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The mechanisms and function of social
recognition in the cooperatively breeding
Southern pied babbler, *Turdoides bicolor*

David John Humphries



A dissertation submitted to Macquarie University in application for
the degree of Doctor of Philosophy

June 2013

Department of Biology

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Summary

For group-living species, where the same individuals interact repeatedly, recognising conspecifics and remembering the outcomes of previous encounters may be critical to regulating group dynamics. In all social species, recognition potentially affects individual decisions about mate choice, dispersal, and inter and intra-group interactions. Recognition may be even more important in cooperative species, where cooperation is often predicated on the ability to detect kin, and where stable cooperation may require the capacity to monitor contributions by collaborators. However, we currently know very little about how cooperative species perceive their social environment. Thus, studies of social recognition are of extreme relevance for understanding how cooperation is maintained. Understanding the mechanisms of social recognition, its development, and its limitations facilitates our understanding of the social interactions we observe within populations, and the life history strategies we see. In this thesis I investigate the occurrence, mechanism, and function of social recognition in the highly cooperative Southern pied babbler, *Turdoides bicolor*. I show that: a) individuals use vocalisations to signal their identity, b) these signals are perceived and discriminated by receivers, both at the level of individual and kin and c) kin are recognised through prior association, but fail to discriminate after years of separation. Finally, I demonstrate the importance of recognition on social behaviour by revealing the influence it has on territorial defence and subordinate dispersal strategies. These findings represent a comprehensive example of the occurrence, development and mechanisms of

social recognition, and demonstrate its use and limitations in mediating social interactions.

Statement of candidate

I certify that the work carried out in this thesis, entitled 'The mechanisms and function of social recognition in the cooperatively breeding Southern pied babbler, *Turdoides bicolor*', has not been previously submitted for a degree, nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University.

I also certify that the thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research and during the preparation of this thesis has been appropriately acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis. The research presented in this thesis was approved by Macquarie University Ethics Review committee, under reference number 2010/032.

David J. Humphries

Student Number: 41936922

June 2013

Preface

Chapters 3, 4, and 6 have been submitted for publication to peer-reviewed journals, with a shortened version of chapter 3 submitted to the journal '*Ethology*', chapter 4 submitted to the journal '*Animal behaviour*', and chapter 6 submitted to the '*Proceedings of the Royal Society: Biological sciences*'. They are formatted here to meet the requirements of the respective journals. Chapter 9 has been prepared in the format of '*Proceedings of the Royal Society: Biological sciences*', and chapter 10 has been prepared for '*PLoS*' although they have not currently been submitted.

The chapters of this thesis were reviewed and revised with the assistance of my academic supervisors, Dr Amanda R. Ridley (University of Western Australia and Macquarie University) and Dr Matthew B. V. Bell (University of Edinburgh).

Although the behavioural observations and experiments included in this thesis were collected by my assistant, Fiona Finch, and myself, we were working within an established research project, founded in 2003 by Dr Amanda R. Ridley. The population was already colour-ringed, and habituated to human presence and this work was certainly aided by the contributions of those that have worked on the research project both whilst I was there, and since 2003.

My personal estimated contribution to each chapter is indicated below:

Chapter 1 - conception 100%, writing 100%,

Chapter 2 – conception 90%, writing 85%

Chapter 3 – conception 90%, data collection 75%, analysis 100%, writing 80%

Chapter 4 – conception 90%, data collection 100%, analysis 100%, writing 80%

Chapter 5 – conception 95%, data collection 50%, analysis 100%, writing 80%

Chapter 6 – conception 75%, data collection 50%, analysis 80%, writing 75%

Chapter 7 – conception 75%, data collection 50%, analysis 100%, writing 80%

Chapter 8 – conception 75%, data collection 50%, analysis 100%, writing 80%

Chapter 9 – conception 100%, data collection 50%, analysis 80%, writing 70%

Chapter 10 – conception 100%, data collection 100%, analysis 100%, writing 90%

Chapter 11 – conception 100%, writing 100%

In addition to my academic supervisors and field assistant, this work was aided by a number of individuals including:

Dr Martha Nelson-Flower (University of Cape Town), who ran the microsatellite analysis to determine parentage and developed the pedigree used within this thesis. She also worked on the genetic sexing of the population. Additionally, Martha provided feedback on the writing of chapters 6 and 9.

In chapter 9, the durations of inter-group interactions were recorded by all observers at the field site including: Alex Thompson, Elizabeth Wiley, and John Forecast.

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I have had a fantastic time over the last three and half years and genuinely enjoyed the process of bringing this thesis together. I have had the opportunity to travel to some amazing places, and work with some incredible people, and on top of that, I have had an enormous amount of fun along the way. The enjoyment I have gained from undertaking this thesis owes a lot to the quality of the individuals I have been surrounded by and the hard work they have put in. I am certainly indebted to many, and below I will attempt to list and thank some of those people that have helped so much over the years.

I'll start from the top with the key figures in this thesis, my supervisors, Mandy Ridley, Matt Bell and Simon Griffith. All of whom have contributed significantly towards bringing this thesis together.

Mandy has been critical to this thesis from the very start, helping to secure the funding for this project and in inspiring many of the original ideas within it. She has been instrumental in bringing this thesis together and has been through an awful lot of drafts over the last three and half years. These drafts were always returned with comments within days, and often within hours. It is impossible to state the value of this rapid feedback and I know how fortunate I have been to have had such brilliant assistance throughout my time. I have also been the receiver of enormous amounts of generosity from Mandy, who lent me her personal car throughout both of my field seasons, as well as field equipment and I am enormously grateful for her generosity.

Equipment doesn't come cheap, and this thesis has functioned on the generosity of those I have managed to beg, steal and borrow from. Matt Bell stepped-in repeatedly during my time in the field, offering me the use of his personal equipment. Aside from his generosity, I owe Matt an enormous thanks for the contributions he has made to the chapters of this thesis. He is an incredibly bright mind and his contributions have brought out the best of these chapters.

Simon Griffith has been invaluable in my time at Macquarie. His knowledge of the inner workings of university life has consistently aided my path through the PhD and he has shown me a lot of trust over the years. I am enormously grateful for his assistance during my time at Macquarie.

The next group of people I would like to thank are all those that I worked with in South Africa. The field work was a collaborative effort, and I was fortunate to be surrounded by a superb team of people including: Alex Thompson, Martha Nelson-Flower, Adam Britton, Kate Du Plessis, Elizabeth Wiley, and John Forecast. All of whom helped to maintain the population, but more importantly, provided so much entertainment over the two years. I also owe them thanks for taking the time and effort to record the additional data on inter-group interactions that I use in Chapter 9. We were all busy, and I am enormously grateful for that extra time and effort made by all of you on my behalf.

I also owe thanks to the staff and volunteers at the KMP for their assistance. The constant feedback provided by all those that worked on the reserve regarding the location of the pied babbler groups was fantastic. In addition to the practical details, I have an awful lot of fond memories of parties, sports and

social events from my time in South Africa and owe thanks to everyone we worked with for making the whole time so enjoyable. A special thanks to Jamie Samson, Nathan Thavarajah, Michiel Jooste for their excellent management of the reserve while I was there.

This PhD has been an enormous learning curve, and I have been fortunate to have had a number of individuals impart their knowledge on me including: Enrico Sorato, who helped guide me through the processes of mapping territories and pointed out the respective merits of each of the available techniques. He also assisted me in my early ventures into model averaging. I also would like to thank Simon Townsend, Miya Warrington, Mike Finnie, Roger Mundry, and Marta Manser who have guided me through the dizzying politics that surround the field of bioacoustics. I would like to thank all of them for providing me with their thoughts and discussion on the topic of acoustic analysis and for reading through the early drafts of the acoustic chapters of this thesis. I would also like to thank Eirik Søvik for his advice, coffee and support and for making my time at Macquarie so enjoyable.

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I am indebted to my partner Fiona Finch, who had the unenviable position of being my field assistant over the two seasons. The numerous adventures of 'data-less Dave' and 'failure Fi' have paid off and we really did achieve an

extraordinary amount in the two field seasons we did. The numbers speak for themselves, with over 450 successful playbacks, over 2,000 calls recorded, 120 IGIs timed, as well as managing to compile a database of over 50,000 GPS points. It was an exceptional achievement and I owe that success to Fiona. Fiona added essential levels of organisation to the field work, and the constant remarks of 'are your batteries charged', 'have you got your mealworms' and 'do you have your binoculars' (I probably didn't) are what saved the field seasons and made them so fruitful. We must have clocked-up hundreds of kilometres in walking while searching for dispersed birds for the kin recognition experiment. She put in an awful lot of hard work in to making this thesis happen. I would like to thank Fiona for her un-wavering support and hard work throughout my PhD, both while we were in the field work and during the write-up process.

This PhD was funded through an '*International Macquarie University Research Excellence Scholarship*' (iMQRES)

Chapter 1

Introduction



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1.1 An introduction to social recognition

The ability to recognise social partners is a central feature to our understanding of many of the behaviours we observe within animal societies. From the formation of stable, cohesive groups, where individuals identify and remain in proximity to a fixed set of individuals (Waser 1977; Rasoloharijaona et al. 2006), to social monogamy, where faithful breeding partnerships require mates to recognise one another over time (Wanker et al. 1998; Clark et al. 2006), recognition plays a key role in regulating group dynamics. Recognition can enable social hierarchies to form within groups (Barnard & Burk 1979; Weiß & Scheiber 2012), and can reduce the frequency of agonistic aggression between individuals by memory of previous encounters (Karavanich & Atema 1998; Gherardi & Atema 2005). Additionally, the ability to recognise and avoid kin as mating partners can enable costly inbreeding to be avoided (Blouin & Blouin 1988; Pusey & Wolf 1996; Charlesworth & Charlesworth 1999), and allow the expression of kin-directed helping behaviour (Hamilton 1964; Komdeur et al. 2008). Kin selection is the most widely supported theory to explain the occurrence of helping behaviour, but pivotal to the theory is the assumption that individuals can discriminate kin from non-kin (Hamilton 1964; Agrawal 2001; Mateo 2002; Komdeur et al. 2008). Therefore, how individuals perceive their social environment is a hugely important area of research because of its potential to explain many of the behavioural interactions we observe in social species.

The importance of recognition may be even more pronounced in highly cooperative animal societies, where the ability to recognise individuals and monitor their contributions in cooperative tasks may be critical for building

reputations (both for reliability and social prestige) and for identifying 'cheats' who do not reciprocate in cooperative interactions (Zahavi 1990; Crowley et al. 1996; Nowak & Sigmund 1998). While some cooperative interactions may be self-serving (e.g. by-product mutualisms (Hamilton 1971), and positive pseudo-reciprocity (Connor 1986)), in many of the cooperative interactions we observe, there are likely to be positive pay-offs to the actor for cheating and reducing their investment in cooperative tasks (reviewed in Brosnan et al. 2010). When cooperative interactions are open to exploitation by uncooperative individuals (Brown 1983), the evolutionary stability of cooperation within animal societies may be dependent upon the ability to recognise and monitor individuals (Crowley et al. 1996; Nowak & Sigmund 1998). Yet, despite the far-reaching implications of social recognition in animal societies, we know very little about how recognition is achieved. The ability to recognise individuals (Beer 1971; Balshine-Earn & Arnon 1998), and kin (Nam et al. 2010; McDonald & Wright 2011) within social interactions has been identified at a broad level (e.g. the presence or absence of recognition; Payne et al. 1988; Price 1999; Akçay et al. 2013), however, the underlying mechanisms through which recognition is achieved have seldom been explored.

Understanding the operative mechanisms of recognition is likely to be important as each mechanism may differ in its development, reliability and resolving power. For instance, if we look at the possible mechanisms for recognising kin, there are four main hypotheses: 1) prior association, 2) phenotype matching, 3) recognition alleles, and 4) spatial recognition (Sherman & Holmes 1985; Waldman 1987), which may be innate (e.g. recognition alleles) or learnt (Tang-Martinez 2001), dependent upon familiarity (e.g. prior association) or not

(Hepper 1986; Waldman 1987). These variations have consequences for when and where we may expect to see kin discrimination occurring within a species, both when it is used for nepotism (Hamilton 1964; Agrawal 2001; Komdeur et al. 2008) or inbreeding avoidance (Blouin & Blouin 1988; Pusey & Wolf 1996). It is only through understanding these nuances of recognition that we will be able to identify when behavioural discrimination can occur, which will ultimately enlighten our understanding of the social interactions we observe within a species.

Recognition is a two-way interaction involving a 'signaller', who must reliably display cues and signals to their identity (often collectively termed identity 'labels' or 'signatures'), and a 'receiver' that must detect and infer identity from the information available (Sherman et al. 1997). Both sides of this interaction (the signalling and the receiving) are highly variable between taxa and social systems in their occurrence, development and reliability. Identity labels can vary depending upon the social context in which they are given, for example, in vocal communication, individuality can vary between the call types in a vocal repertoire, occurring within some call types but not others (or being more distinctive in some) as a consequence of their social function (Price et al. 2009; Bouchet et al. 2012). It may only be beneficial to reveal identity under certain ecological conditions (Johnstone 1997; Dale et al. 2001), and we may only expect selection to favour the production of identity labels when the interactions with a receiver are routinely beneficial, or conversely, routinely agonistic (Johnstone 1997). Thus, there is variation in the production of identity labels depending upon a range of spatial, temporal, or social factors (Waldman 1987).

It is therefore important to explore when and where species are producing identity labels in order to understand when recognition can occur.

The way in which receivers recognise social partners may also be highly variable. For instance, recognition may occur through generalised relationships dependent upon spatial cues (e.g. many avian species use the location of the nest to recognise their chicks; Komdeur & Hatchwell 1999), or by direct recognition mechanisms where individuals are assessed on their phenotypic cues and signals (Sherman et al. 1997; Komdeur & Hatchwell 1999; Bradbury & Vehrencamp 2011). Even when recognition is based upon phenotypic cues, it can extend from broad recognition mechanisms such as familiarity (where recognition occurs, but without the recall of source information; Mandler 1980), to fine-scale recognition such as kin recognition or individual recognition, where identity labels are matched to information stored in memory so that each individual has a unique significance and personality to the perceiver (Mandler 1980; Johnston 2008). This variation in how conspecifics are perceived by receivers will ultimately influence when and where behavioural discrimination can occur within a species which will impact upon the social interactions we observe.

It is the highly variable nature of the mechanisms of recognition, and the associated variation in the ontogeny, reliability, and resolving power of each mechanism that make current studies exploring only the presence or absence of recognition limited in their ability to draw conclusions about when and where we may expect to see behavioural discrimination occurring within animal societies. What is required is a holistic approach to the study of recognition, exploring: a) the production of reliable cues and signals to identity by a

signaller, b) the ability of the receiver to recognise and discriminate these identity labels, c) an exploration of the underlying mechanisms through which recognition is occurring, which in turn will be informative for d) the ontogeny and the long-term reliability of recognition and e) interpreting the behavioural patterns we observe (e.g, dispersal and mate attraction). Our understanding of all of these fundamental areas of recognition is currently fragmented, dispersed over taxa and study species. We repeatedly draw conclusions on the ability of species to behaviourally discriminate conspecifics (e.g. tests for kin selection through kin-directed helping behaviour (Clarke 1984; Curry 1988; Emlen & Wrege 1988; Marzluff & Balda 1990; Komdeur 1994; Dickinson et al. 1996; Painter et al. 2000; Russell & Hatchwell 2001; Browning et al. 2012) – yet we know little about how species perceive their social environment. By providing a complete picture of the resolving power and reliability of the recognition mechanisms in operation, we can hope to develop an understanding of the behavioural interactions we observe within animal societies.

Here I explore the mechanisms of recognition within the cooperatively breeding Southern pied babbler, *Turdoides bicolor*. This species may be under particularly strong selection for recognition, because it displays many of the social behaviours where recognition has previously been found to be important. For example, pied babblers live within stable social groups (Ridley et al. 2007), where breeding is monopolised by a monogamous dominant pair (Nelson-Flower et al. 2011). Subordinate individuals come and go from the natal territory, using it as a 'safe haven' between dispersal events (Raihani et al. 2010), where they appear to search for breeding opportunities while avoiding breeding with familiar kin (Nelson-Flower et al. 2012). Pied babblers are also

highly cooperative, working together to defend a territory (Golabek et al. 2012), engaging in cooperative predator detection and defence (Bell et al. 2010; Hollén et al. 2011), and cooperative breeding (Raihani & Ridley 2007; Ridley & Heuvel 2012). I aim to advance our understanding of recognition by exposing not only the presence of recognition, but also identifying the underlying mechanisms through which recognition is achieved within the pied babbler, and how this influences social behaviour. In this thesis I aim to:

- a) Investigate whether the pied babbler produces vocal identity signatures that may facilitate recognition.
- b) determine whether recognition exists, and at what level of discrimination this occurs (i.e. individual, kin or group)
- c) determine the underlying mechanism of kin recognition within this species (based on four main hypotheses (1) prior association, 2) phenotype matching, 3) recognition alleles, and 4) spatial recognition (Sherman & Holmes 1985; Waldman 1987))
- d) determine the consequences of recognition on individual behaviour

1.2 Thesis outline

In chapters 3 and 4 I explore the production of identity labels in pied babbler vocalisations, investigating whether they produce both individual identity signatures and group labels within their calls which may facilitate recognition. In chapter 3, I also explore the long-term reliability of identity labels.

In chapters 5 and 6 I focus on testing for the ability to recognise individuals (chapter 5) and kin (chapter 6). When testing for kin recognition, I investigate the underlying mechanisms of kin recognition.

In chapters 7 and 8 I explore the limitations of recognition, looking at both when recognition develops in young (Chapter 7), and the ability of adult individuals to maintain recognition over extended periods of separation (Chapter 8).

Finally, in chapters 9 and 10 I look at how kin recognition influences pied babbler social interactions, in particular with regards to their interactions with neighbouring groups. Kinship in the pied babbler does not appear to influence helping at the nest (Nelson-Flower 2010). However, indiscriminate helping behaviour is common in cooperative breeding societies that live in kin-structured family groups, as spatial correlates to kinship may be sufficient for kin selection to occur via indiscriminate behaviour (West et al. 2007; Cornwallis et al. 2009). In this thesis I focus on how kinship is affecting interactions with individuals that occur outside of their social group, where spatial cues to relationships may be less reliable. I then focus on how kinship is affecting aggression between neighbouring groups (Chapter 9) and how relatedness is influencing the strategies employed by subordinates for mate attraction (Chapter 10).

In this thesis I aim to comprehensively explore the process of recognition in the pied babbler, identifying the production of identity labels by the signaller and the mechanisms that enable receivers to discriminate conspecifics. Through developing our understanding of pied babbler social recognition, I will advance our understanding of the behaviours we observe within this species.

Chapter 2

General Methods



2.1 Study species

The Southern pied babbler, *Turdoides bicolor*, (herein referred to as the 'pied babbler') is a medium sized (75-95g), cooperatively breeding passerine, endemic to the Kalahari region of Southern Africa. It is a member of the family *Leiothruidae*, and one of the 29 species that make up the *Turdoides* genus. Pied babblers live in stable social groups of 2-15 individuals (Ridley et al. 2007). Pied babblers, with their distinctive pied colouration (white bodies and black tail and wing feathers), are sexually monomorphic in both physical size and plumage. The pied babbler is not considered to be threatened, and is classified as 'least concern' on the IUCN red list of threatened species (IUCN, version 3.1).

2.1.1 Breeding behaviour

Pied babblers typically breed between September-March (Ridley & Raihani 2008), when both temperature and rainfall are highest (see Figure 5). Breeding within the social group is monopolised by a dominant pair (Nelson-Flower et al. 2011), whose breeding attempts are assisted by all adult subordinate group members (Ridley & Heuvel 2012). Helpers participate in the incubation and feeding of dependent young, as well as defending the nest and young from predators (Ridley & Heuvel 2012). These subordinate helpers are usually (although not always) retained offspring (Raihani et al. 2010). Lone breeding pairs are able to breed, but both reproductive success and fledgling growth are improved with the assistance of additional helpers (Ridley & Raihani 2007c; Ridley & Heuvel 2012). Prior to breeding, dominant pairs will court each other over a 2-6 day period (Figure 1; Ridley & Thompson 2011), presenting nesting



Figure 1 – Male pied babblers court females with the presentation of nesting material.

material, allopreening and calling to one another, before starting to build a nest together. Nests are built by the dominant pair (except in complex groups where competing individuals will both build the nest; Nelson-Flower et al. 2013), predominantly from dried grasses and other soft vegetation, in a variety of tree species including camelthorn (*Acacia erioloba*), grey camelthorn (*Acacia haemotoxylon*), Buffalo Thorn (*Ziziphus mucronata*), and blackthorn (*Acacia mellifera*). The dominant female will typically lay clutches of 3 eggs (range 2-5; Ridley & Thompson 2011) into a cup-shaped nest, where the eggs will be incubated for 14-15 days. All adult individuals of the social group will participate in the incubation of the eggs throughout the day, however, overnight incubation is exclusively carried out by the dominant female (Ridley & Raihani 2008).

Once hatched, young remain in the nest for between 13-19 days before fledging into the natal territory where they will remain well beyond reaching sexual maturity (Raihani & Ridley 2007b). The age of fledging is affected by the number of individuals within the social group, where small groups tend to fledge

young early, a tactic thought to counteract the higher predation risk of nests within small groups (Raihani & Ridley 2007b). Fledged young are dependent upon the provision of food by adults for up to 3 months post-fledging (Ridley & Raihani 2007b; Thompson & Ridley 2012; Thompson et al. 2013). Pied babblers are considered sexually mature at one year old (since hatching; Raihani et al. 2010).

2.1.2 Territorial defence

Pied babblers are highly territorial, defending territories of $\sim 0.75\text{km}^2$, and frequently engage in ritualised, territorial disputes with neighbouring groups (Chapter 9; Golabek et al. 2012). During these ritualised encounters, groups typically take up positions in opposing trees, taking it in turns to vocalise at one another (Chapter 9; Golabek et al. 2012) where males and females adopt sex-specific call types (Golabek & Radford 2013). These encounters may therefore provide a useful point of information exchange about the composition of neighbouring groups. Territorial encounters will occasionally escalate into chases and physical fighting between groups, although aggression is rare (Chapter 9). Territories are defended the year round, however the frequency of territorial encounters often declines during the driest times of the year, when food is at its most scarce, suggesting an energetic cost to territorial defence (Golabek et al. 2012).

2.1.3 Communication

Like many avian species (Halpin 1991), pied babblers utilise vocalisations extensively for communication, producing a range of context-specific vocalisations to facilitate their social interactions. For instance, the 'purr'

vocalisation is only used by adults during interactions with dependent young (Raihani & Ridley 2008). Adults teach young to respond to these calls by associating the vocalisations with the provision of food (Raihani & Ridley 2007a; Raihani & Ridley 2008). These calls can then be used to move fledglings between foraging patches, or to safe locations (Raihani & Ridley 2008; Thompson et al. 2013).

Vocalisations play an integral role in pied babbler social behaviour, with previous research demonstrating that they are used to coordinate cooperative behaviours, (such as sentinel behaviour; Bell et al. 2010), and to defend territorial boundaries (Golabek et al. 2012). They are also used to signal potential threats, and pied babblers produce several types of alarm call when a threat has been detected, signalling the presence of both terrestrial and aerial predators (Bell et al. 2009; Flower 2011).

During foraging, pied babblers continually vocalise to one another using several types of close call (Radford & Ridley 2008; Golabek 2010). These calls facilitate the spacing and social cohesion of foraging individuals (Radford & Ridley 2008). An elevated form of the close call is produced upon food discovery, particularly if it is a divisible food source, which may signal opportunities to share resources (Golabek 2010). Pied babblers also produce a range of other vocalisations such as 'loud' calls (that occur in a wide range of social contexts, from territorial disputes to prospecting for breeding opportunities; Golabek & Radford 2013), 'mobbing' calls, which are used to harass predators (A. R. Ridley, unpublished data), and 'sentinel calls', used to signal the presence of a sentinel to the rest of the group (Hollén et al. 2008; Hollén et al. 2011a).

2.1.4 Cooperation and predation

The pied babbler is a highly cooperative species, with group members working together to raise offspring and defend a territory. Additionally, group members work together to both detect predators and to deter them from remaining within the territory. Pied babblers face a number of both aerial predators (including gabar goshawk (*Melierax gabar*), pale chanting goshawk (*Meliarax canorus*), black-shouldered kite (*Elanus caeruleus*), greater kestrel (*Falco rupicoloides*), lanner falcon (*Falco biarmicus*)) and terrestrial predators including several mammalian species (such as wildcat (*Felis silvestris*), slender mongoose (*Galerella sanguinea*), and yellow mongoose (*Cynictis penicillata*)) and reptilian species (including the cape cobra (*Naja nivea*) the puff adder (*Bitis arietans*) and the rock monitor (*Varanus albigularis*) (Ridley et al. 2010; Ridley et al. 2013). Both the nests and fledged young may face additional threats, with nests being destroyed by species such as the wattled starling (*Creatophora cinerea*) (Ridley & Thompson 2011), or parasitized by the Jacobin cuckoo (*Clamator jacobinus*) (Ridley & Thompson 2012), and newly fledged young are occasionally captured and eaten by the meerkat (*Suricata suricatta*; Personal observation), a predominantly insectivorous species. Predator detection can take several forms including: 1) vigilance, where individual pied babblers will scan for predators, alarm calling when a threat has been identified, 2) utilising the alarm calls of other species to identify the location of predators (Bell et al. 2009), and 3) cooperative sentinel activity to scan for predators (Bell et al. 2010; Hollén et al. 2011a; Hollén et al. 2011b; Ridley et al. 2013). Sentinel behaviour is characterised by a group member adopting an exposed, elevated position while scanning the surrounding area for potential threats (Bell et al.

2010; Hollén et al. 2011a; Hollén et al. 2011b; Ridley et al. 2013). Their elevated position means that sentinels are often the first to spot a predator (Ridley et al. 2010). Sentinels continuously vocalise (through the use of 'sentinel calls'(Hollén et al. 2008)), conveying information about the risk of predators in the surrounding environment. These calls enable foragers to invest less in personal vigilance, and consequently, they experience improved foraging success (Hollén et al. 2008).

Upon sighting a predator, groups can adopt one of two behavioural strategies, 1) fleeing for cover, typically in the direction of dense woody vegetation (Ridley et al. 2013), or 2) mobbing – where predators are surrounded by the group and harassed through continual vocalising (and may occasionally escalate to pecking).



Figure 2 – Southern pied babbler 'mobbing' a cape cobra.

2.1.5 Diet

Pied babblers are primarily terrestrial foragers, using their long curved bill to dig through the substrate in search of food items, or to glean food items from vegetation (Child et al. 2012). They eat a wide range of ground-dwelling invertebrates, predominantly focusing on subterranean larvae and pupae, but they have also been known to take larger food items such as skinks and lizards (Child et al. 2012). Pied babblers require woody vegetation for both nesting and foraging, spending much of their time foraging for food items in the sand and litter at the base of trees and bushes (Thiele et al. 2008).

2.1.6 Routes to breeding

The dominant pair within the social group are monogamous and extra-pair paternity is rare (Nelson-Flower et al. 2011). As a consequence, competition for dominant breeding positions is high, because subordinate members must find a dominant position within a group in order to achieve reproductive success (Raihani et al. 2010; Nelson-Flower et al. 2011). When vacancies do occur, either through the death of dominant individual, or by divorce, (where a dominant leaves a group to take up a breeding position within another group), the breeding vacancies are rapidly filled (A. Ridley, unpublished data). However, pied babblers avoid inbreeding (Nelson-Flower et al. 2012), and because most groups are highly kin-structured, vacancies are typically filled by an unrelated individual dispersing into the group (Raihani et al. 2010; Nelson-Flower et al. 2012). Dispersal in the pied babbler does not appear to be sex-biased, and both subordinate males and females will prospect for breeding vacancies in neighbouring groups (Chapter 10; Raihani et al. 2010).

2.2 Study site

The pied babbler population is located at the Kuruman River Reserve, in the Northern Cape, South Africa (26°58'S, 21°49'E; Figure 4). The site is approximately 3200 hectares, and spans the dry riverbed of the Kuruman River. The field site lies 27km west of the town of Van Zylsrus, and 17km south of the Botswana border.

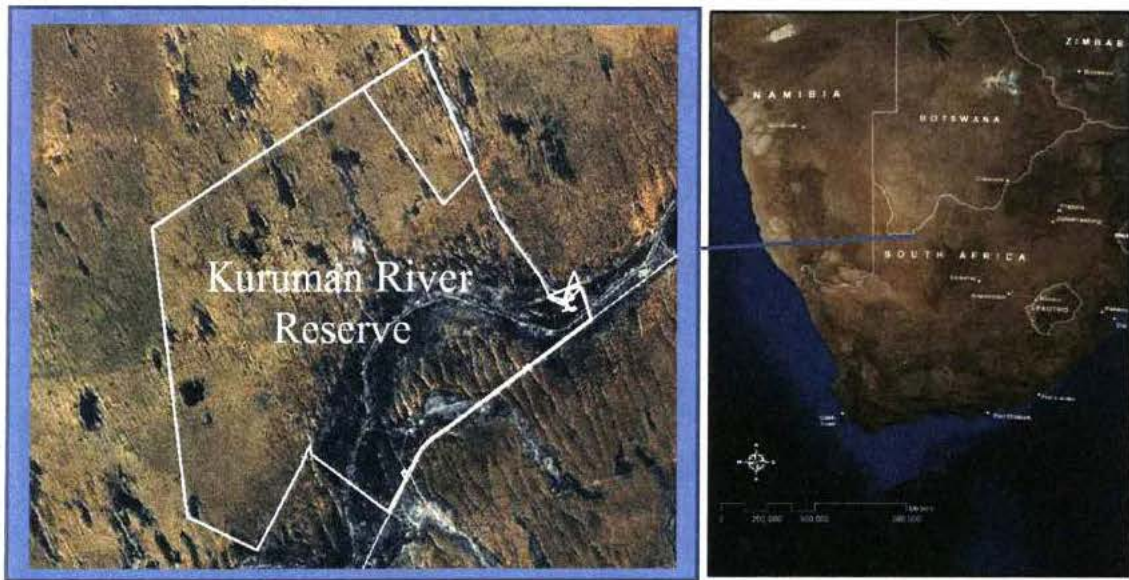


Figure 4 – The Kuruman River Reserve is a ~3200 hectare site located in the Northern Cape, South Africa. Maps drawn in ArcGIS 9.3.1.

2.2.1 Climate

The site is in a semi-arid zone typified by hot wet summers (between September and March) and cool, dry winters (between April and August). The site receives extreme changes in weather over the year, with temperatures regularly reaching over 35°C in December and January, but falling well below freezing in the winter months (June-July; Figure 5).

2.2.2 Habitat

The Kuruman River Reserve is classified as Kalahari thornveld (Leistner & Werger 1973), but encompasses a range of micro-habitats, from the open

woodland of acacia trees that line the dry riverbed (typified by camelthorn and Buffalo Thorn, as well as the invasive glandular Mesquite (*Prosopis glandulosa*)), to flat terraces covered in small shrubby vegetation, such as velvet brandybush (*Grewia flava*) and drie doring (*Rhigozum trichotomum*). The site also has areas of sand dune, whose slopes are typically covered in perennial grasses (such as *Aristida*, *Eragrostis*, *Stipagrostis* and *Schmidtia* Spp.), and sparsely covered by trees such as the grey camelthorn, shepherd's tree (*Boscia albitrunca*) and blackthorn.

The Kuruman River Reserve is a former farm, previously used in the production of domestic cattle and goats, but was purchased in 1993 and the domestic livestock were subsequently removed. Since 1999, a range of herbivores have been introduced onto the site including: eland (*Taurotragus oryx*), blue wildebeest (*Connochaetes taurinus*), springbok (*Antidorcus marsupialis*), gemsbok (*Oryx gazella*), and red hartebeest (*Alcelaphus buselaphus*).

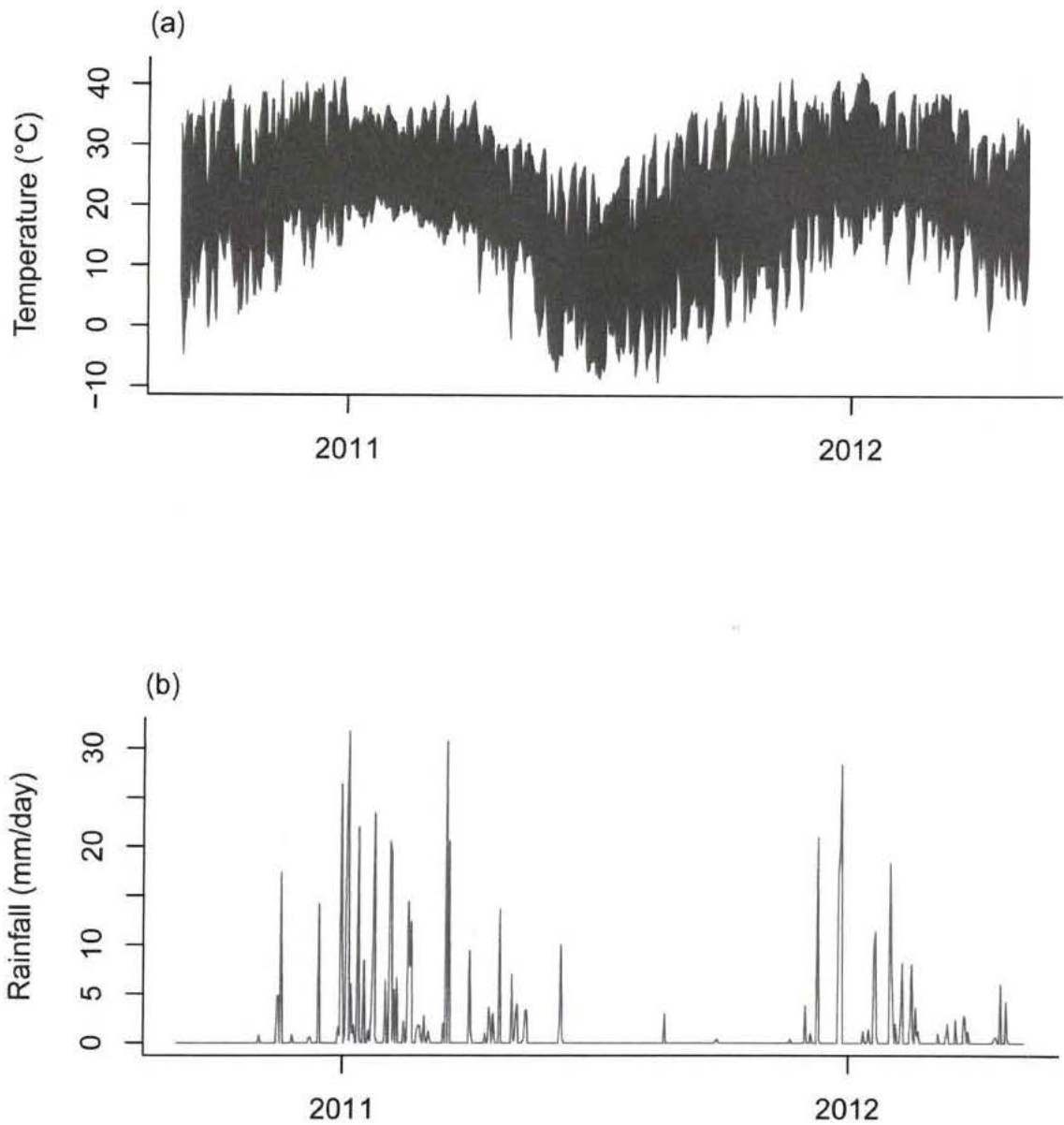


Figure 5 – (a) The daily temperature range (daily maximum and minimum temperature), and (b) the daily rainfall between September 2010 – April 2012 (the period of this study) at the Kuruman River Reserve, Northern Cape, South Africa. Data collected from the on-site weather station.

