Kinship and its consequences in the cooperatively breeding Southern Pied Babbler *Turdoides bicolor*

Martha J. Nelson-Flower

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Abstract

Cooperative breeding occurs when more than two individuals help to raise offspring that are not their own, and has been the focus of empirical and theoretical research for over forty years. Of central importance to this work are the fitness costs and benefits of helping, and the factors limiting the reproduction of helpers. To understand these, the genetic relationship between individuals must be known. In this thesis, I use genetic and observational data to explore kinship between individuals in groups of wild Southern Pied Babblers *Turdoides bicolor*. Nine polymorphic microsatellite loci were used to genotype 321 individuals and, following parentage analysis, a pedigree was constructed. I used this information first to characterize the breeding system and show that it is almost completely monogamous, and successful subordinate reproduction is rare. Individuals in the group are generally closely related to one another, resulting in groups that are separate genetic entities. Although subordinates rarely successfully breed, many groups contain potential breeding partners for subordinates due to the relatively common immigration of dominants. Next, I show that inbreeding is very rare: individuals avoid sexual behaviour with known relatives, and subordinates inherit dominant breeding positions within groups only when the prospective partner is unrelated. Thus inbreeding avoidance is an important constraint on subordinate reproduction. Sex-biased dispersal does not function as an inbreeding avoidance mechanism; rather, recognition of those individuals known from the natal group (associative learning) appears to drive inbreeding avoidance. I then explore reproductive conflict within groups. Subordinates show interest in breeding only when there is a suitable unrelated potential breeding partner in the group. Physical condition does not affect whether potentially competitive subordinates show interest in breeding, although female subordinates were less likely to engage in competition when they were closely related to the dominant female. Female subordinates that are potential competitors impose a cost on dominants through delays in the onset of the breeding season, likely by way of the destruction of the dominant female's eggs. Dominants of both sexes respond to subordinate competition for reproduction by increased aggression during the fertile period. Finally, I show that changes in relatedness between donors and recipients of help do not affect the amount of food that is given to chicks, thus providing no support for indirect kin-selected benefits as a primary cause of helping behaviour in this species.

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Chapter 1

Introduction

In 1859, Darwin's seminal publication "The Origin of Species" introduced the revolutionary idea of 'natural selection' as the process driving evolution; it also stated that each individual should be selected to maximize its own reproductive output. This hypothesis caused a revolution in our understanding of the natural world, but some naturalists noticed that individuals in cooperatively breeding species appeared to help to raise the offspring of others rather than pursue their own independent breeding opportunities (Skutch 1935). This behaviour was interpreted as altruism, because in many cases the helping individuals did not go on to breed themselves. As Darwin himself acknowledged (1859), this apparent altruism in cooperatively breeding societies contradicted natural selection, which implied that helpers should pursue their own independent breeding opportunities or not help at all. Thus there is a seeming inconsistency between the apparent altruism of helpers and the imperatives of natural selection, and therefore a great deal of attention has been focused on the evolutionary benefits of helping behaviour (Brown 1987).

Despite the apparent evolutionary paradox it represents, cooperative breeding has been observed in a broad range of species, from insects to fish, mammals and birds. It is estimated that approximately 9% of all bird species display cooperative breeding behaviour (Cockburn 2006). The challenge remains to determine, within an evolutionary framework, exactly what benefits promote the occurrence of cooperative breeding, and what costs are incurred by individuals. Cooperative breeding has been defined as occurring when three or more individuals contribute to raise young and it is certain that some individuals cannot be parents of some of the young they are helping to rear (Brown 1987, Koenig *et al.* 1992, Cockburn 2004). Research into the evolution of cooperative breeding has first asked why individuals help to raise offspring that are not their own (Ekman *et al.* 2004). Secondly, many cooperatively breeding societies are characterized by high reproductive skew in one or both sexes, in that a small number of individuals monopolize reproduction (Keller and Reeve 1994, Cockburn 2004). Therefore another major research question asks why some helpers in groups do not breed, or are less successful in breeding: what factors are responsible for variation in reproductive skew (Johnstone 2000)? These questions lie at the heart of our understanding of the occurrence of cooperative breeding behaviour and the properties of complex animal societies, and, although hotly debated over the past several years, these questions still await resolution.

Helpers are usually subordinates in social groups and are often, but not always, retained natal offspring that delay dispersal (Emlen 1995). Why should these individuals help to raise young that are not their own? Rather than being purely altruistic, it is expected that they must benefit in some way from this behaviour. In 1964, Hamilton proposed the idea of kin selection: that individuals help to raise the offspring of relatives instead of breeding independently because fitness can be gained by furthering the spread of shared genes. Specifically, individuals will be selected to help when the cost of help to the donor (C) is less than the benefit of help to the receiver (B), weighted by the relatedness between the donor and receiver (r), such that $rB > C$ (Hamilton 1964). In this way, kin selection was proposed to drive the evolution and maintenance of cooperative breeding behaviour (Hamilton 1964). Although kin selection was initially embraced as a complete explanation for helping behaviour, as time passed many argued that kin selection might not fully account for the maintenance of cooperative breeding in all species, particularly among vertebrates (Clutton-Brock 2002). Rather, individuals may help in order to increase their own immediate or future chances of survival or reproduction, thereby deriving direct benefits from this behaviour (reviewed by Dickinson and Hatchwell 2004). By helping, individuals can increase the size of the group they live in ('group augmentation' – Brown 1987, Kokko *et al.* 2001), which may result in an increase in the amount of future help received ('delayed reciprocity', Kokko *et al.* 2001, Clutton-Brock 2002), and increase current survival and future reproduction due to increased vigilance and larger coalition sizes at dispersal (reviewed by Heinsohn and Legge 1999, Russell 2004). Additionally, through helping, individuals may increase their breeding "experience" or "skills" (Skutch 1961, Hatchwell *et al.* 1999), or improve future access to mates (Reyer 1990, Zahavi 1995). It is vital to continue investigations of how levels of helping by individuals in cooperatively breeding species may change according to the amount of indirect fitness benefits that are available to them, which lends more evidence to the debate over the importance of kin selection.

