sticklebacks (Gasterosteus aculeatus)', *Behaviour*: 351-71.

Weston, MA, Emily M McLeod, DT Blumstein, and P-J Guay. 2012. 'A review of flight-initiation distances and their application to managing disturbance to Australian birds', *Emu*, 112: 269-86.

Wey, Tina W, Orr Spiegel, Pierre-Olivier Montiglio, and Karen E Mabry. 2015. 'Natal dispersal in a social landscape: considering individual behavioral phenotypes and social environment in dispersal ecology', *Current Zoology*, 61: 543-56.

Zajonc, Robert B. 1965. 'Social Facilitation', *Science*, 149: 269-74.

CHAPTER 5

Within-group social structure and cooperative activities in an obligate cooperatively breeding bird, the white-winged chough (*Corcorax melanorhamphos*)

Abstract

The social environment of animals may affect them as much as their physical environment. For cooperative breeders, exploring how their social organisation functions can be key to understanding the interaction between their social relationships and cooperative behaviour. I examined the social environment of an obligate cooperatively breeding bird the white-winged chough (*Corcorax melanorhamphos*) using social network analysis, a widely used approach to examine animals' social structure. I looked at patterns of associations among individuals in groups during the breeding season in order to explore if association preferences were linked to age, sex or relatedness. I also explored individual levels of cooperation using feeding fledglings and proximity to them, and how these measures were affected by genetic relatedness to the group, life history traits (age, sex) and social position. I found that in most groups, unrelated adults of the opposite sex showed strong associations, and discuss how this probably reflects the importance of inbreeding avoidance within chough groups. Social position, measured as associations to others, did affect cooperation, with more connected individuals spending more time close to the fledglings. I did not find that relatedness was associated with higher cooperative behaviour within groups, and suggest that cooperation is as much related to the direct benefits individuals can gain from group living, such as future breeding positions, than it is to indirect fitness benefits.

Introduction

The social environment of group-living animals is characterized to a great extent by the complex and non-random associations and interactions among individuals (Sih, Hanser et al. 2009, Hasenjager and Dugatkin 2015). The synthesis of these associations and interactions among individuals of the same species can be defined as a population's social structure (Whitehead 2008). For cooperatively breeding animals, defined as those that breed in groups of three or more, their social environment is connected to most aspects of their lives, influencing their behaviours and fitness outcomes differently (Hasenjager and Dugatkin 2015). Furthermore, cooperating implies social interactions among individuals, which may denote not everyone in a group interacting with everyone to the same extent; with these interactions being different among individuals and with different numbers of individuals (Krause, Croft et al. 2007). Consequently, interactions have important consequences for population level processes, including in the development and maintenance of cooperation (Krause, Croft et al. 2007, Croft, Krause et al. 2009).

Cooperative breeding is enigmatic from an evolutionary perspective because some individuals forgo breeding to help others to reproduce (Hatchwell and Komdeur 2000). Many evolutionary mechanisms have been proposed to account for its emergence, with kin selection presented as one of the main mechanisms at work. Kin selection can occur when individuals help close relatives to rear additional young because they increase their genetic representation in the next generation without reproducing themselves (Hamilton 1964, Griffin and West 2003). The advent of genetic tools that enabled the detection of variable relatedness within social groups has provided new insights into the mechanisms promoting the maintenance of cooperation, including both kin and non-kin directed cooperation in many cooperatively breeding birds' species (Hatchwell 2010, Riehl 2013). Since obligate cooperative breeders constantly find

themselves in social groups, studying their social environment is important for understanding the evolutionary mechanisms underlying and maintaining this social system.

Traditionally, the methods for determining social complexity among animals have focused on measures of group size, and the study of their mating systems. However, these methods do not directly address highly variable relationships between individuals, which have been assumed to be homogeneous (Wey, Blumstein et al. 2008). Social network analysis, a technique used by sociologists since the 1930s to study human relationships, considers the associations and interactions between individuals and uses quantitative measures of these relationships. These quantitative measures allow hypotheses to be tested statistically relating to an individual's phenotypic or demographic features and their position in the social network (Wey et al., 2008; Croft et al., 2011).

Integrating genetic studies of relatedness within groups, and social network analyses describing the social structure of an animal society, has made it possible to examine the importance of kinship and group living in different animals' social structures (Wey and Blumstein 2010, Madden, Nielsen et al. 2012, Carter and Wilkinson 2015). If the relationship between relatedness and social position is complemented with information on relative cooperative effort of individuals, I can also estimate the influence social factors have on cooperative behaviours.

This study focuses on the patterns of associations, relatedness and cooperation within groups of obligately cooperative white-winged choughs (*Corcorax melanorhamphos*). Choughs are an endemic Australian passerine bird that always breeds in groups of three to 20 individuals. Pairs cannot breed unassisted and groups normally include many non-reproductive helpers who remain sexually inactive until they are four years old. Every individual cooperates in all tasks to rear the brood including nest building, incubation, and feeding and defending nestlings and

fledglings (Rowley 1978, Heinsohn and Cockburn 1994). Their closed groups are usually highly related, but relatedness among group members is variable (Beck, Peakall et al. 2008) , which makes them an ideal model to explore the link between kin structured societies and cooperation.

Taking advantage of data for genetic relatedness within five groups of choughs, I first explored their network structure during the breeding season just after the young had fledged. I looked at preferred associations within the groups through spatial proximity, and examine how individual connections vary with individual traits; including relatedness, cooperativeness, age and sex.I then looked at the relationship between cooperative behaviour and social relationships, exploring two cooperative behaviours towards the fledglings and the variation in effort among individuals. I linked variation in cooperative efforts to individual life history traits, relatedness to the group, and to two social network metrics that offer information about individuals' social position within the group. I hypothesised, based on kin selection theory, that individuals highly associated to others in their social group would be more cooperative.

Methods

Study site and population

I studied in detail the social connections of five groups of choughs comprising 44 individuals during the breeding season of 2017-2018, in suburbs and adjoining nature reserves in Canberra. Data collection was designed to be intensive over a relatively short period to control for as many extrinsic factors as possible and was concentrated on the chough groups for which the best genetic data were available. I thus selected five groups of choughs for which the data collected was reliable, being collected by the same investigator each time; uniform, classifying each behavior following the same rules for each group; and therefore comparable. Data were collected over the three-week period after each group had fledged their young so that all group members were moving together over their enlarged home ranges (Rowley 1978). For description of study site and catching methods see Chapter 2.