2.3 The pied babbler population

The pied babbler population at the Kuruman River Reserve was established by Dr Amanda Ridley in 2003 and has been under continuous observation since then, currently containing 115 colour-ringed individuals. Each individual within the population was ringed with a uniquely numbered metal ring provided by

SAFRING (under license numbers 1263 and 1328), and 3 additional plastic colour rings for identification. Birds were ringed either as chicks in the nest, at 11 days post hatching, or as adults, caught using a walk-in trap. Walk-in traps are baited with mealworms, and triggered by hand by an observer. Walk-in traps are never left unattended, and are placed away from the social group to avoid distressing other group members. During ringing, blood samples (ca 50 μ L) were collected through brachial venipuncture for use in genetic sexing and microsatellite analysis. Blood samples were stored at 4°C in 700 μ L of Longmire's Solution Blood Lysis Buffer. All research was conducted under permits provided under the Northern Cape Conservation Authority and with ethical clearance provided by the University of Cape Town (approved under ethics number R2012/2006/V15/AR). The number of groups under observation since 2003 has fluctuated between years with a median of 18 groups, ranging between 12-26 groups per year.

The population is habituated to the presence of humans, allowing behavioural observation and data collection from close quarters (within 2-3m) without causing any perceivable behavioural change or stress (Ridley & Raihani 2007a). Behavioural observation sessions occurred twice a day, with a morning session (from dawn, running for 3-4 hours) and an evening session till dusk (~3hrs in duration). At both the start and end of every session, the weights of each individual within the social group were recorded. The birds have been trained to stand upon a flat-panned scale in exchange for a small reward of boiled egg, and mealworm, *Tenebrio molitor* (weighing accuracy ± 0.1 g). The habituation aims to ensure that the observer is not perceived as a threat, nor as a source of food, and consequently the birds act naturally in the presence of an

observer. This study was carried out between September 2010 and April 2012, across two field seasons (both between September and April).

2.3.1 Determining sex and social relationships

The pied babbler is phenotypically monomorphic, with no consistent difference in either the mass or plumage between the sexes. They do however, produce sex-specific vocalisations (Golabek & Radford 2013). To confirm the sexes, the population was sexed genetically from blood samples collected during ringing (using the technique outlined in Griffiths et al. 1998). Genetic sexing was carried out by M. Nelson-Flower and N. Munes at the University of Cape Town.

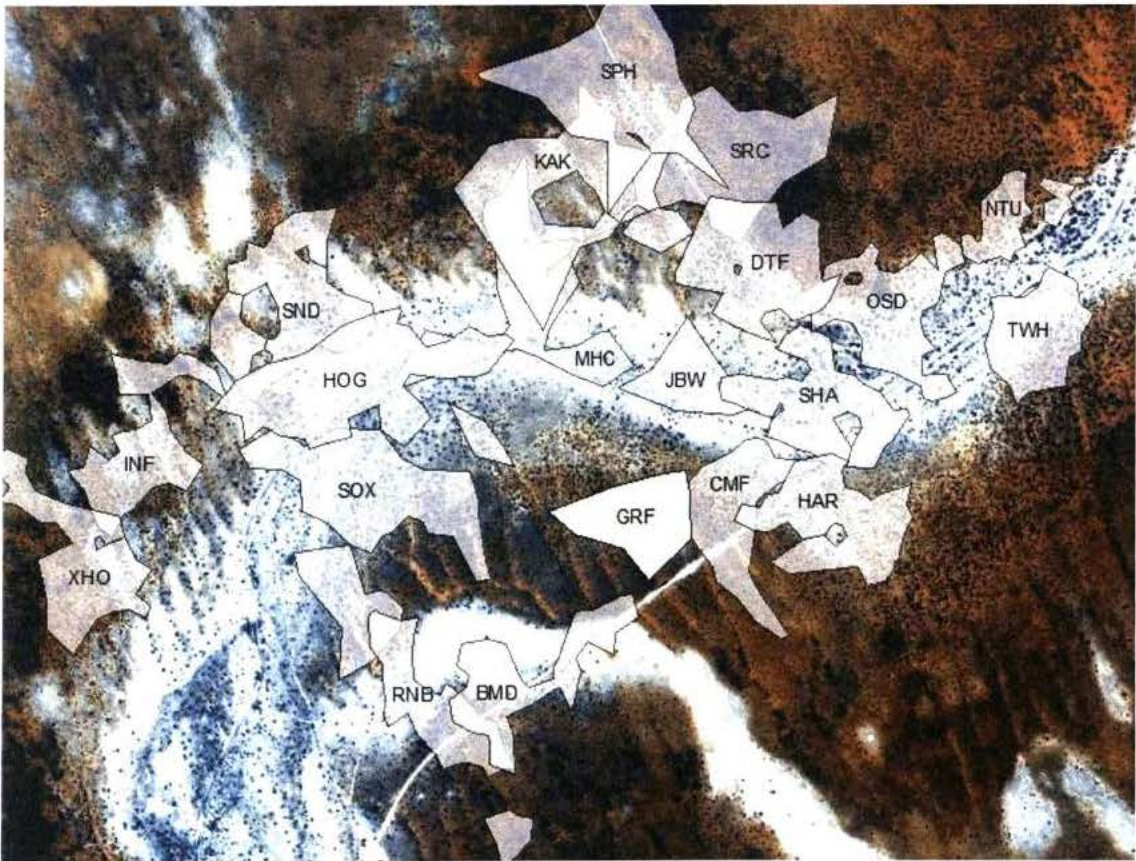


Figure 7 – The territories of each of the habituated groups at the study site between September 2011 and April 2012. Territories were established using the 'a-LoCoH' methodology in R 2.15.2 (see Chapters 9 and 10 for more details). Image created in ArcGIS 9.3.1. A colour filter has been added to highlight the dry riverbed running through the study site.

Dominance within pied babbler social groups can be determined through a combination of both behavioural observations of aggression and breeding behaviour (Raihani 2008; Ridley & Raihani 2008). Dominant individuals assert their dominance over subordinate individuals through pecking behaviour and physical attacks, to which subordinate individuals will respond with submission (bill-gaping, crouching, rolling over, or fleeing, (Raihani 2008)). Dominance can also be established from breeding behaviour, with observations of courting, nest building, or overnight incubation (which is performed by the dominant female) being useful proxies of dominance within the group (Nelson-Flower et al. 2013).

Pied babbler breeding pairs are monogamous and parentage can be reliably assigned from observations of breeding behaviour and activity at the nest (Nelson-Flower et al. 2011). One exception to this is within social groups that have two co-dominant females that are unrelated to the dominant male (Nelson-Flower et al. 2011). In these situations, both females may lay eggs within the nest (Nelson-Flower et al. 2011). We developed a pedigree for the population based upon behavioural observations of breeding activity and dominance within each group. These relationships were confirmed by microsatellite genotyping (Nelson-Flower et al. 2011; Nelson-Flower et al. 2012).

2.4 Data Collection

2.4.1 Sound recording and analysis

Throughout this thesis I utilise recorded vocalisations from the pied babbler, both for bio-acoustic analysis in chapters 3-4, and for acoustic playback in chapters 5-8. We recorded 1044 different 'loud' calls (as defined by Golabek &

Radford 2013), and 473 'chuck' contact calls (as defined by Radford & Ridley 2008). All of these calls were recorded using a Marantz PMD660 data recorder (2008 D&M Holdings Inc.) and a Sennheiser ME66 shotgun microphone with a K6 power module (2004 Sennheiser). The microphone was housed in a Rycote pistol grip with a windshield to reduce background wind noise. For recordings of the 'loud call' vocalisations, a sampling rate of 44.1 kHz was used, but for the 'chuck' contact calls, this was adjusted to 48.0 kHz to ensure we captured the higher frequencies in this call type. All calls were recorded to 16-bit WAVE files (.wav).

Calls were cut from the audio files and readied for either playback or acoustic analysis in the audio editing software package 'Adobe Audition 3.0' (2007, Adobe Systems Inc.). All playback files were normalised prior to playback to standardise the amplitude during playback (to -0.1dB).

For acoustic analysis, calls were measured in the bio-acoustic analysis programme 'Raven Pro v1.4' (Cornell lab of Ornithology, www.birds.cornell.edu/raven). For analysis, spectrogram windows were drawn in a Hamming window (512 point, with an overlap of 96.9%).

ACTIVITY		13:09
REST	SENTINEL	
VIGILANT	FEED	
LONG CALL	GRP CHORUS	
PLAYFIGHT	BILL WIPE	
OTHER	PAUSE	
START	END	
SELF PREEN	FORAGE	
ALLOPREEN	MOVE	

Figure 8 – A screenshot of the focal program created in 'cybertracker' for behavioural data collection using a Palm TX. See Table 1 for a description of each of the behaviours.

2.4.2 Behavioural data collection

During behavioural observations, we recorded each behaviour as it occurred to an electronic handheld device (Palm TX; Palm Inc., Sunnyvale, CA, U.S.A.) using a program created in ‘cybertracker’ (www.cybertracker.org; Figure 8). This program enabled us to record the duration of each behaviour made by a focal individual, and was used in chapters 6-8 to record the behaviours observed before and after a playback (a list of the behaviours recorded is described in Table 1). Additionally, in chapter 5, the behavioural responses to a playback were recorded using a hand-held video camcorder (Canon Legria FS46e). These videos were scored blind in ‘Windows Movie Maker 6.0’ (Microsoft Corporation, 2006), scoring vigilance and movement behaviour.

Table 1 – The description for each of the behaviours recorded during focal data collection

Behaviour	Description
Rest	Periods with no obvious behaviour observed. Rest in the pied babbler is often associated with heat dissipation behaviours such as wing spreading and sitting in the shade (du Plessis et al. 2012).
Sentinel	Vigilance, often from an elevated or exposed position, whilst producing ‘sentinel calls’ that signal sentinel activity to other groups members (Hollén et al. 2008).
Vigilant	Vigilant individuals have their head up, scanning the surrounding environment.
Feed	The provision of food to dependent young.
Long Call	Solo loud (or long-distance) calling behaviour. Pied babblers produce eight acoustically distinct loud calls (Golabek & Radford 2013). Three of these call types are given predominantly as a solo call by just one individual.
Group Chorus	Group chorusing - where multiple individuals produce atonal loud call types repetitively together in a coordinated vocal display (Golabek & Radford 2013).
Play fight	Engaging in play. Play may take a variety of forms including chasing, hanging upside down and wrestling, where individuals kick and grapple each other.

Bill wipe	Bill-wiping, where birds drag their bill repeatedly over branches and other objects, is used by birds to clean and hone the bill (Cuthill et al. 1992). However, there is evidence to suggest that avian species increase this behaviour when under stress or as a symptom of misplaced aggression (Evans 1984; Goodson et al. 1998). As a consequence, bill-wiping behaviour can be a useful proxy to stress and aggression in avian species.
Auto preen	Where individuals clean their own feathers. Pied babblers dedicate a significant proportion of their time to maintaining their brilliant white and black plumage.
Forage	The time spent in search of food. Pied babblers are predominantly terrestrial foragers and spend much of their foraging time sifting through sand and leaf litter looking for subterranean invertebrates (Child et al. 2012). However, they will also take food items from the surface and they will glean prey items from vegetation.
Allopreen	Where individuals clean the feathers of social partners. Allopreening in birds can serve a dual function, used for both parasite removal and for social affiliation (Radford & Du Plessis 2006). Allopreening can increase both before and after aggressive encounters (Radford 2008; Radford 2011) and therefore can provide a useful proxy for stress.
Moving	A combined measure of all moving behaviour including both flight and hopping between sites.

2.4.3 Playback protocol

Throughout this thesis (Chapters 5-8), I utilise acoustic playback of pied babbler vocalisations to initiate responses from focal individuals. Playbacks followed two formats including, 1) a 50 second playback composed of ten chuck contact calls (with 5.5 seconds silence between calls), played back at 40dB from a speaker placed on the floor to mimic the terrestrial foraging behaviour associated with this call (chapter 5), and 2) a five second atonal chatter loud call, played back at 70dB, from a speaker mounted to a tripod 1.5m high (chapters 6-8). In both cases, vocalisations were played back from a Sony Walkman NWZ-E345 using an Altec Lansing Orbit (iM227) speaker (which was painted brown and green to aid concealment). Amplitudes were measured from 5m with a Voltcraft SL100 (Voltcraft, Barking, UK) sound level

meter. Between September 2010 and April 2012 we carried out a total of 83 chuck contact call playbacks, and 402 loud call playbacks, ensuring that no individual was exposed to the same playback twice.

2.4.4 Establishing the territories of each group

Every researcher at the pied babbler study site since 2003 has recorded the location of the group using a handheld GPS (accuracy <10m), at fifteen-minute intervals throughout their data sessions. This has led to an extensive database of location points for each social group over time. We collated these points into a database of >50,000 location points.

Pied babblers need woody vegetation for foraging (Thiele et al. 2008), and seldom forage within the open flat terraces of the reserve. As a consequence, some territory plotting techniques, such as minimum convex polygons, poorly predict land use patterns by the pied babbler because they fail to exclude areas that are not in use. Here we use the 'adaptive sphere of influence localised convex hull' (a-LoCoH) technique for plotting the home ranges of the pied babbler, a technique that is particularly well adapted to excluding unused areas (Getz et al. 2007).

Territories were plotted using a random selection of 300 points collected over a breeding year (September-August), using the a-LoCoH technique carried out in the 'adehabitat' package (Calenge 2007) in R 2.15.2 (R core development team, 2012). 95% density isopleths were exported as shapefiles (.shp), and projected into ArcGIS 9.3.1 (ESRI, 2009) for further analysis. Measurements of territory size and overlap with neighbouring territories were measured using the 'Hawths Tools' extension for ArcGIS (Beyer, H. L., 2004. Hawth's Analysis Tools for ArcGIS. Available at <http://www.spatialecology.com/htools>).

2.5 Statistics

This thesis incorporates a range of different statistical techniques, the majority of which were carried out in 'R' versions 2.12.1 to 2.15.2 (R core development team, 2013), although we also used the statistical package SPSS statistics, version 19.0 (SPSS Inc., IBM 2012). See Table 2 for a list of the statistical techniques employed in this thesis.

Table 2 – a brief description of the statistical techniques employed throughout this thesis.

Statistical Test		
<i>Discriminant Function Analysis (DFA)</i>	<i>Chapters 3 and 4</i>	<i>SPSS</i>
<p>The discriminant function analysis (DFA) is used to predict a categorical dependent variable based upon one or more predictor variables. The DFA is used widely in the study of bioacoustics to determine whether call properties can predict a categorical feature of interest, for instance individual identity (e.g. Rukstalis et al. 2003). I utilise the DFA here to explore whether call parameters are more similar among the calls of an individual than between the calls of different individuals.</p>		
<i>Binomial test</i>	<i>Chapters 3 and 4</i>	<i>SPSS</i>
<p>The binomial test is used to explore the significance of an observed distribution from a theoretically derived expected distribution. I used it here as a follow up analysis to the DFA, exploring whether the number of calls correctly classified by the DFA after leave-one-out cross-validation (% of calls correctly classified), differs from an expected classification rate (if calls had been assigned to an individual at random).</p>		

Permutation-based DFA (pDFA)

Chapter 4

R

The DFA has limitations when testing higher-order grouping variables such as sex or social group as it fails to capture the within-individual variation that can occur (because only one call per individual can be used; Mundry & Sommer 2007). The nested permutation-based discriminant function analysis (pDFA) has been created for the analysis of two-factorial data sets (Mundry & Sommer 2007). I used the pDFA to explore whether call parameters can be classified by the social group of an individual, when controlling for individual variation in these calls. The pDFA was run in R using a script written for this purpose (R. Mundry, *personal communication*).

Mantel test

Chapter 3

R

The mantel test is used to test for correlations between two matrices. I used the mantel test to explore call similarity values derived from spectrographic cross-correlation (SPCC) with a hypothesis matrix (within or between individuals). The SPCC output inherently has an element of pseudoreplication because every pair of calls in a data set is compared, making the values mathematically interrelated. I used Mantel's randomisation test (Mantel 1967) to account for this, a technique used previously for SPCC analysis (Sharp & Hatchwell 2005; Sharp & Hatchwell 2006). This test incorporates a randomisation procedure to avoid the pseudoreplication inherent in the data. Mantel's randomisation test was carried out in R using the 'Vegan' package (Oksanen et al. 2012).

GLMMs with AICc

Chapters 5, 6 and 9

R

When conducting experiments with wild animals, a range of environmental (e.g. rainfall and food abundance) and social factors (e.g. identity, age, sex, or dominance) can come into play, and are potentially important for explaining

response terms. Generalised linear mixed models (GLMMs) provide the ability to simultaneously explore multiple explanatory terms and statistically control for both random and fixed effects (reviewed in Bolker et al. 2009). I have used GLMMs where repeated measures have occurred with individuals or social groups over time as this technique accounts for this known variation within the dataset. Best models were chosen based upon Akaike's information criterion adjusted for small sample sizes (AICc; Hurvich & Tsai 1989) weighting (lowest AICc value). Global model sets were reduced down to a top model set by removing all models with AICc weighting >5 from the best model. Estimates of effect sizes and significance for explanatory terms were determined by model averaging of this top model set. Model averaging was performed in R using the 'AICcmodavg' package (Mazerolle 2012). Model averaging is a technique that allows robust parameter estimates to be obtained when there is best model uncertainty (AICc weighting for the best model < 0.9 ; Grueber et al. 2011). Model averaging calculates a weighted average of each parameter estimate, so that parameter estimates reflect the respective AICc weightings of the models in which the terms appear (i.e. terms that appear in the best models are given the highest weighting)(Grueber et al. 2011).

GEEs

Chapter 9

SPSS

Generalised estimating equations (GEEs) are an extension of a generalised linear model. However, the two techniques differ in their focus, with GEEs focused on population averages rather than subject-specific responses (as is the case with GLMMs). I have used GEEs to address the frequency of fighting behaviour between groups, where I was interested in general trends in fighting behaviour between groups within the population (rather than in fighting

behaviour between specific groups). Best models were chosen based upon lowest quasi-AIC values (QICc). Model averaging is currently unavailable for QICc, and we therefore adopted an alternative approach to determining the importance of model terms. I deemed explanatory terms as important if they were present in the best models. Best models were all those within two QICc of the best model (the model with the lowest QICc value). I have opted for a more conservative approach to model selection for the GEEs because of the unavailability of model averaging.

Paired T-test *Chapters 7,8,9 and 10* *R*

The paired t-test is used to compare the difference between two normally distributed, matched samples. Specifically, I use a ‘repeated measures’ or ‘paired sample’ t-test, where measures are taken from the same individual (e.g. body mass, behaviour) before and after an event of interest.

Chi-square *Chapter 10* *R*

Chi-square tests can be used to explore whether an observed dataset differs significantly from a pre-determined hypothesis data set. I use Chi square in chapter 10 to explore whether observed calling rates by subordinate individuals differ significantly from the rate expected if calls were distributed evenly throughout their territory.

Chapter 3

Vocal cues to identity: pied babblers produce individually distinct but not stable loud calls



Co-authored by: Fiona M. Finch, Matt B.V. Bell and Amanda R. Ridley

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3.1 Abstract

Reliable cues to identity are an important component for the successful coordination of social behaviours in group living animals. Coordinating social behaviours over long distances becomes problematic, as cues to identity are often limited to one or two sensory modalities. This limitation can often select for strong individuality in those cues used for long distance communication. Pied babblers, *Turdoides bicolor*, produce a number of different types of 'loud calls' which are frequently used to signal to individuals beyond the range of visual or olfactory pathways of communication. Here we show that three of these 'loud call' types: the v-shaped chatter, the double note ascending chatter, and the atonal chatter, are each individually distinct. We hypothesise that individuality in the three loud call types tested here may represent a possible pathway to social recognition in this species that may have important consequences for social interactions. However, we also found that the atonal chatter was unstable between years suggesting that this particular call type may not be a reliable long-term indicator to identity.

3.2 Introduction

The ability to recognise and classify individuals either as kin, mate, neighbour or rival is likely to be advantageous (Sherman et al. 1997). Correct recognition of these classes may reduce the cost of agonistic competition, increase the opportunity for kin directed altruism, and decrease the risk of costly inbreeding (Barnard & Burk 1979; Tibbetts & Dale 2007). It has been suggested that animals that engage in complex social behaviours display distinctive phenotypic characteristics to facilitate recognition (Tibbetts 2004; Pollard & Blumstein 2011). In birds, vocalisations are often the dominant form of communication (Halpin 1991) and 'vocal signatures' to both identity (Price 1998; Seddon et al. 2002; Sharp & Hatchwell 2005; McDonald et al. 2007; Kennedy et al. 2009) and kinship have been found (Sharp & Hatchwell 2006; McDonald & Wright 2011). Reliable cues to identity may be particularly important in animals that engage in cooperative tasks with others, as it can allow individuals to maximise their direct or indirect fitness by recognising and avoiding cheats or by preferentially assisting kin (Bradbury & Vehrencamp 2011).

Among highly social birds, individuality has been found in a variety of vocalisation types including contact calls (Sharp & Hatchwell 2005), lost calls (Seddon et al. 2002), mobbing calls (Kennedy et al. 2009), provisioning calls (McDonald et al. 2007), and song (Price 1998). Individuality in these calls may play an important role in coordinating social behaviours. For example, long-tailed tits, are able to recognise familiar kin from their vocalisations, and use these cues to preferentially assist at the nests of close relatives (Sharp et al.

2005). Recognition speed and accuracy may be improved by combining information from multiple sensory modalities (Amedi et al. 2005). With 'loud call' vocalisations (also referred to as 'long distance calls'), the receiving individual may often be out of range to perceive visual or olfactory cues of identity and the receiver is therefore reliant on the identity signals within the vocalisation in order to evaluate caller identity (Schleidt 1973; Mitani et al. 1996; Darden et al. 2003; Slabbekoorn 2004). Vocal individuality, where inter-individual call variation is greater than intra-individual variation (Falls 1982), may be under particularly strong selection in loud calls due to: (a) its function in the coordination of social behaviours, and (b) the limits on the number of communication pathways available over long distances.

The social interactions that occur between individuals may be interspersed by extended periods without contact. Here it is not just important that the signalling individual produces a cue to identity, but also that those cues remain stable through time. For instance, the black-legged kittiwake produces individually distinct loud calls that are used for mate recognition and may be used to relocate a breeding partner at the beginning of each breeding season (Wooller 1978; Aubin et al. 2007). The use of vocalisations to relocate breeding partners after months of separation may necessitate selection for identity cues that are reliable from year to year. However, in a number of studies where vocalisations have been found to be individually distinct over short periods, those vocal characteristics that defined an individual changed through time (Jorgensen & French 1998; Ellis 2008). It is therefore important to ascertain how stable cues to identity are through time.

The Southern pied babbler, *Turdoides bicolor*, is a highly social and territorial species from southern Africa that produces a range of different loud call vocalisations (Golabek & Radford 2013). Here we investigate whether the loud calls of the Southern pied babbler are both (a) individually distinct when collected within one week of each other, and (b) distinctive from one year to the next. Previous work has identified that pied babblers produce eight acoustically distinct loud call types that are used in a variety of both inter- and intra-group social situations (Golabek & Radford 2013). These loud calls are characteristically one or two syllables that are repeated for up to 80 seconds in duration (see methods). Loud calls can be given by any member of the social group, but all eight call types are most commonly produced by a dominant group member (Golabek & Radford 2013). Here we have focused our analysis on three of these loud call types, the 'v-shaped chatter', the 'double note ascending chatter', and the 'atonal chatter' (see Figure 1). These three call types were chosen as they were the most frequently observed and recorded of the eight call types. We also investigate the stability of one of the loud calls, the atonal chatter, to test how reliable it may be as a cue to identity through time. The atonal chatter was chosen because it was the most frequently observed of the call types across the two observation years. Given that loud calls are often meant for long distance communication, and that pied babblers are a highly social species, we hypothesise that these three call types will have lower intra-individual call variation than inter-individual call variation, which may facilitate the correct recognition of individuals. We also expect these calls to be reliable indicators of identity through time by having lower call variation from one year to the next than variation between individuals.

3.3 Methods

3.3.1 Study population and sound recording

We recorded the loud calls from a population of pied babblers located at the Kuruman River Reserve in the southern Kalahari desert, South Africa (26°57'S 21°49'E) (see Ridley & Raihani 2007 for more details about the study site). Each member of the study population is individually identifiable using a unique combination of colour bands. These medium-sized (75-95g) cooperatively breeding passerines are habituated to close observation, allowing sound recordings to be collected within 5-10 metres of the calling bird. Vocalisations were recorded between October 2010 and April 2012 using a Marantz PMD660 data recorder (2008 D&M Holdings Inc.) and a Sennheiser ME66 shotgun microphone with a K6 power module (2004 Sennheiser), housed in a Rycote pistol grip with windshield to reduce background wind noise. Recordings were collected at a sampling rate of 44.1 kHz, to 16-bit WAVE files (.wav). We recorded a minimum of six loud call vocalisations of the same call type from an adult bird within a seven day period. This was to try to minimise any acoustic changes that may have been brought on by changes in physical condition, age or environmental conditions. To test whether calls were reliable indicators to identity through time, we re-recorded individuals a minimum of one year on, again collecting a minimum of 6 calls within seven days. None of the individuals that were re-recorded experienced a change in dominance status, a factor that has been found to affect vocal characteristics in other species (Rukstalis et al. 2003). All calls were collected during the wet season (September-April) to minimise acoustic changes resulting from seasonal variation in physical condition. We also compared the weights of the birds at the time of recording

across the two field seasons as a measure of change in physical condition. Focal birds were habituated to the use of a weighing scale by rewarding this behaviour with small amounts of egg and mealworm. Weights were collected for each focal bird using an Ohaus CS200 flat-topped weighing scale (Ohaus, UK) at the start of each recording session.

3.3.2 *The three call types*

The three loud call types analysed, the v-shaped chatter, the double note ascending chatter and the atonal chatter, were all given in a variety of social contexts. However, we have limited our analysis to calls of the same call type given in the same social context.

The v-shaped chatter is given predominantly as a solo call by the dominant male in both inter and intra-group social contexts (Golabek & Radford 2013). We observed that strings of v-shaped chatter calls lasted for 7.37 ± 0.46 seconds on average (mean \pm SD; range 1.8-59.0). Our acoustic analysis of the v-shaped chatter was conducted on a total of 81 v-shaped chatter calls collected from 8 individuals (average number of calls per focal bird 10.13 ± 5.17 SD).

The double note ascending chatter is mostly frequently observed as a solo call by the dominant male in both inter and intra-group social contexts (Golabek & Radford 2013). We observed calling bouts of the double note ascending chatter lasting 8.02 ± 0.44 seconds on average (mean \pm SD; range 1.1 – 40.1). For the double note ascending chatter, we were able to collect 87 calls from 8 different

individuals for our analysis. We measured a minimum of six calls from each focal bird, with an average of 10.87 ± 6.73 (mean \pm SD) calls per focal bird.

The 'atonal chatter' can be given by either sex, and is the most common female solo loud call in the pied babbler (Golabek & Radford 2013). It is typically given in intra-group social interactions (Golabek & Radford 2013). Calling bouts of the atonal chatter were 6.12 ± 0.33 seconds long on average (mean \pm SD; range 2-25). Our analysis was conducted on 147 atonal chatter calls collected from 15 individuals. We collected an average of 9.73 ± 3.43 (mean \pm SD) calls per focal bird in the first year of recording. We recorded the atonal chatter calls from seven individuals at least one year on. 64 calls were collected from these seven individuals in year one (average number of calls per individual 9.14 ± 3.28 SD) and 57 calls in season two (with an average of 8.14 ± 4.18 calls per individual; mean \pm SD).

3.3.3 Sound Analysis

Acoustic analysis was carried out in the bio-acoustic software package 'Raven Pro v1.4' (Cornell lab of Ornithology, www.birds.cornell.edu/raven). For the 'v-shaped chatter' and the 'atonal chatter' we took the 20th call in the call sequence, and for the 'double note ascending chatter' we cut the 15th pair of syllables, taking the long and short syllables separately for analysis. If these calls were marred by background noise we cut the next clear call in the sequence. The calls in the call sequence are typically erratic for the first few seconds of calling. We have chosen the 20th and 15th syllables as these appeared to represent points of consistent stability in the respective call

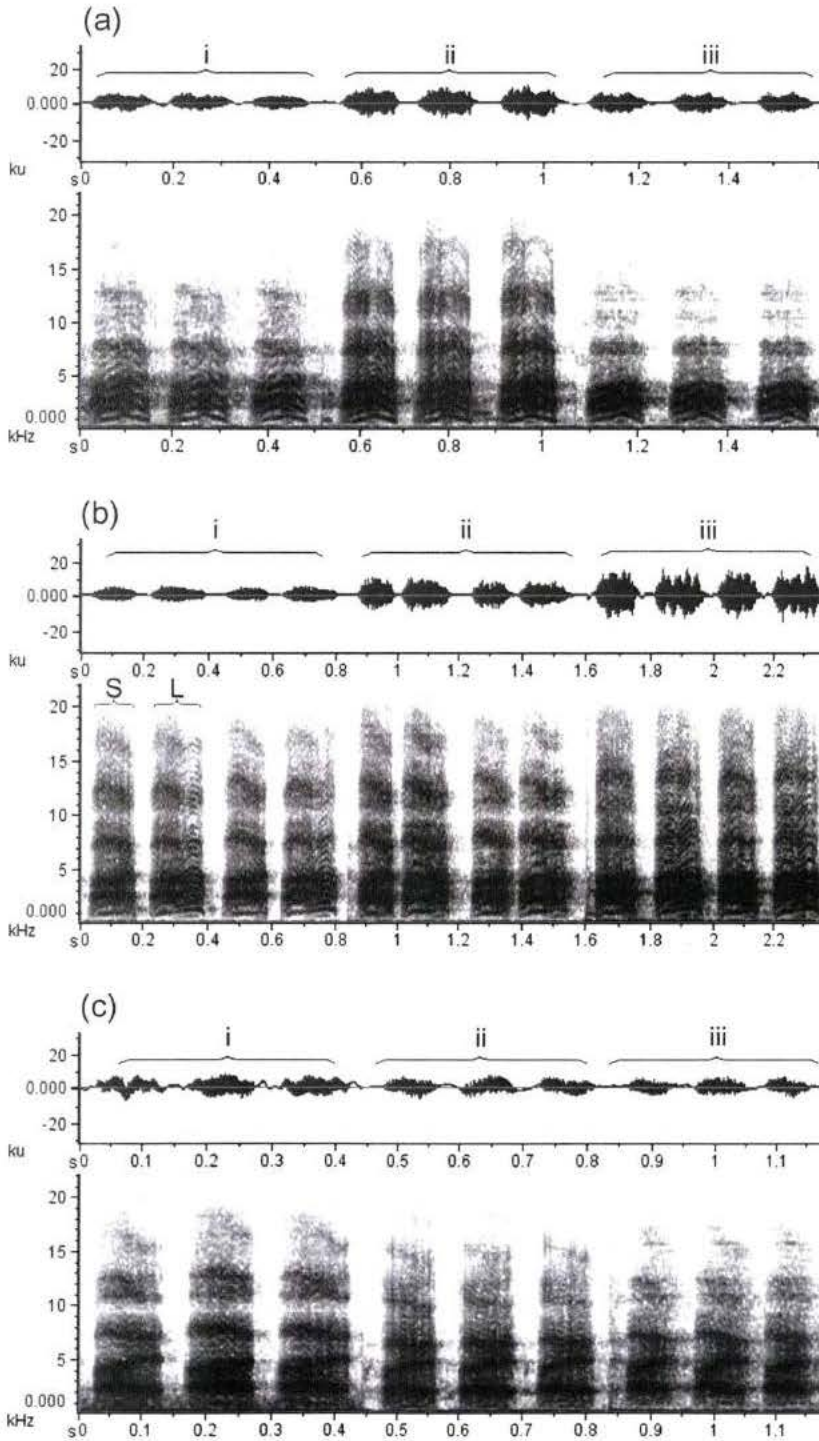


Figure 1. Spectrogram and waveform views of the three loud call types, a) the 'v shaped chatter', b) the 'double note ascending chatter' and c) the 'atonal chatter' as defined by Golabek and Radford (2013). For the double note ascending chatter, (S) denotes the 'short' syllable and (L) the 'long' syllable section of this call. We present three examples of each call type (two for the double note ascending chatter) collected from three different individuals, denoted by 'i', 'ii', 'iii'. Spectrogram windows are drawn in a Hamming window (512 point, with an overlap of 96.9%). Grey scale represents a 65dB range.

sequences. Spectrogram windows were drawn in a Hamming window (512 point, with an overlap of 96.9%). A band pass filter between 500Hz and 22050 kHz was used to eliminate any low frequency noise in the recordings. Each syllable was manually selected and 10 parameters were automatically measured. The 10 measurements were: first quartile frequency, third quartile frequency, aggregate entropy, average entropy, bandwidth 90%, centre frequency, frequency 5%, frequency 95%, peak frequency and inter-quartile range bandwidth (see Charif et al. 2009 for more information on these call parameters). Call duration and inter-call duration were measured by hand, resulting in a total of 12 measurements for each call.

3.3.4 Spectrographic cross-correlation

Call parameter analysis is widely used and is a proven effective method of identifying individuality in calls (Terry et al. 2001), but there is inherently a risk of ignoring important features if those parameters that make the call biologically meaningful to the species are not measured (Cortopassi & Bradbury 2000). We therefore decided to follow up the call parameter analysis with a second technique, spectrographic cross correlation (SPCC), to confirm our findings. SPCC provides a measure of similarity between two calls; it works by passing the two spectrograms past each other along the time axis until a peak correlation score is reached (Charif et al. 2009). The correlation scores range between zero and one, with zero indicating that there is no correlation and one representing a perfect match. The calls used previously for call parameter analysis were cut from the call sequence into separate WAVE files using Raven. A batch correlation was carried out, correlating all files against each other, using the bio-acoustic software package 'Raven Pro v1.4'. Spectrogram

windows were drawn in a Hamming window (512 point, with an overlap of 96.9%). All calls were normalised before correlation. A band pass filter between 500Hz and 22050 kHz was used to remove low frequency noise in the recordings.

3.3.5 Statistics

Call parameter measurements were used to test for individuality using discriminant-function analysis (DFA) performed in the statistical package SPSS statistics, version 19.0 (SPSS Inc., IBM 2012). Our sample sizes here were limited to a minimum of six calls per individual. The DFA has a tendency to overestimate classification when the number of parameters exceeds the minimum number of cases (Tabachnick & Fidell 2001). We therefore limited the number of call parameters in each analysis to just five of the 12 call parameters. We included first quartile frequency, aggregate entropy, the centre frequency, peak frequency and call duration in our analysis. These call parameters were chosen because they showed a lack of outliers and were not collinear with the other terms included (variance inflation factors < 7; Allison 1999). All call parameters that were excluded showed at least one of the above traits (either outliers or co-linearity with other terms). The percentage of correctly classified cases after leave-one-out cross-validation from the DFA was tested for significance using a binomial test performed in SPSS.

To test for the consistency of vocal identity signatures, a DFA was run on the atonal chatter calls collected in the first year of study. The discriminant functions developed from those calls were then used to assign a predicted calling individual to the calls collected in the second year. We then established

the percentage that had been assigned to the correct individual and followed this up with a binomial test performed in SPSS (testing observed classification rate versus what we would expect by chance). The average weights for each focal bird from the first year of recording were compared against the weights of the second year using a paired t-test to test for changes in the mass of the recorded birds between years.

We used the correlation values from SPCC to determine if within-individual variation in correlation coefficients differed significantly from between-individual variation. The SPCC output inherently has an element of pseudoreplication because every pair of calls in a data set is compared, making the values mathematically interrelated. We used Mantel's randomisation test (Mantel 1967) to account for this, a technique used previously for SPCC analysis (Sharp & Hatchwell 2005; Sharp & Hatchwell 2006). This test incorporates a randomisation procedure to avoid the pseudoreplication inherent in the data. The SPCC similarity matrix was converted to a distance matrix (distance = 1 - similarity) (Legendre & Legendre 1998, p275). This distance matrix was tested against a second binary hypothesis matrix, with a '1' representing correlations between calls given by the same individual, and a '0' for inter-individual correlations (see Schnell et al. 1985 for a detailed discussion of the technique). Mantel's randomisation test was carried out in R 2.15.0 (R Development Core Team 2012) using the 'Vegan' package (Oksanen et al. 2012).