A second major research question asks why some helpers in groups do not breed. Subordinate helpers may not breed, or may breed less successfully than dominants, for many reasons. First, in many cooperatively breeding species, subordinates are the offspring of the dominant pair (Emlen 1995), and may be constrained from breeding by a lack of unrelated partners in the group, i.e. through inbreeding avoidance (Koenig *et al.* 1998). Once the need for inbreeding avoidance is removed as a result of the immigration of an unrelated dominant or subordinate, subordinates may begin to compete with dominants for reproduction (Balcombe 1989, Emlen 1997). If subordinate reproduction (or subordinate competition for reproduction) is costly to dominants, dominants may attempt to suppress it through aggression or mate-guarding, resulting in reproductive conflict (Young *et al.* 2006). A large body of literature (reproductive skew theory) addresses the struggle between dominants and subordinates for reproduction (reviewed by Johnstone and Cant 2009). First developed by Vehrencamp (1983) and Emlen (1982b), reproductive skew theory encompasses a series of models that ask (a) whether control of the division of reproduction is settled by conflict, or if one party has complete control; (b) whether "outside options" such as the forced or voluntary removal of the competitor affect reproductive skew; and (c) whether factors like group productivity or relatedness affect reproductive skew (reviewed by Johnstone and Cant 2009). Unfortunately, testing models of reproductive skew empirically is very difficult, because small changes to a model's assumptions may greatly impact the predictions it makes (Hodge 2009, Koenig *et al.* 2009), and all of a model's assumptions (even those not explicitly stated) must be met by the study system before a meaningful test can be performed (Magrath *et al.* 2004, Hodge 2009). To move forward in understanding reproductive skew, it is therefore essential to conduct empirical studies that examine the key processes underlying the distribution of reproductive success in model systems (Hodge 2009). Understanding these processes represents one of the most intriguing problems in the study of social behaviour and animal societies (Keller and Reeve 1994).

Kinship – are helpers breeding?

Before any other questions can be asked about a cooperatively breeding species, it is first necessary to know the true existence and extent of successful subordinate breeding, and this may differ markedly from field observations. For example, subordinate Seychelles Warbler *Acrocephalus sechellensis* females often lay in the dominant female's nest (Richardson *et al.* 2001). Until recently, sexual monogamy was assumed in most socially monogamous avian species, including cooperative breeders, such that social dominants were presumed to monopolise reproduction.

However, the advent of molecular techniques has revealed that extra-pair reproduction is far more common than previously supposed (reviewed by Cockburn 1998, Griffith *et al.* 2002). For example, extra-group young were found in 95% of Splendid Fairy-Wren *Malurus splendens* broods (Mulder *et al.* 1994) and 40% of Seychelles Warbler broods (Richardson *et al.* 2001), while extra-pair (but within-group) young were found in 63% of Mexican Jay *Aphelocoma ultramarina* broods (Li and Brown 2000). Such a high frequency of extra-pair parentage could not have been predicted from field observations alone, and genetic data have become essential for an accurate assessment of kinship in cooperatively breeding societies (Koenig and Dickinson 2004). Kinship thus has important and far-reaching implications for our understanding of cooperative breeding behaviour. Through understanding kinship, we can measure the direct fitness benefits of attaining a dominance position, the direct benefits that subordinates may gain from reproduction, and the potential indirect benefits of helping as a subordinate. These benefits play a key role in the evolution and maintenance of cooperative breeding.

Consequences of kinship – inbreeding and inbreeding avoidance

Avoidance of inbreeding is an important factor influencing the partitioning of reproduction in cooperatively breeding societies (Koenig *et al.* 1998), but until recently, the existence of inbreeding depression and inbreeding avoidance mechanisms in the wild was questioned (reviewed by Keller and Waller 2002). In some species where detailed pedigrees are known, inbreeding has been shown to occur, albeit rarely (Szulkin and Sheldon 2008), but in most species, especially cooperative breeders, the extent of inbreeding has proven difficult to establish, despite recent advances in molecular techniques used to determine kinship (McRae and Amos 1999, Koenig and Haydock 2004). This is because parentage prediction programs are sometimes confounded by the genetic similarity between true parents and older full-siblings, because all may share the same rare alleles (McRae and Amos 1999). In wild populations of cooperative breeders, inbreeding is thought to be avoided through (a) increased dispersal distances from natal groups, (b) sex-biased dispersal or (c) kin recognition (Koenig and Haydock 2004), but these mechanisms too may be difficult to show. Combining field observations with genetic data can provide a new perspective on the existence of inbreeding and inbreeding avoidance mechanisms in cooperatively breeding species. For example, breeding female Southern Pied Babblers

Turdoides bicolor experience body mass changes during egg-laying (Chapter 4); this information can confirm or refute predictions of inbreeding inferred from parentage analysis, a conclusion which has not been possible in the past (McRae and Amos 1999). Although sex-biased dispersal may function (in part) as an inbreeding avoidance mechanism (Greenwood 1980), in some cooperatively breeding species there is no evidence to support this (e.g. White-winged Choughs *Corcorax melanorhamphos*, Beck *et al.* 2008). Rather, dispersal itself, and/or the recognition (and active avoidance) of kin by associative learning, may be the means by which individuals avoid inbreeding (Komdeur and Hatchwell 1999, Szulkin and Sheldon 2008). It is vital to identify the extent of inbreeding and inbreeding avoidance in cooperatively breeding species to understand better the role of inbreeding in the partitioning of reproduction, and the role of inbreeding avoidance in driving dispersal patterns.

Consequences of kinship – reproductive conflict and skew

Empirical investigations of reproductive skew in cooperatively breeding bird societies are very valuable, because this is an area of research that remains mostly theoretical, controversial and highly active. The information required to assess models of reproductive skew thoroughly is extensive, and to measure the required parameters precisely may prove impossible (Magrath *et al.* 2004). Empirical research should investigate several key processes underlying variation in reproductive skew in model systems (Hodge 2009). First, it is important to understand the contexts in which subordinates compete for reproduction: subordinates are expected to compete when they have access to potential unrelated breeding partners (Emlen 1997), or when they possess characteristics that make it more difficult for dominants to suppress them (Clutton-Brock *et al.* 2001a). Second, the cost to dominants of subordinate reproduction (or subordinate competition for reproduction) must be established; if it is costly, reproductive suppression of subordinates is likely to ensue (Young *et al.* 2006). Finally, the reaction of dominants to reproductive competition by subordinates must be measured. When subordinates compete for reproduction, dominants may become more aggressive (Williams 2004, Ratnieks *et al.* 2006) or guard their mates more intensively (Mumme *et al.* 1983a, Piper and Slater 1993). Once this information is known, the fundamental assumptions of reproductive skew models can be better assessed (Magrath *et al.* 2004). This in turn leads to better understanding of the social transactions that lead to observed patterns of skew, and allows identification of further avenues for research to examine the interactions that underpin cooperative societies.