Choughs from the analysed groups were banded and genotyped (see Chapter 2 methods). Exceptions to this were as follows. One individual from group LR1 was unbanded but I was able to infer through mating observations and relatedness to the offspring that this individual was the breeding female of the group. Another group (Earle) had two individuals that were taken out of all analyses because they were not banded and it was not possible to distinguish between them. GIO group contained two unbanded individuals, but they could be reliably distinguished by eye colour because one was a subadult and the other an adult. They were however left out of analyses that needed sex and relatedness information. The only unbanded chough from LR3 group was also excluded from analyses that needed sex and relatedness information.

For each individual I determined the age up to five years by iris colour (see Chapter 2 methods). I classified choughs as subadults if they were aged three years or younger (sexually immature) and adults if they were aged four years or older (sexually mature). Using genetic data, I determined sex, mean relatedness towards the group (excluding fledglings), and breeding status (breeders or helpers). All methods to determine sex, relatedness and parentage in choughs are described in detail in Chapters 2 and 3.

Data collection

I used spatial proximity between individuals to measure social associations among individuals (Farine 2015). Following other authors (Whitehead 2008) I used the group scan method for sampling. I considered that two birds were associated if they were less than three body lengths apart, a data collection method known as the "chain rule" (Croft, James et al. 2008). I defined my sampling periods as one-minute intervals, considering choughs are constantly busy and moving after the young fledge, in order to feed them. Therefore, after months of observation, one-minute intervals were considered a reasonable amount of time to capture preferred associations, without overrating or underestimating individual connections".

In order to build a robust representation of a social network, a minimum of 20 potential dyadic observations are required (Franks, Ruxton et al. 2010, Davis, Crofoot et al. 2018). Potential observations refer to the recording of two individuals either associated or not associated at the moment the data is collected.

I collected a total of 18 hours and 8 minutes of data for social network analyses and cooperation data across all five groups, which is equivalent to 1088 sampling periods. Data were recorded in intervals ranging from 28 to 104 minutes, with a mean of 56.5 minutes. (Table 1).

Fledglings were left out of social network analyses, because I was interested in the associations among helpers and breeders, and associations and interactions with fledglings were used separately to measure cooperative behaviour.

I recorded two behaviors directed by group members to the fledglings and classified these as cooperative. The first was feeding fledglings, which is considered costly in white-winged choughs and other cooperative breeders (Boland, Heinsohn et al. 1997, Heinsohn and Legge 1999). I recorded feeding events as the number of times an individual placed a food item in the fledgling's beak. For within group comparisons, feeding behaviour was expressed as a proportion relative to their group. I calculated individual feeds per total number of feeding events within the group. Then I calculated an average of the individuals' feeding proportions, and all individuals above the mean where classified in the "high cooperative level", and those below in the "low cooperative level". For comparisons between groups, cooperation by feeding was calculated as a rate of feeding events per minute (see methods in statistical models section). I did not record enough fledgling feeding events for group BT, so I did not analyse their feeding data.

The second behaviour I considered cooperative was the extent to which individuals stayed in close proximity to the young, which is also considered costly in this and other cooperative vertebrates (Clutton-Brock, Gaynor et al. 1998, Heinsohn and Legge 1999). Group members were considered to be in close proximity to the fledglings when they were three or less body lengths apart. This was recorded as a frequency at which birds were next to the fledglings at the end of every one-minute interval. Overall proximity to fledglings was expressed as the proportion of times individuals were close to fledglings each different day observations were collected.

Table 1. Group size without fledglings, the number of fledglings per group, and the number of sampling periods used to build their social networks.

Group name	Group size without fledglings	Number of fledglings	Number of sampling periods
Earle	8	2	245
GIO	10	2	302
BT	6	1	233
LR1	5	2	157
LR2	6	2	135

Data analysis

Social network analyses

I used the R package *asnipe* (version 1.1.11) (Farine 2013) to build five social networks, one for each chough group, using the simple ratio index to define the edges (relationships between individuals), since I observed almost all associations among individuals when collecting data (Farine and Whitehead 2015). In order to test if the general structure of each network was not random, and considering that social network data are not independent, I generated a null model for comparison using 10000 randomized networks generated by permutations with the R package *asnipe* (Farine 2017). I compared the observed network and the random network through a Welch Two Sample t-test.

I conducted a community detection analysis using the R package *igraph* (version 1.2.2) (Csardi and Nepusz 2006) to detect sub-groups of individuals that associate with each other preferentially within the rest of the group (Newman 2006). To identify likely variables that

explained these structured associations, I use the R package *assortnet* (version 0.12) (Farine 2016).

To better understand the relationship between each individual's attributes and its network position, I built networks per sex, age category, cooperative level (classified as high or low, depending if the rate was above or below average), and breeding status per group; comparing mean edge weight per network. I first calculated a test statistic value for the observed networks. I then generated 10000 networks using data-stream permutation in *asnipe* R package, for which 10000 test statistics were calculated. Finally, I calculated the test significance by counting how many times the simulated test statistic was larger or smaller than the observed test statistic, which was divided by 10000, the number of null models used in order to obtain a p-value (Farine 2017). I used the same method to generate p-values for the assortativity analyses detailed below.

To determine if individuals within groups were positively or negatively assorted by their traits, I calculated an assortativity coefficient, a correlation coefficient of associations among individuals that are phenotypically similar or different (Farine 2014), of each group of choughs in relation to sex, age category, cooperative efforts (high or low) and mean relatedness to the rest of the group. If the assortment is positive, it suggests that individuals that share certain phenotype(s) are more likely to associate/interact, and if it is negative it suggests avoidance between similar individuals (Newman 2010, Farine and Whitehead 2015). In order to see if relatedness was correlated with individuals spending more time in proximity, I performed a Mantel test between edge weight and relatedness between individuals.