3.4 Results

3.4.1 The 'v-shaped chatter'

The DFA was able to correctly classify the v-shaped chatter in 50.0% of cases after leave-one-out cross-validation (DFA, Wilks Lamda = .100, $X^2_{35} = 167.250$, $P < 0.001$) indicating significant individuality in the parameter measurements recorded. Using 6561 comparisons, the SPCC confirmed that there was less variation within calls of the same individual (average call similarity 0.459 ± 0.135 SD; range 0.119-0.871) than between individuals (average 0.397 ± 0.109 SD; range 0.111-0.724; Mantel test, 10000 permutations, $r = -0.2187$, $p = 0.05$; see Figure 2a).

3.4.2 The 'double note ascending chatter'

For the double note ascending chatter, both syllables proved to be individually distinct. The short syllable could be correctly classified in 53.2% of cases after leave-one-out cross validation (DFA, Wilks Lamda = 0.121, $X^2_{30} = 151.103$, $P < 0.001$), and SPCC correlation values were higher within an individual 0.440 ± 0.101 (mean \pm SD, range 0.15-0.768) than between individuals 0.377 ± 0.096 (mean \pm SD, range: 0.087-0.686; Mantel test, 10000 permutations, $r = -0.267$, $p = 0.039$; see Figure 2b). The long syllable could be correctly classified in 61.5% of cases after leave-one-out cross-validation (DFA, Wilks Lamda = .159, $X^2_{30} = 130.512$, $P < 0.001$), and call similarity was greater within an individual (average 0.381 ± 0.126 SD; range 0.060-0.781) than between individuals (average 0.264 ± 0.098 ; range: 0.046-0.793; Mantel test, 10000 permutations, $r = -0.427$, $p = 0.042$; see Figure 2b).

3.4.3 The 'atonal chatter'

The DFA was able to correctly classify these calls 42.7% of the time using leave-one-out cross validation (DFA, Wilks Lamda = 0.057, $X^2_{70} = 377.947$, $P < 0.001$). Using 23,104 comparisons, the SPCC confirmed that there was less variation within calls of the same individual (mean \pm SD = 0.410 \pm 0.130, range 0.088-0.831) than between individuals (mean \pm SD = 0.320 \pm 0.110, range 0.05-0.79; Mantel test, 10,000 permutations, $r = -0.210$, $p = 0.024$; see Figure 2d).

3.4.4 Consistency of individual call signatures

Using a subset of the atonal chatter calls from year one, they were again found to be individually distinct and could be correctly classified in 43.8% of cases after leave-one-out cross-validation (DFA, Wilkes Lamda = .167, $X^2 = 101.959$, $df = 30$, $P < 0.001$). Additionally, the calls collected one year on in the second season were also individually distinct and could be correctly classified in 56.1% of cases after leave-one-out cross-validation (DFA, Wilkes Lamda = .093, $X^2 = 118.696$, $df = 30$, $P < 0.001$). However, calls collected in the second year were only classified in 12.3% of cases by the discriminant functions produced from the calls of the first year (binomial test, $P = 0.288$). This demonstrates that there is as much variation within the calls collected from an individual between two different years as exists between individuals and suggests that the atonal chatter may be an unreliable cue to identity through time. The change in vocalisations occurred despite no significant change in the weights of the calling birds between the two recording sessions (paired t-test, $P = 0.86$).

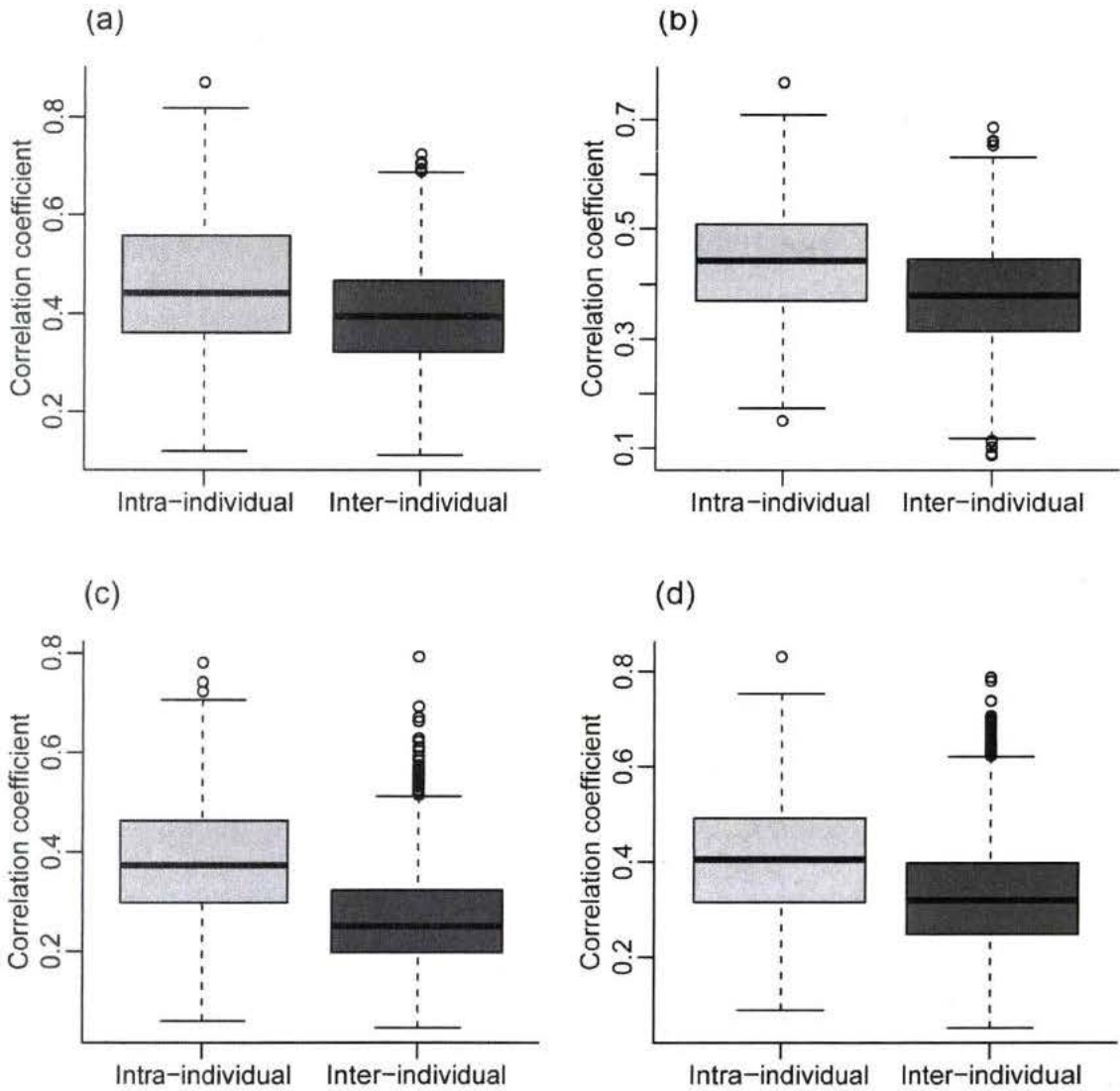


Figure 2. Correlation coefficients from SPCC for within individual and between individual comparisons for: (a) the v-shaped chatter, (b) the short syllable of the double note ascending chatter, (c) the long syllable for the double note ascending chatter, and (d) the atonal chatter.

3.5 Discussion

Vocal individuality, where variation within the calls of an individual is lower than variation among individuals (Falls 1982), was found in all three of the loud call types tested here (the v-shaped chatter, the double note ascending chatter and

the atonal chatter). Distinctive cues to identity are the foundation of recognition and are required for the identification of individuals, kin, neighbours, parent-offspring, rivals, and species (Sherman et al. 1997). Our findings that at least three of the call types of the pied babbler are individually distinct suggest a potential pathway to social recognition in this species that may be used to facilitate social interactions. Social recognition allows individuals to be selective in whom they cooperate with, which can both reduce cheating in mutualistic interactions, as well as increasing indirect fitness benefits when preferentially assisting kin (Crowley et al. 1996; Nowak & Sigmund 1998; Bradbury & Vehrencamp 2011).

Recognition has been described as a three-step process in which firstly a signalling individual must produce reliable cues to identity, and then a receiver must both detect these cues and make an appropriate connection between the cue and the identity (Sherman et al. 1997). The production of vocal cues to identity can facilitate recognition at many levels, allowing both individual recognition as well as the recognition of familiar relatives (Halpin 1991). For example, in emperor penguins, *Aptenodytes forsteri*, individuality in parental calls allows parents and offspring to relocate one another in a crowded colony (Robisson et al. 1993), and in the cooperatively breeding long-tailed tit, individually distinct calls are used to recognise familiar kin and direct helping behaviours towards closely related individuals, which is likely to have inclusive fitness benefits (Hatchwell et al. 2001; Sharp & Hatchwell 2005; Sharp et al. 2005). Pied babblers coordinate many of their social behaviours, such as the spacing between foraging individuals, and the coordination of sentinel bouts

through vocalisations (Radford & Ridley 2007; Hollén et al. 2008; Bell et al. 2010). Our findings that pied babblers produce vocal cues to identity demonstrates a potential pathway to recognition of both individuals and kin in this species which may help further facilitate the coordination of social interactions, although whether they can discriminate between these calls remains to be tested.

Vocalisations are often highly plastic and acoustic structures may change in response to age, physical (Gouzoules & Gouzoules 1990; Bertucci et al. 2012), social (Farabaugh et al. 1994; Mathevon et al. 2010), motivational (Morton 1977), and environmental factors (Patricelli & Blickley 2006; Slabbekoorn & den Boer-Visser 2006). Our findings that the atonal chatter was not a stable long-term indicator to identity demonstrated that this call is also plastic, changing over the course of a year. The changes in the atonal chatter may represent a form of honest signalling where vocalisations change in response to changes in the physical and social status of the calling bird. We found no significant changes in the body mass of the focal birds between the two seasons, but vocal changes may correlate with other physical factors such as age (Green 1981; Blumstein & Munos 2005; Ey et al. 2007) or fatigue (Vannoni & McElligott 2009). Voice breaking has been noted in several species of birds and it is possible that the vocal shifts observed in the atonal chatter may correspond to the ageing of the birds (Radford 2004; Klenova et al. 2010). Here we controlled for social factors by only using calls from individuals that were subordinates in both recording seasons, but it is possible that the changes in the identity signals reflected changes in social status within the subordinate ranks.

Instability in the atonal chatter may have important consequences for its reliability as an identity cue over the long term. This could have an impact on the social behaviour and may require either frequent contact between individuals, or alternative cues to identity to be used in order for long-term recognition to occur. The atonal chatter call is most often observed in intra-group social interactions (Golabek & Radford 2013). The use of the atonal chatter call within the social group and the frequent contact that occurs between group members may keep group members updated on changes occurring within individual signatures. However, atonal chatter calls have also been observed from prospecting individuals (D. Humphries, *personal observation*). In the pied babbler, long-term recognition is likely to be important for inbreeding avoidance because they are a long-lived species and may need to find mating partners many years after initial dispersal from the natal territory (Nelson-Flower et al. 2012). Unstable identity labels could potentially lead to costly recognition errors such as inbreeding, if kin recognition in this species is based on prior association. However, research has indicated that inbreeding is rare in this species (Nelson-Flower et al. 2012), and therefore it is possible that other cues (such as different call types or signals) may act to allow inbreeding avoidance in this species.

To conclude, we have found that pied babblers produce three individually distinctive call types that have the potential to act as cues for social recognition. We also found that the atonal chatter was not a reliable indicator to identity from one breeding season to the next, although the causality of these vocal changes currently remains unclear.

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Chapter 4

Group and identity signatures in pied babbler contact calls



Photo by Fiona Finch

Co-authored by: Fiona M. Finch, Matt B.V. Bell and Amanda R. Ridley

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4.1 Abstract

The ability to recognise social partners is thought to carry wide-ranging benefits and can help to reduce aggression, support cooperation and maintain social cohesion within animal societies. Given the benefits of social recognition, individuals are expected to have developed mechanisms for signalling their individual identity. For group-living animals, it may also be beneficial to produce cues and signals for group membership. Doing so can help to develop social affiliations as well as providing an alternative pathway to social recognition. Here we show that the 'chuck' contact calls of subordinate Southern pied babblers, *Turdoides bicolor*, are individually distinct. Additionally, calls also contained a 'group label', where calls were more similar among group members than they were to non-group members. The production of vocalisations that are informative of individual identity and group membership provide pathways for social recognition, which may facilitate the social behaviour of this group-living, cooperatively breeding species.

4.2 Introduction

In group-living societies, where individuals make repeated interactions with a limited number of conspecifics, the ability to recognise group members and retain information about previous encounters is likely to carry substantial benefits (Tibbetts & Dale 2007). Remembering the outcomes of previous aggressive encounters reduces the need to repeatedly interact, saving both time and energy, and limiting the risk of injury (Barnard & Burk 1979). Social recognition allows cooperation to develop through mutualistic interactions, because 'cheats' who do not reciprocate can be identified and avoided (Bradbury & Vehrencamp 2011; Crowley et al. 1996). It can also play a vital role in maintaining social cohesion, allowing groups members to locate one another (Waser 1977). Given the benefits of social recognition, we expect individuals to have developed mechanisms for signalling their identity, and for detecting and recognising the identity cues and signals of others (Tibbetts & Dale 2007).

In avian species, vocalisations are believed to be the dominant mechanism for communication (Beecher 1988; Halpin 1991). Investigations into whether vocalisations carry the 'individual signatures' required for recognition have been fruitful, revealing that the calls of many species are more similar within an individual than between individuals (Aubin et al. 2007; Kennedy, Evans & McDonald 2009; Kondo, Izawa & Watanabe 2010; Lessells, Rowe & McGregor 1995; Robisson, Aubin & Bremond 1993; Sharp & Hatchwell 2005). Identity signatures do not necessarily occur in isolation, and may occur alongside a number of other cues and signals within vocalisations, such as those for sex (Ballintijn & Cate 1997; Eda-Fujiwara et al. 2004; Lessells et al. 1995; Price

1998; Radford 2004), physical quality (Hardouin et al. 2007; Klenova, Zubakin & Zubakina 2011; Woodgate et al. 2012), aggressive state (Morton 1977), and dominance status (Kroodsma 1979). The ability of vocalisations to carry a multitude of information simultaneously mean they are not just limited to advertising individual identity, but they can also carry other cues and signals to facilitate recognition including information about the social group an individual belongs to (Gillam & Chaverri 2012; Townsend, Hollén & Manser 2010), the relatedness between individuals (McDonald & Wright 2011; Price 1998; Sharp & Hatchwell 2006), or the geographical area an individual is from through regional dialects (Baker & Cunningham 1985; Wright 1996).

The production of 'group labels' provides an indication to the broader social affiliations of an individual. For instance, in budgerigars, *Melopsittacus undulatus*, vocalisations signal both breeding partnerships and the social group to which an individual belongs, and are an important factor for the formation and maintenance of social bonds (Hile, Plummer & Striedter 2000; Hile & Striedter 2000). They may also play an important role in facilitating recognition. The speed and accuracy of recognition can be improved when multiple cues and signals are used for identification (Amedi et al. 2005). The production of group labels alongside individual identity signatures may therefore function to improve the accuracy of social recognition. However, little is known about the production of group signatures, or how widespread this behaviour is because the statistical procedures required to test for them have only recently emerged (e.g. Mundry & Sommer 2007).

Here we investigate whether vocalisations produced by the Southern pied babbler, *Turdoides bicolor*, contain both individual signatures and group labels.

Pied babblers are a cooperatively breeding, medium sized (75-95g) passerine, of the Southern Kalahari that live in year-round stable social groups (Ridley, Child & Bell 2007). Social groups are comprised of a dominant breeding pair, who monopolise breeding activity within the group, and non-breeding subordinate helpers of both sexes who are usually retained offspring (Nelson-Flower et al. 2011).

Vehrencamp et al. (2003) identified group-specific labels in the contact calls of the orange fronted conures, *Aratinga canicularis*, and concluded that group labels may be of particular importance in calls used to maintain social cohesion. We therefore focused our investigation on the 'chuck' contact call of the pied babbler, because this call type has previously been found to maintain social cohesion and regulate the spacing of foraging individuals in this species (Radford & Ridley 2008). The 'chuck' call of the pied babbler is a short close call given during foraging (Radford & Ridley 2008). Previous research has shown that several long-distance vocalizations within the pied babbler vocal repertoire are individually distinct (Humphries et al., Chapter 3), and we therefore predict that individual signatures will also be present in the short-distance contact calls. We also predict that due to the high relatedness and time spent in social affiliation with other group members, that group members may share call characteristics, and that a group label will be present in their contact calls.

4.3 Methods

4.3.1 Study population and sound recording

Contact calls were collected from a study population of free-ranging, habituated pied babblers at the Kuruman River Reserve, South Africa (26°57'S 21°49'E) (see Ridley & Raihani 2007 for more details about the study site). We recorded the contact calls from 17 colour-banded adult subordinates from 6 different social groups between October 2011 and February 2012. Dominants were excluded from the analysis to avoid the potential of dominance signals within the calls exaggerating vocal differences among callers. We recorded a minimum of 10 calls from each bird (mean 16.24 calls; range 10-30). Pied babblers can produce an 'elevated chuck call' which is higher in pitch after food discovery (Golabek 2010). We excluded these from the analysis and used only the non-elevated 'chuck' contact call collected in the context of moving between foraging bouts. Each of the focal birds was habituated to close observation by humans and recordings could be collected within 3m of the calling bird. Calls were collected using a Sennheiser ME66 shotgun directional microphone with a K6 power module (2004 Sennheiser), housed in a Rycote pistol grip with windshield to reduce background noise. Calls were recorded at a sampling rate of 48 kHz to 16 bit WAVE files (.wav) to a Marantz PMD660 data recorder (2008 D&M Holdings Inc.).

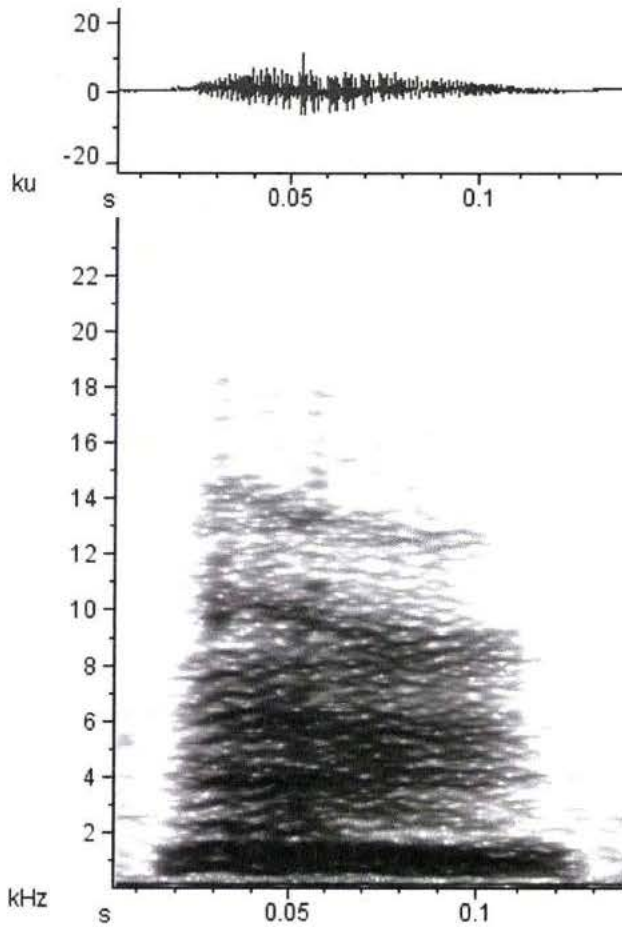


Figure 1. Spectrogram and waveform views of a typical pied babbler contact call. The spectrogram window was drawn in a Hamming window (512 point, with an overlap of 96.9%). Grey scale represents a 60dB range.

4.3.2 Contact call measurement and analysis

Acoustic analysis was carried out in the bio-acoustic software package 'Raven Pro v1.4' (Cornell lab of Ornithology, www.birds.cornell.edu/raven).

Spectrograms were drawn in a Hamming window (512 point, with an overlap of 96.9%). A band pass filter between 500 and 24,000 kHz was used to eliminate any low frequency noise in the recordings. The start and end of each 'chuck'

call was manually selected from the spectrogram and six parameters were automatically measured by the Raven program. The six parameters were; first quartile frequency, average entropy, centre frequency, frequency 95%, and peak frequency (see Charif, Waack & Strickman 2009 for more information on these call parameters). Call duration was measured by hand, resulting in a total of seven parameters for each call. Individuality in the contact call was assessed using a Discriminant Function Analysis (DFA) of the call measurements. The DFA was performed in SPSS statistics, version 19.0 (SPSS Inc., IBM 2012). All variables were checked for outlying values and for co-linearity with the other terms (variance inflation factor < 7 ; Allison 1999). A log transformation was used to normalise peak frequency. The percentage of correctly classified cases after leave-one-out cross-validation from the DFA was tested for significance using a binomial test performed in SPSS.

DFA has limitations when testing higher-order grouping variables such as sex or social group because only one call per individual may be used (Mundry & Sommer 2007). Using multiple calls from an individual would violate the assumptions of independence of the DFA (Mundry & Sommer 2007). This limits the analysis by failing to capture much of the within-individual variation that exists within a call type (Mundry & Sommer 2007). To test for group signatures within the contact calls we therefore used a nested permutation-based discriminant function analysis (pDFA) which has been created for the analysis of two-factorial data sets (Mundry & Sommer 2007). The pDFA was run in R 2.15.1 (R Development Core Team 2012) using a script written for this purpose (R. Mundry, *personal communication*). To ensure that group separation was not a by-product of age differences between the individuals of different groups, we

ran another pDFA nesting individuals into three age categories of (1) 300-600 days, (2) 600-900 days, and (3) >900 days old. A third pDFA was run using only calls collected from females to control for the potential effect of caller sex. Pied babblers are sexually monomorphic and were therefore sexed genetically from blood samples collected during ringing (following the methods described in Griffiths et al. 1998). The female-only analysis used calls collected from eight females belonging to four different social groups. There was an insufficient sample size to repeat the test for males.

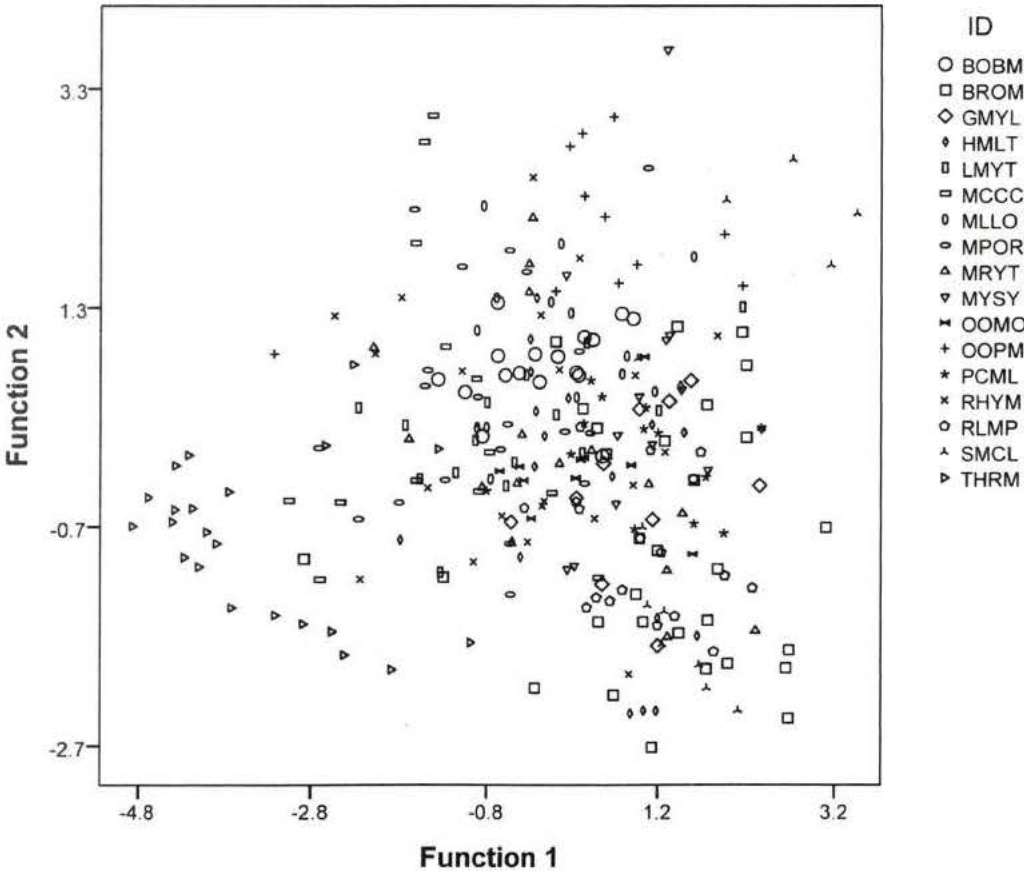


Figure 2. Graphical representation of the first two functions in the DFA showing the individual differences between chuck call acoustic measurements from 17 individuals.

4.4 Results

Contact calls were individually distinct and could be correctly classified in 29.3% of cases after leave-one-out cross-validation (DFA, Wilks Lamda = 0.127, $X^2_{96} = 544.010$, $P < 0.001$). When controlling for individuality in the chuck calls, calls could be classified by the social group of the calling individual, indicating that calls were more similar among members of the same social group than between social groups (pDFA, $P = 0.001$). We found that call similarity did not correlate with age, indicating that the group labels we observed were not a by-product of differences in the age of group members from different groups (pDFA, $P = 0.072$). The presence of a group signature was also maintained when the calls from females were analysed separately (pDFA, $P = 0.031$), ruling out the effects of sex on call similarity among group members.

4.5 Discussion

Our analysis found pied babbler contact calls contain information about both the identity of the caller and the social group to which the individual belongs. Identity signatures are the first step in achieving social recognition, and our findings provide evidence for a potential pathway to social recognition in the pied babbler. Recognition can be seen as a three-step process in which; firstly, a signaller produces one or more cues and signals to identity, and then a receiver both detects those cues and signals, and then makes a cognitive connection between the identity label and the individual from which it came. Our findings provide clear evidence for the first stage of the recognition process, with pied babblers vocally providing information about both their individual identity, as well as the identity of the social group from which they belong.

Whereas individual signatures may occur as a by-product of individual differences in physical size or tracheal morphology (Suthers 1994), group signatures usually develop when call elements are learned from a shared social environment (Bradbury & Vehrencamp 2011). Many bird species develop their vocalisations based upon the vocalisations they hear in their environment (Eales 1985; Greig, Taft & Pruett-Jones 2012; Lehongre, Aubin & Del Negro 2009; Marler 1970; Sharp et al. 2005; Waser & Marler 1977), and in some cases, where birds spend time in mixed species flocks, vocal learning may even explain the apparent vocal mimicry of other species (Kelley et al. 2008). Our observation that pied babbler contact calls are more similar among group members than among individuals *between* groups indicates that the pied babbler may also learn and develop some of the characteristics of the contact calls from the social environment. However, some vocalisations may also have a genetic component (Forstmeier et al. 2009). Pied babbler group members usually have high relatedness to one another (Nelson-Flower 2010), and vocal similarity in the contact calls among group members may have developed as a by-product of a shared genetic and social environment (Deecke et al. 2010).

Group signatures have been observed in a number of other highly social bird species including the long-tailed tit, *Aegithalos caudatus*, (Sharp & Hatchwell 2006) and the splendid fairy-wren (Greig et al. 2012). In the long tailed tit, cross-fostering experiments have demonstrated that vocal similarity among siblings arises from vocal learning in a shared rearing environment rather than a result of genetic influence (Sharp et al. 2005). It seems likely, therefore, that

vocal similarity among group members in the pied babbler may have arisen as a product of vocal learning.

The production of group labels can help to maintain social cohesion among group members (Bradbury & Vehrencamp 2011; Knörnschild et al. 2012), and may facilitate the recognition of non-group members. This may be particularly important in the highly territorial pied babbler, as aggressive interactions with groups in neighbouring territories are common (Golabek, Ridley & Radford 2012). The production of group labels within pied babbler vocalisations may facilitate the early detection of foreign group members within a territory, and may limit the costly exploitation of resources within the territory from non-group members (Davies & Houston 1981; Stamps 1984). Our findings indicate that pied babblers vocally display information about individual and group identity, and that the chuck contact call could potentially be used for social recognition. Social recognition may be particularly important in cooperative species where the benefits of cooperation can vary depending on who is being cooperated with (Crowley et al. 1996). Our findings therefore provide an important first step in understanding how cooperation could be communicated, coordinated and maintained.

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Chapter 5

Individual recognition in the cooperatively breeding Southern pied babbler



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5.1 Abstract

The ability to recognise individual social partners and retain information in memory about previous encounters is likely to be advantageous in social species. Recently the study of individual recognition has been advanced by the introduction of cross-modal experiments, which highlight the cognitive associations formed between different identity signals to demonstrate recognition that is specific to the individual. Here we suggest that these experiments are at risk of being confounded if identity is not the only point of association between the presented signals. Visual cues, as well as being informative of individual identity, are also informative of a host of other information including the sex, size, race and age of the individual. Importantly, the selection for multi-modal signalling can mean that this information may also be present in other sensory pathways including vocal and olfactory routes. Consequently, there are multiple points of association between two signals independent of individual identity. Here we suggest the use of one sensory signal set against a spatial cue to identity as an alternative route to displaying individual recognition. We test for individual recognition in the Southern pied babbler, *Turdoides bicolor*, using an experimental design that investigates the association between an individual's current location and its vocalisations. We played back 'chuck' contact calls from familiar siblings that were either: 1) currently in the foraging group, 2) currently incubating, or 3) had recently dispersed from the group. We found that responses to 'chuck' calls from familiar kin were elevated when the playback individual was incubating. Elevated responses to the calls of incubating individuals can only be explained by individual recognition as we have controlled for broader recognition

mechanisms such as familiarity, kinship, or absence from the group. This experiment highlights the cognitive associations that have been formed between the spatial information and the vocalisation.

5.2 Introduction

The capacity to recognise specific individuals and assign value to relationships based on previous experience may provide substantial benefits in social species (Tibbetts & Dale 2007; Bradbury & Vehrencamp 2011). The potential importance of individual recognition in animal societies has stimulated research into the diversity of recognition across a variety of taxa (Tibbetts & Dale 2007). These studies of individual recognition are often based upon testing the ability to discriminate between the identity signals of two or more individuals, either through choice tests (e.g. Miller 1979; Kondo et al. 2010), habituation-dishabituation experiments (e.g. Mateo 2006; McDonald 2012) or violation-of-expectation experiments (e.g. Townsend et al. 2012). Whilst the ability to discriminate between identity signals is of importance, the biological significance of individual recognition lies in the ability to link information that is held in memory about a specific individual (for instance, the outcomes of previous agonistic encounters) to its identity label (Johnston 2008). Tests for 'true individual recognition' require two additional features to be demonstrated on top of the ability to separate identity labels. Firstly, an integrated memory of identity developed from multiple sources of information, and secondly, an association between the sensory information and additional information held in memory that is specific to that individual, e.g. memory of previous interactions or the individuals current location (Johnston 2008).

Recently, studies have demonstrated these cognitive associations through cross-modal experiments (Johnston & Jernigan 1994; Adachi & Fujita 2007; Adachi et al. 2007; Proops et al. 2009; Adachi & Hampton 2011; Kondo et al. 2012), where experiments expose the associations formed between the different sensory cues and signals of an individual. For example, Adachi and Hampton (2011) demonstrated that Rhesus monkeys (*Macaca mulatta*) are able to correctly associate images of conspecifics presented on a screen, to their vocalisation. This combination of visual and auditory information demonstrated the cognitive associations between multiple sensory modalities that the test subject had developed of the individual (Adachi & Hampton 2011). Cross-modal recognition has now been found in several species under captive and laboratory conditions (Johnston & Jernigan 1994; Adachi & Fujita 2007; Adachi et al. 2007; Adachi & Hampton 2011; Sliwa et al. 2011; Kondo et al. 2012; Proops & McComb 2012).

We believe, however, that cross-modal experiments are often at risk of being confounded, as individual identity is not the only point of association between the presented cues and signals. For illustration, the vocalisations of animals are not just informative of individual identity, they can also indicate an array of information including sex (Ballintijn & Cate 1997; Radford 2004), age (Klenova et al. 2010), size (Sanvito et al. 2007), geographic region of origin (Wright 1996; Bradbury et al. 2001), social group (Sharp & Hatchwell 2006; Townsend et al. 2010), and social rank (Kitchen et al. 2003). This additional information may be present in multiple information pathways as selection often favours multi-modal presentation of features (Rowe 1999). As a consequence, two cues or signals, in different sensory modalities may be associated

independently of individual identity. This has important consequences for cross-modal experiments as expectations can be formed and violated on simpler associations than individual identity (i.e. large males may be expected to sound like large males). The multiple points of association between cues and signals generate the potential for discrimination without individual recognition, and raise the possibility of discrimination without familiarity. For instance, humans are able to determine the race of a speaker (determined from the visual cue of skin colour) purely from auditory information, and this can be achieved without familiarity (Walton & Orlikoff 1994). We believe it is important to carry out cross-modal studies in situations where identity is the only point of association between the presented cues and signals.

This can be achieved through a violation-of-expectation experimental design (Bates et al. 2008; Proops et al. 2009). For instance, Bates et al. (2008) demonstrated that the expectations of African elephants, *Loxodonta africana*, could be violated through the presentation of urine samples depending upon the current location of the sampled individual. When urine samples were presented to a moving group of elephants, focal individuals invested more time in investigating urine samples when they came from group members that were currently walking behind them (Bates et al. 2008). This experimental design demonstrates the cognitive association between spatial and olfactory information. Here the use of spatial information is effectively acting as the second cue in the cross-modal experiment. Utilising spatial cues to identity in cross-modal experiments is advantageous as it can circumvent the complications of multiple points of association between cues and signals

(where spatial location is not defined by other features, such as age or dominance).

Here we use a violation-of-expectation cross-modal experimental design to expose the cognitive associations between vocal and spatial cues in the Southern pied babbler, *Turdoides bicolor*. Pied babblers live in groups of 2-15 individuals, where all adult group members work to raise the young of the dominant pair (Raihani & Ridley 2007). Using playback experiments we tested whether subordinate individuals could discriminate between the vocalisations of individuals that were either: a) current group members and in the foraging unit, b) current group members away from the group and incubating, c) former group members that had dispersed, and d) unfamiliar, unrelated individuals (included as a control). All adult pied babblers of either sex are involved in incubation (Ridley & Raihani 2008; Ridley & Heuvel 2012), and can disperse from the natal group (Raihani et al. 2010). As a consequence, there is unlikely to be any association other than identity that can be formed between an individual's spatial location and the vocalisation. By playing back the vocalisations of related, familiar birds in differing locations, we intended to expose information held in memory about the whereabouts of the individual. All of the experiments simulate an unexpected scenario. However, there is likely to be variation in the significance of each situation. Unattended nests are at higher risk of predation (Rothenbach & Kelly 2012), and therefore the test subject may attribute greater significance to the calls of incubating birds. Should we observe discrimination between the calls of dispersed birds and incubating individuals, this could not be explained by broader recognition mechanisms such as kinship, familiarity or absence from the group and would therefore represent individual recognition.

5.3 Methods

We tested for individual recognition in a habituated, colour-ringed, population of pied babblers located at the Kuruman River Reserve, in the Southern Kalahari (26°57'S 21°49'E) (see Ridley & Raihani 2007 for more details about the study site). We exposed focal individuals to the 'chuck' contact calls (Radford & Ridley 2008) of siblings. Chuck calls are a short call used to facilitate social cohesion during terrestrial foraging (Radford & Ridley 2008). We recorded the chuck calls between October 2010 and October 2011 to a Marantz PMD660 (2008 D&M Holdings Inc.) using a Sennheiser ME66 shotgun microphone with a K6 power module (2004 Sennheiser), housed in a Rycote pistol grip with windshield to reduce background noise. Recordings were collected at a sampling rate of 48.0 kHz, to 16-bit WAVE files (.wav). Calls were collected from subordinates of independent age (at least 300 days old; mean \pm SD 644 \pm 330 days; range 306-1448) whilst foraging on the ground. Sound recordings were collected within 3m of the calling bird. For playbacks, we cut ten clear chuck calls from the recordings and added 5.5 seconds of silence between calls in Adobe Auditions 3.0 (Adobe, USA). 5.5 seconds represents the average contact call rate of the pied babbler (Golabek 2010). All playback files were then normalised (to -0.1dB) in Adobe Auditions 3.0.