Consequences of kinship – kin selection

Kin selection theory suggests that the high indirect fitness benefits gained by those individuals helping their relatives to breed could drive the evolution and maintenance of cooperative breeding (Hamilton 1964). In some species, however, direct benefits, rather than kin selection, may provide a better explanation for the occurrence of cooperative breeding behaviour (Clutton-Brock 2002). To determine whether kin selection and indirect benefits contribute to the evolution of helping behaviour, it must be shown that help leads to inclusive fitness benefits by increasing (a) the fitness of the related recipient (the nestling or fledgling) and/or (b) the fitness of the related breeder (reviewed by Dickinson and Hatchwell 2004). However, this does not conclusively demonstrate that kin selection drives helping behaviour, because increases in the fitness of the recipient or the breeder may also result in direct benefits to the helper. To demonstrate conclusively that kin selection influences helping behaviour, helpers must be shown to vary the extent to which they help, depending upon their relatedness to the recipient of that help (Griffin and West 2003). While many studies have investigated this, few have been able to compare the behaviour of individuals both before and after dominant-breeder dispersal events, which can result in major changes in relatedness between the focal individual and the brood they help to raise. This approach provides a direct comparison between an individual's relative provisioning of full- *vs* half-siblings. If subordinate helpers vary the degree to which they help based on differences in relatedness between potential recipients, this provides unqualified support for kin selection as a primary force driving the evolution and maintenance of cooperative behaviour in this species.

Study site

The Pied Babbler Research Project was initiated in June 2003 at the Kuruman River Reserve in the southern Kalahari Desert. The Reserve is located in the Northern Cape Province of South Africa, 27 km west of Van Zylsrus and 17 km south of the Botswana border (26°58'S; 21°49'E). The Reserve was converted from ranchland in 1993 and is owned by the Kalahari Research Trust. It encompasses a stretch of the (generally) dry bed of the Kuruman River and covers 2800 hectares of desert scrubland or “thornveld”. The study site is characterized by sandy dunes 10-30 m high, separated by valleys of up to 300 m across, and small saltflats. The area is sparsely vegetated with perennial and annual grasses, perennial shrubs and in some areas, trees. Trees found on the reserve include *Acacia erioloba, A. haemotoxylon* and *Boscia albitrunca*, while common shrubs include *Rhigozum trichotomum* and *Grewia* spp. Common perennial grasses are *Eragrostis* spp., *Aristida* spp., *Stipagrostis* spp. and *Schmidtia* spp. The region is classified as semi-arid with low and unpredictable rainfall. Mean annual rainfall is ~250 mm per year; daily rainfall was measured using a standard rain-gauge (mm). The winter months of May to September are generally cold and dry, and the months of October to April comprise the hot, wet summer. This is when the bulk of the rainfall for the year falls and the babbler breeding season occurs.

Study species: the Southern Pied Babbler

The Southern Pied Babbler *Turdoides bicolor* is a sexually monomorphic, medium sized passerine, weighing 75 – 95 g. It is a member of the family Timaliidae; the genus *Turdoides* comprises 27 species, all of which live in social groups. Southern Pied Babblers are not considered to be threatened, and permission to work on groups of wild Southern Pied Babblers was granted by the Northern Cape Conservation Authority. Babblers live in groups year-round; group size over the course of this study was 5.6 ± 0.1 birds (range 2 – 15). Groups usually consist of one behaviourally dominant male and female and their subordinate helpers. Individuals can be classified as ‘dominant’ or ‘subordinate’ based on the observation of dominance assertions (pecks and other attacks), to which subordinate individuals respond with submission. These submissive reactions include bill-gaping (begging), crouching or rolling over, looking away or fleeing (Raihani 2008). Helpers are generally natal offspring of the group, although immigrant subordinate helpers sometimes occur. Groups defend year-round territories of 1 – 3 km^2. When neighbours are encountered, the birds enter into ritualized vocal displays that continue until one group retreats. Group members roost together and leave the roost tree at first light, spending the day foraging together as a cohesive group. Southern Pied Babblers are primarily terrestrial foragers, spending > 95% of their foraging time on the ground (Ridley and Raihani 2007a). They forage mainly by digging into the substratum with their beaks to uncover small invertebrates; they also glean food items such as termites, ants and beetles from the surface. During the hottest part of the day, the birds cease foraging and shelter in the shade of trees and shrubs. All adults in the group contribute to cooperative behaviours such as vigilance (Hollén et al. 2008), incubation of eggs and feeding of nestlings and fledglings (Ridley and Raihani 2008). Only the dominant female incubates eggs and broods nestlings at night. Brood sizes range in size from 1 - 4 offspring; the mean brood size for this study was 2.38 ± 0.1 chicks. Incubation lasts 14.9 ± 0.3 days (range 13 – 17 days), and chicks fledge *ca* 16 days after hatching (range 13 – 19 days - Raihani and Ridley 2007a). Chicks are dependent on adults for post-fledging care: although chicks first attempt independent foraging at about 17 – 29 days of age, these attempts are always unsuccessful (Ridley and Raihani 2007b). The period of post-fledgling care ranges from 40 – 97 days with a mean of *ca* 59 days; the extent of this period depends on the adult:fledgling ratio of the group (Ridley and Raihani 2007b).

The Southern Pied Babbler study population at the Pied Babbler Research Project offers remarkable insights into cooperative breeding societies. Study animals are habituated to close observation (< 2 m), and detailed behavioural observations can be collected without affecting the birds' natural behaviour. The babblers have been trained to jump onto scales such that their daily body mass can be measured, which allows the costs and benefits of various cooperative behaviours to be quantified in terms of lost or gained body mass. This population of babblers has been studied extensively for six years, and comprehensive behavioural observations of breeding attempts, reproductive competition, reproductive success, dispersal attempts, and nestling and fledgling provisioning by group members have been collected. Blood samples have been taken from all birds, allowing detailed genetic analysis of relatedness between both individuals and groups. The study population encompasses contiguous habituated groups, and dispersers are commonly resighted prospecting at or having successfully dispersed into non-natal groups. This allows valuable analyses of the role that dispersal plays in inbreeding avoidance. Similar to other cooperative breeders (Emlen 1997), turnover of dominants in Southern Pied Babblers is relatively common, leading to natural variation in the composition of groups, with some groups comprising nuclear families ('simple' groups) and others containing dominants that are not related to some or all subordinates ('complex' groups) (Raihani 2008). These 'complex' groups provide possible breeding partners for subordinates in the form of step-parents, and contain young that vary in relatedness to adult subordinate helpers.

This variation presents exceptional opportunities to study the patterns, costs and consequences of competition for reproduction between breeders and helpers, and the relationship between relatedness and investment in young by adult subordinates.

Thesis Structure

In this thesis, I combine behavioural and genetic data to investigate kinship and its consequences in groups of cooperatively breeding Southern Pied Babblers. I am interested primarily in which individuals breed successfully (i.e. the extent of reproductive skew), and the factors that influence variation in reproductive skew across groups. In Chapter 2, I describe the techniques used both in the laboratory and in the field to collect the data, and detail the polymorphic microsatellite loci used to determine kinship. Chapter 3 explores the extent of reproductive skew and describes the breeding system. This chapter also examines intra-group relatedness and immigration to non-natal groups by dominants and subordinates. Factors which may affect reproductive skew in groups are inbreeding avoidance and reproductive conflict: these are covered in Chapters 4 and 5. In Chapter 4, I present data on the prevalence of inbreeding, and examine two possible mechanisms of inbreeding avoidance using both genetic and behavioural data. Chapter 5 explores the response of subordinates to reproductive opportunities in the group, and investigates the costs to dominants of subordinate competition for reproduction, and dominant suppression tactics. Chapter 6 addresses the effect of kinship on levels of investment in young by helpers, offering insights into the extent and importance of kin-selected benefits in this species. Finally, Chapter 7 summarizes my findings and discusses their implications with respect to current understanding of cooperative breeding behaviour.