I calculated two node metrics for each chough, weighted degree and closeness centrality, with the R package *igraph*. Weighted degree measures individuals' gregariousness, and is calculated by adding all edge weights directly connected to that individual (Wey, Blumstein et al. 2008,

Farine and Whitehead 2015), and it highlights the direct connections to others in the group. Closeness centrality can be understood as the potential of a node to reach or be reached by all the rest of the nodes in the network, so it can be used as an indicator of influence. It measures a node's social connections to all the other nodes based on the shortest path lengths (Friedkin 1991, Wey and Blumstein 2010), and therefore it takes into account indirect connectivity to others. Both metrics were calculated to be used in the general mixed linear model describe next, in order to explore the link between cooperativity and individual traits.

Statistical models

To determine if cooperation was associated with individual attributes, I used RStudio (RStudio 2018) and the R package lme4 to build a general mixed linear model of individual cooperation, using a binomial distribution. I used counts of sampling periods (per minute) in which individuals fed a fledgling per individual, against counts of sampling periods with non-feeding events. I did the same for accompanying fledglings, with number of sampling periods in which the bird spent time next to the fledglings against the number of times it was not. I made one model for feeding fledglings, and another for accompanying fledglings, as a function of sex, age category, (adult or subadult) mean relatedness to the rest of the group, breeding status (breeder or helper), weighted degree and closeness centrality. The random effects were group ID and individual ID.

To test whether individuals differed in their cooperative rates, I used a likelihood ratio test. This means I compared the full model described above including the fixed effect individual ID with a null model, excluding individual ID, using an ANOVA test. I did the same with the random effect group ID to determine if cooperative rates differed among groups.

Results

i. Measures of network structure

I found that in four out of five groups, individual associations within groups were non-random when compared to networks generated randomly by permutation (Table 2).

Table 2. Comparison of each observed network with 10000 randomized networks generated by permutation to determine if the association among individuals within groups were random. In all groups except BT, the observed network had non-random patterns of associations among individuals.

Group ID	t	df	p
Earle	4.127	63	< 0.01
GIO	6.337	99	< 0.01
BT	0.298	35	0.768
LR1	3.127	24	< 0.01
LR2	2.890	35	< 0.01

a. Are there preferred associations within groups of choughs?

The community detection analysis showed that all five groups had two distinct sub-groups within. In one group (GIO), one male adult chough spent less time associating with all other group members. In group LR1 the breeding pair were more closely associated with each other than the rest of the group, while in group BT the most associated individuals were the breeding pair of a previous (but not the current) year. In the Earle group the male breeder was more closely associated with an unrelated adult female (not the breeder), while the rest of the group were closely associated with each other. Group LR2 showed two distinct sub-groups: one formed by both breeders and an adult female, and another one containing two adult females and one subadult male (Figure 1).

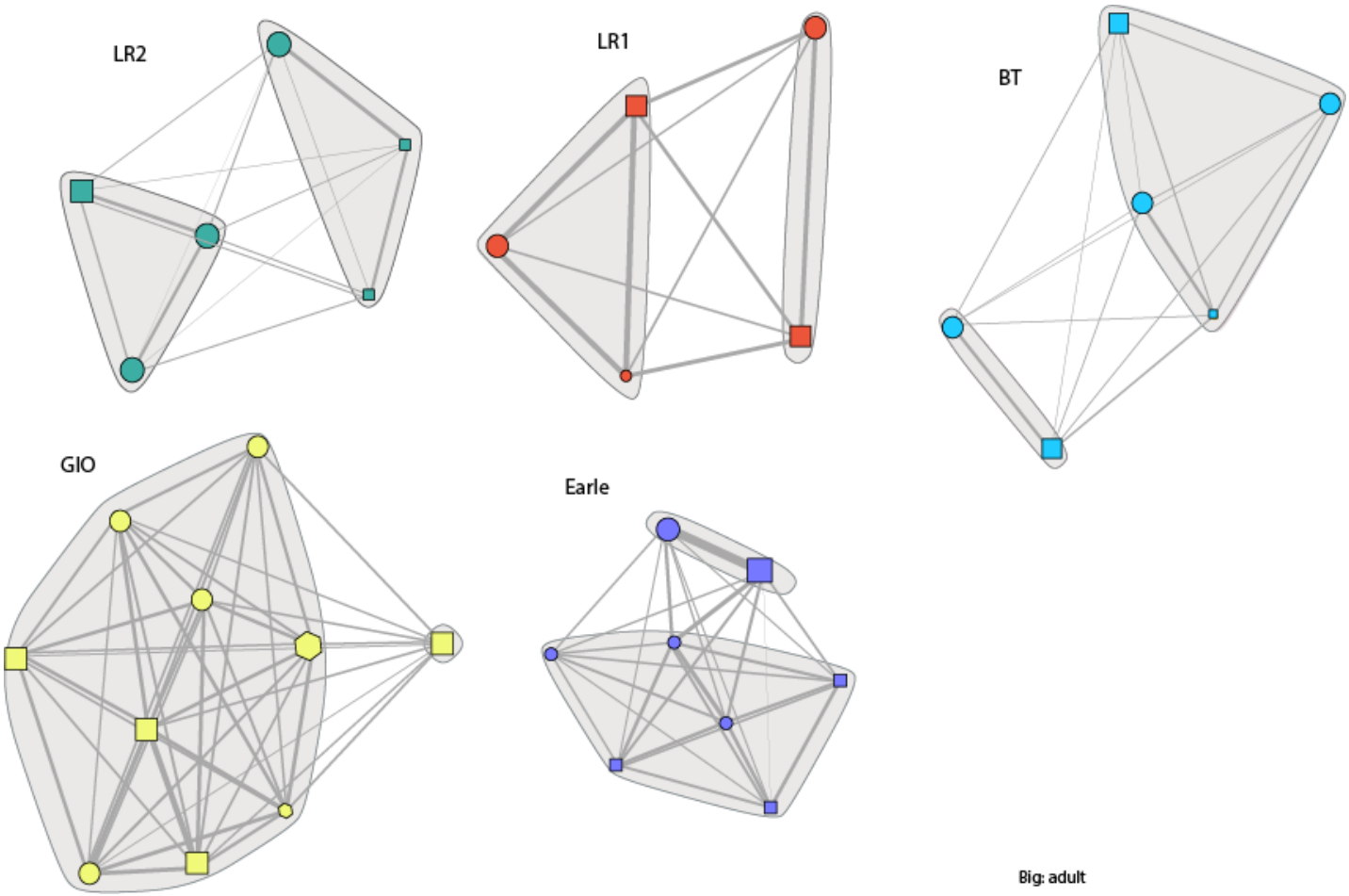


Figure 1. Community detection within groups of choughs. Sub-groups are delimited by grey areas. Colours refer to five different social groups. Line thickness refers to mean edge weight, with thicker lines representing higher connections between individuals. Shapes refer to sex, with squares denoting males, circles females and hexagons unknown sex. Shape size refers to the age category, with small ones representing sub-adults and big ones adults.

b. Are associations within groups stronger among individuals that share the same traits?