Playbacks were carried out to 16 subordinate adults (mean age was 654 days; range 328-903), from eight different social groups between November 2011 and April 2012. Playbacks were done opportunistically as each test situation arose. We carried out a total of 49 playbacks including 16 from familiar kin (siblings; $r=0.5$) that were in the foraging group, eight from familiar kin that were incubating, nine from familiar kin that had dispersed (<100 days prior to the

playback; mean \pm SD = 63.0 \pm 38.9 days; range 1-100), and 16 from unrelated individuals where no record of contact existed between the two individuals ($r=0$). During incubation the group typically forages away from the nest tree, out of auditory range of the incubating bird (barring 'loud calls'; A. Ridley, *unpublished data*). We carried out all playbacks of incubating birds over 150m from the nest tree to ensure that the incubating individual was out of auditory range of the experiment. Relationships between individuals were established based upon behavioural observations of parentage. Parentage can be reliably assigned from behavioural observations of breeding behaviour and activity at the nest (Nelson-Flower et al. 2011). All individuals received at least two of the playbacks, and three received all combinations. The average number of days between recording and the playback was 65.96 \pm 48.85 days (mean \pm SD; range 2-165). Playbacks occurred at 40dB, 4m from the focal bird from a Sony Walkman NWZ-E345 using an Altec Lansing Orbit speaker (iMT227) placed on the ground. The speaker was placed on the ground to mimic the terrestrial foraging behaviour of the pied babbler. Playbacks were carried out when the focal bird was foraging with at least half of the group members. Playbacks lasted for 50 seconds with responses recorded to a camcorder (Canon Legria FS46e). Responses during this 50 second window were scored blind from the video footage, scoring both the amount of time spent in vigilance behaviours and a binary measure of whether the focal bird approached the speaker in Windows Movie Maker 6.0 (Microsoft Corporation, 2006).

Responses to playbacks were analysed using a series of generalised linear mixed models (GLMM's). Alongside the test treatment (location of the playback individual) we included the following terms in to the models; the age of the focal

bird (the number of days since hatching), the amount of rainfall that had fallen in the past 14 days (in ml), the number of adult birds in the foraging party, the sex of the focal bird, and the number of previous contact call playbacks the focal bird had been exposed to. We included the identity of the focal bird and the social group in the models as random terms. Pied babblers are sexually monomorphic and were therefore sexed genetically from blood samples collected during ringing (Griffiths et al. 1998).

5.3.1 Statistical analysis

GLMM's were carried out in R 2.15.1 (R core development team, 2012) using the 'lme4' (Bates et al. 2011), 'MuMIn' (Barton 2012) and 'AICcmodavg' (Mazerolle 2012) packages. Predictive terms were centralised by subtraction from the mean. Linear predictors were further standardised by dividing by the standard deviation (Schiegg 2010). This allowed comparisons to be made of effect sizes within and between models. A global model with all possible combinations of predictive terms was created using the 'dredge' function in the 'MuMIn' package. A top model set was then created by excluding all models where $\Delta AICc > 5$ from the top model. Models that were excluded were deemed to be poor predictors because of their low AICc weighting. Model averaging was performed on the top model set using the 'AICcmodavg' package. The effect sizes of predictive terms were established from the model averaged estimates and confidence intervals. Predictive terms were regarded as important when the confidence intervals did not include zero.

5.4 Results

We observed greater vigilance in response to the playbacks of the vocalisations of incubating birds than to calls from both current group members that were in the foraging unit and to former group members that had dispersed (Table 1, Figure 1). Vigilance in response to the contact calls of incubating birds was elevated to the level observed for calls from an unfamiliar, unrelated individuals (Figure 1). Focal individuals were also more likely to approach the speaker when the playback was from either an incubating bird or an unfamiliar individual (Figure 2, Table 2).

Table 1 – Top model set of GLMM’s investigating the factors that affect the time spent vigilant during a playback.

Rank	Parameter	K	AICc	Δi	w_i	Cum w_i	Dev
1	Test condition	7	219.58	0.00	0.46	0.46	-101.39
2	Test condition + Group size	8	221.61	2.03	0.17	0.62	-100.96
3	Test condition + age	8	222.29	2.71	0.12	0.74	-101.30
4	Test condition + Experience	8	222.46	2.88	0.11	0.85	-101.39
5	Test condition + rain	8	222.46	2.88	0.11	0.96	-101.39
6	Experience	5	224.25	4.67	0.04	1.00	-106.41

Model Averages				
Parameter	Estimate	Unconditional SE	CI	Sum of w_i
Intercept	1.68	0.61	(0.48, 2.87)	
Dispersed*	1.33	0.81	(-0.26, 2.91)	0.96
Incubating	2.37	0.83	(0.74, 4.01)	0.96
Unfamiliar	2.47	0.71	(1.08, 3.87)	0.96
Age	1.01	0.97	(-0.88, 2.91)	0.12
Rainfall	-1.32	1.22	(-3.72, 1.07)	0.11
Group size	1.54	0.93	(-0.27, 3.35)	0.17
Experience	0.81	1.07	(-1.28, 2.91)	0.04

Group and individual ID were included as random terms in the model. *K* = parameters; AICc is Akaike’s information criteria corrected for small sizes; Δi = Delta AICc; w_i = Akaike weights; Dev (deviance) is the -2 log likelihood of the model; CI = confidence intervals. Significant results are highlighted in bold. *Current group members foraging with the group was set as the reference category.

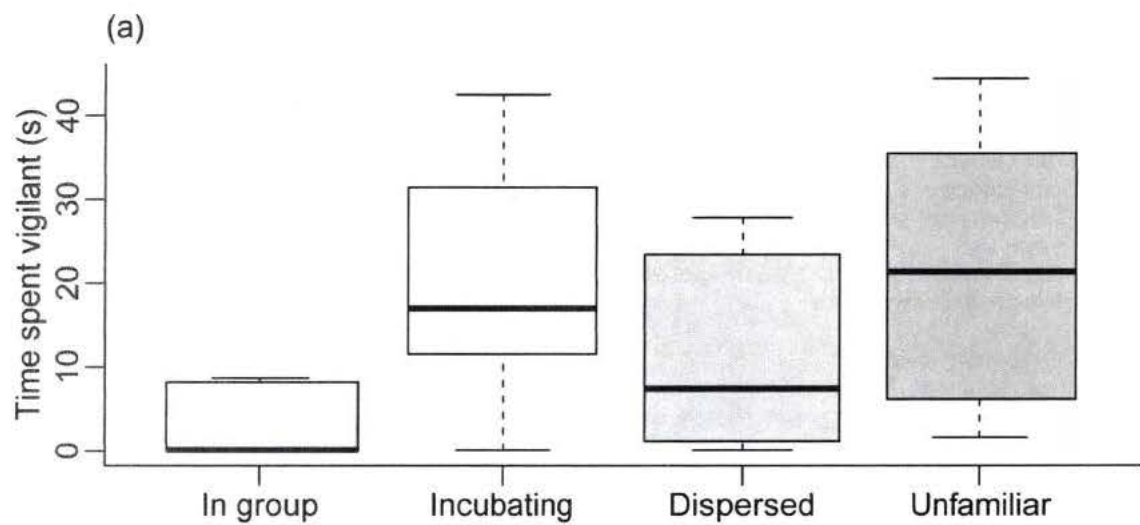


Figure 1 – The time spent vigilant (out of a maximum of 50 seconds) in response to ten contact calls played from individuals from different locations. Graph drawn from the raw data.

Table 2 – Top model set of GLMM's investigating the factors that affect whether the playback speaker was approached.

Rank	Parameter	K	AICc	Δi	w_i	Cum w_i	Dev
1	Test condition	6	64.75	0.00	0.18	0.18	-25.35
2	Test condition + group size	7	64.76	0.01	0.18	0.35	-23.98
3	Test condition + group size + age	8	65.80	1.05	0.10	0.45	-23.06
4	Test condition + rainfall	7	65.97	1.22	0.10	0.55	-24.59
5	Test condition + group size + rainfall	8	66.66	1.91	0.07	0.62	-23.48
6	Test condition+ age	7	67.05	2.30	0.06	0.67	-25.13
7	Test condition + experience	7	67.18	2.43	0.05	0.73	-25.19
8	Test condition + group size+ experience	8	67.34	2.59	0.05	0.77	-23.82
9	Test condition + group size + age + rainfall	9	67.79	3.04	0.04	0.81	-22.53
10	Experience	4	68.26	3.51	0.03	0.84	-29.66
11	Group size + experience	5	68.34	3.59	0.03	0.87	-28.45
12	Test condition + age + rainfall	8	68.34	3.59	0.03	0.90	-24.32
13	Test condition + experience + rainfall	8	68.63	3.88	0.03	0.93	-24.47
14	Test condition + group size + age + experience	9	68.68	3.93	0.02	0.95	-22.97
15	Test condition + group size + sex	9	69.41	4.66	0.02	0.97	-23.34
16	Test condition + group size + experience + rainfall	9	69.42	4.67	0.02	0.99	-23.34
17	Test condition + age + experience	8	69.70	4.95	0.01	1.00	-25.00
Model Averages							
Parameter		Estimate	Unconditional SE	CI	Sum of w_i		
Intercept		-2.91	1.35	(-5.54, -0.27)			
Dispersed*		2.59	1.45	(-0.26, 5.44)	0.96		
Incubating		3.70	1.54	(0.68, 6.72)	0.96		
Unfamiliar		3.48	1.38	(0.78, 6.18)	0.96		
Age		1.01	0.97	(-0.88, 2.91)	0.26		
Rainfall		-1.32	1.22	(-3.72, 1.07)	0.29		
Group size		1.54	0.93	(-0.27, 3.35)	0.53		
Experience		0.81	1.07	(-1.28, 2.91)	0.24		
Sex – Male [†]		-0.71	0.84	(-2.35, 0.93)	0.02		
Sex - Unconfirmed		-1.76	1.80	(-5.30, 1.78)	0.02		

Group and individual ID were included as random terms in the model. *K* = parameters; AICc is Akaike's information criteria corrected for small sizes; Δi = Delta AICc; w_i = Akaike weights; Dev (deviance) is the -2 log likelihood of the model; CI = confidence intervals. *Current group members foraging with the group was set as the reference category. [†]Females were set as the reference category.

5.5 Discussion

Studies of cross-modal individual recognition have the following purpose, firstly, to expose discrimination of the individual that cannot be explained by a broader level of recognition, and secondly, to demonstrate that the identity signal is matched to information stored about the individual in memory (Proops et al. 2009). The elevated vigilance and rates of approach we observed in response to the calls of unfamiliar, unrelated individuals can be explained by both differences in kinship and familiarity. However, the elevated vigilance observed here in response to playbacks from incubating birds cannot be explained by kinship, familiarity or absence from the group. Kinship was the same for all of the playbacks of familiar individuals. We observed stronger responses to calls from incubating birds than to calls from dispersed birds ruling out the effect of absence from the group. By presenting vocalisations from individuals that were in differing locations we have exposed not only the individual recognition of the contact call, but also the information held in memory about the individual, and its current location.

The low response to the calls of dispersed individuals may seem surprising. However, dispersing individuals appear to use their natal territory as a 'safe haven' between prospecting bouts (Raihani et al. 2010). Prospecting for dispersal opportunities is energetically costly, and returning to the natal group may allow individuals to recover from the energetic costs of this activity (Ridley et al. 2008; Raihani et al. 2010). Prospecting is common among subordinate individuals of reproductive age (Raihani et al. 2010), and the coming-and-going from the social group associated with prospecting behaviour may explain why we observed limited responses to calls of dispersed individuals.

Pied babblers coordinate many of their social activities, including sentinel behaviour and spacing, through vocalisations (Radford & Ridley 2008; Bell et al. 2010; Hollén et al. 2011). The elevated responses to playback calls from birds that were 'supposed to be' incubating not only provide evidence for individual recognition, but also demonstrate an awareness of the activity of the individual. This may represent a possible mechanism for coordinating incubation in a group living bird where incubation is shared among a number of individuals (Raihani & Ridley 2007; Ridley & Raihani 2008).

Individual recognition in social species plays an important role in many aspects of behaviour, from helping to maintain social cohesion (Waser 1977), to developing reciprocity in cooperative tasks (Crowley et al. 1996). It can help to maintain dominance hierarchies at minimal energetic cost (Barnard & Burk 1979), and enable kin recognition through prior association (Sherman et al. 1997). Kin recognition can help to avoid costly incestuous mating (Pusey & Wolf 1996) or to direct cooperative behaviours in favour of kin (Hamilton 1964). Pied babblers are highly social, repeatedly interacting with individuals both within the group and in neighbouring groups, and individual recognition may mediate many of their social interactions. The importance of individual recognition, and the information held in memory about previous encounters with individuals (e.g. regarding hierarchies, investment in cooperative tasks, or breeding success), is an important area of future research that will develop our understanding of social interactions and cooperation in animal societies.

The ability to monitor the contributions of social partners to cooperative tasks may be critical for the evolutionary stability of cooperative interactions (Crowley et al. 1996; Nowak & Sigmund 1998). Our study has demonstrated that the

pied babbler is able to monitor which group member is incubating.

Consequently, we have provided evidence for a process which may ultimately be critical in our understanding of how cooperative interactions are coordinated and maintained within animal societies.

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Chapter 6

Testing for vocal kin recognition in the cooperatively breeding Southern pied babbler



Southern pied babbler feeding a Jacobin cuckoo, *Clamator jacobinus*

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6.1 Abstract

Studies of vocal kin recognition in avian systems have typically tested responses to vocal signals based upon either familiarity or relatedness, but rarely have studies used a combined approach that tests for the effects of both familiarity *and* relatedness. Doing so is important as it enables conclusions to be drawn about mechanisms of kin recognition in operation. Here we outline that the experimental conditions required (familiar and unfamiliar, kin and non-kin) occur naturally in the Southern pied babbler. To our knowledge, no study of vocal kin recognition has ever played back vocalisations from familiar and unfamiliar, kin and non-kin in an avian system without the use of cross-fostering. This approach exposes not only the presence of vocal kin recognition, but also the *underlying mechanisms* of kin recognition. We demonstrate that vocal kin recognition in this species is based upon prior association during a period of associative learning. This enables the identification of close kin among familiar individuals. However, we observed no discrimination of the kin signals from unfamiliar kin (c.f. unfamiliar non-kin), suggesting this species is at risk of forming costly incestuous mating partnerships with unfamiliar kin.

6.2 Introduction

The ability to recognise close relatives can be advantageous in two ways.

Firstly, costly incestuous breeding attempts can be averted by recognising and avoiding mating with close relatives [1-3], and secondly it can increase the indirect fitness of an individual by allowing it to discriminate social behaviours in favour of kin [4-6]. In avian systems, the influence of kinship can be seen across a wide spectrum of social interactions, from kin-biased alarm calling [7] and spatial clustering [8, 9]; to mate choice decisions [10, 11] and helping at the nest [12-21].

Kin recognition can be achieved through several mechanisms, four are commonly cited in the literature: (1) recognition by prior association (also referred to as familiarity); (2) phenotype matching; (3) recognition alleles and (4) spatially-based recognition [22, 23]. These mechanisms are broadly defined and variation exists within each of these classifications. For instance, prior association can apply broadly to all familiar individuals, or can be limited to those encountered during a critical period of associative learning [23].

Additionally, phenotype matching can occur either through matching kin labels to a template developed from known kin or through 'self-referent phenotype matching', where labels are matched to the receiver's own kin labels [also referred to as 'the armpit effect'; 24]. Testing for kin recognition can be problematic for several reasons: firstly, the recognition process is cognitive, making it invisible to the observer. Tests for kin recognition are therefore dependent on instigating a behavioural change in the test subject [referred to as kin discrimination; 25]. Secondly, the test must disentangle which kin recognition mechanism is in operation [26]. On this second point, the

mechanisms of avian kin recognition are less well understood, with few studies using an approach that can distinguish the operative mechanism of kin recognition [27]. For instance, several studies have identified the ability to discriminate the kin labels of familiar kin from the labels of unfamiliar individuals (both kin and non-kin) [28, 29]. Yet, without including kin labels from familiar non-kin, we are unable to tell whether recognition is based broadly upon familiarity, or whether kinship itself is being directly assessed.

Although the mechanisms of kin recognition are not necessarily mutually exclusive [5], it is important to separate out the underlying mechanisms of kin recognition because they can differ in their resolving power. This is particularly true for kin recognition by prior association, which can only discriminate kinship among familiar individuals [23]. This limitation is likely to have implications for when and where we see kin discrimination during social interactions. Kin recognition studies are able to identify the mechanisms of kin recognition in operation by exposing dependence upon familiarity. This is achieved by testing the ability to discriminate between the kin labels of both familiar and unfamiliar, kin and non-kin. Species that recognise kin by either phenotype matching or recognition alleles should discriminate between the kin labels of related individuals regardless of familiarity, whereas kin recognition that is dependent on prior association should show no discrimination between the kinship signals of unfamiliar kin and non-kin [23].

It is important therefore that studies of kin recognition use an experimental design that exposes a test subject to kin labels from both familiar and unfamiliar, kin and non-kin. There are two routes through which this can be achieved: firstly, through natural instances where familiar and unfamiliar kin

and non-kin exist to a test subject, and secondly through cross-fostering experiments [26]. Cross-fostering experiments are a powerful tool in the study of kin recognition, used to address a range of topics, including: (1) to explore whether the ability to recognise kin is innate [30], (2) to address whether kin labels are learnt or innately produced [31], or (3) for separating the mechanisms of phenotype matching from self-referent phenotype matching [32]. However, when addressing the primary question of whether a species is able to recognise kin, particularly with regard to vocal kin recognition, the cross-fostering technique is not always suitable. This is because cross-fostering, when used to identify the presence of kin recognition, assumes that kin labels are not transferred or learnt in the rearing environment [26]. However, there is considerable evidence that vocalisations are often developed in early life and are based upon what is heard in the surrounding social environment [31, 33-37]. Cross-fostering results in chicks being reared in an environment surrounded by unrelated individuals, which can potentially lead to offspring developing a modified kin label (resulting in them sounding more like their adopted family than their actual kin) [31, 37]. Previous research revealing that birds can learn to recognise kin by developing a kin template from those individuals associated with in early life [31], suggests that cross-fostering could disrupt this learning process [38]. For these reasons cross-fostering may be unsuitable for testing vocal kin recognition because potentially both the kin label *and* kin template may be modified. We believe that this could lead to both type I and type II error, where species that are able to recognise unfamiliar kin by phenotype matching may not discriminate cross-fostered unfamiliar kin or

may falsely recognise familiar non-kin due to a modification of the kin-label or kin template.

The alternative route to testing for vocal kin recognition is to study naturally occurring instances where familiar and unfamiliar kin and non-kin exist for a test subject. While natural instances in which these criteria occur may be rare, there are several behavioural characteristics that make studying vocal kin recognition feasible in the Southern pied babbler, *Turdoides bicolor*. Pied babblers are a group-living, cooperatively breeding passerine of semi-arid Southern Africa [39]. Some of the life history and behavioural characteristics that enable vocal kin recognition study in the pied babbler are: (1) Pied babblers have a year-round territorial system with aggressive territorial defence [40]. Year-round aggressive territoriality both limits the number of individuals that a test subject can be familiar with, as well as allowing familiar neighbours and group members to be reliably identified. (2) Pied babblers are a long-lived species (>10 years; A.R. Ridley, *unpublished data*), with a monogamous breeding system [41] between unrelated dominant breeding pairs [42]. This ensures that successive broods contain full siblings, and when coupled with the dispersal of offspring, provides the natural scenario for unfamiliar kin to occur. (3) Dispersal is typically around 1.7km, taking them outside the contact range of most close relatives [42]. Dispersal of offspring beyond the contact range of the natal territory is important so that siblings from successive broods, separated by dispersal, are truly unfamiliar. (4) Unrelated individuals are observed dispersing into groups [43], creating a situation where non-kin are familiar to the test subject. Exposing the test subject to vocalisations from familiar non-kin is vital for ruling out broader recognition mechanisms such as

familiarity. These qualities make the pied babbler suited to studying vocal kin recognition in a non-cross-fostered experimental design. To our knowledge, no study of vocal kin recognition in birds has ever played back vocalisations from the full complement of familiar and unfamiliar kin and non-kin (it is worth noting that Payne et al. [29] used all categories except familiar non-kin; and that Price [44] used all categories but without a formal separation for the effects of familiarity). Here we test for vocal kin recognition in an avian system without the use of cross-fostering, using a technique that exposes not only the ability to discriminate kinship, but also exposes the mechanism of kin recognition in operation.

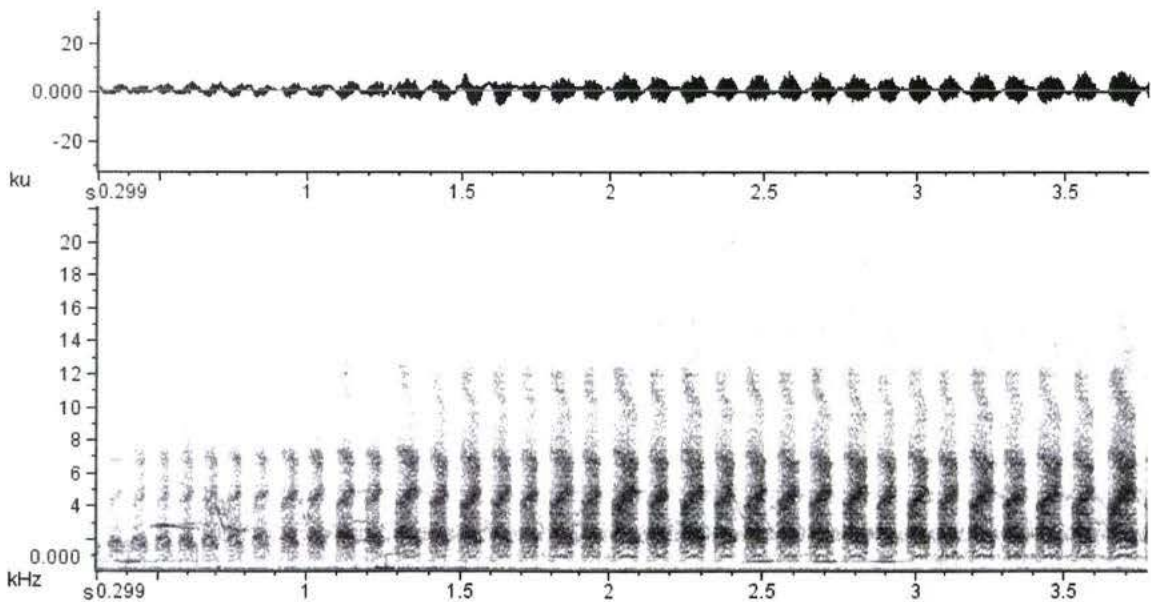


Figure 1. Spectrogram and waveform views of atonal chatter calls. Spectrogram drawn in a hamming window (512 point, with a 96.9% overlap). Greyscale represents a 65dB range.

6.3 Methods

6.3.1 Study population and determining relatedness

The pied babbler research project is located in the Kuruman River Reserve, in the Southern Kalahari, South Africa (26°57'S 21°49'E) [see 45 for more details about the study site]. The study population has been under continuous investigation since 2003 and extensive life-history data exists for each of the study groups. The population is colour-ringed for individual identification and habituated to the presence of humans, allowing close behavioural observation and sound recording. Each pied babbler group contains a dominant pair that are both socially and sexually monogamous, and parentage of a brood can be reliably matched to dominance behaviours within the social group [41]. We measured relatedness at three levels: (1) where $r=0.5$ (parent/offspring and siblings), (2) where $r=0.25$ (half-siblings), (3) where $r=0$ (where no detectable relatedness exists between two individuals). Pedigrees were developed from behavioural assignments of relationships. Relationships were confirmed with parentage analysis using microsatellite genotyping [41].

6.3.2 Experimental protocol

We conducted playback experiments of the atonal chatter call [46] from familiar and unfamiliar kin and non-kin to 20 dominant female individuals belonging to 21 different social groups (the number of groups is higher than the number of individuals because of dispersal between trials). The atonal chatter call was chosen because it was the most frequently observed of the loud call types (D. Humphries, unpublished data). We limited our study to dominant females for two reasons: 1) to rule out the effects of dominance or sex on responses to

playbacks; and 2) we observed the greatest variation in relatedness and familiarity of individuals in the population for dominant females. Here we term 'familiar' as former group members who dispersed no more than 180 days before the playback. We focused on former group members to rule out current group membership as an additional factor that could potentially affect the responses of the dominant female. Unfamiliar individuals were regarded as those where no record of contact existed between the two individuals, and that lived in territories that were at least 2km away from the focal individual.

Playbacks were carried out between November 2010 and April 2012.

Atonal chatter calls were recorded within 15m of the calling bird to a Marantz PMD660 data recorder (2008 D&M Holdings Inc.) using a Sennheiser ME66 shotgun microphone with a K6 power module (2004 Sennheiser), housed in a Rycote pistol grip with windshield to reduce background noise. Recordings were collected at a sampling rate of 44.1 kHz, to 16-bit WAVE files (.wav). The atonal chatter is a short call (approx. 0.09 seconds in duration) that is performed in strings up to 80 seconds in duration (mean \pm SD 4.53 \pm 3.07; range 0.7-80.0 seconds; Figure 1). For playbacks we used atonal chatter strings five seconds in duration to mimic the natural average duration of this call type. Playback files included ten seconds of silence, followed by a five second atonal chatter sequence. The ten seconds of silence was added to allow the observer time to move away from the speaker before the playback. We used 47 atonal chatter calls collected from 40 different individuals. Calls were cut and normalised (to -0.1 dB) and saved to WAVE files for playback using the audio software package 'Adobe Audition 3.0' (2007, Adobe Systems Inc.).

Playback experiments consisted of a 'before playback' and a 'during playback' twenty-minute focal observation in which the behaviours of the focal individual were recorded to a Palm TX (Palm Inc., Sunnyvale, CA, U.S.A.) using a purpose built program created in 'cybertracker' (<http://www.cybertracker.org>). The behaviours recorded were: times spent (s) foraging, moving, resting, vigilance, sentinel [where individuals were vigilant from an elevated position whilst producing 'sentinel calls'; 47], loud calling, group chorusing, play-fighting, autopreening, allo-preening, and bill wiping. The focal observation period was followed by the playback of an atonal chatter call. Atonal chatter calls were played back at 70dB, 15m from the focal bird from a Sony Walkman NWZ-E345 using an Altec Lansing Orbit (iM227) speaker mounted to a tripod (1.5m high). 70dB represents the natural calling volume of the atonal chatter call, when measured from 5m with Voltcraft SL100 (Voltcraft, Barking, UK) sound level meter. The responses to the playback were recorded through a second twenty-minute focal observation period, which started parallel to the playback starting. We carried out 17 playbacks of familiar kin where $r=0.5$, eight for familiar kin where $r=0.25$, 12 for familiar non-kin, 13 for unfamiliar kin (due to low sample sizes for unfamiliar kin, relatedness of 0.5 and 0.25 were combined; sample size of 13 results from two playbacks where $r=0.5$ and 11 playbacks where $r=0.25$), and 15 for unfamiliar non-kin. Playbacks were carried out opportunistically as each situation arose. We used four different measures of response to playback, including: (1) the time taken to resume foraging after the playback; (2) the time spent moving between when the playback was initiated and when the focal individual returned to foraging (referred to here as 'time in pursuit' - measuring movement separately removes the effect of autopreening

and vigilance response behaviours on the time to resume foraging); (3) a binary measure of whether the focal bird replied to the playback with a vocalisation (either its own loud call, or a group chorus); and (4) the change in vigilance behaviour (personal vigilance and sentinel behaviours combined) between the first and second focal.

6.3.3 Statistics

We used generalised linear mixed models (GLMM's) to investigate the factors affecting the responses to playbacks. GLMM's with maximum likelihood were performed in R 2.15.1 [R Core 48] using the 'lme4' [49], 'MuMIn' [50], and 'AICmodavg' packages [51]. We used an information theoretic approach to model selection, identifying best models based upon the lowest Akaike's information criterion (adjusted for small sample sizes) value [AICc; 52]. The time to resume foraging, the time spent in pursuit and the change in guarding behaviour were square root transformed to achieve normality. Model terms included were; relatedness and familiarity of the playback individual to the focal bird ('relatedness'), the age of the focal bird (number of days since hatching), the amount of rainfall that had fallen in the previous 14 days (in ml – this term was included because previous research has indicated that the responsiveness to playbacks may be affected by body condition in this species, with lower responsiveness in dry periods [40]), the number of adult birds in the group at the time of the playback ('group size'), and the number of playbacks of the atonal chatter call the focal bird had previously been exposed to ('experience'). Predictive terms were centralised by subtraction from the mean. Linear predictors were further standardised by dividing by the standard deviation. This allowed comparisons to be made of effect sizes within and between models

[53]. A global model, with all combinations of predictive terms, was generated using the 'dredge' function in the 'MuMIn' package [50]. The global model was reduced to a top model set by removing all models with $\Delta\text{AICc} > 5$ from the top model [54]. Excluded models were deemed to be poor predictors because of low AICc weighting. Model averaging was performed on the top set of models [because AICc weights for top models < 0.90 ; 55] to establish the relative importance of each term in the models. The effect of predictive terms was concluded from the model-averaged estimates and confidence intervals (those that did not encompass zero were considered significant). All graphics were drawn in R 2.15.1.

6.4 Results

In all but five of the 67 playbacks of an atonal chatter a response was generated. The time to resume foraging after a playback was on average lower for calls from familiar individuals than all other categories (Figure 2a). Playback responses were limited for familiar kin where $r=0.5$, compared with kin where $r=0.25$, suggesting recognition of different levels of kin (Figure 2a, Table 1). Relatedness was the sole predictive term in the top model for the factors affecting the time to resume foraging (Table 1). Model-averaged estimates indicate that individuals returned to foraging sooner when relatedness to the playback individual was high ($r = 0.5$), compared to all other categories of playback. The amount of time spent in pursuit of the playback was also lowest when the playback was from a familiar relative (where $r=0.5$, Table 2, Figure 2b). Based upon AICc weighting, vocal responses to playbacks were most affected by the number of adult birds in the group, with birds less likely to vocalise in large groups (Table 3). The model term 'relatedness' did not appear

in any of the models in the top model set for vocal responsiveness. We interpreted this as relatedness being a poor predictor of whether playbacks received a vocal response from the focal individual. The behavioural changes in guarding behaviour following a playback indicated that individuals were less likely to become vigilant when listening to playbacks of familiar kin (where $r=0.5$; Table 4).

Table 1 – GLMM output from the top model set on the factors affecting the time to resume foraging after a five second playback of an atonal chatter call. Analysis was conducted on the responses to 67 playbacks by 20 focal individuals.

Rank	Parameter	K	AICc	Δi	w_i	Cum w_i	Dev
1	Relatedness	8	456.66	0.00	0.38	0.38	-219.09
2	Relatedness + rainfall	9	458.20	1.54	0.18	0.56	-218.52
3	Relatedness + age	9	459.29	2.63	0.10	0.66	-219.07
4/5	Relatedness + group size	9	459.32	2.66	0.10	0.76	-219.08
4/5	Relatedness + experience	9	459.32	2.66	0.10	0.86	-219.08
6	Relatedness + rainfall + experience	10	460.90	4.24	0.05	0.91	-218.49
7	Relatedness + rainfall + age	10	460.92	4.26	0.05	0.96	-218.49
8	Relatedness + rainfall + group size	10	460.97	4.31	0.04	1.00	-218.52
Model Averaging							
Parameter	Estimate	Unconditional SE	CI	Sum of w_i			
Intercept	4.59	1.64	(1.38, 7.80)	1.00			
Familiar kin 0.25*	11.75	2.41	(7.02, 16.48)	1.00			
Familiar non-kin	6.53	2.38	(1.86, 11.19)	1.00			
Unfamiliar kin	11.75	2.41	(7.02, 16.48)	1.00			
Unfamiliar non-kin	9.36	2.23	(4.99, 13.73)	1.00			
Age	0.39	1.84	(-3.21, 3.99)	0.15			
Rainfall	1.68	1.56	(-1.38, 4.75)	0.32			
Group size	-0.14	1.71	(-3.50, 3.23)	0.14			
Experience	0.06	0.41	(-0.73, 0.86)	0.15			

Group and individual ID were included as random terms in the model. Dev (deviance) is the -2 log likelihood of the model; K = parameters; AICc is Akaike's information criteria corrected for small sizes; Δi = difference in AICc values from top ranked model; w_i = Akaike weights. *Familiar Kin 0.5 was set as the reference category.

Table 2 – GLMM output from the top model set on the terms affecting the time spent in pursuit of the playback. Analysis was conducted on the responses to 67 playbacks by 20 focal individuals.

Rank	Parameter	K	AICc	Δi	w_i	Cum w_i	Dev
1	Relatedness	8	338.40	0.00	0.33	0.33	-159.96
2	Relatedness + group size	9	340.09	1.69	0.14	0.47	-159.47
3	Relatedness + age	9	340.24	1.84	0.13	0.60	-159.54
4	Relatedness + rainfall	9	340.91	2.51	0.09	0.69	-159.88
5	Relatedness + experience	9	340.94	2.54	0.09	0.78	-159.89
6	Relatedness + age + order	10	342.12	3.72	0.05	0.84	-159.09
7	Relatedness + experience + group size	10	342.46	4.06	0.04	0.88	-159.27
8	Relatedness + rainfall + group size	10	342.47	4.07	0.04	0.92	-159.27
9	Relatedness + age + group size	10	342.51	4.12	0.04	0.96	-159.29
10	Relatedness + rainfall + age	10	342.78	4.39	0.04	1.00	-159.43
Model Averaging							
	Parameter	Estimate	Unconditional SE	CI	Sum of w_i		
	Intercept	1.85	0.70	(0.47, 3.23)			
	Familiar kin 0.25*	3.77	1.02	(1.78, 5.76)	1.00		
	Familiar non-kin	2.18	0.98	(0.25, 4.10)	1.00		
	Unfamiliar kin	3.77	1.02	(1.78, 5.76)	1.00		
	Unfamiliar non-kin	2.87	0.92	(1.08, 4.67)	1.00		
	Age	-0.73	0.80	(-2.30, 0.83)	0.26		
	Rainfall	-0.33	0.66	(-1.62, 0.96)	0.17		
	Group size	-0.69	0.70	(-2.06, 0.68)	0.26		
	Experience	0.10	0.18	(-0.25, 0.46)	0.13		

Group and individual ID were included as random terms in the model. Dev (deviance) is the -2 log likelihood of the model; K = parameters; AICc is Akaike's information criteria corrected for small sizes; Δi = difference in AICc values from top ranked model; w_i = Akaike weights. *Familiar Kin 0.5 was set as the reference category.