Chapter 2

Materials and Methods

Field Methods

Behavioural data collection

Wild babbler groups were habituated to the close presence of a human observer through following the group for six to twelve weeks: habituation was considered complete when observers could slowly approach and stay with the group without causing apparent alarm or distress. All behavioural data were collected using a Psion hand-held data logger (Psion Teklogix Inc., Ontario, Canada). GPS trackers (Garmin, UK) were used to record geographic locations and the daily movements of each group. GPS waypoints were recorded at the locations of roosts, nests, and territorial interactions between groups. Waypoints were also recorded every 15 minutes when following groups of babblers. Babblers were followed for roughly four hours after they left the roost at first light, and for roughly three hours in the afternoon until they went to the roost at dusk.

The weight of each individual in each group was recorded as soon as they left the roost in the morning, at the end of the morning observation session, at the start of the afternoon session and just before the group went to the roost. Individuals were weighed by enticing them onto a portable scale with crumbs of boiled egg yolk (< 0.5 g). After weighing, at least 15 minutes was allowed to pass before behavioural observations were resumed. Weights that were acquired within 20 minutes of leaving the roost were termed morning weights; weights acquired within 20 minutes of birds returning to the roost at night were termed evening weights. All other weights were termed mid-day weights.

For some analyses in this thesis, data were collected by all Pied Babbler Project researchers from 19 groups over the breeding seasons from September to May from 2003 until 2009. Other analyses used my own observational data collected during field seasons which took place from September 2006 to April 2007, and October 2007 to March 2008. Observations on a variety of behaviours (specifically described in each chapter) were recorded using *ad libitum* and focal sampling methods (Altmann 1974). Overall, I collected 517 hours of *ad libitum* behavioural data and 114 dominant female mate-guarding focals (see Chapter 5) over two field seasons.

Collection of blood samples

Blood samples were collected from 321 Southern Pied Babblers from 23 groups over 5 years. Ninety-two adults, juveniles and fledglings were captured using walk-in baited traps, while 229 nestlings were removed from the nest at the age of 11 days for the purposes of ringing and acquiring blood samples. Most of the individuals sampled were members of stable groups, but 3 of them were solitary floaters. The birds were ringed with SAFRING metal bands and individually identifiable plastic colour-ring combinations, under the SAFRING licenses 1263 and 1328. Metal rings had a unique code so that birds could be identified even if the colour rings were lost. A blood sample (*ca* 50 μL) was collected from each bird through brachial venipuncture, stored in 700 μL of Longmire's Solution Blood Lysis Buffer, and kept at 4°C.

Lab methods

DNA extraction and quantification

DNA was extracted from each blood sample using the following salt-extraction technique (Richardson *et al.* 2001). 150 μL of the blood-lysis buffer mixture was added to 300 μL of Longmire's blood lysis buffer and 20 μL of 20 mg/mL Proteinase K (Roche). The mixture was incubated overnight at 37°C with constant agitation. After the incubation, half a volume of 5M NaCl was added and the solution was thoroughly mixed and shaken for 20 minutes at room temperature. One volume of 24:1 chloroform:iso-amyl alcohol was added and the solution was vortexed, then shaken at room temperature for a further 10 minutes. The solution was then centrifuged at 8000 *g* for 10 minutes and the supernatant removed to a new tube. 600 μL of ice-cold isopropanol was added, the solution was mixed, and then left at -70°C for 30 minutes. The tube was then centrifuged at 8000 *g* for 10 minutes, and the supernatant was removed. 1 mL of ice-cold 70% ethanol was added and the tube was centrifuged again at 8000 *g* for 5 minutes. The supernatant was removed and the DNA pellet was allowed to dry. The pellet was dissolved in 50 μL of TE buffer. DNA concentration was measured using a NanoDrop 1000 spectrophotometer (Thermo Scientific) according to manufacturer's instructions. 10-30 μL of each DNA extraction was then diluted to make a working DNA solution of 50 ng/μL, stored at 4°C. Each DNA extraction was stored at -20°C.

Development of polymorphic microsatellite loci in the Southern Pied Babbler

1. Testing passerine microsatellite loci

Microsatellite loci are the marker of choice to elucidate population diversity and structure and parentage of offspring. Cross-species amplification of microsatellite loci has been reported for a number of closely related taxa (FitzSimmons *et al.* 1995, Primmer *et al.* 1996). A vast array of passerine microsatellite loci has been isolated; primers for 204 passerine microsatellite loci were tested to identify polymorphic microsatellite loci for the babblers. Test primers were received from Dr. Deborah Dawson at the Sheffield Molecular Genetics Facility at the University of Sheffield. PCR was used to check if the primers amplified any products in Southern Pied Babbler DNA. Each primer set was initially tested at the annealing temperature suggested by the literature or at an estimate of annealing temperature based on the primer sequence as estimated by the 'Oligonucleotide Properties Calculator' (www.basic.northwestern.edu/biotools/oligocalc.html). Each primer was initially tested at three different $MgCl_2$ concentrations (1.0 mM, 1.5 mM and 2.0 mM) with the DNA of four different individuals. The individuals were selected from distant geographical parts of the population and were not thought to be related to one another. Polymerase chain reaction (PCR) was done with 50 ng DNA, buffer supplied by the manufacturer diluted to 1X, one of 1.0 mM, 1.5 mM or 2.0 mM $MgCl_2$, 10 picomoles of forward and reverse primer, 4 picomoles of each dNTP, and 0.5 units of GoTaq DNA polymerase enzyme (Promega) in a 20 µL reaction. PCR cycle settings were: a denaturation step of 5 minutes at 95°C, amplification of 35 cycles of 95°C for 30 seconds, the specific annealing temperature for 45 seconds, and 72°C for 45 seconds, followed by an extension step of 72°C for 10 minutes. Five µL of the PCR product was electrophoresed on a 1.5% agarose gel to check amplification; if a clearly defined product was present, the PCR products from the reactions of all four individuals were electrophoresed on a 4% acrylamide gel in an ABI 373 DNA sequencer to see if the microsatellite locus was monomorphic or polymorphic in this babbler population. If there were several PCR products present per individual, the annealing temperature was increased; if there were no products, the annealing temperature was decreased. Some primer sets that produced a positive result but that were found to be monomorphic were tested on a further three to five individuals. In other cases, if the four individuals had clearly monomorphic results, the primer set was not tested further.