Within groups, edges by age category, cooperation level and breeding status were not significantly different in any of the groups analysed. Mean edge weight for males was significantly higher than for females in group BT. (Table 3).

Table 3. Difference in edge weight within groups in networks built by sex, age category, cooperation level, and breeding status. Grey boxes were used to highlight p-values, and hatched boxes indicate groups that did not have enough data for comparison in that category. Significance is shown in bold and by * and calculated by comparing the observed edge weight values with those in networks generated by permutation (see methods).

	Mean edge weight by		Earle	GIO	BT	LR1	LR2
Network	sex	male	0.119	0.142	0.062	0.114	0.083
		female	0.134	0.127	0.038	0.145	0.075
		p-value	0.285	0.611	**0.050***	0.338	0.709
	age category	adult	0.289				0.095
		subadult	0.155				0.099
		p-value	0.451				0.928
	cooperation level proximity to fledglings	high cooperation level	0.167	0.171	0.087	0.236	0.053
		low cooperation level	0.184	0.132	0.102	0.147	0.087
		p-value	0.846	0.056	0.785	0.174	0.269
	cooperation level fledgling feeding	high cooperation level	0.131	0.132	0.037	0.236	0.124
		low cooperation level	0.146	0.148	0.065	0.147	0.108
		p-value	0.947	0.998	0.266	0.168	0.414
	breeding status	breeder		0.146	0.043	0.147	0.111
		helper		0.153	0.077	0.236	0.078
		p-value		0.868	0.490	0.172	0.422

c. Are individuals within groups positively or negatively assorted by their traits?

I did not find that individuals were significantly assorted by sex, age category, cooperation rates and relatedness in any group. Correlation among relatedness and edge weight within groups was not significant (Table 5).

Table 4. Assortativity of edge weight by sex, age, cooperation and relatedness.

	Assortativity edge weight by									
	Sex		Age		Cooperation				Relatedness	
					proximity to baby		feeding			
Group ID	r	p value	r	p value	r	p value	r	p value	r	p value
GIO	-0.183	0.18	only one Sub-adult		-0.127	0.69	-0.197	0.142	0.053	0.388
Earle	-0.212	0.44	0.154	0.2	0.080	0.746	-0.124	0.752	-0.079	0.648
BT	-0.386	0.513	-0.280	0.166	0.141	0.857	-0.30	0.33	-0.282	0.833
LR1	-0.350	0.206	only one Sub-adult		-0.0328	0.789	-0.033	1	0.353	0.175
LR2	-0.184	0.618	-0.004	0.929	-0.219	0.549	0.198	0.524	-0.081	0.64306

Table 5. Correlation between proximity associations (edge weight) and relatedness among individuals within each group of choughs.

Mantel test between edge weight and relatedness		
Group ID	r coefficient	p value
GIO	0.053	0.388
Earle	-0.079	0.648
BT	-0.282	0.833
LR1	0.353	0.175
LR2	-0.081	0.643

d. Is cooperation associated with individual traits?

I found that cooperation rates were significantly different between individuals for accompanying fledglings ($\chi^2 = 109.64$: $p < 0.01$) and feeding fledglings ($\chi^2 = 11.88$; $p < 0.01$). Subadults, birds from smaller groups, and individuals with higher weighted degree measures were significantly more cooperative when it came to staying close to the fledglings (Table 6) (p-value <0.05). Individuals with high closeness centrality showed a tendency of being more cooperative accompanying fledglings, but not significantly. I found that individuals that were significantly more cooperative at feeding fledglings were from smaller groups (p-value < 0.05), and also helpers were more active in these measures ('cooperative') than breeders, although not significantly (Table 6). Sex and relatedness to the group did not have a significant effect in any of the cooperative behaviours.

Table 6. Associations between cooperative behaviors of accompanying (proximity to fledglings) and feeding fledglings according to sex, age category (adults or subadults), mean relatedness to the rest of the group, breeding status, weighted degree and closeness centrality (social network node metrics). Significance is shown in bold and by "*" if < 0.05.

Cooperative behaviour:	Proximity to fledglings			Feeding fledglings		
Traits:	z	df	p-value	z	df	p-value
sex	0.170	1	0.865	0.413	1	0.68
age category	2.234	1	**0.026***	0.211	1	0.833
relatedness	-0.954	1	0.34	1.071	1	0.284
breeding status	0.737	1	0.461	1.729	1	0.084
group size	2.630	1	**0.009***	3.090	1	**0.002***
weighted degree	2.027	1	**0.043 ***	0.681	1	0.496
closeness centrality	1.766	1	0.077	1.033	1	0.302

Discussion

The importance of the social environment in group-living species has been increasingly realised in the last two decades, with more studies focusing on group social structure by examining the interactions among individuals (Lusseau 2003, Wolf, Mawdsley et al. 2007, Madden, Drewe et al. 2009, Schürch, Rothenberger et al. 2010, Aplin, Farine et al. 2013, Wey, Burger et al. 2013). These studies show that the patterns of these social relationships are far from random, and that they can be associated with different individual traits, and that ultimately they will have an impact on evolutionary processes (Krause, James et al. 2015). In this study, I focused on associations among white-winged choughs within their breeding groups based on proximity, when interactions can potentially occur (Farine 2015). White-winged choughs are obligate cooperative breeders, meaning they have to breed in groups, and therefore live in closed and defined groups, making them an ideal model to investigate the relationship between individual traits, social connectedness and cooperative behaviour. In general, network analysis of choughs showed that, within groups, proximity associations did not significantly reflect any preferred or avoided associations according to any traits examined including sex, age or relatedness. However, there was a tendency to spend time with those with opposite attributes, such as unrelated males associating with females.