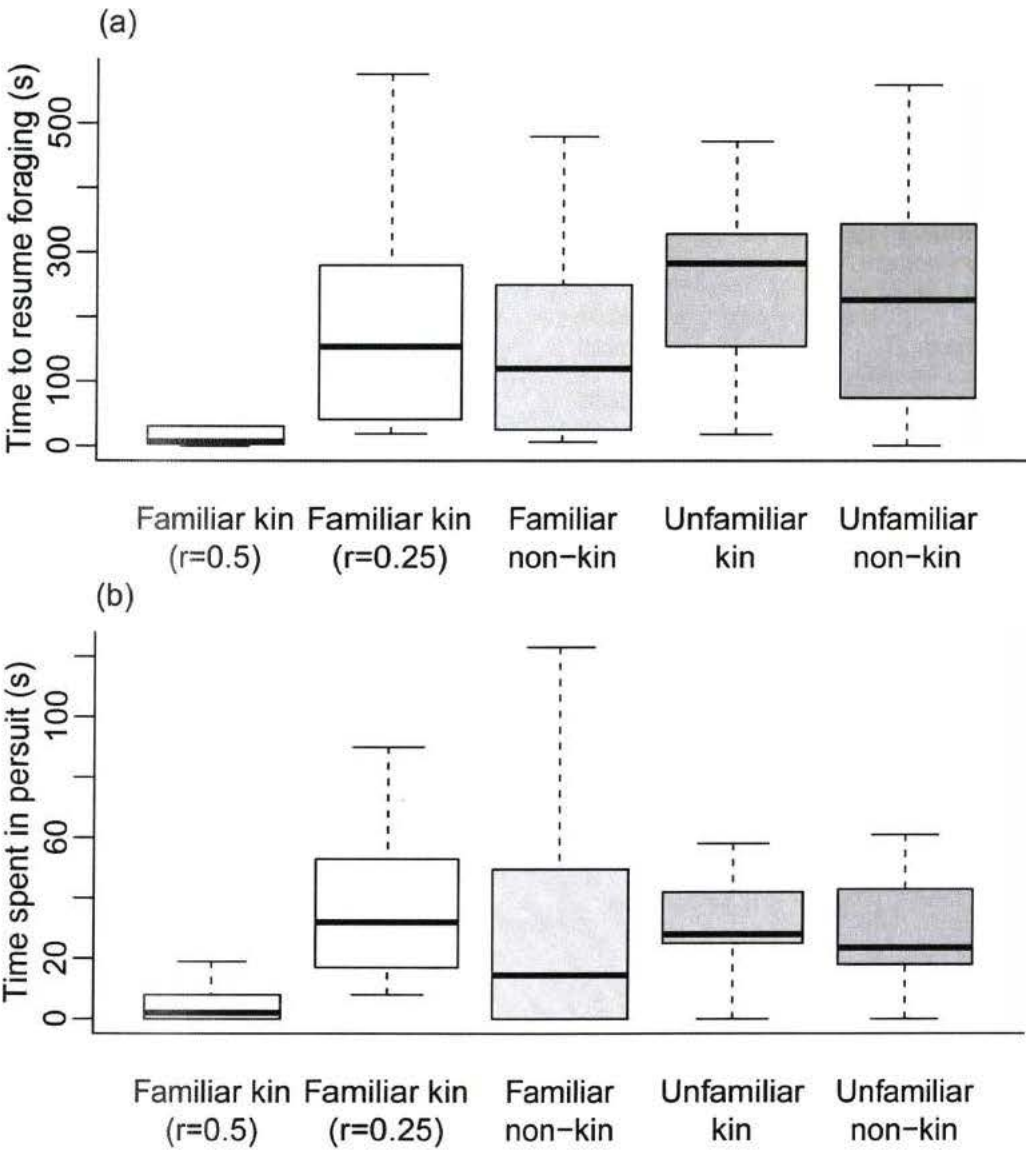


Figure 2. (a) The time to resume foraging in seconds after a playback of an atonal chatter call at each level of relatedness and familiarity. (b) The time spent in pursuit after the playback (the sum of the time spent moving before returning to foraging) of calls at each level of relatedness. Graphs are generated from the means derived from raw data.

Table 3 – GLMM output from the top model set for the factors affecting whether birds responded to playbacks with a vocalisation. Analysis was conducted on the responses to 67 playbacks by 20 focal individuals.

Rank	Parameter	K	AICc	Δi	w_i	Cum w_i	Dev
1	Group size	4	90.21	0.00	0.34	0.34	-40.78
2	Rainfall + group size	5	91.74	1.53	0.16	0.50	-40.38
3	Experience + group size	5	92.23	2.01	0.13	0.63	-40.62
4	Age + group size	5	92.32	2.11	0.12	0.75	-40.67
5	Basic	3	93.77	3.56	0.06	0.81	-43.70
6	Experience + rainfall + group size	6	93.83	3.62	0.06	0.86	-40.22
7	Age + rainfall + group size	6	93.97	3.76	0.05	0.92	-40.29
8	Rainfall	4	94.26	4.05	0.05	0.96	-42.81
9	Age + experience + group size	6	94.58	4.37	0.04	1.00	-40.59
Model Averaging							
	Parameter	Estimate	Unconditional SE	CI	Sum of w_i		
	Intercept	-0.67	0.39	(-1.43, 0.09)			
	Age	0.26	0.60	(-0.91, 1.43)	0.21		
	Rainfall	0.56	0.55	(-0.53, 1.64)	0.32		
	Group size	-1.59	0.70	(-2.95, -0.22)	0.90		
	Experience	0.07	0.13	(-0.18, 0.32)	0.23		

Group and individual ID were included as random terms in the model. Dev (deviance) is the -2 log likelihood of the model; K = parameters; AICc is Akaike’s information criteria corrected for small sizes; Δi = difference in AICc values from top ranked model; w_i = Akaike weights. *Familiar Kin 0.5 was set as the reference category.

Table 4 – Top model set from a series of GLMM's testing the change in vigilance behaviours following a playback. Analysis was conducted on the responses to 67 playbacks by 20 focal individuals.

Rank	Parameter	K	AICc	Δi	w_i	Cum w_i	Dev
1	Relatedness + rainfall	9	373.49	0.00	0.18	0.18	-175.91
2	Relatedness + experience + rainfall	10	373.98	0.49	0.14	0.31	-174.70
3	Relatedness + experience	9	374.11	0.61	0.13	0.44	-176.22
4	Relatedness	8	374.43	0.94	0.11	0.55	-177.77
5	Basic	4	376.03	2.54	0.05	0.60	-183.64
6	Relatedness + rainfall + group size	10	376.27	2.78	0.04	0.65	-175.84
7	Relatedness + age + rainfall	10	376.33	2.84	0.04	0.69	-175.87
8	Relatedness + age + experience + rainfall	11	376.69	3.20	0.04	0.73	-174.54
9	Relatedness + age + experience	10	376.85	3.36	0.03	0.76	-176.13
10/11	Rainfall	5	376.91	3.41	0.03	0.79	-182.89
10/11	Experience	5	376.91	3.41	0.03	0.82	-182.89
12	Relatedness + group size	9	376.93	3.43	0.03	0.85	-177.63
13	Relatedness + age	9	376.95	3.45	0.03	0.89	-177.64
14	Relatedness + experience + group size	10	376.99	3.50	0.03	0.92	-176.20
15	Relatedness + experience + rainfall + group size	11	377.01	3.52	0.03	0.95	-174.70
16	Group size	5	377.69	4.20	0.02	0.97	-183.28
17	Experience + rainfall	6	378.15	4.66	0.02	0.98	-182.27
18	Age	5	378.42	4.92	0.02	1.00	-183.64

Model Averaging				
Parameter	Estimate	Unconditional SE	CI	Sum of w_i
Intercept	2.38	2.15	(-1.84, 6.60)	
Familiar kin 0.25*	4.61	2.15	(0.39, 8.82)	0.83
Familiar non-kin	5.91	2.02	(1.96, 9.86)	0.83
Unfamiliar kin	5.98	2.02	(2.01, 9.94)	0.83
Unfamiliar non-kin	5.45	1.79	(1.93, 8.97)	0.83
Age	0.11	1.85	(-3.50, 3.73)	0.16
Rainfall	2.31	1.29	(-0.22, 4.84)	0.52
Group size	-0.53	1.44	(-3.36, 2.30)	0.15
Experience	-0.54	0.33	(-1.18, 0.11)	0.45

Group and individual ID were included as random terms in the model. Dev (deviance) is the -2 log likelihood of the model; K = parameters; AICc is Akaike's information criteria corrected for small sizes; Δi = difference in AICc values from top ranked model; w_i = Akaike weights. *Familiar Kin 0.5 was set as the reference category.

6.5 Discussion

When we exposed dominant female pied babblers to loud calls from familiar and unfamiliar kin and non-kin, they returned to foraging faster, spent less time in pursuit, and were less vigilant when the playback was from a familiar relative (where $r=0.5$). This suggests a level of recognition that is not based solely on broader classifications of recognition such as familiarity, and that kinship is being directly discriminated. The discrimination between playbacks from familiar kin where $r=0.5$ against familiar kin where $r=0.25$ also suggests that the pied babbler is capable of fine-scale kin discrimination, discriminating not only relatedness, but also levels of relatedness. In addition, we observed that dominant females did not discriminate between the loud calls of unfamiliar kin and unfamiliar non-kin, indicating that neither phenotype matching nor recognition alleles are likely to operate for vocal kin recognition in this species. This inability to discriminate unfamiliar kin may have profound implications for when and where we would expect to see kin-biased interactions occurring in the pied babbler. For instance, we may expect kin to be avoided as mating partners because reproductive success often declines when breeding with relatives [56]. However, inbreeding avoidance through kin recognition may be limited in the pied babbler to only avoiding familiar relatives.

The fine-scale discrimination of kinship among familiar individuals can be achieved through two routes. Firstly, the cues of familiar kin may be learnt during a period of associative learning [5]. By learning the kin signals of familiar individuals during a period of associative learning, individuals may monitor any subsequent changes in group composition that occur after this learning period, such as changes in breeding positions within the group (and their resultant

offspring), or immigration events, and assess the relatedness of these incumbent individuals separately. Secondly, a vocal kin label that signals relatedness may exist in the atonal chatter call. Kin labels have been observed in many avian vocalisations [44, 57, 58], and can be used to discriminate kinship among unfamiliar individuals [44, 57]. However, we found no evidence of kin discrimination for unfamiliar individuals, suggesting that kin recognition is likely to be achieved through associative learning in the pied babbler. Our findings support those of previous investigations into kin recognition in avian species where associative learning appears to be the operative kin recognition mechanism in both the long-tailed tit, *Aegithalos caudatus* [31], and the Seychelles warbler, *Acrocephalus sechellensis* [59]. Our observations that pied babblers did not discriminate between the atonal chatter calls of familiar kin (where $r=0.25$) from familiar non-kin support our notion that kin recognition is dependent upon associative learning, and that recognition is sensitive to immigration events and changes in group structure. Half-siblings (familiar kin $r=0.25$) occur when a change in breeding position within the group has occurred. Our observations that the atonal chatter calls of all new arrivals, including half-siblings, are subject to greater inspection and vigilance than the calls of close kin (where $r=0.5$) suggest that pied babblers are sensitive to immigration and changes in breeding position within the group.

Although our findings indicate that vocal kin recognition is based upon associative learning, it is possible that the mechanism of kin recognition in operation may alter at varying life history stages [5]. For example, in bank swallows, *Riparia riparia*, a spatially-based recognition system is used until the chicks are fifteen days old, after which a direct recognition system is used to

recognise offspring [60]. Successful parasitism of the pied babbler by the Jacobin cuckoo, *Clamator jacobinus*, indicates that a spatially-based mechanism of kin recognition is likely to be used to recognise young in the nest [61].

Previous investigations into the effect of kinship on pied babbler social behaviour have revealed that relatedness does not influence contributions to helping at the nest [62]. However, kin recognition is likely to be important for inbreeding avoidance [42]. Genetic data has indicated that pied babbler dominant pairings are not inbred, and that inbreeding avoidance is likely to be occurring through a combination of both dispersal and avoidance of familiar kin as mating partners [42]. Our findings that pied babblers are able to recognise familiar kin (where $r=0.5$) demonstrates a potential mechanism for avoiding breeding with familiar relatives. Further, our observations that pied babblers are unable to recognise unfamiliar kin help us to understand why pied babblers also adaptively use dispersal as a means of avoiding inbreeding, where initial dispersal from the natal group tends to be further than subsequent dispersal between non-natal groups [42, reviewed in 63]. Our work supports the conclusions of previous research on inbreeding in pied babblers [38]: that inbreeding is avoided through a combination of recognising and subsequently avoiding familiar kin, and dispersal from the natal territory.

We have provided evidence that pied babblers have a fine-scale kin recognition system, and that this enables them to not only identify familiar kin, but also separate levels of relatedness among familiar individuals. Our work supports the findings of previous investigations into avian kin recognition that have concluded that kin recognition is learnt through association [30, 31, 59]. The

behavioural ecology of the pied babbler has provided the opportunity to conclusively demonstrate the mechanisms of vocal kin recognition in an avian species without the use of cross-fostering. This has allowed us to both understand the operative mechanisms of vocal kin recognition, and importantly, understand the limits of vocal kin recognition in this species.

6.6 References

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Chapter 7

The ontogeny of vocal recognition in the Southern pied babbler



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7.1 Abstract

Avian young typically learn to recognise care-givers by using either spatial cues to identity, such as the location of the nest, or by direct recognition mechanisms, where care-givers are identified by their phenotypic cues and signals. Which of these mechanisms is operative appears to correspond to the environment and the frequency of encounters between non-group members. The ontogeny of direct recognition is thought to be conservatively selected for, and if spatial cues are reliable, spatial recognition tends to be the dominant recognition mechanism because of the risk of falsely rejecting true parents and helpers should direct recognition mechanisms fail. As a consequence, the ontogeny of direct recognition is often delayed until spatial cues become unreliable cues to identity (usually just prior to fledging from the nest and the corresponding increased mobility of young). However, in highly territorial species, we may expect spatial cues to remain reliable even post-fledging, as encounters with individuals who are not parents or helpers are rare. Here we show that the ontogeny of direct recognition in the highly territorial, group-living, Southern pied babbler, *Turdoides bicolor*, is delayed until individuals are at least 118 days old, well beyond the age of fledging from the nest (13-19 days). We suggest that direct recognition in this species is delayed because year-round territoriality results in young encountering few non-group members in the first three months post-fledging, making spatial cues reliable indicators to identity. This study shows the direct recognition can develop well beyond the age of fledging, and represents to our knowledge, the oldest recorded age at which direct recognition develops within an avian species.

7.2 Introduction

For altricial young that are dependent upon parents and helpers (referred to hereafter as 'care-givers') for nutrition and predator defence, there is a need for mechanisms to accurately identify care-givers to ensure maximum levels of care during the period of offspring dependence. Maintaining contact with caregivers is likely to be dependent upon the use of mechanisms for recognising these individuals. Recognition of social partners can occur through two, broadly defined, sets of mechanisms, 1) by using indirect recognition mechanisms to define relationships, such as their spatial location. For example, anyone who attends the nest may be considered a parent or helper, or 2) through direct recognition, where individuals use the phenotypic cues and signals of social partners for recognition (reviewed in Penn & Frommen 2010). For most avian species, parent-offspring interactions are typically dependent upon spatial recognition whilst young are in the nest (Komdeur & Hatchwell 1999), before displaying a facultative shift to a direct recognition just prior to fledging (Evans 1970b; Evans 1970a; Shugart 1978; Colgan 1983; Medvin & Beecher 1986). While young are in the nest, selection is thought to have favoured indiscriminate parent-offspring interactions (Beecher et al. 1981; with the exception of species where direct recognition has developed as a defence against brood parasites López-Sepulcre & Kokko 2002; Lyon 2003). This is because the spatial location of the nest can provide a reliable signal to relationships, whereas there is a risk of falsely rejecting true parents or helpers should direct recognition mechanisms fail (Beecher et al. 1981). As a consequence, the ontogeny of direct parent-offspring recognition is often

delayed until spatial recognition and the use of indiscriminate behavioural interactions become unreliable.

The reliability of spatial cues for identifying care-givers is often influenced by the environment and the behavioural ecology of the species. For instance, the highly colonial Bank Swallow (*Riparia riparia*) and Cliff swallow (*Hirundo pyrrhonota*) show strong parental discrimination as chicks, whereas parental discrimination is weaker in the non-colonial barn swallow (*Hirundo rustica*) (Stoddard & Beecher 1983; Beecher et al. 1985; Medvin & Beecher 1986; Leonard et al. 1997). Here, the need for an accurate discrimination mechanism to detect parents in a crowded colonial environment may be selecting for reliable direct recognition mechanisms (Beecher et al. 1985; Medvin & Beecher 1986). The effect of environmental selection for direct recognition mechanisms can even be observed within a species. For instance, ground-nesting herring gulls (*Larus argentatus*) will not accept cross-fostered young after five days post-hatching (Tinbergen 1953), but in cliff-nesting herring gulls, where the cliff environment offers less opportunity for young to drift into neighbouring nests, cross-fostering can occur after the chicks are one week old (von Rautenfeld 1978). The ontogeny of vocal recognition is well studied in the chicks of colonial species, including various species of swallow (Beecher et al. 1981; Medvin & Beecher 1986; Leonard et al. 1997), finch (Mulard et al. 2010), gull (Beer 1969; Beer 1970), and penguin (Davis & McCaffrey 1989; Jouventin et al. 1999; Aubin & Jouventin 2002), however, little is known about the development of vocal recognition at the other social extreme, where young encounter few non-parents or helpers post-fledging. This can occur in highly territorial species, where young develop within areas that are exclusively defended, and where

contact with individuals who are not parents or helpers is limited. In such species, we may expect the dependence upon spatial cues to remain reliable even post-fledging, and therefore, a delayed ontogeny in direct recognition mechanisms.

We investigated the ontogeny of recognition in the Southern pied babbler, *Turdoides bicolor*. Pied babblers are a cooperatively breeding passerine of the southern Kalahari that live in year-round stable social groups of 2-15 adult individuals (Radford & Ridley 2007). Pied babblers aggressively defend territories of around 0.75 km² (Golabek et al. 2012), and encounters between non-group members outside of territorial disputes are rare (Raihani et al. 2010). Young pied babblers develop into adults in their natal territory, remaining there beyond sexual maturity (Raihani et al. 2010). By adulthood (at least one year in age) pied babblers can discriminate kin from their vocalisations (Humphries et al., Chapter 6), and are able to discriminate the atonal chatter loud calls of familiar kin (where $r=0.5$) from unfamiliar non-kin (Humphries et al, Chapter 6). Here we tested the ability of juvenile pied babblers, less than one year in age, to discriminate between the atonal chatter calls of familiar kin from unfamiliar non-kin. We tested discrimination during five different age ranges: 56-117, 118-179, 180-241, 242-303, and 304-365 days since hatching. To draw comparisons between the different age categories, we have limited our study to birds that were foraging for themselves. Post-fledging care is highly variable and may last up to nine weeks (Ridley & Raihani 2007; Thompson & Ridley 2012). We used 56 days as the lower limit for our age ranges because this represents the earliest age that we observed young foraging exclusively for

themselves. We then evenly divided the time between 56 and 365 days in to five 61 day time periods.

Current research suggests that parent-offspring recognition develops in response to the risk in the rearing environment of making recognition errors (Beecher et al. 1985; Medvin & Beecher 1986). Pied babblers do not have to pick parents and helpers out from a crowd, and they will rarely encounter non-group members during their development. We predict that if the development of recognition is influenced by the social environment, then the ontogeny of recognition may be slower in highly territorial cooperative species than what has previously been witnessed in colonial avian species (Beer 1969; Beer 1970; Beecher et al. 1981; Medvin & Beecher 1986; Davis & McCaffrey 1989; Leonard et al. 1997; Jouventin et al. 1999; Aubin & Jouventin 2002; Mulard et al. 2010).

7.3 Methods

7.3.1 Study site and sound recording

Sound recording and playback experiments were carried out on a population of pied babblers located at the Kuruman River Reserve, in the Southern Kalahari, South Africa (26°57'S 21°49'E) (see Ridley & Raihani 2007 for more details about the study site). The population has been under observation since 2003, and is habituated to close observation (2-3m) allowing detailed behavioural observations to be made. Each individual in the population is colour ringed for individual identification. Here we played back the atonal chatter calls from familiar kin (either siblings or parents; where $r=0.5$), and unfamiliar non-kin (where $r=0$). Parentage can be reliably assigned from behavioural observations

of dominance within the group, breeding behaviour and activity at the nest (Nelson-Flower et al. 2011). We used behavioural observations of parentage to develop a pedigree for the population and establish the relatedness between the caller and the focal individuals in this experiment. As with previous kin recognition studies in the pied babbler, we term familiar here as former group members that have dispersed (Humphries et al., Chapter 6). We limited the playback of familiar individuals to instances where both the playback and the dispersal event occurred whilst the focal individual was within one of our five age brackets. We define unfamiliar individuals as those that live in territories at least 2km apart, and where we have no record of contact between the two individuals (Humphries et al., Chapter 6).

The atonal chatter call is one of eight loud call types produced by the pied babbler (Golabek & Radford 2013). It is a short call that is produced repetitively for up to 80 seconds in duration (Humphries et al., Chapter 3). It is typically given by dominant individuals of both sexes during intra-group communication for instance, to relocate the group following incubation (Golabek & Radford 2013). Atonal chatter calls were collected from familiar and unfamiliar individuals within 5-10 metres of the calling bird to a Marantz PMD660 data recorder (2008 D&M Holdings Inc.) using a Sennheiser ME66 shotgun microphone with a K6 power module (2004 Sennheiser). The microphone was housed in a Rycote pistol grip with windshield to reduce background noise. Recordings were collected at a sampling rate of 44.1 kHz, to 16-bit WAVE files (.wav). We recorded 51 atonal chatter calls from 39 individuals. In keeping with previous studies, we used atonal chatter strings five seconds in duration (Humphries et al., Chapter 6). Playback files included ten seconds of silence,

followed by a five second atonal chatter sequence. The ten seconds of silence was added to allow the observer time to move away from the speaker before the playback. Calls were cut and normalised (to -0.1 dB) and saved to WAVE files for playback using the audio software package ‘adobe audition 3.0’ (2007, Adobe Systems Inc.).

Table 1 – The mean age and sample sizes for each of our five age ranges.

Age range (number of days since hatching)	Mean ± SD age (days since hatching)	Playbacks of familiar Kin (n)	Playbacks of unfamiliar non-kin (n)
56-117	77.58±19.03	10	10
118-179	138.14±17.79	9	11
180-241	206.06±21.53	6	11
242-303	283.25±18.05	6	11
304-365	336.05±16.63	9	9

7.3.2 Playback experiment

Playbacks were carried out between November 2010 and April 2012 (see Table 1 for sample size details). We played back the atonal chatter calls of familiar kin and unfamiliar non-kin to focal individuals while they were foraging. Playbacks consisted of a twenty minute focal observation in which the behaviours of the focal individual were recorded to a Palm TX (Palm Inc., Sunnyvale, CA, U.S.A.) using a purpose built program created in ‘cybertracker’ (<http://www.cybertracker.org>). The behaviours recorded were: foraging, moving, resting, vigilance, sentinel (vigilance in an elevated position and producing ‘sentinel calls’; Hollén et al. 2008), loud calling, group chorusing, play-fighting, auto-preening, allo-preening, and bill wiping. The focal was followed by the playback of an atonal chatter call. Atonal chatter calls were played back at 70dB, to mimic natural calling amplitude, 15m from the focal bird from a Sony Walkman NWZ-E345 using an Altec Lansing Orbit (iMT227) speaker mounted

to a tripod (1.5m high). A second twenty minute focal was started parallel to the start of the playback to record any behavioural changes in the focal bird.

7.3.3 Statistics

Previously we have observed that guarding behaviour (the combined time in seconds spent in vigilance, and sentinel behaviour) is elevated in response to atonal chatter calls from unfamiliar non-kin, but not from familiar kin in adult pied babblers (Humphries et al., Chapter 6). We therefore explored the changes in guarding and foraging behaviour between the first and second focal in young pied babblers in each of the five age ranges. We tested the time spent guarding or foraging in the first focal with the time invested in these behaviours in the second focal (depending on whether the playbacks was from either familiar kin or unfamiliar non-kin) using a paired t-test carried out in R 2.15.2 (R Core development team, 2012).

Table 2 – Output from a series of paired t-tests investigating the behavioural change in foraging and guarding behaviour between two twenty minute behavioural focals, separated by a playback of a five second atonal chatter call. The tests examine the effect of the age of the focal individual and familiarity of the playback individual. Significant results are highlighted in bold.

Age	Activity	Familiar			Unfamiliar		
		t	df	P	t	df	P
56-117	Foraging	0.809	9	0.439	0.609	9	0.558
	Guarding	-1.012	9	0.388	-1.668	9	0.130
118-179	Foraging	-0.974	8	0.359	2.561	10	0.028
	Guarding	-0.259	8	0.803	-4.660	10	0.001
180-241	Foraging	-1.458	5	0.205	3.488	10	0.006
	Guarding	0.739	5	0.493	-3.621	10	0.005
242-303	Foraging	-2.066	5	0.094	3.005	10	0.013
	Guarding	0.677	5	0.528	-3.783	10	0.004
304-365	Foraging	-0.239	8	0.818	2.566	8	0.033
	Guarding	0.450	8	0.665	-5.379	8	<0.001

7.4 Results

The guarding and foraging behaviour of young pied babblers in the youngest age bracket (56-117 days old), did not change following the playbacks of atonal chatter calls from either familiar kin or unfamiliar non-kin (Table 2). In contrast, all other age categories tested showed a significant increase in guarding behaviour and a reduction in foraging behaviour in the 20 minutes following the playback of an unfamiliar, unrelated individual (Table 2, Figures 1 and 2).

These behavioural changes were not observed when atonal chatter call playbacks were from familiar kin, indicating that young pied babblers are discriminating atonal chatter calls after they are 118 days old post-hatching (Table 2, Figures 1 and 2). Our findings suggest that young pied babblers less than 117 days post-hatching, are not discriminating between the vocalisations of unfamiliar non-kin from familiar kin.

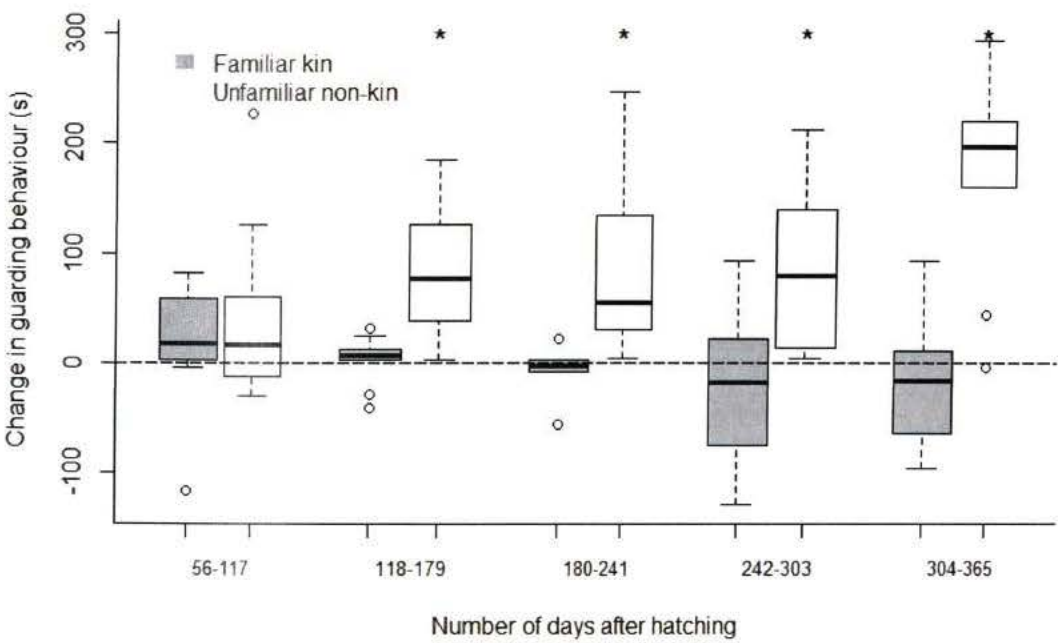


Figure 1 – The difference in guarding behaviour between two, twenty minute behavioural focals separated by a five second playback of an atonal chatter call (guarding post-playback minus the guarding behaviour prior to the playback). The graph demonstrates the effect of age and familiarity with the calling individual on behavioural responses. Significant change in guarding behaviour, as established in Table 2, is denoted using the ‘*’ symbol.

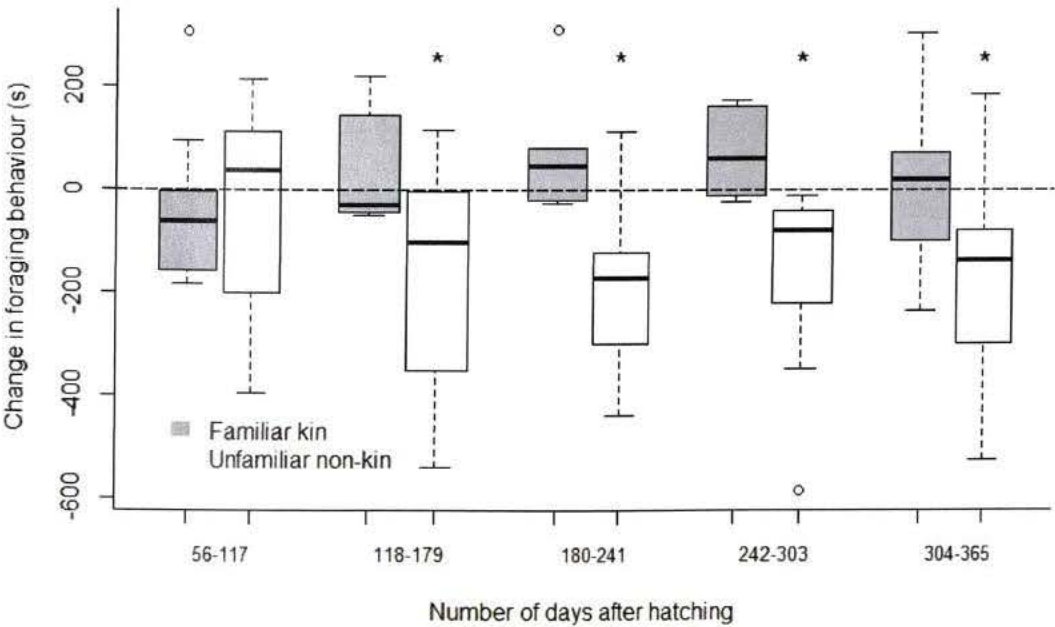


Figure 2 – The difference in foraging behaviour between two twenty minute behavioural focals separated by a five second playback of an atonal chatter call (behaviour post-playback minus the behaviour prior to the playback). The graph demonstrates the effect of age and familiarity with the calling individual on foraging behaviour. Significant change in foraging behaviour, as established in Table 2, is denoted using the ‘*’ symbol.

7.5 Discussion

Discrimination of the atonal chatter call develops remarkably late in the pied babbler, with discrimination taking over 117 days to develop. When we compare this with observations of the ontogeny of recognition in other avian species, we can get a sense of how unusually late 117 days is. For instance, laughing gulls, *Leucophaeus atricilla*, recognise the calls of parents at six days post-hatching (Beer 1970), while in cliff and barn swallows it occurs by 18 days (Beecher et al. 1985; Medvin & Beecher 1986), and in the Pinyon jay, *Gymnorhinus cyanocephalus*, between 14 and 20 days (McArthur 1982). Parent-offspring recognition is thought to develop in most avian species just before the onset of mobility (Evans 1970b; Evans 1970a; Shugart 1978; Colgan 1983; Medvin & Beecher 1986). Pied babblers typically fledge between 13-19 days after hatching (Raihani & Ridley 2007; Ridley & Heuvel 2012), and we may therefore have expected recognition to occur around this period, well before the first time period that we found recognition in young pied babblers. Recognition can occur at many levels, from fine scale individual recognition, to broader classes such as kin recognition, or familiarity (Bradbury & Vehrencamp 2011). In this experiment, there were differences between the two playbacks in both their familiarity and their relatedness to the focal individual. Yet, we observed no discrimination in our youngest age category, suggesting that even broad recognition systems have not developed in young pied babblers at this age.

One possible reason for the slow development of vocal recognition is the environment the young birds fledge into. Pied babbler young fledge into large, actively defended territories (Golabek et al. 2012) and will encounter few non-

group members outside of territorial disputes (Raihani et al. 2010). Even when encounters with neighbouring groups do occur, young are often left behind in the protection of dense vegetation whilst the inter-group interaction takes place (A. Ridley, unpublished data). High levels of territoriality (Golabek et al. 2012; Humphries et al., Chapter 9) may isolate the young, resulting in recognition not being required until later in development, since there is limited risk of encountering a non-natal individual or group. For most avian species, recognition is dependent upon spatial cues (such as the location of the nest), during the early stages of development (Komdeur & Hatchwell 1999). For young pied babblers, territoriality may mean that spatial cues are reliable well beyond the age of post-fledging mobility.

A key problem with recognition studies is inferring results when no discrimination occurs (Waldman et al. 1988). When a test subject fails to discriminate, it does not necessarily imply that the test subject has failed to recognise. Barnard (1991) used the following anecdote to highlight the problems facing recognition studies, 'recognising a fruit as an orange does not necessarily lead to us eating it'. Failure for young babblers to discriminate between the atonal chatter calls of other individuals may be due to limited discrimination rather than limited recognition. The lack of behavioural discrimination by young pied babblers between 56-117 days old of calls from familiar and unfamiliar individuals may be attributable to two causes, (1) that pied babblers between 56-117 days old are failing to recognise, or (2) that they do recognise but do not discriminate. Young individuals may not get involved in inter-group interactions until they are more physically developed. There is, however, evidence that recognition is genuinely absent in young pied babblers.

Individuals up to 64 days have been observed to be successfully kidnapped by neighbouring groups (A. Ridley et al, unpublished data). Observations of kidnapping, and the fact that discrimination is occurring in older juvenile pied babblers, lead us to believe that there is a genuine failure to recognise the vocalisations of group members in young pied babblers.

It has been suggested that the social environment has implications for the ontogeny of recognition in young birds (Beecher et al. 1985; Medvin & Beecher 1986). Young pied babblers face the challenges of learning the identity signals of many individuals, not just those of one or two parents, and they also fledge into a territory where encounters with non-group members are rare. It seems that territoriality in the pied babbler, and its consequences for limiting contact with non-group members, may mean that, unlike in many other avian species, recognition does not need to develop at the onset of mobility. The defence of a large territory may result in spatial cues to identity being reliable for up to three months post-fledging. It is perhaps informative that three months is approximately the age at which young start to participate in territorial disputes with neighbouring groups and begin to regularly encounter non-group members (A. Ridley, unpublished data). Given that direct recognition mechanisms are likely to be imperfect (Waldman 1987), and the inherent risk of falsely rejecting true group members when using direct recognition mechanisms, selection may favour indiscriminate behaviour when spatial cues are a good proxy to identity and relationships. Direct recognition is only likely to become important in pied babblers when either: a) they start to begin their involvement in territorial defence, and start to regularly encounter non-group members, and b) when they start to explore reproductive and dispersal opportunities. Our work

supports the hypothesis that direct recognition mechanisms are most likely to develop when needed (Beecher et al. 1985; Medvin & Beecher 1986), and that the social environment may play an important role in determining the ontogeny of recognition.

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Chapter 8

Limits to long-term recognition in the Southern pied babbler



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8.1 Abstract

Encounters between individuals within animal societies are often interspersed with periods without contact. Maintaining vocal recognition between encounters is potentially problematic as identity labels within vocalisations may change over time, and the cognitive associations that are formed between identity labels and the calling individual may be forgotten if individuals are not in regular contact. Previously we have demonstrated that the Southern pied babbler, *Turdoides bicolor*, is able to discriminate between the atonal chatter loud calls of former, related, group members and unfamiliar, unrelated individuals. Here we tested if this ability was lost when individuals had been separated for two years. Critically, vocalisations were recorded within three months of the playback to ensure that any changes that had occurred to the vocal characteristics of the calling individual over the two years was present in the playback. This test therefore assumes both stable identity labels by the signaller *and* long-term-memory of these identity labels by the receiver. We found that focal individuals no longer discriminated between the vocalisations of familiar kin from unfamiliar non-kin when these individuals had been separated for two years. When we explored the underlying mechanisms of long-term recognition (both the stability of identity labels and long-term memory for identity labels), we found that individuals could discriminate the atonal chatter calls of familiar kin after one year of separation if those calls were recorded at a time when the individuals were still associating with one another. This suggests that pied babblers have a long-term memory for identity labels. We have previously observed that the identity labels within loud calls are unstable between years. We suggest that long-term recognition is therefore

likely to be limited by unstable identity labels rather than by limited memory in this species.

8.2 Introduction

Vocalisations are the dominant mode of social communication in birds (Halpin 1991), and often encode identity cues allowing conspecific recognition (Sharp & Hatchwell 2005; McDonald et al. 2007; Levréro et al. 2009; Bradbury & Vehrencamp 2011). However, maintaining recognition over time may be problematic as the specific characteristics of an individual's calls may change over time in relation to changes in age (Radford 2004; Klenova et al. 2010), physical size (Suthers 1994), and social environment (Farabaugh et al. 1994; Hile et al. 2000; Hile & Striedter 2000). Receivers may also forget the cognitive associations that enable them to match identity labels to their owner if they are not in regular contact (Kendrick et al. 2001).