Of the 204 primer sets tested, 13 did not amplify any products, and 28 resulted in non-specific products, despite extensive adjustment of PCR conditions. Six of the primer sets had products that were too large for analysis on the ABI 373 DNA sequencer. Of the remaining 157 primer sets with clear results, 135 were monomorphic and 22 primer sets were polymorphic. Of these 22 polymorphic primer sets, 13 were found to have only two alleles. Ideally a usefully polymorphic locus will have several alleles in the population, so these 13 were rejected from further investigation. This left nine loci with three or more alleles each. Five of these nine loci (Ase55, GCGATA10, Pij15ZFS, PmaTGAn42 and Ppi2) did not amplify reliably in each individual, so the primers were redesigned to improve amplification, described below. A summary of the results from the tests is shown in Table 2.1.

Table 2.1: Results of tests of 204 passerine microsatellite loci primer sets on Southern Pied Babbler DNA.

Result	N primers
No amplification	13
Non-specific products	28
Product too large	6
Monomorphic	135
Polymorphic: 2 alleles	13
Polymorphic: 3 or more alleles	9

2. Cloning PCR products

Some of the PCR primers sets were polymorphic for the babbler DNA, but did not amplify reliably in every sample. These primers were redesigned by first cloning and then sequencing the PCR product of interest. In cloning, the DNA to be cloned is ligated into a vector containing ampicillin resistance and the cloning site is located in a *lacZ* gene, which is then introduced into *lacZ*$^{-}$ *E. coli* cells. These cells are grown in an ampicillin-enriched medium, ensuring only those containing a vector are able to grow. In addition, the media also contains the sugar X-gal. Clones containing vectors that have foreign DNA ligated into the *lacZ* gene are unable to process the X-gal and are a white colour, while those with a vector but no insert are blue. This allows further investigation and sequencing of only those clones that are likely to contain the DNA fragment of interest. For the ligation step, the PCR products of several different individuals were mixed together and the concentration of the DNA was measured with the NanoDrop 1000 spectrophotometer. The mixed PCR products were then ligated into pGEM-T vector (Promega). The amount of insert DNA to use in the reaction was calculated according to the manufacturer's instructions. This product was mixed with 2.5 µL of 2X Buffer, 0.5 µL of pGEM-T vector, 0.5 µL of ligase and dH_2O to 10 µL. The reaction was gently mixed and incubated at 4°C for at least 24 hours. For the cloning step, high-efficiency competent cells were used (Promega—JM109 cells). The tube of cells was thawed on ice for 5 minutes and very gently stirred by flicking. 2 µL of ligation reaction was added to 20 µL of cells and gently stirred with the pipette tip, and then incubated on ice for 20 minutes. The cells were then heat-shocked at exactly 42°C for 45 to 50 seconds, and returned to ice for a further 2 minutes. Finally 980 µL of room temperature SOC medium was added to the cells and they were incubated at 37°C for 90 minutes while shaking at 100 rpm. Luria Broth (LB) plates were prepared with 100 µg/mL ampicillin and allowed to set. Thirty minutes before cells were spread on the plates, 100 µL of 100 mM IPTG and 20 µL of 50 mg/mL X-gal was aseptically spread on the plates and allowed to dry in a fumehood. Finally, between 20 µL and 100 µL of cells per reaction were aseptically spread on plates and allowed to dry in the fumehood before incubation overnight at 37°C. White clones were picked and further grown in LB broth with ampicillin. The insert was checked using PCR: 1 µL of the colony broth was used as template DNA, buffer supplied by the manufacturer (Promega) diluted to 1X, 1.5 mM $MgCl_2$, 10 picomoles of M13 forward and reverse primers, 4 picomoles of each dNTP, and 0.5 units of GoTaq DNA polymerase enzyme (Promega) in a 20 µL reaction. PCR cycle settings were: a denaturation step of 5 minutes at 95°C, amplification of 29 cycles of 95°C for 30 seconds, 70°C for 30 seconds, and 72°C for 55 seconds, then an extension step of 72°C for 10 minutes. PCR products were electrophoresed on an agarose gel to check the size of the insert. If the insert was the expected size, the DNA was sent for sequencing.

3. Alkaline lysis miniprep of plasmid DNA

Alkaline lysis miniprep was used to increase the concentration of the insert DNA from cloning, in order to increase sequencing success, using a procedure modified from Sambrook *et al.* (1989). Selected colonies were grown overnight in 1 mL of LB-ampicillin at 37°C while shaking at 100 rpm. Each culture was centrifuged at 1500 *g* for 5 minutes and the supernatant was removed. The bacterial pellet was resuspended in 200 µL of Solution A (50 mM glucose, 25 mM Tris pH 8, 10 mM EDTA). The mixture was incubated at room temperature for 5 minutes, after which 400 µL of freshly prepared Solution B was added (0.2 N NaOH, 1% SDS) and the tube was inverted several times to mix. After 5 minutes further incubation on ice, 300 µL of Solution C was added (3 M NaOAc). Further gentle mixing was followed by incubation on ice for 10 minutes. The tube was centrifuged at 10 000 rpm for 5 minutes and the supernatant was carefully transferred to a clean eppendorf tube; the pellet was discarded. 0.6 volumes of ice-cold isopropanol was added, the solution was mixed and then incubated for 10 minutes at room temperature. It was then centrifuged at 21 000 rpm for 15 minutes. The supernatant was then discarded and 1 mL of ice-cold 70% ethanol was added. The pellet was not disturbed and the tube was centrifuged again at 21 000 rpm for 10 minutes. The supernatant was removed and the pellet was dried. Finally the pellet was dissolved in distilled water and the final DNA concentration measured using the NanoDrop 1000 spectrophotometer.

4. Sequencing the PCR product

Sequencing was performed by PCR using 10 ng of template DNA, 3.2 picomoles of primer, and 2 µL of sequencing mix (Big Dye pink juice) in a 10 µL reaction. The PCR conditions were: 25 cycles of 94°C for 30 seconds, 50°C for 5 seconds, and 60°C for 4 minutes. These sequencing reactions were then electrophoresed at the University of Stellenbosch on an ABI 373 sequencer (Applied Biosystems).

5. Re-designing primers

Sequences of microsatellite loci from several individuals were aligned and the original primer sites were noted. New primer sites were designed to areas with no sequence variability a short distance from the original priming site. Primer design aims were: 50% G/C, similar annealing temperatures for the forward and reverse primer pairs, no self-annealing or hairpin sequences, and several C or G at the 3’ end of the primer. Several of these primer parameters could be checked through the ‘Oligonucleotide Properties Calculator’ (www.basic.northwestern.edu/biotools/oligocalc.html). In general, the Forward primer was labeled with the fluorescent dyes FAM or HEX. The Reverse primer was given a 5’ GTTTCTT pigtail which decreases problems with genotyping such as A overhang. If the primer pair did not work, a Reverse primer without the pigtail was used, although the pigtailed Reverse primer worked better in 4 of the 5 redesigned primer sets. After the above steps, the five re-designed primers, along with four unaltered primers, were ready for use to genotype the Southern Pied Babbler population.