I explored the patterns of individual proximity within chough groups, and found that they were non-random when compared to networks generated by permutations in all observed groups, except in one group (BT). The outstanding group was unlikely to be due to sample size as the number of observations was larger than the amount collected for other groups (Table 1). However, the fact that randomness could not be rejected in this network does not mean it cannot be analysed for preferred or avoided relationships. For instance, I found patterns within the BT group that were not observed in other groups, such as stronger associations (higher

mean edge weight) among males than females. The three females in BT group were all adults and closely related (first order relatives), while the two adult males were first order relatives, and the subadult male was, respectively, a first and second order relative to each adult. As found by Heinsohn, Dunn et al. (2000), within groups, females with or without relatives can successfully become breeders, whereas males without relatives often fail to gain breeding positions. This is relevant because males' stronger social ties can be related to the importance of gaining social support to become breeders compared to females. Another pattern that might be captured here is the stronger relationship that the only subadult of the group, a male bird in its second year, had with an adult male in the group. In this species, younger individuals benefit from staying in close proximity to adults both for feeding (Heinsohn 1991a) and defense (Heinsohn 1987).

Choughs were not significantly assorted by any other measured trait. However, all groups revealed negative assortativity by sex even though non-significant. These observations were consistent with the community detection analysis (Figure 1, Table 4) in which four out of five groups showed strong associations between unrelated adult individuals of the opposite sex. It is worth noting that the group not showing this effect (GIO) was the only group in which the breeders were closely related (parent-offspring or siblings), and the only group in which the community analysis did not show stronger associations among unrelated individuals of the opposite sex. Therefore I propose that preferred spatial proximity during the breeding season captures the importance of inbreeding avoidance in choughs, a species where group members are often highly related (Beck, Peakall et al. 2008); especially during the breeding season when mating behavior is occurring, (e.g. barnacle geese *Branta leucopsis* (Kurvers, Adamczyk et al. 2013))".

GIO group was the only one in which individuals that showed high levels of cooperation towards fledglings by staying in close proximity, were also significantly more associated in proximity to each other, than less cooperative individuals. This could be explained by group members being closer to each other, simply because they are close to the fledglings at the same time. In many cooperative breeders, ties among group members and fledglings are very close (e.g. Florida scrub-jays (*Aphelocoma coerulescens*) (McGowan and Woolfenden 1990), white-throated magpie-jays (*Calocitta formosa*) (Langen 1996), and pied babblers (*Turdoides bicolor*) (Raihani and Ridley 2007), and this is not different in white-winged choughs (Heinsohn 1991a). It could also be reflecting a widely studied aspect in the evolution of cooperation, which is that more cooperative individuals tend to associate with each other more frequently in structured populations, in order for cooperation to prevail (Ohtsuki, Hauert et al. 2006, Kurvers, Krause et al. 2014).

Finally, I checked which individual traits were most likely to be related to cooperative behaviours across groups. I found that individuals that were more cooperative by staying in close proximity to fledglings showed significantly higher weighted degree (more direct connections to others) and higher closeness centrality. Closeness centrality is a measure of individuals' connectedness to others through the shortest paths. High closeness centrality means that their distance to others is lower and is thus a measure of their social influence (Friedkin 1991, Wey and Blumstein 2010). As above, since choughs that spend more time in close proximity to fledglings are also more likely to encounter other group members that spend more time next to fledglings, the probabilities of establishing higher connections among them are higher. However, considering that I did not find assortativity by cooperation in any group, more central individuals are associating with all individuals within their groups, including the less cooperative ones. Sub adults cooperated more by staying in closer proximity to fledglings than adults, suggesting that younger choughs may gain benefits from staying close to fledglings. Since young choughs are poor foragers (Heinsohn 1991a), they may occasionally

receive food items and preening from other group members. Further observations and quantifications of this events would be necessary to confirm this hypothesis.

For both feeding and accompanying fledglings, choughs from smaller groups were more cooperative than choughs from larger groups. Group size is considered to be critical in this species, with larger groups being able to successfully produce more offspring than smaller groups (Heinsohn 1992). In this case, if larger groups are cooperating less per individual than members from smaller groups, larger group size can be reflecting another advantage for these birds. This is also consistent with the Heinsohn and Cockburn (1994) study, in which they observed that in smaller groups all group members help equally with the breeding tasks, while in bigger groups adults contribute as much as adults from smaller groups, but younger individuals contribute less in incubating. This is related to the higher costs young individuals incur when cooperating compared to adults (Heinsohn and Cockburn 1994).

Future research could include other individual attributes that may be relevant for understanding the determinants of individual levels of associations and cooperation. For instance, it has been observed that chimpanzee relationships are stronger among individuals with similar boldness and sociability traits (Massen and Koski 2014). I showed in chapter 4 that choughs within groups do not differ significantly in boldness measured by flight initiation distance, so I did not include these attributes here. However, other personality traits may differ among individuals within groups and be relevant for understanding their associations and cooperative behaviour.

It is established that chough populations are highly structured, and most of their social groups are comprised of highly related individuals (see Beck, Peakall et al. (2008) and Chapter 2). Therefore, from a population perspective kin selection has played a major role in the evolution

of chough cooperation, mainly by retaining offspring from previous years as it is common in many other cooperatively breeding vertebrates (Hatchwell 2010). However, looking at individual cooperative efforts within social groups, in which members vary in their relatedness, it is possible to establish that cooperating is not only associated with relatedness. At this scale, it is possible to identify that there are other mechanisms and benefits promoting their cooperative behaviour, as it is the case of other cooperatively breeding birds (Riehl 2013). In the case of obligate cooperative breeders, like white-winged choughs, a study by Riehl (2013) recognized that in these species non-kin cooperation is more frequent than in facultative cooperatively breeding species, in which social groups often contain unrelated individuals. Thus, if independent breeding is extremely constrained, direct benefits of group living may present an alternative to potential kin-selected benefits (Riehl 2013). Social network analysis is now established as a powerful approach to investigate animal populations' social structure and its influence in the evolution of cooperation in social species (Wey, Blumstein et al. 2008, Madden, Drewe et al. 2009). Now that genetic analyses are more accessible, establishing preferred relationships among individuals by kin and other traits will continue to advance our understanding of the mechanisms driving individual patterns of cooperation within social groups (Covas and Doutrelant 2019). In the case of cooperatively breeding species, focusing on the relationships among individuals seems essential for disentangling the evolutionary processes and for understanding the proximate causes behind the formation of their complex societies. Nevertheless, the study of the link between cooperation and social structure of cooperative breeders through social networks is still very limited.