Temporal variation in individual call characteristics and the deterioration of long-term memory are both likely to limit long-term vocal recognition. However, social relationships often involve unpredictable periods of separation, where there may be pronounced benefits to be gained from recognising previous social partners. The duration of separation can vary extensively, from short-term separations of a few hours, such as incubation, to longer-term separations of months or years, such as migration, dispersal or prospecting events. For instance, the monogamous wandering albatross (*Diomedea exulans*) forms life-long mating partnerships (Jouventin et al. 2007) but only comes to land to breed biannually, with breeding pairs potentially separated from one another in the years between breeding attempts as their foraging requirements lead them

to traverse vast areas of open ocean (Weimerskirch & Wilson 2000). The ability to recognise individuals over these extended periods without contact will affect the expression of a broad spectrum of social interactions, from mate choice decisions to the re-uniting of social partners after periods of separation. For instance, many species appear to avoid mating with individuals they associated with in infancy as a strategy for avoiding costly inbreeding (Westermarck 1891; Pusey & Wolf 1996; Lieberman & Symons 1998; Charlesworth & Willis 2009). However, there is often a delay between leaving the natal environment and reaching sexual maturity. For example, young toucan barbet (*Semnornis ramphastinus*) disperse from the natal environment at around nine months of age, but do not begin to seek reproductive partners until they are 18 months old at the earliest (Del Hoyo et al. 2002).

Long-term recognition requires a combination of both stable identity labels by the caller, and long-term memory by the receiver (Charrier et al. 2003).

Studying the latency of recognition therefore requires a three-pronged approach: Firstly, it is important to identify whether individuals are able to discriminate between the identity labels of social partners. Secondly, it is necessary to test whether recognition between former social partners is lost when individuals are separated without contact for extended periods (Boeckle & Bugnyar 2012). If in following these first two steps, recognition is found to break down when individuals are apart, it is then informative to separate out whether this is due to unstable identity labels by the calling individual or limited memory of identity labels by the receiver.

There is good evidence that birds have the capacity for long-term recognition of individuals (Godard 1991; Boeckle & Bugnyar 2012). For instance the hooded warbler (*Wilsonia citrina*) is able to recognise the calls of individuals in neighbouring territories (Godard 1991). This ability to discriminate the vocalisations of neighbours can be maintained even when those neighbours have been separated for eight months (Godard 1991). In another example, Boeckle and Bugnyar (2012) were able to demonstrate long-term vocal recognition in ravens (*Corvus corax*) by recording the calls of individuals that had been separated for almost three years. This experimental design of recording the individual long after the initial separation provides evidence for both long-term memory *and* stable identity signatures in this species. Long-term recognition may be important for the dynamic social system of the raven, where non-breeding individuals live in fission-fusion societies and may regularly be separated from social partners for extended periods (Boeckle & Bugnyar 2012). The ability to recognise conspecifics over extended periods of separation has been identified in both birds (Miller 1979; Godard 1991; Boeckle & Bugnyar 2012), and mammals (Hepper 1994; Insley 2000; Charrier et al. 2003; Briefer et al. 2012), however, few studies of recognition latency have been run until the cessation of recognition. Consequently, our understanding of both when recognition fails and the underlying mechanisms that cause recognition to fail remain limited.

Here we tested for recognition latency in the Southern pied babbler, *Turdoides bicolor*, a cooperatively breeding passerine of the semi-arid southern Kalahari biome. Pied babblers live in year-round stable social groups of 2-15 adult individuals (Radford & Ridley 2008) that defend exclusive territories of around

0.75km² (Golabek et al. 2012). Adult pied babblers are able to discriminate both individual group members and kin (Humphries et al., Chapters 5 and 6) from their individually distinctive vocalisations (Humphries et al., Chapters 3 and 4). However, long-term recognition of some calls, such as the atonal chatter loud call (as defined by Golabek & Radford 2013) may be limited, with recent research revealing that the atonal chatter loud call is unstable between years (Humphries et al., Chapter 3). Here we investigated the ability of pied babblers to discriminate atonal chatter calls after extended periods of separation focusing on two key areas: Firstly, we aim to understand whether the ability to discriminate atonal chatter calls of previously familiar individuals is lost over time. We tested this hypothesis by playing back the atonal chatter calls of individuals that had been separated for two years (720 ± 1 days). This duration was chosen because dispersed offspring, who utilise their natal territory as a 'safe haven' between dispersal attempts (Raihani et al. 2010), have not been observed returning to their natal group beyond 717 days (A. Ridley, unpublished data). Importantly, we recorded the calls less than three months prior to the playback to ensure that any potential change within the vocalisations over time would be accounted for in the recognition tests. Given the instability of the atonal chatter call between years (Humphries et al., Chapter 3), we predict that when these calls are recorded almost two years after the last known contact, that changes in the identity signals will result in previously familiar individuals failing to discriminate these calls from those of individuals that have never been encountered.

Secondly, we explore the underlying mechanisms of long-term recognition, testing for the presence of long-term memory of identity labels. Testing for long-

term memory of vocal identity labels can be achieved by recording the calls at a time when focal individuals were in association, and then exposing a test subject to these signals at a later stage. As these tests use signals encountered during the period of familiarity between test subjects, they make no assumptions of stable identity labels and test purely for memory. This experimental design has been used to demonstrate long-term memory in Northern fur seals, *Arctocephalus tropicalis*, which continue to respond to the calls of their pups that had been recorded up to four years previously (Insley 2000). Here we play back the atonal chatter calls of previously familiar individuals at one year (360 ± 4 days) of separation. Critically, these calls were recorded whilst the individuals were together in the social group, or within a few days of separation. The aim here is to test for long-term memory of identity signals over the same time span that we have previously observed instability in the identity labels (Humphries et al., Chapter 3). This study aims to identify whether pied babblers are capable of long-term recognition, and identify the mechanisms that either enable or limit long-term recognition.

8.3 Methods

8.3.1 Study population and sound recording

We investigated the effects of separation on responses to atonal chatter call playbacks in a population of pied babblers located at the Kuruman River Reserve, in the Southern Kalahari, South Africa ($26^{\circ}57'S$ $21^{\circ}49'E$) (see Ridley & Raihani 2007 for more details about the study site). The study population is habituated to close observation, allowing close (within 2-3m) behavioural observations and sound recording. The study population has been under

investigation since 2003, and detailed life history information exists for each individual within the population. The atonal chatter loud call is a short, one syllable call given repeatedly for up to 80 seconds in duration (mean \pm S.D 4.53 \pm 3.07; range 0.7-80.0 seconds; Humphries et al., Chapter 3). It is one of eight loud call types produced by the pied babbler and it is given by both sexes primarily in intra-group social situations (for instance, to relocate the group after incubation; Golabek & Radford 2013). We focus here on the atonal chatter call as it is the loud call most frequently heard being given as a solo call (D. Humphries, unpublished data), and because pied babblers have previously been shown to be able to discriminate between the atonal chatter calls of familiar kin from unfamiliar non-kin (Humphries et al., Chapter 6).

Vocalisations were recorded between October 2010 and April 2012 using a Marantz PMD660 data recorder (2008 D&M Holdings Inc.) and a Sennheiser ME66 shotgun microphone with a K6 power module (2004 Sennheiser), housed in a Rycote pistol grip with windshield to reduce background wind noise. Recordings were collected at a sampling rate of 44.1 kHz, to 16-bit WAVE files (.wav). Here we used 40 different atonal chatter calls, collected from 29 individuals. Recordings were collected within 5-10m of the calling individual, when background noise (wind, singing cicadas etc) was low. For playback, we used strings of atonal chatter calls that were five seconds in duration (to mimic the natural duration of this call type; Humphries et al., Chapter 6). Recorded calls were cut and normalised (to -0.1 dB) and saved to WAVE files (.wav) for playback using the audio software package 'Adobe Audition 3.0' (2007, Adobe Systems Inc.). We added 10 seconds of silence

before the call to allow the observer time to move away from the speaker before the playback started.

8.3.2 Experimental design

8.3.2.1 Is recognition lost over time?

Breeding within pied babbler groups is typically monopolised by the dominant pair (Nelson-Flower et al. 2011). Dominant pairs are sexually monogamous, and parentage can be reliably assigned from behavioural observations of dominance and breeding behaviour within the group (Nelson-Flower et al. 2011). Behavioural observations were used to develop a pedigree of the population and establish the relationships between individuals. Here we set out to investigate whether pied babblers are able to discriminate between the atonal chatter calls of unfamiliar non-kin and previously familiar, adult kin (either parents-offspring or full sibling relationships) that have been separated for two years. To test for recognition latency rather than long-term memory, we recorded the calls of these individuals within three months of the playback to ensure that any change in the callers vocal characteristics over the two years the individuals had been apart was present in the playback. We located familiar kin in the population that had been separated without any recorded subsequent contact. We required playback individuals to live in territories at least 2km away to ensure that these individuals were out of contact. We carried out nine playbacks of atonal chatter calls from familiar kin that had been separated, and 32 playbacks of unfamiliar non-kin. Playbacks were carried out opportunistically as each test situation arose between November 2010 and April 2012. Playbacks were carried out to 30 individuals from 18 different social groups.

Playbacks consisted of a twenty-minute focal observation in which the behaviours of the focal individual were recorded to a Palm TX (Palm Inc., Sunnyvale, CA, U.S.A.) using a purpose-built program created in 'cybertracker' (<http://www.cybertracker.org>). The behaviours recorded were: foraging, moving, resting, vigilance, sentinel (vigilance from an elevated position whilst producing 'sentinel calls'; Hollén et al. 2008), loud calling (for more information on these call types see; Golabek & Radford 2013), group chorusing, play-fighting, auto-preening, allo-preening, and bill wiping. The first focal period was followed by the playback of an atonal chatter call. Atonal chatter calls were played back at 70dB, 15m from the focal bird from a Sony Walkman NWZ-E345 using an Altec Lansing Orbit (iMT227) speaker mounted to a tripod (1.5m high). Atonal chatter calls were played back at 70dB (determined as the natural calling volume, when measured from 5m with Voltcraft SL100 (Voltcraft, Barking, UK) sound level meter. The responses of focal individuals to the playback were recorded during a second twenty-minute focal, which started parallel to the playback starting. We used two measures of response to playbacks including: (1) the difference in guarding behaviour (combined vigilance and sentinel behaviour) between the first and second focal, and (2) the difference in foraging behaviour between the first and second focal. These two measures of response have previously been effective in demonstrating discrimination between the atonal chatter calls of familiar kin from unfamiliar non-kin (Humphries et al. Chapters 6 and 7).

8.3.2.2 Testing for long-term memory of identity signals

We investigated long-term memory for identity signals in the pied babbler by testing whether individuals discriminated between the atonal chatter calls of

unfamiliar individuals and former group members that had been separated for one year. We used one year of separation because previous research on the atonal chatter loud call has revealed that the identity labels within this call are unstable over this time period (Humphries et al, Chapter 3). In this experiment we aim purely to test the ability of focal individuals to remember the identity labels of former social partners. Playbacks followed the same protocol as described for the recognition latency experiment above. We carried out 11 playbacks of atonal chatter calls from previously familiar, related individuals (parents and offspring or siblings), and 32 from unfamiliar non-kin. Playbacks were carried out to 33 individuals from 17 different groups.

For both experiments presented here, we analysed the behavioural change in both guarding and foraging behaviour in response to the vocalisations presented. Responses to playbacks were analysed using paired t-tests carried out in R 2.15.1 (R Core development team, 2012).

8.4 Results

8.4.1 *Is recognition lost over time?*

When we exposed individuals to five second atonal chatter calls from individuals that were either familiar kin that had been out of contact for two years, or unfamiliar non-kin, we found that in both cases the focal birds showed significant increases in guarding behaviour, and a reduction in foraging behaviour (Table 1, Figure 1). The lack of discrimination we observed indicates that recognition of familiar kin is likely to have ceased when individuals have been separated for two years.

Table 1 – Paired t-test output investigating the behavioural changes between two twenty minute focals, separated by a five second atonal chatter call from either, a) a familiar relative ($r=0.5$) that has been out of contact for 720 ± 1 days, or b) an unfamiliar and unrelated individual.

Playback	Activity	t	df	P
Familiar kin (separated for 720 ± 1 days)	Foraging	2.964	8	0.018
	Guarding	-3.886	8	0.005
Unfamiliar non-kin	Foraging	4.205	36	<0.001
	Guarding	-6.572	36	<0.001

8.4.2 Long-term memory of the atonal chatter call

We found that focal individuals discriminated between the atonal chatter calls of familiar kin that had been separated for a year (when those calls were collected at the time of dispersal), and the atonal chatter calls of unfamiliar non-kin (Table 2, Figure 1). Whilst guarding behaviour was elevated and foraging behaviour reduced following the playback of an atonal chatter call from an unfamiliar, unrelated individual, no significant difference was observed in response to calls from familiar kin that had been separated for a year. This suggests that the pied babbler is able to memorise the identity labels of familiar kin for up to a year following separation.

Table 2 – Paired t-test output investigating the behavioural changes between two twenty minute focals, separated by a five second atonal chatter call from either, a) a familiar relative ($r=0.5$) that has been out of contact for 360 ± 4 days, or b) an unfamiliar and unrelated individual.

Playback	Activity	t	df	P
Familiar kin (separated for 360 ± 4 days)	Foraging	-0.057	10	0.956
	Guarding	0.067	10	0.948
Unfamiliar non-kin	Foraging	4.205	36	<0.001
	Guarding	-6.572	36	<0.001

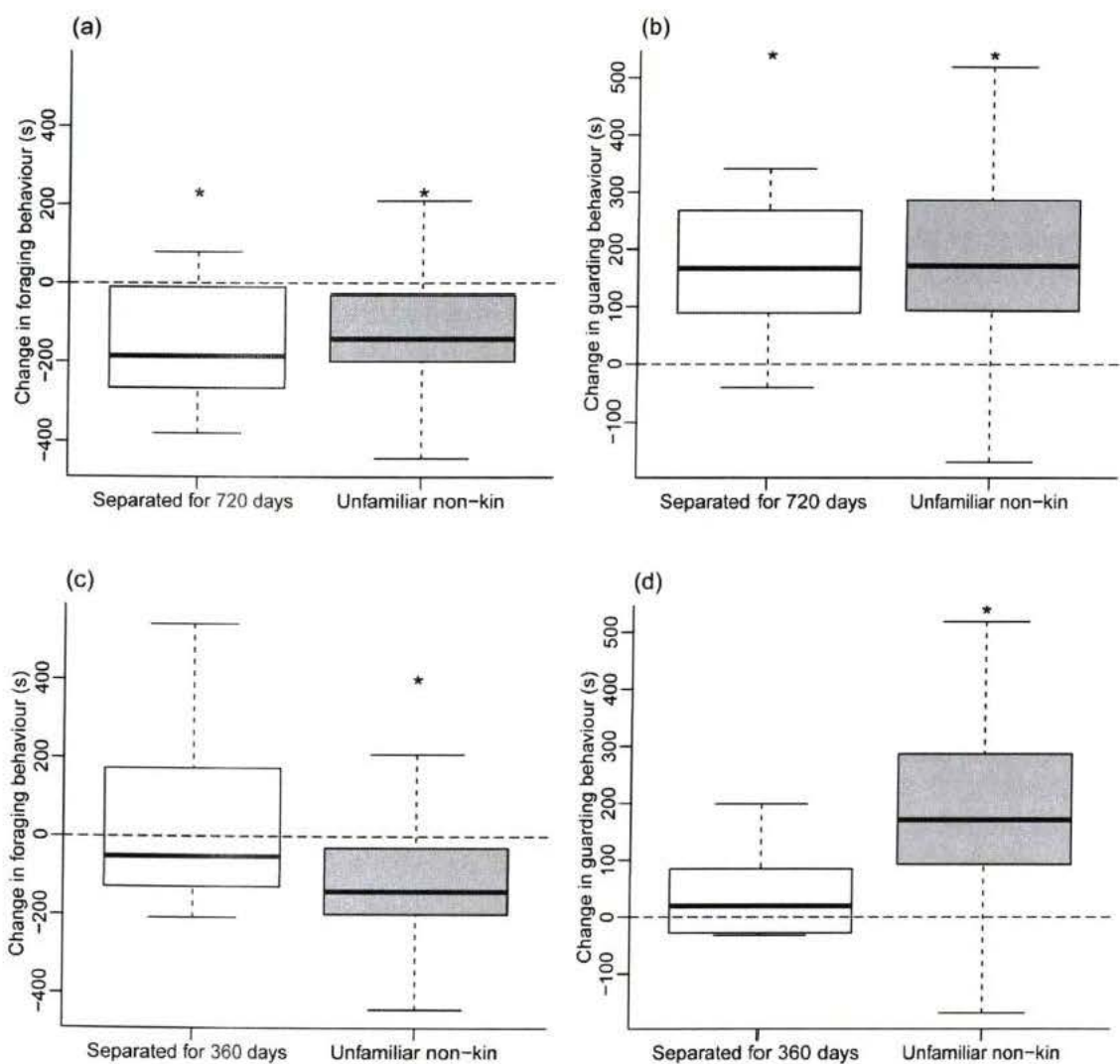


Figure 1 - The change in time spent foraging (graph (a)) and guarding (graph (b)) in the twenty minutes following a playback of an atonal chatter vocalisation from either a familiar relative who has been out of contact for 720 ± 1 days or an unfamiliar, unrelated individual. Graphs (c), and (d) demonstrate the change in foraging and guarding behaviour when the playback was from either a familiar relative who has been out of contact for 360 ± 4 days or an unfamiliar, unrelated individual. Critically, these calls were recorded at the time of initial dispersal and therefore graphs (c), and (d) are testing memory rather than long-term recognition. Significant change in foraging and guarding behaviour, as established in Tables 1 and 2, is denoted using the '*' symbol.

8.5 Discussion

Although pied babblers have previously been shown to discriminate between the atonal chatter calls of familiar kin, from those of unfamiliar non-kin (Humphries et al., Chapter 6), we found that this ability is lost when individuals have been separated for two years. When we investigated the underlying mechanisms of long-term recognition (the stability of identity labels (Humphries et al., Chapter 3) and the presence of long-term memory), we found that pied babblers are able to memorise the identity labels of familiar kin for up to a year. This suggests that long-term recognition is likely to be limited by unstable identity labels rather than by limited memory of identity labels in the pied babbler. Our previous observations that identity labels within the atonal chatter call are unstable between years (Humphries et al., Chapter 3), support this conclusion.

Loss of recognition over time may have profound implications for the social behaviours that species display, particularly in reference to mate choice and inbreeding avoidance. Inbreeding can increase the risk of genetic diseases among offspring through greater expression of deleterious recessive alleles (Charlesworth & Charlesworth 1999). This in turn can reduce offspring survival and therefore parental fitness (Charlesworth & Charlesworth 1987; Daniels & Walters 2000; Nielsen et al. 2012). Inbreeding in the pied babbler is rare and is thought to be avoided through a combination of dispersal and kin recognition by prior association (Nelson-Flower et al. 2012). A breakdown in recognition between individuals that are out of contact could potentially lead to recognition errors and costly incestuous mating. While inbreeding in the pied babbler is rare, they do occasionally form incestuous breeding partnerships (Nelson-

Flower et al. 2012), and the break down in recognition between individuals that have been physically separated over extended periods may explain the formation of these incestuous breeding associations.

Another implication for limited long-term recognition in the pied babbler is its consequences for dispersal tactics by subordinate group members.

Subordinate group members of reproductive age begin to leave the natal group to prospect for reproductive opportunities within non-natal groups (Raihani et al. 2010), but utilise the natal territory as a 'safe haven' between dispersal attempts to minimise the costs of this activity (Kokko & Ekman 2002; Ridley et al. 2008; Raihani et al. 2010). This safe haven tactic can be used for up to a year after initially leaving the natal group (there is one instance of an individual returning after 717 days; A. Ridley, unpublished data). Limits to long-term recognition potentially set a maximum limit to the duration of this 'safe haven' strategy, with 'unfamiliar' individuals almost always chased off the territory by group members (Raihani et al. 2010).

The capacity for long-term memory has been previously observed in avian species, from remembering the spatial locations of cached items for many months (Hitchcock & Sherry 1990; Balda & Kamil 1992; Bednekoff et al. 1997; Roth et al. 2012), to the ability to recall both colour and learnt colour combinations over time (Bogale et al. 2012; Weiß & Scheiber 2012). Our findings that pied babblers are able to discriminate between the vocalisations of unfamiliar, unrelated individuals and those of familiar kin that had not been encountered for one year provides further evidence for the capacity for long-term memory. Importantly, this does not rule out the possibility of long-term recognition in the pied babbler through a stable identity signalling mechanism.

Avian species can utilise a range of visual (Stoddard & Beecher 1983; Dale et al. 2001), olfactory (Bonadonna & Sanz-Aguilar 2012; Krause et al. 2012), and vocal signals to recognise conspecifics (Sharp et al. 2005; Levréro et al. 2009; McDonald & Wright 2011). While we found that one of the loud call types was unstable between years (Humphries et al., Chapter 3), the stability of other communication pathways in the pied babbler is not currently understood, and we therefore cannot rule out the possibility of long-term recognition through other communication pathways.

While recognition latency has been widely studied (Godard 1991; Insley 2000; Mateo & Johnston 2000; Kendrick et al. 2001; Mathevon et al. 2004; Mateo 2010; Murai et al. 2011; Boeckle & Bugnyar 2012), the influence of signal stability or memory on recognition has been poorly explored. This study provides the first example to use a holistic approach to long-term recognition and has demonstrated not only a limit to long-term recognition, but has also identified signal instability as the likely limiting factor.

8.6 References

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Chapter 9

Kinship and costly combat: the effects of relatedness on inter-group aggression in a cooperative breeder



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9.1 Abstract

Many species aggressively hold territories to defend resources such as breeding sites and foraging patches. Yet, there can be considerable variation within a species in the extent to which a territory is exclusive and neighbouring territories overlap. In many species, the overlap between territories appears to be affected by kinship, with greater overlap occurring when territory holders are close relatives. Here we investigate whether there are behavioural changes when interacting with kin on territory boundaries and whether this has an effect on overall territorial overlap. We also determine the energetic costs or benefits that might be associated with having relatives in neighbouring social groups in the Southern pied babbler, *Turdoides bicolor*. We found that territories overlap more when neighbouring groups are closely related and that encounters between related neighbouring groups tended to be shorter in duration. We show here for the first time that territorial defence is costly, resulting in significant loss of body mass. However, we also found that neither the frequency of costly, inter-group interactions nor the rate of physical fighting were reduced between related neighbouring groups. It therefore seems unlikely that kin-biased overlap is a by-product of greater tolerance of kin. Contrary to previous suggestions that kin-biased overlap may be driven by kin-selected benefits, we suggest that it is instead the result of changes in intruder pressure with kinship.

9.2 Introduction

The defence of a territory can help to ensure access to sites for breeding or foraging when these resources are limited and in demand [1]. However, there is considerable variation in the extent to which territories are exclusive and the intensity with which territory holders attempt to repel incursions by others [2-4]. Any tolerance of territorial overlap or incursion requires explanation, since it is likely to deplete the resources available to the territory holder, with negative consequences for foraging success [5, 6], with potentially long-term consequences for growth [5]. The costs of territorial overlap, however, must be balanced against the costs of territorial defence [7, 8]. Investment in territorial defence is often dynamic, changing in response to intruder pressure (the number of intruder visits per territory) [9, 10], or the quality of the habitat being defended [11, 12]. Relatedness is another factor known to affect the extent of territorial exclusivity, with greater overlap sometimes observed between the territories of related neighbours [13-17].

Kin-biased territory overlap is a wide-spread phenomenon, occurring across a variety of taxa including fish [14], birds [18], and mammals [13, 15-17]. Yet, the causes of kin-biased territory overlap remain poorly understood. It has been touted as a possible example of kin selection [15, 19], where territory holders endure the costs of territory overlap because the beneficiaries are close relatives, however it is difficult to empirically show any energetic or fitness benefits from these interactions that may be indicative of inclusive fitness [19]. For example, in prairie voles, *Microtus ochrogaster*, kin-biased territory overlap does not appear to influence reproductive success [13]. One issue with understanding kin-biased territory overlap is that there is currently very little

information about how kinship affects the frequency of territorial disputes between neighbours, or affects investment in territorial defence. As a consequence, it is difficult to rule out other potential explanatory factors such as changes in intruder pressure when neighbours are related [which can effect territory overlap; 9, 10] or to attribute any energetic benefits that may indicate the presence of kin selection.

Here we examine the extent of territorial overlap between neighbouring groups with regards to kinship and assess the effects of relatedness on the intensity of inter-group interactions (IGIs) in the cooperatively breeding Southern pied babbler, *Turdoides bicolor*. Further, we quantify the costs of territorial defence in this species to investigate the potential costs or benefits that may arise from having kin in neighbouring territories. Pied babblers are medium sized (75-95g) passerines of the semi-arid Kalahari Desert [20]. They live in stable groups consisting of a dominant breeding pair and a variable number of non-reproductive helpers and dependent chicks [mean number of adults 4.73 ± 1.48 SD, 21]. Social groups hold year-round territories that are frequently defended from neighbouring groups (on average one IGI every 4.4 hrs of observation). These border interactions vary widely in aggression, from purely vocal, ritualized border defences to physical attacks [22]. As with many cooperatively breeding species [23], dispersal distances in the pied babbler are typically low and closely related neighbouring groups are common within the study population [24]. Here we set out to: (1) investigate how kinship affects territorial overlap; (2) explore the factors that influence the frequency and intensity of territorial defence; (3) understand the interaction between territorial defence

and territorial overlap; and (4) examine the consequences of variation in territorial overlap on the energetic costs of territorial defence.

9.3 Methods

9.3.1 *Study site and species*

We observed a colour ringed, habituated population of pied babblers at the Kuruman River Reserve, southern Kalahari desert, South Africa (26°58'S, 21°49'E) [see 20 for more details about the study site]. The population was observed continuously between 2003 and 2012, with the number of groups observed each year ranging between twelve and 26 (median 18 groups). Groups were visited on average 1.39 times per week, either in the morning (from dawn, average observation time 2.23hrs \pm 20mins) or in the afternoon (until dusk, average observation time of 1.19hrs \pm 27mins). Birds were trained to step on an electronic balance for a mealworm reward, and were weighed at the start and end of every observation session (accuracy \pm 0.1g).

9.3.2 *Establishing inter-group relatedness*

Pied babbler groups are typically composed of a dominant breeding pair and their retained offspring [25]. Dominant pairs are monogamous, and extra-pair paternity and maternity is extremely rare [25]. As a consequence, relatedness between the dominant individuals of neighbouring groups provides a useful proxy for relatedness between the groups. We identified two categories of relatedness between neighbouring groups: (i) Unrelated: the dominant pairs of neighbouring groups are completely unrelated; (ii) Related: at least one dominant of one group is closely related (either parents and offspring or siblings) to at least one dominant of the neighbouring group (we removed

instances where neighbouring groups had intermediary relationships from the data set). Pedigrees of the population were constructed using behavioural assignments of relationships and parentage analysis with microsatellite genotyping. For full information on the pied babbler parentage analyses see Nelson-Flower et al. [24, 25].

9.3.3 Territorial overlap

9.3.3.1 Measuring territorial overlap between neighbouring groups

We measured the territorial overlap between 15 groups (representing 35 different territory boundaries) taken from eight different years of observation at the study site. Group territories were established using 300 GPS points collected during each breeding year (breeding year defined as Sept 1 – Aug 31), representing a minimum of 60 hours of observation per group per year. GPS points were recorded with hand-held GPS devices, at 15min intervals during observation sessions, from the centre of the foraging group. Territory sizes were calculated using the ‘adaptive sphere-of-influence local convex hull’ (a-LoCoH) methodology outlined by Getz et al. [26]. A-LoCoH was performed in R 2.15.2 (R Development Core Team 2008) using the ‘adehabitat’ package [27]. 95% density isopleths were exported from R in to ArcGIS 9.3.1 (ESRI, 2009) where territory sizes were measured using the ‘Hawths tools’ extension (Beyer, H. L., 2004. Hawth's Analysis Tools for ArcGIS. Available at <http://www.spatialecology.com/htools>). Overlap between territories was measured using the ‘polygon-in-polygon’ analysis available in Hawth's tools. We converted the area of overlap between neighbouring territories to a percentage of the whole territory area. The percentage of overlap was calculated once per

boundary (established from the territory that was the smallest in area of the two neighbouring groups). We investigated the degree of territory overlap at 35 territory boundaries, consisting of 19 between unrelated groups and 16 between related groups. The percentage of overlap between neighbouring groups was analysed using a series of generalised linear mixed models (GLMMs), using Akaike's information criterion adjusted for small sample sizes [AICc; 28] to identify the best models (see analysis section for more details). We included relatedness (related or unrelated), the difference between the number of adult birds (>365 days since hatching) in neighbouring groups (model term: 'group size difference'; largest group size-smallest group size), and the total amount of rainfall across a season as explanatory terms in the candidate models (in ml; 'rainfall'). To control for variation in the number of adult birds within a group over the year (as immigration, dispersal, death and young group members reaching adulthood can affect the group size over time), group size was calculated as a mean for the year (from the number of adult individuals that were present within a group for each day of the breeding year). We included breeding year into the model as a random term.

9.3.4 Characteristics of territorial defence

Pied babbler inter-group interactions (IGIs) tend to be highly ritualised, predominantly consisting of vocal displays [22]. Displaying groups occupy positions in opposing trees on the territory boundary, taking it in turns to chorus ('choruses' classified as calling bouts involving more than one group member, lasting for more than one second, and with breaks of more than one second between choruses) [22]. However, occasionally IGIs escalate to active chases and physical fights.

9.3.4.1 The frequency of territorial encounters

For all IGIs observed during the period Sept 2007–April 2012, we recorded the location, group size (number of adult birds from each group), and outcome (retreat, advance, fight, ritualised display). We examined the frequency of IGIs at 37 territorial boundaries, of which 17 were boundaries between related groups. Variables influencing the frequency of IGIs were tested using a series of GLMMs, using AICc to identify the best models. Potential explanatory variables included: group size difference; percentage of overlap between territories; and total rainfall over the previous fourteen days. Rainfall in the Kalahari induces insect emergence [29], and therefore provides a proxy for food abundance for the pied babbler [examples of other studies that have included prior rainfall include: 30, 31]. We included the breeding year and the territory boundary in all models as random terms.

9.3.4.2 Duration of encounters

During the period January 2011–April 2012, we recorded the duration of all IGIs observed, recording: (i) the time in seconds from the start of the first chorus produced by the initiating group until half the adults in that group had resumed foraging; and (ii) the number of choruses produced by the initiating group during the encounter. We recorded the duration of 127 IGIs, of which 79 were between unrelated groups. The duration of IGIs and the number of choruses involved were tested using a series of GLMMs with model averaging. Models contained the following explanatory terms: relatedness, group size difference, and the total amount of rainfall (mm) in the fortnight prior to the interaction. The percentage of overlap between neighbouring territories was also included as an

explanatory term. Breeding year and the identity of the initiating group were included in all models as random terms.

9.3.4.3 The frequency of fighting behaviour

To determine the factors that lead to aggression during inter-group interactions, we investigated the proportion of all IGIs that occurred between September 2007 and April 2012 that escalated into physical fights. 498 IGIs were recorded between groups where the relatedness was well understood, of which 199 occurred between unrelated groups, and 299 between related groups. The proportion of interactions escalating to physical fighting was tested using a series of generalized estimating equations [GEE; 32]. Best GEE models were chosen using corrected quasi-likelihood under independence model criterion (QICc). As well as relatedness, we included the difference between the number of adult birds in an interaction, the percentage of overlap between the two territories, and the amount of rainfall in the previous fourteen days as explanatory terms in the models. We included the breeding year and the identity of the initiating group as random terms in all models.

9.3.5 The cost of territorial defence

In order to assess whether investment into territorial defence is costly, we investigated the daily weight gain of 30 individuals across two paired sessions. Daily weight gain was established as the number of grams gained per hour between the first recorded weight, collected as the birds came off roost, and a second weight collected at the end of the session (at least 1.5 hours after the first weight was collected). We compared daily weight gain between days where an IGI had occurred (the minimum duration for an IGI was set at five

minutes; mean \pm SD 13m16s \pm 5m02s), against a second session where no border interaction had taken place. Paired sessions (IGI and non-IGI) occurred within one week of each other, and all paired sessions occurred before breeding began and prior to the arrival of the first rains of the wet season (so that environmental conditions were unlikely to affect the weight differences measured between the two sessions). Data were collected from individuals from 11 different social groups. The difference in weight gain across paired sessions was analysed using a paired t-test.

9.3.6 Analysis and model selection

GLMMs were carried out in R 2.15.2 (R core development team 2012) using the 'lme4' [33], 'MuMiN' [34], and 'AICcmodavg' packages [35]. Explanatory terms were centralised by subtraction from the mean. Additionally, continuous explanatory variables were divided by the standard deviation to further standardise these terms [36]. This allows direct comparisons of model estimates and effect sizes to be drawn within and between models.

Global models, containing all possible combinations of explanatory terms were created using the 'dredge' function in the 'MuMiN' package. The global model set was reduced to a top model set by eliminating all models with $\Delta\text{AICc} > 5$ from the best model (the model with the lowest AICc value). Models eliminated at this stage were considered poor predictors of the response term [37]. Model averaging was performed on the top set of models [because AICc weights for best models < 0.90 ; 38], using the 'AICcmodavg' package. The importance of each explanatory term in the top model set was judged upon model averaged estimates and confidence intervals (those that did not encompass zero).

GEEs were carried out in SPSS 19 (SPSS, IBM, USA). We created a set of models based upon a *priori* hypotheses. This was reduced to a top model set by removing all models with $\Delta QICc > 5$ from the best model. The best model was deemed important when $\Delta QICc > 2$ from the second best model [37]. All graphics were drawn in R 2.15.2.

9.4 Results

9.4.1 Territorial overlap

Neighbouring groups shared proportionally more of their territory when they were closely related (Table 1, Figure 1).

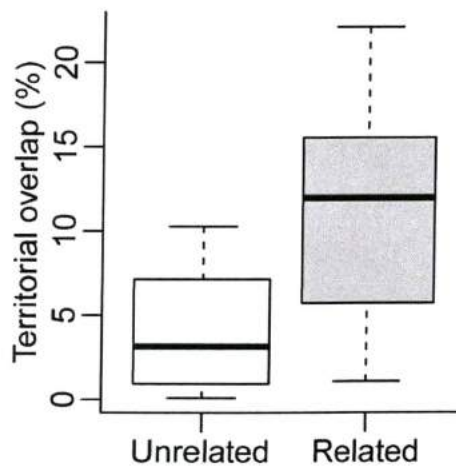


Figure 1 - The effect of relatedness on territorial overlap between neighbouring groups (calculated as a percentage from the smallest territory).

Table 1 – Top model set from a series of GLMMs investigating the terms that predict the percentage of territorial overlap between neighbouring groups.

Rank	Model	<i>K</i>	AICc	Δi	<i>w_i</i>	Cum <i>w_i</i>	dev
1	Relatedness	5	113.77	0.00	0.64	0.64	-50.85
2	Relatedness + Group size difference	6	116.07	2.30	0.20	0.85	-50.54
3	Relatedness + Rainfall	6	116.62	2.85	0.15	1.00	-50.81
Model averaging results							
Predictor		Estimate	Unconditional SE	CI	Relative Importance		
Intercept		1.77	0.24	(1.30, 2.33)	1.00		
Related*		1.39	0.35	(0.70, 2.08)			
Group size difference		-0.28	0.35	(-0.97, 0.41)	0.20		
Rainfall		-0.10	0.36	(-0.80, 0.60)	0.15		

Breeding year (defined as 1st September-31st August) was included in all models as a random term. *K* = parameters; AICc is Akaike’s information criteria corrected for small sizes; Δi = change in AICc from the model considered most parsimonious; *w_i*= Akaike weights; Cum *w_i*= cumulative Akaike weights; Dev (deviance) is the -2 log likelihood of the model; CI = confidence interval. *Unrelated groups were set as the reference category.