Genotyping the Southern Pied Babbler study population

1. PCR reactions for genotyping

Nine microsatellite loci were polymorphic and useful for genotyping the babbler population (Table 2.2). PCR was done with 50 ng of DNA, buffer supplied by the manufacturer (Promega) diluted to 1X, one of 1.0 mM, 1.5 mM or 2.0 mM $MgCl_2$, 10 picomoles of forward and reverse primer, 4 picomoles of each dNTP, and 0.5 units of GoTaq DNA polymerase enzyme (Promega) in a 20 µL reaction. PCR cycle settings were: a denaturation step of 5 minutes at 95°C, amplification of 35 cycles of 95°C for 30 seconds, the specific annealing temperature for 45 seconds, and 72°C for 45 seconds, then an extension step of 72°C for 10 minutes.

Table 2.2: Primer sequences and conditions required for genotyping at nine polymorphic loci in the Southern Pied Babbler.

Name	Primer sequences 5' to 3'	**$MgCl_2$ (mM)**	**T_a (°C)**	**Size (bp)**
Ase55_TB*	F 5’-GTGTGGACTCTGGTGGCC-3’ R 5’-GAATGACAAGACGTGGTTCAAGG-3’	1.5	55	222-240
Calex08	F 5’-AAGAGGGCCAATGTGCTTCTC-3’ R 5’-AAGCGGAATATTAAGTAGAGGCTTCC-3’	2.0	60	200-214
GCGATA10_TB*	F 5'-TCCGTTGTCTTTCCAGTCCCATGG-3' R 5'-GTTTCTTTCTCAGTACAGCATCCATAGC-3'	1.5	58	168-348
GCGATA13	F 5'-GGAGACCAAACTCCTGCCTGC-3' R 5'-GTTTCTTTTCTCTCCAGGAAACCCTTGC-3'	1.0	58	256-332
GCGATA15	F 5'-CAGAGTTTTCACAAAGCCTCTGC-3' R 5'-GTTTCTTGACTATGGAAAATCAATCAAGG-3'	1.5	60	168-208
Pgm3	F 5’-CACTGGGATGAAAAGACCTG-3’ R 5’-TCTCCAGAGCTGGCTATAAAC-3’	2.0	55	203-233
Pij15ZFS_TB*	F 5'-AACCCTTCACCTGCAGCTCTTCC-3' R 5'-GTTTCTTTCGCAAGGTGCTGAACAC-3'	1.5	58	156-160
PmaTGAn42_TB*	F 5'-ACTTCCACATGCCAGTTTCC-3' R 5'-TGTTAAGGCAGAGAGGTGGG-3'	1.5	57	285-345
Ppi2_TB*	F 5’-TGTCCTGTAGCCACTTTCAC-3’ R 5’-GTTTCTTAGAGTGTGAACACAACAGGG-3’	2.0	55	181-199

* Primers redesigned for *T. bicolor* DNA

2. Genotyping individuals

Samples were prepared for scanning on the ABI 373 gene sequencer by electrophoresis of 5 µL of each PCR product on a 1.5% agarose gel to check amplification. PCR product (3 μL) was mixed with 0.5 μL ROX-350 size standard (Applied Biosystems), 1 μL loading buffer (Applied Biosystems) and 2.5 μL formamide. The mixture was flicked gently to mix and heated at 95 °C for 5 minutes to denature the DNA, then snapped cool on ice for a few minutes before loading into a 4% acrylamide gel. Sequencing gels were electrophoresed at 900 to 1200 V for three to four hours. Sequence results were collected with GENESCAN COLLECTION (Applied Biosystems) and analyzed with GENESCAN ANALYSIS (Applied Biosystems). Using the nine polymorphic loci, 319 Southern Pied Babbler individuals were genotyped at 8.9 ± 0.02 loci per individual (Table 2.3). The average number of individuals typed per locus was 317 ± 0.6 and the mean proportion of individuals typed was 0.99. Seven of the nine loci showed moderate to high variation with up to 20 alleles, with an average of 9.7 ±1.9 alleles per locus. Expected heterozygosity (H_E) is a measure of the informativeness of a locus: H_E of less than 0.5 per locus is not optimal for parentage analysis (CERVUS v. 3.0.3 help file, Kalinowski *et al.* 2007). Mean H_E of the nine loci was 0.76 ± 0.04. The mean observed heterozygosity (H_O) was 0.78 ± 0.04, which was slightly but not significantly larger than the mean H_E (paired t-test, t = 2.06, d.f. = 8, P = 0.074). When H_O is found to be significantly higher than H_E, more heterozygotes than expected are present in the population. The polymorphic information content (PIC), a measure related to H_E, was 0.72 for the nine loci (Botstein *et al.* 1980; Hearne *et al.* 1992; CERVUS v. 3.0.3 help file, Kalinowski *et al.* 2007).

Table 2.3: Polymorphic loci used for genotyping the Southern Pied Babbler population with number of individuals genotyped per locus (N), number of alleles per locus (k), observed heterozygosity (H_O), expected heterozygosity (H_E) and *P* values for Hardy-Weinberg equilibrium.

Name	**Originally from sp**	**Reference**	**N**	**k**	H_O	H_E	***P*** value (whole pop)	***P*** value (subset pop)
Ase55_TB	*Acrocephalus sechellensis*	Richardson *et al.* 2000	318	9	0.673	0.709	0.0089	0.097
Calex08	*Charadrius alexandrinus*	Kupper *et al.* 2007	319	3	0.755	0.661	0.0007	0.089
GCGATA10_TB	*Garrulax canorus*	Huang *et al.* 2000	316	16	0.934	0.9	0.2003	0.730
GCGATA13	*Garrulax canorus*	Huang *et al.* 2000	316	20	0.93	0.923	0.8184	0.963
GCGATA15	*Garrulax canorus*	Huang *et al.* 2000	319	10	0.925	0.843	0.0005	0.640
Pgm3	*Petroica goodenovii*	Dowling *et al.* 2003	314	7	0.764	0.747	0.0022	0.802
Pij15ZFS_TB	*Phylloscopus ijimae*	Saito *et al.* 2005	318	3	0.607	0.562	0.4321	0.936
PmaTGAn42_TB	*Parus major*	Saladin *et al.* 2003	315	11	0.844	0.839	<0.0001	0.483
Ppi2_TB	*Pica pica*	Martinez *et al.* 1999	318	8	0.632	0.628	0.0747	0.749
Mean ±SD			317 ±0.6	9.7 ±1.9	0.78 ±0.04	0.76 ±0.04		