Finally, I highlight the value of studies conducted in the wild and in the long-term, in order to disentangle the relationship between cooperation and factors such as fitness benefits,

environmental variation (Covas and Doutrelant 2019), and other aspects that would affect long-lived social animals (Wasser and Sherman 2010), such as white-winged choughs.

References

Aplin, LM, DR Farine, J Morand-Ferron, EF Cole, A Cockburn, and BC Sheldon. 2013. 'Individual personalities predict social behaviour in wild networks of great tits (Parus major)', *Ecology letters*, 16: 1365-72.

Beck, R Peakall, and R Heinsohn. 2008. 'Social constraint and an absence of sex-biased dispersal drive fine-scale genetic structure in white-winged choughs', *Molecular ecology*, 17: 4346-58.

Boland, Christopher RJ, Robert Heinsohn, and Andrew Cockburn. 1997. 'Deception by helpers in cooperatively breeding white-winged choughs and its experimental manipulation', *Behavioral Ecology and Sociobiology*, 41: 251-56.

Carter, Gerald G., and Gerald S. Wilkinson. 2015. 'Social benefits of non-kin food sharing by female vampire bats', *Proceedings of the Royal Society of London B: Biological Sciences*, 282.

Clutton-Brock, TH, D Gaynor, R Kansky, ADC MacColl, G McIlrath, P Chadwick, PNM Brotherton, JM O'riain, M Manser, and JD Skinner. 1998. 'Costs of cooperative behaviour in suricates (Suricata suricatta)', *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265: 185-90.

Covas, Rita, and Claire Doutrelant. 2019. 'Testing the sexual and social benefits of cooperation in animals', *Trends in ecology & evolution*, 34: 112-20.

Croft, Darren P, Richard James, and Jens Krause. 2008. 'Exploring animal social networks.' in, *Exploring animal social networks* (Princeton University Press).

Croft, Darren P, Jens Krause, Safi K Darden, Indar W Ramnarine, Jolyon J Faria, and Richard James. 2009. 'Behavioural trait assortment in a social network: patterns and implications', *Behavioral Ecology and Sociobiology*, 63: 1495-503.

Csardi, Gabor, and Tamas Nepusz. 2006. 'The igraph software package for complex network research', *InterJournal, Complex Systems*, 1695: 1-9.

Davis, Grace H, Margaret C Crofoot, and Damien R Farine. 2018. 'Estimating the robustness and uncertainty of animal social networks using different observational methods', *Animal behaviour*, 141: 29-44.

Farine, D. 2016. "assortnet: calculate the assortativity coefficient of weighted and binary networks. R package version 0.12." In.

Farine, Damien R. 2013. 'Animal social network inference and permutations for ecologists in R using asnipe', *Methods in ecology and evolution*, 4: 1187-94.

Farine, Damien R. 2014. 'Measuring phenotypic assortment in animal social networks: weighted associations are more robust than binary edges', *Animal Behaviour*, 89: 141-53.

Farine, Damien R. 2015. 'Proximity as a proxy for interactions: issues of scale in social network analysis', *Animal Behaviour*, 104: e1-e5.

Farine, Damien R. 2017. 'A guide to null models for animal social network analysis', *Methods in ecology and evolution*, 8: 1309-20.

Farine, Damien R, and Hal Whitehead. 2015. 'Constructing, conducting, and interpreting animal social network analysis', *Journal of Animal Ecology,* 84(5):1144-1163.

Franks, Daniel W., Graeme D. Ruxton, and Richard James. 2010. 'Sampling animal association networks with the gambit of the group', *Behavioral Ecology and Sociobiology*, 64: 493-503.

Friedkin, Noah E. 1991. 'Theoretical foundations for centrality measures', *American journal of Sociology*: 1478-504.

Griffin, Ashleigh S, and Stuart A West. 2003. 'Kin discrimination and the benefit of helping in cooperatively breeding vertebrates', *Science*, 302: 634-36.

Hamilton, William D. 1964. 'The genetical evolution of social behaviour. II', *Journal of theoretical biology*, 7: 17-52.

Hasenjager, Matthew J, and Lee Alan Dugatkin. 2015. 'Chapter Three-Social Network Analysis in Behavioral Ecology', *Advances in the Study of Behavior*, 47: 39-114.

Hatchwell, and Komdeur. 2000. 'Ecological constraints, life history traits and the evolution of cooperative breeding', *Animal Behaviour*, 59: 1079-86.

Hatchwell, Ben J. 2010. 'Cryptic Kin Selection: Kin Structure in Vertebrate Populations and Opportunities for Kin-Directed Cooperation', *Ethology*, 116: 203-16.

Heinsohn, Robert, and Andrew Cockburn. 1994. 'Helping is costly to young birds in cooperatively breeding white-winged choughs', *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 256: 293-98.

Heinsohn, Robert, Peter Dunn, Sarah Legge, and Michael Double. 2000. 'Coalitions of relatives and reproductive skew in cooperatively breeding white-winged choughs', *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 267: 243-49.

Heinsohn, Robert G. 1987. 'Age-dependent vigilance in winter aggregations of cooperatively breeding white-winged choughs (Corcorax melanorhamphos)', *Behavioral Ecology and Sociobiology*, 20: 303-06.

Heinsohn, Robert G. 1991a. 'Slow learning of foraging skills and extended parental care in cooperatively breeding white-winged choughs', *American Naturalist*: 864-81.

Heinsohn, Robert G. 1992. 'Cooperative enhancement of reproductive success in white-winged choughs', *Evolutionary Ecology*, 6: 97-114.

Heinsohn, Robert, and Sarah Legge. 1999. 'The cost of helping', *Trends in ecology & evolution*, 14: 53-57.

Krause, Jens, DP Croft, and Richard James. 2007. 'Social network theory in the behavioural sciences: potential applications', *Behavioral Ecology and Sociobiology*, 62: 15-27.

Krause, Jens, Richard James, Daniel W Franks, and Darren P Croft. 2015. *Animal social networks* (Oxford University Press, USA).