9.4.2 The frequency of territorial defence

The overlap between neighbouring territories was the strongest predictor of the frequency of IGIs between neighbouring groups, with IGIs occurring most frequently when overlap was high (Table 2, Figure 2a).

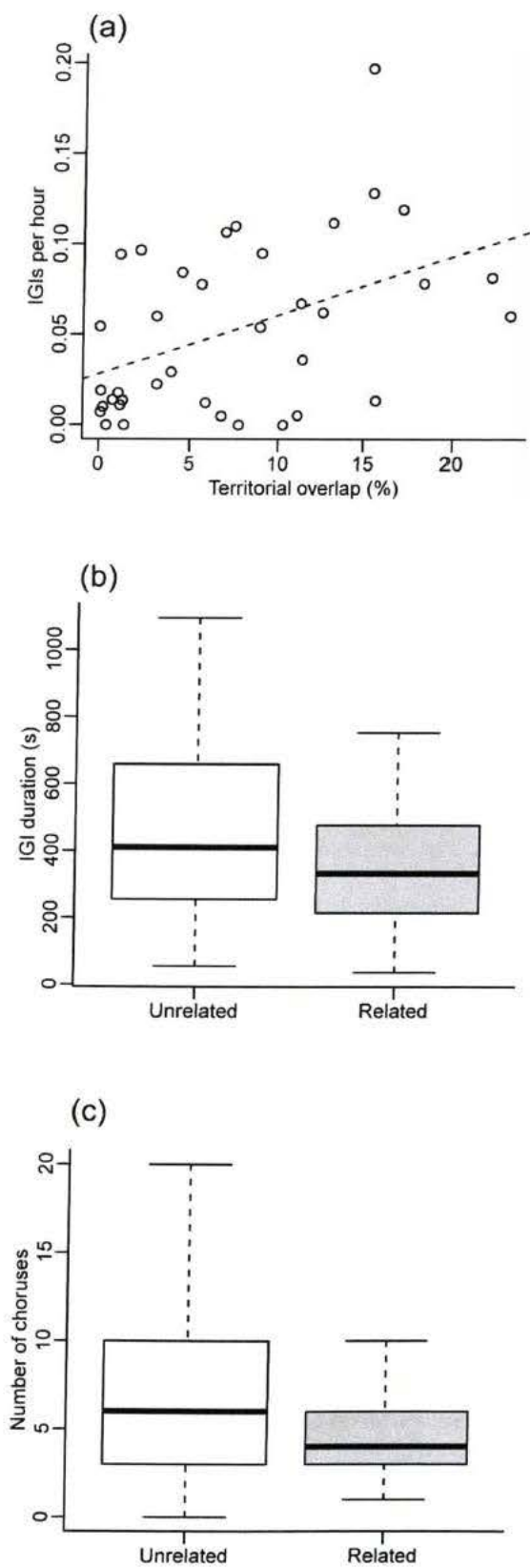


Figure 2 - Graphical representation for: (a) the effect of territorial overlap on the frequency of interactions; (b) the effect of relatedness on the duration of inter-group interactions; (c) the effect of relatedness on the number of choruses in an interaction. Graphs drawn from the raw data.

9.4.3 Relatedness and territorial defence

When opposing groups contained close relatives, IGIs were on average shorter, both in terms of the duration (Table 3, Figure 2b), and the number of choruses that were involved (Table 4, Figure 2c). Fighting behaviour was rare, occurring in just 9.50% of observed IGIs. None of the explanatory terms in the models, including relatedness, affected fighting behaviour (Table 5), with the best model occurring within two Δ QICc of the basic model (random terms only; Table 5).

Table 2 – Top model set from a series of GLMMs investigating the terms that predict the frequency of encounters between neighbouring groups. Analysis was conducted on encounter frequency at 37 different boundaries between 16 groups.

Rank	Model	K	AICc	Δi	w_i	Cum w_i	dev
1	Overlap	5	-55.04	0.00	0.39	0.39	33.49
2	Relatedness + Overlap	6	-53.56	1.48	0.19	0.58	34.18
3	Overlap + Rainfall	6	-52.77	2.27	0.13	0.71	33.78
4	Overlap + Group size difference	6	-52.49	2.56	0.11	0.82	33.64
5	Relatedness + Overlap + Rainfall	7	-51.09	3.95	0.05	0.87	34.48
6	Relatedness + Overlap + Relatedness*Overlap	7	-50.75	4.29	0.05	0.92	34.30
7	Relatedness + Overlap + Group size difference	7	-50.71	4.33	0.05	0.97	34.29
8	Relatedness	5	-50.16	4.88	0.03	1.00	31.05
Model averaging results							
Predictor		Estimate	Unconditional SE	CI	Relative Importance		
Intercept		0.19	0.03	(0.14, 0.25)			
Related		0.04	0.03	(-0.02, 0.11)	0.37		
Group size difference		0.02	0.03	(-0.04, 0.07)	0.16		
Rainfall		0.04	0.05	(-0.06, 0.15)	0.18		
Overlap		0.10	0.03	(0.03, 0.16)	0.97		

Breeding year (defined as 1st September-31st August) was included in all models as a random term. K = parameters; AICc is Akaike's information criteria corrected for small sizes; Δi = AICc; w_i = Akaike weights; Cum w_i = cumulative Akaike weights; Dev (deviance) is the -2 log likelihood of the model; CI = confidence interval. *Unrelated groups were set as the reference category.

Table 3 – Top model set from a series of GLMMs investigating the terms that predict the duration of an encounter between neighbouring groups. Analysis was conducted on the duration of 127 encounters collected between Jan 2011–April 2012.

Rank	Model	K	AICc	Δi	w_i	Cum w_i	Dev
1	Relatedness	5	821.34	0.00	0.44	0.44	-405.42
2	Relatedness + Group size difference	6	822.92	1.58	0.20	0.65	-405.10
3	Relatedness + Rainfall	6	823.52	2.18	0.15	0.79	-405.41
4	Relatedness + Group size difference + Relatedness*Group size difference	7	824.83	3.49	0.08	0.87	-404.94
5	Relatedness + Rainfall + Group size difference	7	825.14	3.80	0.07	0.94	-405.09
6	Relatedness + Rainfall + Relatedness*Rainfall	7	825.29	3.95	0.06	1.00	-405.17
Model averaging results							
Predictor	Estimate	Unconditional SE	CI	Relative Importance			
Intercept	21.26	0.72	(19.85, 22.67)				
Related	-3.41	1.16	(-5.68, -1.14)	1.00			
Group size difference	1.05	1.08	(-1.07, 3.17)	0.35			
Rainfall	-0.16	1.09	(-2.30, 1.97)	0.28			

Breeding year (defined as 1st September–31st August) and the identity of the initiating group were included in to all models as random terms. K = parameters; AICc is Akaike's information criteria corrected for small sizes; Δi = AICc; w_i = Akaike weights; Cum w_i = cumulative Akaike weights; Dev (deviance) is the -2 log likelihood of the model; CI = confidence interval.* Unrelated groups were set as the reference category.

Table 4 – Top model set from a series of LMM's investigating the terms that predict the number of choruses produced by the initiating group in an encounter.

Rank	Model	K	AICc	Δi	w_i	Cum w_i	Dev
1	Relatedness	5	263.45	0.00	0.38	0.38	-126.39
2	Relatedness + Rainfall	6	265.58	2.13	0.13	0.52	-126.32
3	Basic	4	265.60	2.16	0.13	0.65	-128.58
4	Relatedness + Group size difference	6	265.71	2.26	0.12	0.77	-126.39
5	Relatedness + Rainfall + Relatedness*Rainfall	7	267.29	3.85	0.06	0.83	-126.02
6	Rainfall	5	267.57	4.13	0.05	0.87	-128.46
7	Group size difference	5	267.70	4.25	0.05	0.92	-128.52
8	Relatedness + Rainfall + Group size difference	7	267.89	4.44	0.04	0.96	-126.32
9	Relatedness + Group size difference + Relatedness*Group size difference	7	268.03	4.59	0.04	1.00	-126.39
Model averaging results							
Predictor	Estimate	Unconditional SE	CI	Relative Importance			
Intercept	2.45	0.15	(2.16, 2.73)				
Related*	-0.44	0.21	(-0.86, -0.03)	0.77			
Group size difference	0.00	0.20	(-0.38, 0.39)	0.25			
Rainfall	-0.70	0.18	(-0.43, 0.28)	0.28			

Breeding year (defined as 1st September–31st August) and the identity of the initiating group were included in to all models as random terms. K = parameters; AICc is Akaike's information criteria corrected for small sizes; Δi = AICc; w_i = Akaike weights; Cum w_i = cumulative Akaike weights; Dev (deviance) is the -2 log likelihood of the model; CI = confidence interval.* Unrelated groups were set as the reference category.

Table 5 – Model selection output (QICc) from a series of GEEs investigating the terms that affect the proportion of interactions that escalate into physical fighting. Analysis was conducted on information obtained from 498 encounters between 13 different groups.

Model	QICc	Δi	w_i	Parameters	Wald chi-square	df	P
Rain	308.39	0.00	0.14		2.40	1	0.12
Basic	308.75	0.36	0.12				
Overlap	309.51	1.12	0.08		0.81	1	0.37
Rainfall +							
Relatedness*Rainfall	309.58	1.19	0.08	Rainfall	1.96	1	0.16
				Relatedness*Rainfall	0.89	1	0.35
Relatedness*Rainfall	309.58	1.19	0.08		3.09	2	0.21
			0.07				
Group size difference	309.90	1.51			1.18	1	0.28
Relatedness + Rainfall	310.28	1.89	0.06	Relatedness	0.08	1	0.78
				Rainfall	2.42	1	0.12
Relatedness	310.46	2.07	0.05		0.17	1	0.68
Relatedness*Overlap	311.33	2.94	0.03		0.79	2	0.67
Relatedness + Rainfall							
+ Overlap	311.45	3.06	0.03	Relatedness	0.01	1	0.91
				Rainfall	2.14	1	0.14
				Overlap	0.66	1	0.42
Relatedness + Overlap	311.45	3.06	0.03	Relatedness	0.04	1	0.84
				Overlap	0.79	1	0.37
Relatedness + Rainfall							
+ Relatedness*Rainfall	311.50	3.11	0.03	Relatedness	0.06	1	0.81
				Rainfall	1.95	1	0.16
				Relatedness*Rainfall	0.95	1	0.33
Relatedness +							
Relatedness*Rainfall	311.50	3.11	0.03	Relatedness	0.06	1	0.81
				Relatedness*Rainfall	3.14	2	0.21
Relatedness + Group							
size difference	311.63	3.24	0.03	Relatedness	0.17	1	0.68
				Group size difference	1.17	1	0.28
Relatedness + Group							
size difference + Rainfall	311.79	3.40	0.03	Relatedness	0.09	1	0.76
				Group size difference	0.68	1	0.41
				Rainfall	2.08	1	0.15
Relatedness + Group							
size difference +							
Overlap	311.79	3.40	0.03	Relatedness	0.07	1	0.80
				Group size difference	0.70	1	0.40
				Overlap	0.56	1	0.46
Relatedness + Overlap							
+ Relatedness*Rainfall	312.47	4.08	0.02	Relatedness	0.24	1	0.62
				Overlap	0.80	1	0.37

Relatedness + Group size difference + Relatedness*Rainfall	312.97	4.57	0.01	Relatedness*Rainfall	3.29	2	0.19
				Relatedness	0.54	1	0.82
				Group size difference	0.74	1	0.39
Relatedness + Group size difference + Rainfall + Relatedness*Rainfall	312.97	4.57	0.01	Relatedness*Rainfall	2.80	2	0.25
				Relatedness	0.05	1	0.82
				Group size difference	0.74	1	0.39
				Rainfall	1.67	1	0.20
Relatedness + Overlap + Relatedness*Overlap	313.30	4.91	0.01	Relatedness*Rainfall	1.00	1	0.32
				Relatedness	0.03	1	0.87
				Overlap	0.75	1	0.39
				Relatedness*Overlap	0.12	1	0.73
Relatedness + Relatedness*Overlap	313.30	4.91	0.01	Relatedness	0.03	1	0.87
				Relatedness*Overlap	0.79	2	0.68
Relatedness+ Rainfall + Relatedness*Overlap	313.38	4.99	0.01	Relatedness	0.02	1	0.89
				Rainfall	1.96	1	0.16
				Relatedness*Overlap	0.66	2	0.72

GEE's with a binomial error distribution and a logit link function investigating the factors that predict when encounters will escalate into physical fighting. Random terms included the breeding year (defined as 1st September-31st August) and the identity of the initiating group. QICc = corrected quasi likelihood under independence model criterion; Δi = QICc; w_i = QICc weights; df = degrees of freedom; P = significance.

9.4.4 The energetic costs of inter-group interactions

Territorial defence was costly: individuals gained significantly less weight per hour across mornings when there was an inter-group interaction compared to mornings when they did not invest in territorial defence behaviour (Figure 3, paired t-test, $t=4.700$, $df = 29$, $P<0.001$).

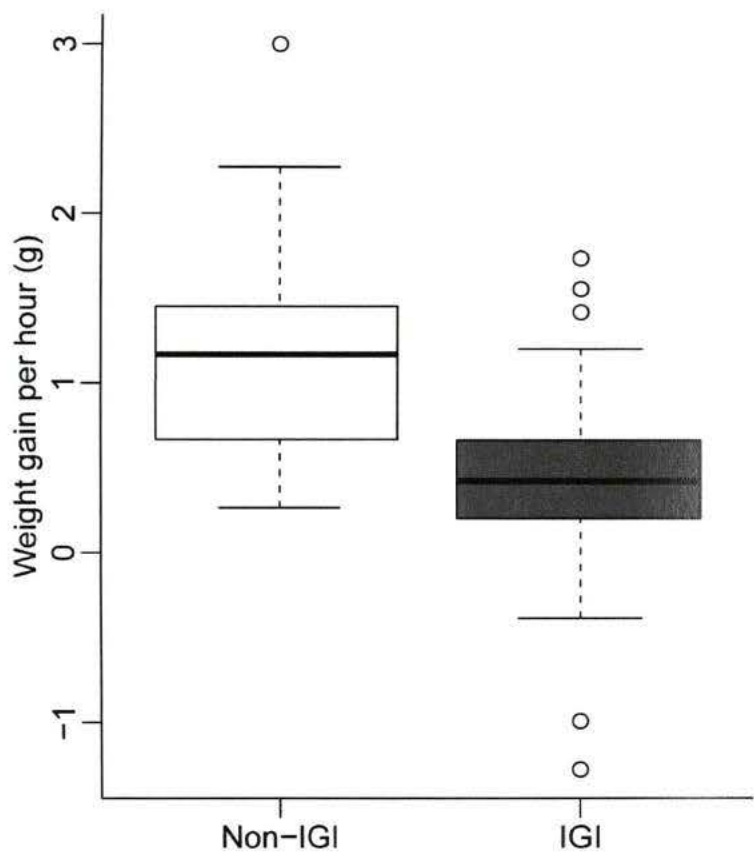


Figure 3 - The average hourly weight gain across a morning. 'Non IGI' represents mornings where no interaction occurred and 'IGI' represents mornings when an inter-group interaction was recorded.

9.5 Discussion

Our observation that kinship between neighbouring groups positively affects territorial overlap in the pied babbler matches the patterns of territory overlap between related neighbours seen in a number of other species [13-18]. When we investigated the effects of relatedness on territorial defence, relatedness *did* influence territorial encounters, but not as we would have expected. We found no evidence that kin-biased territory overlap is driven by a reduction in the

frequency of territorial encounters, nor in the rates of physical fighting. In fact, greater overlap between the territories of neighbouring groups resulted in more frequent interactions. As we have shown IGIs to be costly, related neighbours may therefore face additional costs by interacting more frequently with one another as a consequence of kin-biased overlap. Coupled with the cost of more frequent interactions is the assumed costs of resource exploitation [5, 6] and the limited dispersal and reproductive opportunities for subordinates when neighbouring groups are closely related [as inbreeding is avoided; 23, 24]. However, these costs may potentially be off-set by investing less in territorial encounters when they do occur. We observed that encounters between related groups tended to be shorter (both in terms of time and number of choruses) than IGIs between unrelated groups. Our findings indicate that kinship is affecting interactions between neighbouring groups, but provide little support for the idea that territory overlap is being tolerated, as neither the rate of encounters, nor the rate of physical fighting was reduced between related groups.

Interactions between groups are not necessarily always about displacing neighbours. Encounters between neighbouring groups may also play a role in the transfer of information between groups [39, 40]. In the brown jay for example, individuals are thought to use interactions as a way of gathering information about potential breeding opportunities [40]. Here we observed shorter IGIs between related groups, both in terms of the time taken and the number of choruses involved. The dual function of IGIs as sources of information gathering as well as for territorial defence make it difficult to expose the causality of shorter interactions with kin. IGIs can be shorter between

related groups because of either: a) kin-biased cooperation or altruism, where investment into territorial disputes is reduced with kin, b) shorter IGIs may occur as a by-product of reduced reproductive opportunities between closely related groups. Inbreeding avoidance in the pied babbler [24] may limit investment into IGIs with related groups as they hold few opportunities to scout for potential mating partners. c) IGIs may also become shorter when the encounter rate is high. As related groups tend to interact more frequently as a consequence of kin-biased overlap, we may expect each interaction to become shorter (as information gathering may face diminishing returns with repeated encounters). As a consequence, it remains unclear whether shorter encounters alone can explain the kin-biased overlap we observed here.

Kin selection theory predicts that closely related individuals should be less aggressive towards one another in agonistic competition [41, 42], and this effect has been observed in a number of species [43-47]. However, our findings that neither the frequency of IGIs or rates of physical fighting were affected by kinship match the growing body of literature that have found no link between kinship and aggression [48-50]. Our findings that kinship was not affecting aggression (both the rate of IGIs and fighting), provide little support for greater tolerance towards kin within the territory.

The costs of territorial defence have previously been measured, both from time-energy budgets [51, 52], and by assessing the impact of territorial defence on daily weight gain [7, 8]. However, these previous studies have focused either on intrusions by prospecting individuals or the costs associated with guarding a mate within a territory. To our knowledge, ours is the first study to directly

measure the immediate energetic costs of inter-group interactions. The habituation of the pied babbler population allowed us to conclusively demonstrate the costs of inter-group interactions, with birds gaining less weight during observation sessions where an inter-group encounter had occurred.

Our observation that kin-biased overlap occurs in the face of more frequent, costly IGIs, suggest that there may be benefits to exploiting the territories of related neighbouring groups. Since related neighbouring groups hold few reproductive opportunities, we can assume that this stems from either improved foraging or breeding success. It is been observed that some species demonstrate improved breeding success when breeding positions are gained upon the natal territory [53-55]. One possible explanation for this is that foraging and breeding success can be positively influenced through knowledge of the best sites [55-57]. It is possible that kin who disperse into neighbouring territories seek to remain on the familiar ground of their natal territory, thereby exerting greater intruder pressure on related boundaries.

We found that pied babblers display kin-biased territory overlap, and that kinship alters the nature of interactions between neighbouring groups, which tend to be shorter in duration than interactions between unrelated groups. Yet, whether shorter interactions alone can explain kin-biased overlap is still unclear, owing to the multi-functionality of encounters between groups (used for both territory defence and information exchange). Kin-biased overlap is occurring despite neither encounter frequency, nor rates of fighting being reduced between related neighbours. This suggests that kin-biased overlap is unlikely to be because of greater tolerance of kin within the territory. Instead,

we suggest that kin-biased territory overlap is occurring because of increased intruder pressure by related neighbours.

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Chapter 10

Calling where it counts: subordinate pied babblers target the audience of their vocal advertisements



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10.1 Abstract

For territorial group-living species, opportunities to reproduce on the natal territory can be limited by a number of factors including the availability of resources within a territory, access to non-incestuous mating opportunities, and monopolies on reproduction by dominant group members. Individuals looking to reproduce are therefore faced with the options of either waiting for a breeding opportunity to arise in the natal territory, or searching for reproductive opportunities in non-natal groups. In the cooperatively breeding Southern pied babbler, *Turdoides bicolor*, most individuals who achieve reproductive success do so through taking up dominant breeding positions within non-natal groups. For subordinate pied babblers therefore, searching for breeding opportunities in non-natal groups is of primary importance as this represents the major route to reproductive success. However, prospecting (where individuals leave the group to search for reproductive opportunities within other groups) is costly and individuals rapidly lose weight when not part of a group. Here we demonstrate that subordinate pied babblers adopt an alternative strategy for mate attraction by vocal advertisement from within their natal territories. We show that subordinates focus their calling efforts on the edges of their territory, and specifically near boundaries with neighbouring groups that have potential breeding partners (unrelated individuals of the opposite sex). In contrast to prospecting, calling individuals showed no body mass loss associated with this behaviour, suggesting that calling from within the group may provide a 'cheap' advertisement strategy. Additionally, we show that subordinates use information regarding the composition of neighbouring groups to target the greatest number of potential mating partners.

10.2 Introduction

For species that live in family groups, opportunities to reproduce on the natal territory are limited by both the availability of resources within a territory, and access to unrelated individuals [1-3]. An additional limitation arises when reproduction within the group is monopolised by a single dominant pair [4-6]. For subordinates within these groups, opportunities to reproduce are restricted to either waiting for a breeding opportunity to become available within the natal territory, or searching for breeding opportunities in the surrounding area [7,8].

While subordinate group members of some cooperative species do take over breeding positions within the natal group, these occurrences are usually rare, owing to the high relatedness of group members and the limited number of opportunities available [particularly in long-lived species; 7,8-13]. It is therefore important that subordinates invest in searching for reproductive opportunities outside of the natal group where there are several pathways to reproductive success, including: (1) taking over a breeding position within a non-natal group (either by taking up vacant breeding positions or by taking breeding positions by force)[7]; (2) attempting to found a new group [7]; or (3) engaging in prospecting behaviour to achieve extra-group paternity [14,15]. In all three of these strategies subordinates must signal their intentions to individuals that live outside of the natal territory.

Signalling breeding availability and searching for breeding opportunities beyond the natal territory can be costly [16]. Prospecting, where subordinates of reproductive age leave their natal groups for short periods to search for breeding opportunities in non-natal groups, is a common strategy among

group-living animals [17-21]. However, prospectors are constrained by the energetic costs of being away from the group [22,23]. These costs are thought to result from the increase in movement and vigilance behaviours, decline in foraging activity, risk of attack from territory holders, and the associated stress among individuals that are outside of a group [21-24]. An alternative strategy to prospecting is to advertise breeding availability from within the natal territory. This is a little explored alternative, yet carries the potential for individuals to remain within their social group and continue to receive the benefits of group living [such as a reduced investment in personal vigilance and reduced predation risk; 22,25,26].

For subordinates wishing to advertise their breeding availability, some neighbouring groups may hold greater reproductive opportunities than others, with variation in both the number and quality of potential partners [27]. In cooperatively breeding birds, the combination of delayed dispersal and short dispersal distances can often lead to a high probability of encountering close kin in the local neighbourhood [28]. Inbreeding can be detrimental to reproductive success [29,30], consequently, we might expect individuals advertising for mates to concentrate their efforts on unrelated neighbouring groups. An additional factor for group-living animals is that neighbouring groups may vary in the number of opposite sex individuals they contain. This can result in variation in the number of individuals that can be reached from an advertisement, depending on the location where it is produced. We may therefore expect advertisement calls to occur at locations that reach the greatest number of potential mating partners. Whether subordinates are strategic in their search for breeding opportunities and adopt strategies to

maximise their exposure to potential breeding partners is currently poorly understood in cooperatively breeding species.

We investigated the advertising strategies used by subordinate Southern pied babblers, *Turdoides bicolor*, who begin to produce loud calls from within their natal territories when they reach reproductive age (> 1 year old). Pied babblers are a medium sized (75-95g) passerine endemic to the Kalahari, living in social groups of 2-15 individuals [31]. Breeding within the social group is monopolised by a dominant pair [6], and subordinate individuals will only achieve dominance within their natal territory if they can inherit vacant breeding positions without incest [7,13]. Prospecting in pied babblers is costly [22], and long-term floating is rarely observed (80.0% of prospectors return to their natal group within 30 days; A. Ridley, unpublished data). In some species, prospecting can achieve immediate reproductive success [14,32], however in the pied babbler, the dominant breeding pair are primarily monogamous, and subordinate group members do not breed [6]. Consequently, prospecting is unlikely to represent a significant route to short-term reproductive success [6]. Here we set out to determine whether subordinate individuals adopt strategies to target the audience of their vocal advertisements by a) calling on the edges of their territory, b) focusing their calling efforts near to unrelated groups, and c) focusing their calling efforts near to groups with the greatest number of unrelated, opposite sex, adult individuals. We also assess whether calling behaviour carries observable costs and therefore whether calling from within the natal group provides an energetically costly route to advertising breeding availability.

10.3 Methods

Subordinate advertisements were recorded from a habituated population of pied babblers located at the Kuruman River Reserve, in the Southern Kalahari, South Africa (26°57'S 21°49'E) [see 33 for more details about the study site]. The population is colour-ringed for identification and has been under observation since 2003. We have detailed life history information for each of the groups within the population regarding both the dominance hierarchies and the movement of individuals between groups. We recorded the loud calling behaviour of subordinate individuals between September 2010 and April 2012. Throughout this period, data were recorded twice a day, with a morning session from dawn (mean observation time \pm SD 140.60 \pm 53.72 minutes per group visited), and an afternoon session till dusk (mean observation time \pm SD 82.64 \pm 50.46 minutes per group visited). Pied babblers produce eight acoustically distinct types of loud call, and all are predominantly given by the dominant members of the group [34]. We observed loud calls being given by subordinate members in just 23.85% of cases (249 of 1044 recorded loud calls). This bias towards loud calling from dominant individuals occurs despite the number of subordinates outweighing the number of dominants 2.3 to 1 in the population. Loud calling behaviour can occur in a wide variety of social contexts [34], for instance, during group chorusing, whilst moving fledglings, and to relocate the group should an individual become separated. When loud calling occurs in these contexts, the associated behaviour of the individual makes the causality of calling clear to a trained observer. However, here we focused on the rare instances when subordinates gave solo loud calls when no behavioural context could be observed instigating the calling behaviour. We

deemed these solo loud calls given without perceivable initiating circumstances to be self-advertisements. Pied babblers can breed after their first year [7], and unsolicited loud calling behaviour is observed almost exclusively by subordinate individuals of reproductive age (mean \pm SD 1027 \pm 321 days since hatching; range 327-1536), with only 1.9% of cases observed by individuals under one year of age (since hatching). Prospecting individuals use repeated loud calling during prospecting events, supporting the notion that loud calls can function for self-advertisement (D. Humphries, personal observation). Dispersal in the pied babbler does not appear to be sex-biased [35], and subordinate loud-calling behaviour occurs in both sexes. Of the cases where subordinates were observed calling, only 26.10% had no clear social context, and could be defined as self-advertisement (65 out of 249 recorded vocalisations from 33 individuals belonging to 13 social groups). Each time a subordinate was observed giving loud call advertisements, the location of the calling behaviour was recorded to a handheld GPS (accuracy <10m).

10.3.1 Where do subordinates call within a territory?

We limited our analysis to individuals where we had records of calling from at least six different locations (mean \pm SD 13.00 \pm 4.55; range 6-20) across a breeding year (September through to August). This was to try to capture a distribution of calling events from each individual, resulting in recorded calling locations from seven individuals from five different social groups (n = 91 calls in total). In addition to recording the location of loud calling behaviour, the movements of the whole social group were recorded by all observers every 15 minutes to a handheld GPS during every observation session. Territory sizes were established from 300 GPS points collected across a breeding year. 300

points represents a minimum of 60 hours of observation for each group. Territory sizes were calculated using the 'adaptive sphere-of-influence local convex hull' [a-LoCoH; 36]. A-LoCoH was performed in R 2.15.1 (R Development Core Team 2008) using the 'adehabitat' package [37]. We exported territories at three different densities (50%, 75%, and 95%) from R in to ArcGIS 9.3.1 (ESRI, 2009) for analysis. We investigated whether subordinate calling behaviour followed patterns of group movement. For example, whether half of all loud calling behaviour was observed within the 50% density isopleths, 25% between the 50 and 75% density isopleths, and 20% within the area between the 75 and 95% isopleths. If subordinate calling behaviour did not follow patterns of group movement, it would suggest that subordinates are favouring particular locations (e.g. the border vs the centre of the territory) for calling. We compared observed versus expected calling patterns using a Chi squared test carried out in R.

10.3.2 Does relatedness to neighbouring groups affect where subordinates advertise?

We assessed whether calling behaviour was focused towards borders with neighbouring groups, and in particular whether calling was focused towards groups containing unrelated, potential breeding partners. Using the 'buffer zone' tool in the 'Hawths tools' extension (Beyer, H. L., 2004. Hawth's Analysis Tools for ArcGIS. Available at <http://www.spatialecology.com/htools>) we created a 100m zone around the 95% density isopleths of neighbouring territories. We then established the number of calls occurring within 100m of a neighbouring territory (see Figure 1 for a schematic). The number of calls observed was assessed relative to what would be expected if calling behaviour

was evenly distributed within a territory. The expected number of calls was calculated as the total number of calls given by an individual, divided by the area of the 95% density isopleth (in hectares), multiplied by the area that was in a 100m proximity to a neighbouring group. We classified groups as related when at least one dominant of the neighbouring group was a close relative ($r=0.25$ or closer) to the calling individual. In pied babblers, parentage can be reliably assigned from behavioural observations of breeding behaviour and activity at the nest [6]. To establish relatedness between individuals, pedigrees were developed from behavioural observations of parentage. We compared observed versus expected calling patterns using a Chi squared test carried out in R.

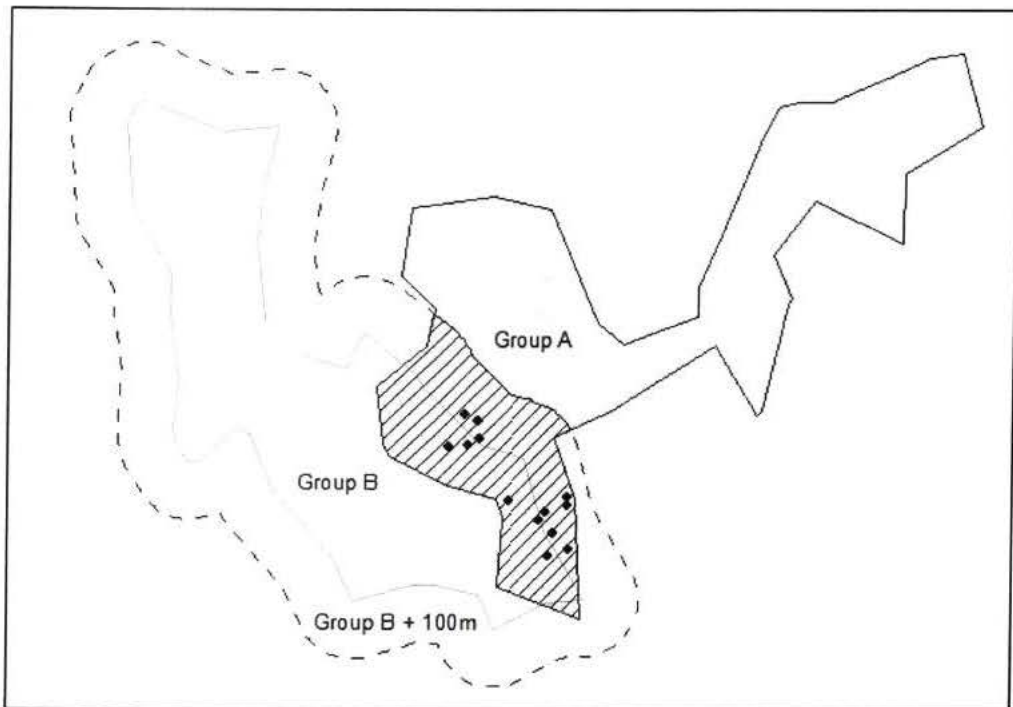


Figure 1 – A schematic demonstrating how we established proximity to neighbouring territories. A 100m buffer zone was created around the 95% density isopleths of neighbouring groups. We then established the number of loud calls (shown here as points) that fell within 100m of the neighbouring territories (inclusion area shown here as a hashed area). Image created in ArcGIS 9.3.1.

We investigated whether the number of calls given by an individual in proximity to a neighbouring group was affected by the number of adult individuals within the neighbouring group. This was to investigate whether loud calling effort was influenced by size of the potential audience. We classified adults as individuals that were at least one year in age (365 days post-hatching). For each of the seven individuals in this study, we explored the number of calls (per hectare) given in proximity to their largest neighbouring group and their smallest neighbouring group. We then compared whether the number of calls per hectare for each focal individual was significantly higher in proximity to the largest vs smallest neighbouring group using a paired t-test carried out in R. The mean difference between the largest and smallest neighbouring group sizes for focal individuals in this study was 1.73 ± 1.39 individuals (mean \pm SD). In addition, we also ran a separate analysis looking at whether the number of unrelated, opposite sex adult individuals within neighbouring groups affected calling behaviour. This was to explore whether a specific audience (potential breeding partners) was being targeted through vocal advertising. Pied babblers are sexually monomorphic and require genetic sexing from blood samples collected during ringing [following the method described in 38]. We tested whether the number of calls per hectare was higher in proximity to the neighbouring group that contained the most unrelated, opposite sex adult individuals, relative to the neighbouring group that contained the least. The mean difference between the maximum and minimum number of unrelated, opposite sex adult individuals within neighbouring groups was 2.32 ± 1.57 individuals (mean \pm SD). We tested our observed calling behaviour using a paired t-test carried out in R.

10.3.3 Costs of calling

The population is habituated to the use of weighing scales and will stand on a top-pan scale (Ohaus CS200; accuracy $\pm 0.1\text{g}$) in exchange for a small reward (small amounts of egg and mealworm). Assessing patterns of daily weight gain provides a useful mechanism for calculating whether activities carry substantial costs, and have previously been used to assess the costs of floating [22], inter-group interactions (Humphries et al., Chapter 9), and extreme heat [39]. To investigate whether advertising from within the social group is a costly mechanism of advertising for mates, we compared daily weight change on days when we observed at least six advertisement calling bouts from an individual (mean number of advertisement calls 11.5, range 6-30) and again on days where no advertisement calling bouts were observed. We recorded the weight change from eight individuals belonging to seven different groups. Comparable weight sessions occurred within two weeks of each other to minimise seasonal effects on weight gain (mean $\pm\text{SD}$ 4.88 ± 3.52 days apart; range 2-11 days). We compared daily weight gain using a paired t-test. All graphs presented were produced in R.

10.4 Results

10.4.1 Does calling behaviour follow group movement?

Calling behaviour differed significantly from patterns of group movement ($\chi^2=6.027$, $\text{df}=1$, $P=0.014$), occurring more frequently on the outer reaches of a territory (between the 75 and 95% density isopleths) (Figure 2) than we would have expected if calling behaviour followed group movement patterns (Figure 2). This effect was not observed in the inner two zones (50% and 75% zones).

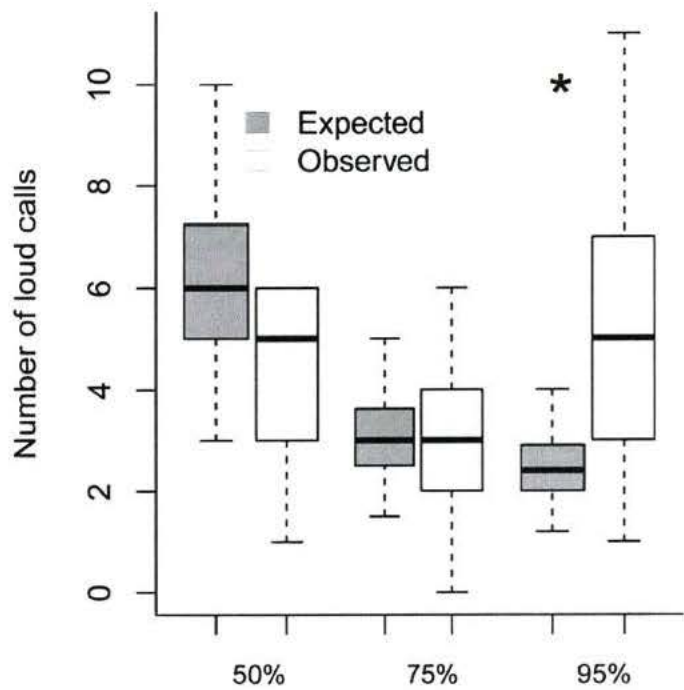


Figure 2 – The observed and expected number of loud calls given by each individual within the following areas (a) within 50% density isopleths, (b) between the 50 and 75% isopleths, and (c) between the 75-95%.