The nine loci were next tested for deviation from Hardy-Weinberg equilibrium. A departure from Hardy-Weinberg equilibrium at one locus could indicate a problem with genotyping, such as a null allele or a failure to distinguish alleles consistently, sex-linkage, or dominant inheritance, rendering the locus unsuitable for parentage analysis (CERVUS v. 3.0.3 help file, Kalinowski *et al.* 2007). Alternatively, departures from Hardy-Weinberg equilibrium at several loci may indicate population substructure, such as several sub-populations or closely related family groups. Five of the nine loci showed significant departure from Hardy-Weinberg equilibrium (see *P* values in Table 2.3). This was expected because, in cooperatively breeding species, helpers in a group are usually the offspring of the breeding pair from previous seasons (Ekman *et al.* 2004), creating group-based population sub-structure. It is, however, important to test that these departures do not signify problems in genotyping, or loci that are unsuitable for parentage analysis. To do this, the population sub-structure was effectively removed by using a sub-set of individuals that represented the population and that were presumed to be unrelated to one another (N = 42). The sub-set included the dominant adults from the first year of study, any subsequent adult immigrants from outside the study population that became dominant, and one chick from each of three groups where no dominants were ringed. This analysis showed that all loci were in Hardy-Weinberg equilibrium, thereby ruling out genotyping error as the source of the deviations (see second set of *P* values in Table 2.3). These nine loci are thus useful for parentage analysis.

Molecular sexing of pied babblers

Because adult Southern Pied Babblers are sexually monomorphic, a PCR-based sexing method was used (Griffiths *et al.* 1998). PCR was done with 50 ng DNA, buffer supplied by the manufacturer (Promega) diluted to 1X, 2.0 $MgCl_2$, 10 picomoles of forward (P2: 5’ – TCTGCATCGCTAAATCCTTT – 3’) and reverse (P8: 5’ – CTCCCAAGGATGAGRAAYTG – 3’) primer, 4 picomoles of each dNTP, and 0.5 units of GoTaq DNA polymerase enzyme (Promega) in a 20 μL reaction. PCR cycle settings were: a denaturation step of 94°C for 2 minutes, amplification of 40 cycles of 94°C for 15 seconds, 50°C for 20 seconds, and 72°C for 25 seconds, then an extension step of 72°C for 1 minute. Birds were sexed according to the presence of the PCR products of *CHD-Z* (357 bases) and *CHD-W* (378 bases): males had one band while females had two bands. Differences between sexes could be seen through electrophoresis of PCR products in 3% agarose gel at 85V for three hours, or through electrophoresis in 4% acrylamide gel at 1200V for three hours.

Statistical techniques

Unless otherwise stated, all analyses presented in this thesis were conducted using Genstat 11.1 (VSN International, Rothamstead, UK), and all tests were two-tailed. Parametric tests were used whenever possible, but if data were not normally distributed (as determined using Kolmogorov-Smirnov normality tests), or could not be transformed to ensure normality, non-parametric tests were used. Generalized Linear Mixed Models (GLMMs) and Linear Mixed Models (LMMs) were used to conduct multivariate analyses; these models allow both fixed and random terms to be included in each model, with the inclusion of random terms controlling for repeated measures such as group, brood or individual (Schall 1991). Random terms were dropped from the model if the effect size associated with the random term was zero, as this indicates that the random term has no effect on model predictions (Crawley 2002). LMMs were used when data were normally distributed, while GLMMs were used when data was binomial or proportional. LMMs had a normal distribution of errors and an identity-link function, while GLMMs had a binomial distribution of errors and a logit link function. The method of fitting models to data followed Crawley (2002). A backwards stepwise approach was used: all terms were initially added to the full model, then were systematically removed, and only replaced when their removal significantly reduced the explanatory power of the model. This resulted in a minimal model, which contained only those terms that had *P*-values < 0.05. *P*-values and Wald statistics for non-significant terms were obtained by adding these terms one at a time to the minimal model, thus ensuring that these terms were indeed non-significant. All two-way interactions were tested, but only those that were significant are presented. When models contained multiple terms that were correlated, only the term with the strongest effect on the distribution of data was retained in the full model.

Relatedness calculations

Relatedness values between individuals were calculated using alleles from nine polymorphic microsatellite loci and an algorithm that is less subject to allele frequency bias (Konovalov and Heg 2008a) using the program KINGROUP v2_090218 (Konovalov *et al.* 2004). Several other algorithms are available and are currently in use for this purpose. However, this particular algorithm retrieved known (parent-offspring) relationships best, and was used for all analyses requiring calculated relatedness values (Fig. 2.1). All analyses using relatedness calculations were repeated using the better-known Kinship estimator (Goodnight and Queller 1999), with no effect on the significance of the results.

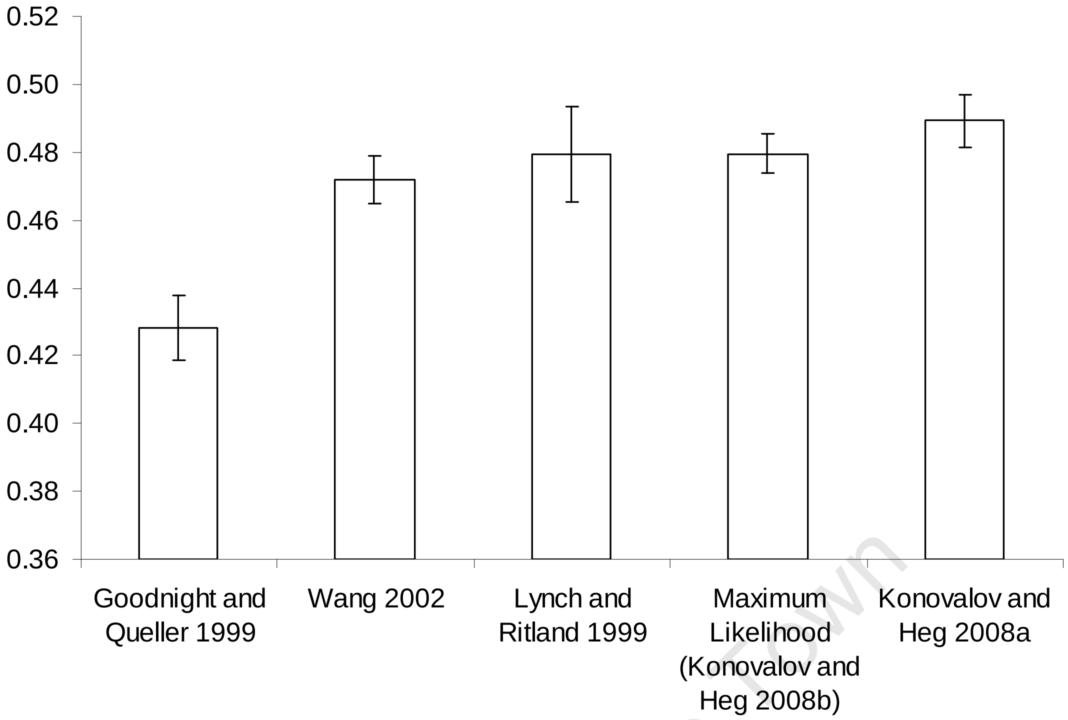

Fig. 2.1: Retrieval of known relationships (parent-offspring, or r = 0.5) by five recently published relatedness estimators. Relatedness was calculated for 128 parent-offspring dyads using each algorithm within the program KINGROUP v2_090218 (Konovalov *et al.* 2004). Means and standard errors of the resulting 128 relatedness estimates are shown. The Konovalov and Heg 2008a relatedness estimator was used in this thesis.