Kurvers, Ralf HJM, Vena MAP Adamczyk, Robert HS Kraus, Joseph I Hoffman, Sipke E van Wieren, Henk P van der Jeugd, William Amos, Herbert HT Prins, and Rudy M Jonker. 2013. 'Contrasting

context dependence of familiarity and kinship in animal social networks', *Animal Behaviour*, 86: 993-1001.

Kurvers, Ralf HJM, Jens Krause, Darren P Croft, Alexander DM Wilson, and Max Wolf. 2014. 'The evolutionary and ecological consequences of animal social networks: emerging issues', *Trends in ecology & evolution*, 29: 326-35.

Langen, Tom A. 1996. 'Skill acquisition and the timing of natal dispersal in the white-throated magpie-jay, Calocitta formosa', *Animal Behaviour*, 51: 575-88.

Lusseau, David. 2003. 'The emergent properties of a dolphin social network', *Proceedings of the Royal Society of London B: Biological Sciences*, 270: S186-S88.

Madden, Joah R, Julian A Drewe, Gareth P Pearce, and Tim H Clutton-Brock. 2009. 'The social network structure of a wild meerkat population: 2. Intragroup interactions', *Behavioral Ecology and Sociobiology*, 64: 81-95.

Madden, Joah R, Johanna F Nielsen, and Tim H Clutton-Brock. 2012. 'Do networks of social interactions reflect patterns of kinship', *Current Zoology*, 58.

Massen, Jorg JM, and Sonja E Koski. 2014. 'Chimps of a feather sit together: chimpanzee friendships are based on homophily in personality', *Evolution and Human Behavior*, 35: 1-8.

McGowan, Kevin J, and Glen E Woolfenden. 1990. 'Contributions to fledgling feeding in the Florida scrub jay', *The Journal of Animal Ecology*: 691-707.

Newman, M. E. J. 2010. *Networks : An Introduction* (OUP Oxford: Oxford).

Newman, Mark EJ. 2006. 'Modularity and community structure in networks', *Proceedings of the national academy of sciences*, 103: 8577-82.

Ohtsuki, Hisashi, Christoph Hauert, Erez Lieberman, and Martin A Nowak. 2006. 'A simple rule for the evolution of cooperation on graphs and social networks', *Nature*, 441: 502-05.

Raihani, Nichola J, and Amanda R Ridley. 2007. 'Adult vocalizations during provisioning: offspring response and postfledging benefits in wild pied babblers', *Animal Behaviour*, 74: 1303-09.

Riehl, Christina. 2013. 'Evolutionary routes to non-kin cooperative breeding in birds', *Proceedings of the Royal Society of London B: Biological Sciences*, 280.

Rowley, Ian. 1978. 'Communal activities among White-winged Choughs Corcorax melanorhamphus', *Ibis*, 120: 178-97.

RStudio, Team. 2018. "RStudio: integrated development for R.(RStudio, Inc., Boston, MA, USA)." In.

Schürch, Roger, Susan Rothenberger, and Dik Heg. 2010. 'The building-up of social relationships: behavioural types, social networks and cooperative breeding in a cichlid', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365: 4089-98.

Sih, Andrew, Sean F Hanser, and Katherine A McHugh. 2009. 'Social network theory: new insights and issues for behavioral ecologists', *Behavioral Ecology and Sociobiology*, 63: 975-88.

Wasser, DE, and PW Sherman. 2010. 'Avian longevities and their interpretation under evolutionary theories of senescence', *Journal of Zoology*, 280: 103-55.

Wey, Tina, Daniel T Blumstein, Weiwei Shen, and Ferenc Jordán. 2008. 'Social network analysis of animal behaviour: a promising tool for the study of sociality', *Animal behaviour*, 75: 333-44.

Wey, Tina W, and Daniel T Blumstein. 2010. 'Social cohesion in yellow-bellied marmots is established through age and kin structuring', *Animal Behaviour*, 79: 1343-52.

Wey, Tina W., Joseph R. Burger, Luis A. Ebensperger, and Loren D. Hayes. 2013. 'Reproductive correlates of social network variation in plurally breeding degus (Octodon degus)', *Animal Behaviour*, 85: 1407-14.

Whitehead, Hal. 2008. *Analyzing animal societies: quantitative methods for vertebrate social analysis* (University of Chicago Press).

Wolf, Jochen BW, David Mawdsley, Fritz Trillmich, and Richard James. 2007. 'Social structure in a colonial mammal: unravelling hidden structural layers and their foundations by network analysis', *Animal Behaviour*, 74: 1293-302.

CHAPTER 6

Conclusions and future directions

Back in the 1970s, Hinde (1976) made an initial formal effort to set up a framework to study non-human animals' social relationships. Back then, the focus was mainly on non-human primates' social structures, and since, countless researchers have focused on studying animals' social structures including all sorts of different taxa. In view of that, this thesis aimed to add meaningful knowledge and understanding to the way social animals are organised, and the factors causing, maintaining and affecting these societies.

The social structure of group living animals can be approached from different perspectives and at different scales. Therefore, during my PhD I explored the social structure of white-winged choughs, Australian birds organized in closed groups of typically highly related individuals, from a genetic, behavioural and demographic perspective, both between and within social groups. During the making of this thesis, I learnt that chough groups are dynamic entities that vary in numbers by reproduction, mortality and dispersal events, and are also affected by environmental factors such as anthropogenic land use and climatic conditions.

In the first data chapter (chapter 2) I began by looking at the choughs' population level structure, and how different climatic scenarios and anthropogenic alteration of habitat affect dispersal and genetic patterns in their populations. From what I learnt, dispersal patterns in choughs are not static, and vary according to environmental conditions. As found by Beck, Peakall et al. (2008), choughs presented non-sex biased dispersal after a period of severe drought, while Heinsohn, Dunn et al. (2000) observed the formation of groups composed only of adult males during another severe drought, suggesting male biased dispersal. Both results contrasted with my results revealing female biased dispersal, obtained using genetic methods and by the observation of the formation of a group composed mainly of female adults previously observed in other groups. One of the most important conclusions from this result is that dispersal patterns in birds should be defined as dynamic processes, which vary according

to the environmental conditions, such as droughts; and demographic states, such as sex or age biased populations. This idea is also relevant for my third chapter, in which I found helpers and breeders' fitness benefits are affected by climatic conditions. From the helpers' inclusive fitness perspective, which is determined by their relatedness to the offspring they take care of, I found that different periods of droughts affected populations differently. A similar conclusion was reached for breeders. The variable inclusive fitness benefits and dispersal patterns according to environmental conditions highlights the fact that predicting the effects climate change will have on cooperative breeders, and other social species, is not straightforward. These effects vary according to many variables that are not easy to account for in social species, which are also influenced by the death or dispersal of other population members, and therefore affect the breeding opportunities in their population.