10.4.2 Is calling behaviour affected by the relatedness of neighbouring groups?

The relatedness of neighbouring groups influenced the calling behaviour of subordinates ($X^2=6.027$, $df = 1$, $P=0.014$), occurring more frequently in proximity to unrelated groups than would be expected if calling behaviour occurred evenly throughout the territory (Figure 3a). However, this effect was not observed for related groups (Figure 3a), suggesting that callers are targeting unrelated groups.

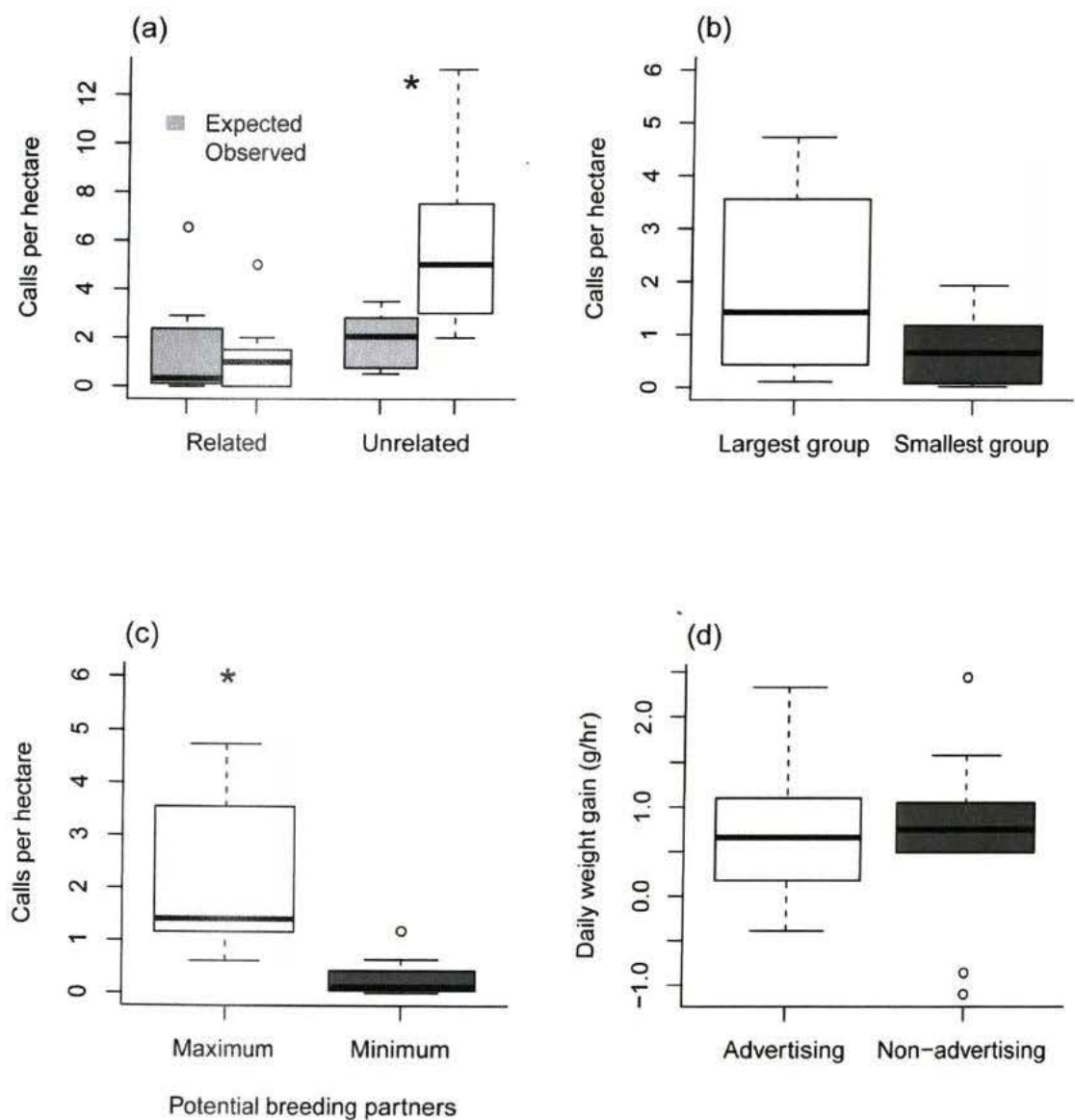


Figure 3 – (a) The expected and observed number of loud calls per hectare occurring within 100m of the territories of both related and unrelated neighbouring groups. (b) The number of calls per hectare occurring in proximity to an individual's largest and smallest neighbouring group, in terms of the number of adult individuals they contain. (c) The number of calls per hectare occurring in proximity to an individual's largest and smallest neighbouring group, in terms of the number of unrelated, opposite sex adult individuals they contain. (d) The daily weight gain of individuals both when we observed at least six loud calling bouts, and when no loud calling behaviour was witnessed.

10.4.3 Is calling focused near groups with the greatest number of unrelated, opposite sex individuals?

We observed a general trend of subordinates focusing their loud calling on boundaries with larger groups, although this trend was not significant (Figure 3b; paired t-test, $t=1.782$, $df=6$, $P=0.125$). However, when we looked at whether subordinates targeted groups that had the highest number of unrelated, opposite sex, adult individuals, we found that they called more in proximity to groups that contained a higher number of potential breeding partners (Figure 3c; paired t-test, $t=3.805$, $df=6$, $P=0.009$). This suggests that they are maximising their exposure to a specific target audience with their advertisements

10.4.4 Cost of calling

We observed no difference in daily weight gain on days when advertisements were observed compared to non-advertising days (Figure 3c; t-test, $t=0.645$, $df=7$, $P=0.540$). This suggests that the cost of advertising from within the natal territory is minimal.

10.5 Discussion

Our findings that subordinate loud calling behaviour is concentrated on the edges of territories, and specifically near to groups containing a number of unrelated, opposite sex individuals suggests that unsolicited loud calling by subordinates functions for mate advertisement. Importantly, it also suggests that pied babblers are capable of discriminating kinship and the *number* of

potential mates within neighbouring groups, and can utilise this information to maximise the audience of their calling efforts.

The ability to discriminate kinship has previously been demonstrated in avian species, which utilise vocal [40-43], visual [44-46], and olfactory signals [47,48] to recognise kin. By avoiding kin as mating partners, an individual can limit the potentially damaging effects of inbreeding depression among resultant offspring, and therefore improve reproductive success [30,49]. Pied babblers are capable of vocal kin recognition by prior association (Humphries et al., Chapter 6), and genetic work has previously suggested that pied babblers utilise kin recognition to avoid inbreeding [13]. Our observations further support the idea that pied babblers both recognise kin and are avoiding close relatives as mating partners.

Our findings indicate that subordinates are maximising their signalling effort in proximity to groups that contain the greatest number of potential mates, and are therefore likely to have a mechanism for determining the composition of neighbouring groups. This information is likely to be obtained through several mechanisms. Firstly, information may be exchanged during inter-group interactions. Baboons, *Papio cynocephalus*, use inter-group encounters to assess the number of opposite sex individuals within neighbouring groups [50]. Pied babblers frequently engage in ritualised inter-group interactions and have many opportunities for information exchange [Humphries et al, Chapter 9; 51]. During inter-group interactions, pied babblers often utilise sex-specific loud calling behaviour [34], which may provide a mechanism for assessing the number of opposite-sex individuals in neighbouring groups.

Secondly, information regarding the composition of neighbouring groups may be obtained from prospecting bouts, with information-gathering considered one of the primary functions of prospecting behaviour [52,53]. Or thirdly, information may be gained through eavesdropping on neighbours [54]. Great tits, *Parus major*, are able to assess the quality of neighbouring males by eavesdropping on their calling behaviour [55]. Eavesdropping may similarly provide a way of obtaining information about the composition of neighbouring groups in pied babblers.

Despite our observations that pied babblers are targeting a specific audience with their vocal displays, the benefits of this behaviour remain unclear. One possibility is that calling serves to initiate encounters with neighbouring groups, facilitating the exchange of information about reproductive opportunities.

Subordinate members of both meerkat, *Suricata suricatta* [56], and banded mongoose, *Mungos mungo* [57] groups are observed leading the social group into encounters with neighbouring groups (although in these species, extra-pair paternity and subordinate reproduction mean that immediate reproductive success maybe gained through encounters [56,57]). Regular information exchange between neighbouring groups may be important for dispersal success [58]. In brown Jays, *Cyanocorax morio*, for example, dispersal occurs most frequently between neighbouring groups where rates of interaction are high [58]. Loud calling behaviour may therefore serve a dual function of both advertising the callers own breeding availability, and encouraging information exchange through inter-group interactions with neighbouring groups.

When we compared daily weight gain on days where we observed subordinate loud-calling to weight gain on days when no calling behaviour was observed, we found no significant difference. This is in contrast to prospecting events, where individuals lose body condition when living outside of a social group [22]. When individuals are away from the social group, they invest more time in vigilance behaviours and experience reduced foraging success [22]. By advertising from within the social group, pied babblers can continue to experience the benefits of living within the social group (such as shared vigilance and better predator detection [22,25,26]), which may explain why we did not observe any difference in their daily weight gain. Advertising from within the natal territory is therefore an energetically cheap route to advertising breeding availability when compared to prospecting.

Here we have described how subordinate pied babblers, in addition to prospecting for breeding opportunities in the wider area [7], also adopt a strategy of vocalising to neighbouring groups from within their natal territory as a mechanism for advertising breeding availability to the local neighbourhood. This strategy is maximised by using information regarding the composition of neighbouring groups to target an audience of potential breeding partners. Importantly, subordinate loud-calling is not just given to any neighbouring group, nor focused towards the largest groups, but subordinate pied babblers are specifically targeting unrelated groups that contain a number of opposite sex individuals. Our findings provide fresh insight into how subordinates within cooperatively breeding societies, that are constrained in their opportunities to breed on the natal territory, appear to use information about the composition of

neighbouring groups to inform the location of their vocal displays to target an audience of potential breeding partners.

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Chapter 11

General Discussion



11.1 Overview

In this thesis I explored conspecific recognition in the pied babbler, investigating the mechanisms by which recognition occurs and the influence it has on social behaviour. I have approached the topic of recognition from both the perspective of the signaller and the receiver, highlighting the production of vocal identity signatures that provide the necessary pathway for recognition to occur, and exposing the ability of receivers to obtain and discriminate those identity labels (both at the level of the individual and kin). I have further explored how recognition develops in young, and the limitations of recognition between adult individuals that become geographically separated without contact. Finally, I have demonstrated the influence kin recognition has upon pied babbler social behaviour, highlighting how kinship affects territorial encounters and subordinate dispersal strategies. By highlighting the underlying mechanisms of recognition and by identifying where recognition is likely to be limited, I have developed our understanding of when and where we may expect to observe behavioural discrimination of social partners within social interactions. Consequently, the social behaviours we observe within the pied babbler can be viewed with greater clarity when considered in the context of how they perceive their social environment.

11.2 The production of vocal identity signatures

The first step in determining whether individual recognition can occur within a species is to address whether there are distinctive cues and signals that may act as a pathway to recognition. Identity signatures are not a feature that is necessarily widespread within vocal communication, and their presence can

vary depending upon the social function of the call (often being absent in alarm calls Charrier et al. 2001; Price et al. 2009; Bouchet et al. 2012; where the ability to discriminate may not be required Schibler & Manser 2007), or the ecology of the species (being selected for in species that live within large groups (Pollard & Blumstein 2011) or in crowded habitats (Mathevon et al. 2003)). I investigated whether individuality existed in pied babbler vocalisations (chapters 3 and 4), by determining whether intra-individual variation was lower than inter-individual variation (Falls 1982). I demonstrated that all three of the most common loud call types (the '*v-shaped chatter*', the '*double-note ascending chatter*', and the '*atonal chatter*') and the '*chuck*' contact call were individually distinct (chapters 3 and 4). The four calls that I investigated occur in different contexts, for instance, the chuck contact call occurs during foraging (Radford & Ridley 2008), the atonal chatter occurs primarily for intra-group communication (e.g. while moving fledglings, or to relocate the group after ending an incubation bout; Golabek & Radford 2013), while the v-shaped chatter and the double-note ascending chatter occur primarily for inter-group communication (Golabek & Radford 2013). Here I provide evidence that individuality exists within each of these calls that occur in differing social contexts, suggesting that individuality may be widespread in the pied babbler vocal repertoire.

Selection is thought to have favoured the display of identity labels whenever individuals are routinely treated favourably by receivers, or conversely, are routinely treated negatively by receivers (Johnstone 1997). This is clearly the case in the pied babbler, where social interactions within the group are highly affiliative, with group members engaging in allo-preening, cooperative predator

detection (Bell et al. 2010; Hollén et al. 2011; Ridley et al. 2013), and territory defence (Golabek et al. 2012), as well as cooperative breeding (Ridley & Heuvel 2012). Conversely, interactions with non-group members tend to be aggressive (chapter 9; Raihani et al. 2010; Golabek et al. 2012). Group-living in the pied babbler, and the subsequent frequent encounters that occur between a limited number of individuals, may therefore promote the production of distinctive vocal cues to facilitate recognition (Pollard & Blumstein 2011).

In chapter 3 I explored the stability of the identity signatures present within the atonal chatter call over time. Vocal communication is likely to be under conflicting selection pressures, where it may be beneficial to modify signals in order to reflect changes in socially important features such as age (Radford 2004; Klenova et al. 2009), physical condition (Clutton-Brock & Albon 1979), dominance rank (Kitchen et al. 2003) and an individual's social affiliations (Hile & Striedter 2000), but it may be equally important to produce reliable and stable indicators to identity in order to maintain recognition in the long-term (Klenova et al. 2012). When I explored the stability of vocal identity labels in the pied babbler (a long-lived cooperative breeder), I found that identity labels were unstable between years, suggesting that selection may act against the production of stereotyped calls in the pied babbler. The consequences of vocal instability for recognition in the pied babbler may be limited because of the amount of time group members spend in social affiliation with one another. By remaining in contact with social partners, the pied babbler may be able to modify identity templates for recognition based on each subsequent call variant. This ability to continually modify identity templates has been observed in Subantarctic fur seals (*Arctocephalus tropicalis*), where mothers are able to

identify each subsequent call type produced by developing pups (Charrier et al. 2003). Loud call instability has also been observed in the Wied's marmoset (*Callithrix kuhlii*), who have a similar social structure to the pied babbler (family units, where breeding is monopolised by a dominant female; Jorgensen & French 1998). They may therefore be exposed to similar selective pressures as the pied babbler that drive the instability of vocal properties (such as the need to acoustically signal physical and social changes) (Jorgensen & French 1998). An important area for future research in the pied babbler is to explore whether these vocal changes that I have identified are reflecting physical and social changes in the calling individual, and to identify what socially important information (such as dominance or body condition) is being signalled in pied babbler vocalisations.

Vocalisations are not just limited to signalling individual identity, and in chapter 4 I found that chuck contact calls, when controlling for individuality within these calls, could be significantly classified to the social group of the calling individual. Group labels may play a functional role within animal societies, signalling social bonds, facilitating the recognition of group members (Hile & Striedter 2000), and helping to identify and exclude non-affiliates from a territory (Knörnschild et al. 2012). Group labels can occur within vocalisations for two reasons: 1) because calls are developed by vocal learning from the calls of social partners (e.g., budgerigar (*Melopsittacus undulatus*) flocks converge on a common call structure (Hile & Striedter 2000)), or 2) because acoustic properties are under genetic control, as has been observed in the zebra finch (*Taeniopygia guttata*) (Forstmeier et al. 2009), and therefore group member vocal similarity is a by-product of the high relatedness of group

members. Pied babbler social groups are typically kin-structured (Nelson-Flower et al. 2011), and as a result, the causes of group-specific vocalisations are unclear because of the high relatedness they have to their social partners. Vocal similarity among group members may represent a form of kin label, enabling the identification of related individuals. Vocal kin labels have previously been observed within cooperative birds (Payne et al. 1988; Sharp & Hatchwell 2006; McDonald & Wright 2011), and can influence helping decisions within cooperatively breeding societies (McDonald & Wright 2011). Whether group labels reflect kinship and can function as a mechanism for identifying kin is a potential avenue for future research. By identifying that call similarity is high among group members in the pied babbler, I have identified a potential pathway through which both group member and kin recognition may occur in this species.

In summary, in chapters 3 and 4 I explored recognition from the signaller's perspective, and found that pied babblers produce vocal signatures to both individual and group identity, therefore providing evidence for the first step in the recognition process.

11.3 Testing for recognition

11.3.1 Individual recognition

In chapter 5, I explored recognition from the receiver's perspective, investigating whether the pied babbler is capable of detecting and recognising the identity labels of individual social partners. Individual recognition can be seen as the cognitive association of memories about previous interactions with identity labels (Mandler 1980; Proops et al. 2009). Demonstrating individual

recognition therefore requires an experimental design that highlights: 1) that test subjects have an integrated memory of multiple sources of information from a signaller. Experiments must therefore demonstrate that what is being recognised is more than just one feature of an individual (this criteria therefore excludes studies where individuals are trained to discriminate a single cue e.g. Bowers & Alexander 1967; Rasa 1973), and 2) that the sensory information is cognitively linked to information held in memory that is specific to the individual, based upon the memories of experiences that two individuals have had (Johnston 2008). The process of testing for individual recognition has been under rapid change over the last few years, with the introduction of cross-modal experimental approaches and violation-of-expectation experiments to identify individual recognition (Johnston & Jernigan 1994; Adachi & Fujita 2007; Adachi et al. 2007; Bates et al. 2008; Proops et al. 2009; Adachi & Hampton 2011; Kondo et al. 2012; Proops & McComb 2012). I argue that some of these experimental designs are at risk of being confounded. The use of two phenotypic cues to identity in some of these experimental designs are at risk of producing type 1 error (false-positive results) if the two cues can be associated independently of individual identity. Multi-modal signalling of a variety of traits (such as the individual's age, sex, size, social affiliations, and dominance rank), mean that two cues and signals may have multiple potential points of association that are independent of individual identity (e.g. large males may sound or smell like large males). I therefore suggest that studies of individual recognition focus on violation-of-expectation experimental designs (Bates et al. 2008; Proops et al. 2009), where associations between spatial and sensory cues are used to expose individual recognition rather than two sensory cues.

In chapter 5 I used the violation of expectation experimental approach to demonstrate that pied babblers are both able to individually recognise group members from their vocalisations, and that they are aware of which group member is involved in a particular cooperative activity (in this case, incubation). Individual recognition and the ability to monitor contributions to cooperative tasks may be critical for the evolution of animal cooperation. For instance, theories of cooperation founded on reciprocity or mutualism may only be evolutionarily stable if participants are able to recognise individual social partners and identify cheats who do not cooperate (Crowley et al. 1996; Nowak & Sigmund 1998). Recognition may also enable unproductive or subordinate individuals to be recognised and coerced into cooperative activity (Raihani et al. 2012), or enable cooperative contributions to be monitored in order to develop social prestige (Zahavi 1990). By demonstrating that the pied babbler is able to individually recognise group members, I have highlighted a potential mechanism through which cooperation may be maintained and coordinated within a cooperative society.

11.3.2 Kin recognition

In chapter 6 I demonstrated not only the ability to discriminate kin, but also determined the *mechanism* of kin recognition in operation, highlighting that kin recognition is likely to occur through prior association, and learnt during a period of associative learning. I have provided, to my knowledge, the first experimental investigation into avian vocal kin recognition that has exposed a test subject to the vocalisations of familiar and unfamiliar kin and non-kin without the use of cross-fostering. This experimental methodology, which enables the operative kin recognition mechanism to be identified from the four

main hypothesis, has been common within other taxa, but an avian example had remained elusive until now (reviewed in Nakagawa & Waas 2004).

Identifying the operative mechanism of kin recognition is important as each of the mechanisms can differ in their resolving power, with kin recognition by prior association limited to identifying familiar kin only (Waldman 1987). The observations I made therefore highlight the limitations pied babblers have in identifying kin that are unfamiliar, which in turn can inform our understanding of when and where we may expect kinship to influence pied babbler social interaction (e.g. kin-biased cooperation or inbreeding avoidance).

The ability to recognise kin is of importance for two key areas of animal behaviour, a) as a mechanism for avoiding costly inbreeding (Blouin & Blouin 1988; Pusey & Wolf 1996), and b) for recognising opportunities for gaining inclusive fitness (Hamilton 1964; Agrawal 2001; Komdeur et al. 2008).

Inbreeding avoidance may be particularly important in pied babblers because they are long-lived, and typically disperse over short distances (Nelson-Flower et al. 2012), increasing the risk of encountering close relatives when searching for mates. My findings that kin are recognised through prior association facilitate our understanding of both how inbreeding in the pied babbler is generally avoided, as well as providing a plausible explanation for why occasional instances of inbreeding between unfamiliar kin occur (Nelson-Flower et al. 2012). Thus, this study of kin recognition is providing key insights into how the pied babbler is avoiding the potential costs associated with inbreeding (Charlesworth & Willis 2009).

I have demonstrated that the pied babbler is able to discriminate levels of kinship among familiar individuals, and I propose that this is because kin

recognition is developed during a critical period of associative learning. Further work is required to explore the ontogeny of kin recognition, and to identify when young start to become sensitive to changes in group composition (which may be a distinct process from the ontogeny of group member recognition I demonstrate in chapter 7). By investigating when this critical window of associative learning is, it would then be possible to identify instances where recognition errors may occur (where half-siblings or non-kin may be treated as full siblings if encountered during this period of associative learning), which in turn would help to promote our understanding of kin-biased cooperative interactions and inbreeding avoidance behaviours in this species. There will always be examples of cooperation between non-kin within cooperative societies (Griffin & West 2002), and many of these examples may potentially be explained by recognition errors. It is therefore important that we explore when recognition is likely to fail as it may ultimately develop our understanding of the cooperative interactions we observe within animal societies.

11.4 Limits to recognition

11.4.1 The ontogeny of recognition

By identifying that kin recognition was not innate, but an acquired skill dependent upon prior association (Chapter 6), it follows that recognition must therefore be both developed in young, and maintained as adults. Young birds commonly develop direct recognition of their parents' identity labels just before fledging (Evans 1970b; Evans 1970a; Shugart 1978; Colgan 1983; Medvin & Beecher 1986), probably because when they leave the nest, spatial cues cease to be reliable cues to identity (Evans 1970b; Evans 1970a; Shugart 1978;

Colgan 1983; Medvin & Beecher 1986). However, the ontogeny of direct recognition can be affected by the social environment, and the frequency of encounters with non-caregivers (Beecher et al. 1985; Medvin & Beecher 1986). The literature on the ontogeny of direct recognition is currently dominated by investigations into colonial species, where young must learn to detect parents in dense crowds (Beer 1969; Beer 1970; Beecher et al. 1981; Medvin & Beecher 1986; Davis & McCaffrey 1989; Leonard et al. 1997; Jouventin et al. 1999; Aubin & Jouventin 2002; Mulard et al. 2010). This means they encounter strangers immediately upon fledging, selecting for rapid development of recognition. By contrast, fledglings in territorial species may not encounter strangers until long after leaving the nest, possibly allowing for slower development of recognition. However, the ontogeny of recognition within a highly territorial species had yet to be explored.

I addressed this literature bias by exploring the ontogeny of recognition within the pied babbler, a species where young fledge into large, well-defended territories and consequently encounter few non-group members until they begin to participate in territorial defence at around three months (A. R. Ridley, unpublished data). I observed that pied babbler young do not develop vocal recognition until several months post-fledging (Chapter 7). This delayed development is the slowest recorded ontogeny of vocal recognition for any bird species. The findings I present therefore support current predictions that the low frequency of extra-group encounters during development affects the ontogeny of social recognition (Beecher et al. 1985; Medvin & Beecher 1986). It is commonly quoted that the extended amount of time young in cooperative societies spend in association with one another may facilitate the development

of kin recognition (Komdeur et al. 2008; Akçay et al. 2013). What I have been able to show is the very opposite effect, where the limited association with extra-group members in highly territorial cooperative societies can ultimately slow the development of recognition.

This delayed ontogeny of direct recognition may have important consequences for pied babbler social behaviour, as they are one of the few social species where successful kidnapping has been observed (A. R. Ridley unpublished data; kidnapping has also been recorded in the white-winged chough, *Corcorax melanorhamphos* (Heinsohn 1991). These kidnapping events may only be possible because pied babbler fledglings are mobile, but unable to identify members of their social group.

11.4.2 Limits to long-term recognition

In chapter 8, I explored the limits to long-term recognition between individuals that were no longer in contact. I demonstrate that vocal recognition in the pied babbler *does* have limits, with individuals failing to discriminate previously familiar individuals after two years of separation. While recognition latency has been previously studied (Godard 1991; Insley 2000; Mateo & Johnston 2000; Kendrick et al. 2001; Mathevon et al. 2004; Mateo 2010; Murai et al. 2011; Boeckle & Bugnyar 2012), the underlying mechanisms of long-term recognition (the production of stable cues and signals to identity by the signaller, and long-term memory for identity labels by the receiver) have been omitted from these investigations. This study has provided the first holistic approach to the study of recognition latency – exploring not only the ability for long-term recognition, but highlighting the causality of recognition failure. I demonstrated that recognition

was likely to be limited by unstable identity signals from the signaller (chapter 3) rather than by limited long-term memory of identity labels by the receiver. By adopting an approach that explores recognition from both the perspective of the signaller and the receiver, I have been able to advance our understanding of the key features that limit long-term recognition within animal societies.

Maintaining recognition over extended periods of separation may be important for the pied babbler for two reasons; firstly, inbreeding is likely to be avoided by not breeding with familiar kin (Nelson-Flower et al. 2012), and secondly, subordinate individuals can utilise the natal territory as a 'safe haven' between prospecting bouts (Raihani et al. 2010). By returning to the natal territory between prospecting forays, subordinates may be able to recover from the energetic expenditure experienced during prospecting (Ridley et al. 2008; Raihani et al. 2010). Failure to maintain recognition over extended periods of separation may set a maximum limit to the duration of prospecting forays, with unfamiliar individuals typically being chased away from groups (Raihani et al. 2010). I have demonstrated that the selection for unstable identity signals in the pied babbler appears to have consequences for long-term recognition, which may ultimately be impacting upon pied babbler behaviour. By demonstrating that long-term recognition is limited if individuals are separated without contact, I highlight the potential importance of frequent encounters between individuals if they are to avoid the potentially costly impacts of inbreeding (Charlesworth & Charlesworth 1999; Charlesworth & Willis 2009) or agonistic aggression from former social partners.

11.5 Kin recognition and social interactions with neighbouring groups

11.5.1 Kin recognition and inter-group interactions

There is considerable taxonomic variation in the use of kin recognition to direct altruism (Cornwallis et al. 2009). In many cooperative breeders, population structure means that spatial cues (such as group membership) may be sufficient to ensure kin directed altruism (West et al. 2007; Cornwallis et al. 2009). Consequently, individuals may often show no fine-scale discrimination between degrees of kinship within groups (as is the case with the pied babbler; Nelson-Flower 2010). This raises the question of whether they retain an ability to identify kin at larger spatial scales, such as in neighbouring groups. I addressed this in chapter 9, investigating how kin recognition influences social interactions with neighbouring groups in territorial encounters. I observed that kin recognition did influence interactions between neighbouring groups, with greater territorial overlap and shorter inter-group interactions between related groups.

Kin-biased overlap has previously been noted in a variety of taxa including fish (Griffiths & Armstrong 2002), birds (Hatchwell et al. 2001), and mammals (Sera & Gaines 1994; Kitchen et al. 2005; Støen et al. 2005; Walker et al. 2008). However, the causality of kin-biased territory overlap is poorly understood. While it has been suggested that it may represent a form of kin selection (Kitchen et al. 2005; Hatchwell 2010), there is currently little evidence to suggest that it does offer inclusive fitness benefits (Hatchwell 2010). I was able to advance our understanding of this phenomenon, and identify that there were unlikely to be inclusive fitness benefits to kin-biased overlap, by exploring how

kin are interacting with one another in territorial disputes. This study was able to demonstrate for the first time that territorial disputes between neighbouring groups are energetically costly. Consequently, I was able to show that groups engaged in costly inter-group interactions more frequently as a consequence of increased territorial overlap (chapter 9). Thus, kin-biased overlap can result in more frequent, costly, inter-group interactions. I also observed no reduction in fighting behaviour between related groups. These extra costs that related neighbouring groups face may potentially be off-set by shorter territorial disputes between related groups, both in duration and in the amount of calling involved. While I have shown that kin recognition does influence the duration of social interactions between neighbouring groups, the fact that kinship did not influence the frequency of interactions or fighting suggest that kin are not permitted within the territories of related neighbouring groups. I found that kin-biased overlap was likely to have multiple costs to territory holders (more frequent interactions and the assumed costs of resource depletion from non-exclusive territories) and therefore I found no evidence that would indicate that kin-biased overlap does represent a form of kin-selection.

I concluded that variation in territorial overlap was likely to occur because of variation in intruder pressure between related groups. Neighbouring groups are typically related in the pied babbler population because an individual has dispersed from its natal territory into a neighbouring group. A logical next step in developing our understanding of why intruder pressure may change in response to kinship would be to address whether reproductive success is higher in individuals who have taken over dominant breeding positions within the natal territory (as has been observed in a number of other group-living

species; Newton & Marquiss 1983; Pusey & Packer 1987; Part 1991) and to explore whether breeding or foraging success is improved through knowledge of the best sites (Greenwood 1980; Part 1991). If it is favourable to occupy a familiar territory, this may explain why neighbouring groups will face the costs of increased territorial disputes to inhabit an area that lies inside the territory of a neighbouring group.

11.5.2 Subordinate advertising

Genetic work on the pied babbler has revealed that rates of inbreeding in this species are low, and that inbreeding is likely to be avoided through a combination of avoiding familiar kin as mating partners, and dispersal from the natal territory (Nelson-Flower et al. 2012). By investigating the advertising behaviour of individuals searching for a mate, I confirmed that pied babblers utilise kin recognition to avoid incestuous mating partnerships. In chapter 10, I demonstrated that subordinate pied babblers adjust their calling behaviour to focus on the edges of their territory, and more importantly, specifically towards neighbouring groups that contain unrelated individuals. I also show that calling behaviour was affected by group composition, where calling effort was not targeted towards the largest groups, but specifically towards groups that contained the most unrelated, opposite sex, adult individuals. This demonstrates an extraordinary capacity to glean information about kinship, sex and the number of individuals within neighbouring groups. While group-living species have been widely thought to assess the composition of neighbouring groups (Henzi et al. 1998; Lazaro-Perea 2001; Hale et al. 2003), there has been little empirical evidence to show that this information is used to adjust behavioural interactions with a group (Henzi et al. 1998). The findings I present

therefore provide a key example of the importance of information-gathering on dispersal tactics and advertising behaviour. Subordinate pied babblers have limited opportunities to breed within their natal territory, both because of the limited number of breeding positions available, and the high relatedness of group members (Raihani et al. 2010). Searching for breeding opportunities within non-natal groups is therefore of primary importance. I have been able to empirically demonstrate that subordinate pied babblers adopt a method of vocal advertisement from within the natal territory that carries lower costs than prospecting. I have demonstrated that kin recognition is playing a key role in the social behaviour of the pied babbler, and that it is used to target a specific audience during vocal displays.

An avenue for future research would be to explore the calling behaviour of dominant individuals who have recently lost their breeding partner. When breeding vacancies emerge, and the position cannot be filled by a remaining group member, the remaining dominant will advertise the vacancy through loud calling (D. Humphries, personal observation). Whereas we may expect a subordinate individual to 'hedge their bets', and advertise to all groups containing unrelated individuals (as breeding vacancies are unpredictable in their occurrence), dominants may potentially target specific known high-quality individuals or groups. This would potentially express itself as targeted displays on a single boundary with a neighbouring group. Such behavioural patterns would provide evidence for the benefits of information gathering (during ritualised territorial displays, or eaves-dropping) for dominant individuals, as well as demonstrating their ability to assess the quality of individuals within neighbouring groups.

11.6 Summary

Previous research has suggested that the ability to recognise individuals and kin may be critical for the evolution of complex social behaviours within animal societies, including the formation of stable cohesive groups (Waser 1977), monogamous mating partnerships (Wanker et al. 1998; Clark et al. 2006), maintaining and coordinating cooperation (Crowley et al. 1996; Agrawal 2001; Komdeur et al. 2008), and for avoiding inbreeding (Blouin & Blouin 1988; Pusey & Wolf 1996). Until now though, comprehensive investigations into the occurrence, type, and ontogeny of recognition within cooperative societies have been rare. I have been able to systematically demonstrate each stage of the recognition process occurring in the pied babbler, following the process through from the production of vocal identity labels by the signaller, to their discrimination by the receiver, to the influence of this on behavioural decisions. I have explored the ontogeny of recognition in young, and its limitations in adults and demonstrated that recognition plays an important role in mediating social interactions, both during territorial disputes and for subordinate tactics for mate attraction. Additionally, by providing insights into the underlying mechanisms through which recognition occurs, I have been able to expose where recognition is limited, showing that: a) individuals are unable to recognise unfamiliar kin, b) that recognition takes several months to develop in young, and c) that recognition breaks down if individuals are separated without contact for extended periods. Understanding the characteristics of pied babbler recognition develops our understanding of the behaviours we observe in this species. For example, by demonstrating that kin recognition is limited to familiar kin, it is possible to understand why inbreeding occasionally occurs between

unfamiliar kin (Nelson-Flower et al. 2012). In addition, the slow development of recognition in this species explains how mobile young are successfully kidnapped from neighbouring groups. By providing this thorough examination of pied babbler recognition mechanisms, I have been able to illuminate our understanding of pied babbler social behaviour and identify when and where we may expect to see behavioural discrimination occurring.

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Appendix

Appendix 1 – Certification of ethical clearance granted for all work undertaken during the production of the thesis “The mechanisms and function of social recognition in the cooperatively breeding Southern pied babbler, *Turdoides bicolor*”.



MACQUARIE
UNIVERSITY

ANIMAL RESEARCH AUTHORITY

AEC Reference No.: 2010/032

Date of expiry: 30 June 2011

Full Approval Duration: 1 July 2010 to 30 June 2013 (36 months)

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Mr James Savage
Dr Claudia Wascher

The above-named are authorised by:
MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:

Title of the project: Kinship and social networks in cooperatively breeding birds

Type of animal research and description of project: Through experiments and molecular work on a number of avian species this project explores the basis of cooperative interactions between individuals, including provisioning of young and predator mobbing. Conducted in the field, individuals will be captured, measured, blood sampled and fitted with identification devices. A captive population will be studied on the university campus.

All procedures must be performed in accordance with the AEC approved protocol.


Numbers Approved:

Species	Sex	Age	Year 1	Year 2	Year 3	Total	Supplier/ Source
Apostlebirds	M/F	Adult and offspring	400	400	400	1200	Fowlers
Chestnut-crowned babbler	M/F	Adult and offspring	1600	1600	1600	4800	Fowlers
Chestnut-crowned babbler	M/F	Adult and offspring	1650	1650	1650	4950	
Beil miners	M/F	Adult	100	100	100	300	Cumberland SF
		offspring	50	50	50	150	Cumberland SF
Noisy miners	M/F	Adult	200	200	200	600	MQ campus
		Offspring	50	50	50	150	
Pied babbler	M/F	Adult and offspring	200	200	200	600	Kalahari, RSA
TOTAL			4250	4250	4250	12,750	

Location of research: Research will be conducted in a variety of locations: Fowlers, Cumberland State Forest, Macquarie University (MQ) Campus and Kuruman River Reserve, Southern Kalahari Desert, South Africa (RSA)

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

This authority remains in force from **1 July 2010 to 30 June 2011**, unless suspended, cancelled or surrendered, and will only be renewed upon receipt of a PROGRESS REPORT before the end of this period.



Prof Michael Gillings
Chair, Animal Ethics Committee

Date: 29th June 2010

Form C (issued under part IV of the Animal Research Act, 1985)