The finding that groups breeding in suburban habitat were significantly less related than those breeding in woodland habitat invites attention to the effect habitat modification can have in social animals. We know from studies in the 1960s and 1980s that chough groups in woodland areas and during non-droughts rarely break up, living mostly in groups that grow from reproduction. When suburban choughs were studied in the 2000s after a severe drought, there were no major genetic differences with their woodland counterparts. This suggested that urban areas act as refuges when water and food are scarce elsewhere, even though predation on young choughs was observed to be higher in suburbs (Beck and Heinsohn 2006). In my study, I confirmed with genetic data that individuals in groups inhabiting woodland areas during non-drought periods are more highly related than those in urban groups. This suggests urbanization may be changing the demography of this bird species. Considering that choughs are long-lived birds, monitoring the long-term fate of individuals in urban social groups would be highly valuable.

This project has confirmed the power of genetic methods in the study of animal populations. Estimating parentage, relatedness and dispersal patterns in animals, using individual observations can be very inaccurate, as well as a tedious and expensive task. I would have needed many more years to establish the patterns I was able to discern using genetic tools, and I could not have known with any certainty exact individual attributes such as sex and relatedness to their social group. The lower costs of recent genetic tools such a SNPs (Single Nucleotide Polymorphisms) also allow us to discover population dynamics that reflect past events, such as pedigree relationships and dispersal events.

Chapter 4 takes a closer look at the influence individual attributes have on chough social organization. More specifically, it examines how boldness, a personality trait, is related to choughs' organization in their population and social groups. The key result that boldness is similar within but different among groups of choughs exposed the underlying effect of both environmental and genetic influence in this behavioural phenotype, as chough groups are highly related and breed both in woodland and suburban areas. These results also emphasize the importance of long-term studies on long-lived species such as choughs, which would benefit from pedigree information in order to establish the evolutionary role of personality traits, and other individual characteristics.

The method I used to measure boldness (flight initiation distance or FID) is a response correlated to predation risk, and there are many other established behavioural phenotypes we could explore and relate to the social structure of choughs. For instance, we could consider individual levels of cooperation as a personality trait by establishing a method to measure cooperative levels in a standardised way across individuals and groups. Considering all the theoretical models around how cooperation evolves in societies, and how its distribution within populations relates to its evolution and maintenance (Lehmann, Keller et al. 2007,

Nowak, Tarnita et al. 2010), we could investigate how chough populations are structured with respect to cooperation. We may find that highly cooperative individuals clustering, as observed in humans by Fehl, van der Post et al. (2011); or on the contrary, that cooperative personalities are equally distributed across groups.

From chapter 5 I learnt that direct benefits of helping may play a more important role in chough society than previously realised. From directly observing cooperative acts within groups, there is no evidence that individuals favour closer kin. This finding, observed in other vertebrates (Clutton-Brock 2009), suggests it may be worthwhile to explore the motivations behind choughs' cooperative interactions. Future research could focus on the exploration of the specific benefits gained by highly cooperative individuals, which may include gaining future allies and breeding positions. Nevertheless, the importance of the role of kin selection in chough societies should still be highly considered. Even though kin recognition does not play a significant role when cooperating to raise young, the importance of kin selection has nonetheless been established by demonstrating high relatedness within chough groups (chapter 2). The data presented in chapter 3 further supports the role of kin selection, in which helpers from larger groups obtain larger inclusive fitness benefits in years of better rainfall.

Finally, I want to stress once more the importance of establishing a long-term study of white-winged choughs. From long term studies of other cooperatively breeding vertebrates, we have immensely expanded our knowledge on the evolution and maintenance of cooperation and sociality (Koenig and Dickinson 2016). Choughs live in stable social groups, in which they exhibit a varied and numerous array of interactions, such as allopreening, vocalisations, feeding, and "wing-wave tail-wag" displays, all of which can be easily observed. In addition, the obligate social and cooperative nature of choughs means they can be constantly assessed from a social perspective. I firmly believe that following and observing choughs for generations has

huge potential for augmenting our knowledge by integrating their ecological, genetic, behavioural and physiological nature, all of which can provide the basis of new ideas and research projects.

References

Beck, Peakall, R. and Heinsohn, R., 2008. Social constraint and an absence of sex-biased dispersal drive fine-scale genetic structure in white-winged choughs, *Molecular ecology,* 17(19):4346-4358.

Beck, N. R. and Heinsohn, R., 2006. Group composition and reproductive success of cooperatively breeding white-winged choughs (Corcorax melanorhamphos) in urban and non-urban habitat, *Austral ecology,* 31(5):588-596.

Clutton-Brock, T., 2009. Cooperation between non-kin in animal societies, *Nature,* 462(7269):51-57.

Fehl, K., van der Post, D. J. and Semmann, D., 2011. Co-evolution of behaviour and social network structure promotes human cooperation, *Ecology letters,* 14(6):546-551.

Heinsohn, R., Dunn, P., Legge, S. and Double, M., 2000. Coalitions of relatives and reproductive skew in cooperatively breeding white-winged choughs, *Proceedings of the Royal Society of London. Series B: Biological Sciences,* 267(1440):243-249.

Hinde, R. A., 1976. Interactions, relationships and social structure, *Man*:1-17.

Koenig, W. D. and Dickinson, J. L., 2016. Cooperative breeding in vertebrates: studies of ecology, evolution, and behavior*,* Cambridge University Press,

Lehmann, L., Keller, L. and Sumpter, D. J., 2007. The evolution of helping and harming on graphs: the return of the inclusive fitness effect, *Journal of evolutionary biology,* 20(6):2284-2295.

Nowak, M. A., Tarnita, C. E. and Wilson, E. O., 2010. The evolution of eusociality, *Nature,* 466(7310):1057-1062.