Chapter 3

Breeding system, immigration, and relatedness within the group

Abstract

In many cooperatively breeding species, animals live in groups where some non-breeding subordinate individuals help to raise offspring that are not their own, and reproduction may be monopolized by only a few, dominant group members. Dominants in these species thus gain large direct fitness benefits, but if subordinates covertly mate within the group, they also gain direct fitness benefits. Alternatively, subordinates can gain fitness benefits through helping, which yields indirect benefits (when help is given to related young), and/or direct benefits (e.g. when help increases group size). Accurate assessment of parentage within the group is essential for any investigation of kin selection or reproductive skew, theories which address issues at the heart of cooperative breeding. Parentage information is vital for understanding the direct benefits of becoming a dominant, the direct and indirect benefits accruing to subordinates through breeding or helping, and the extent and outcome of reproductive conflict within groups. In cooperatively breeding Southern Pied Babblers *Turdoides bicolor*, genetic data show that dominant Southern Pied Babblers monopolise reproduction (94.9% of chicks in 98.5% of broods) and subordinates rarely breed. Subordinate breeding is highly predictable in that it occurs only when unrelated breeding partners are present in the group. However, even in the presence of potential partners, very few subordinates successfully reproduce, providing evidence of high reproductive skew. The strong bias towards monogamy results in groups that are highly kin-structured, and successfully acquiring a dominant breeding position leads to a large fitness benefit. Although subordinates may gain direct fitness benefits from group membership, they rarely gain any from reproduction. Instead, due to high intra-group relatedness, subordinates in Southern Pied Babbler groups gain indirect fitness benefits from helping to raise related young.

Introduction

Cooperative breeding occurs when more than two individuals help to raise young in a single brood, and some of the helping individuals are not parents to any of the young (Cockburn 2004). Understanding the cause of help to non-descendant young is a central point of interest to those investigating the evolution of cooperative breeding (Ekman *et al.* 2004). Forgoing one’s own breeding attempt to help others raise young represents an apparent evolutionary paradox, leading to many theories to explain this behaviour (reviewed by Cockburn 1998). For example, help may be given to non-descendant kin in order to further the success of shared genes and gain indirect fitness benefits, the primary tenet of kin selection theory (Hamilton 1964). Alternatively, helping individuals may acquire direct benefits when help increases group size (‘group augmentation’ – Brown 1987, Kokko *et al.* 2001), leading to increased future help from larger numbers of current recipients (‘delayed reciprocity’, Kokko *et al.* 2001, Clutton-Brock 2002), increased chances of survival due to better vigilance, and larger coalition sizes at dispersal (reviewed by Heinsohn and Legge 1999, Russell 2004). High reproductive skew in one or both sexes, such that a small number of individuals monopolize reproduction, characterizes many cooperatively breeding societies (Keller and Reeve 1994, Cockburn 2004). Therefore, a second major research question investigates the factors that are responsible for variation in reproductive skew (Johnstone 2000). Through investigation of the reasons that some individuals do not breed, and instead help to raise non-descendant young, we can better understand how and why cooperatively breeding behaviour occurs.

As a first step in investigating the cause of help, it is necessary to understand the direct benefits that subordinates gain from reproduction in the group, and the indirect benefits that are available to helpful subordinates. These are affected by the breeding system. For example, groups with a monogamous breeding pair and non-breeding helpers can have high levels of relatedness among group subordinates, as seen in Apostlebirds *Struthidea cinerea* (Woxvold *et al.* 2008), Red-cockaded Woodpeckers *Picoides borealis* (Haig *et al.* 1994), Arabian Babblers *Turdoides squamiceps* (Lundy *et al.* 1998), and Laughing Kookaburras *Dacelo novaeguineae* (Legge and Cockburn 2000). In these closely related, kin-structured groups, dominant breeders gain high direct fitness benefits from reproduction, while subordinate helpers do not; however, subordinates do have the potential to reap large indirect benefits from helping (Hamilton 1964, Maynard-Smith 1964, reviewed by Cockburn 1998, Clutton-Brock 2002). When breeding systems are not monogamous, however, relatedness between helpers and recipients decreases, and thus the indirect fitness benefits available to helpers must also decrease according to Hamilton’s equation (Hamilton 1964). Thus the selective power of kin selection in the evolution and/or maintenance of cooperative behaviour could be limited in these species. When a breeding system includes subordinate reproduction, kin selection may play a lesser role in subordinate helping, since these subordinates may be helping to raise their own young. This is seen in Seychelles Warblers, where subordinate female helpers produce 15% of all offspring, thus accruing direct benefits from reproduction (Richardson *et al.* 2001, 2002). The diversity of breeding systems among cooperatively breeding birds was confirmed only with the advent of molecular techniques (reviewed by Cockburn 1998, Griffith *et al.* 2002). Molecular techniques are now essential in identifying the distribution of parentage (or the breeding system) in cooperatively breeding groups. This information then allows estimates of the fitness benefits available to dominants and subordinates, which can help to reveal the role of kin selection in the evolution and maintenance of cooperatively breeding behaviour.

Extensive research has also addressed the question of how reproduction within cooperatively breeding groups is partitioned (Johnstone 2000). The majority of cooperatively breeding species are characterized by offspring that delay dispersal (Langen 2000, Ekman *et al.* 2004), and, consequently, subordinates are often sexually mature adults that may be constrained from breeding in the natal group by the absence of unrelated breeding partners (Koenig and Pitelka 1979). This is because closely related individuals usually avoid breeding together, presumably because of the risk of inbreeding depression (the decrease in fitness that occurs when deleterious recessive alleles are exposed – Pusey 1987, Pusey and Wolf 1996). Inbreeding avoidance may thus allow the dominant pair to monopolise group reproduction, resulting in high reproductive skew (Cooney and Bennett 2000). However, dominants may not be able to monopolise reproduction if subordinates have access to unrelated breeding partners within the group. When this occurs, helpers may attempt to breed alongside the dominant pair, with a concomitant reduction in reproductive skew (Keller and Reeve 1994, Emlen 1995, Cooney and Bennett 2000). For example, in Damaraland Mole-Rats *Cryptomys damarensis*, adding unrelated males to colonies stimulates breeding attempts by subordinate females (Cooney and Bennett 2000). Similarly, subordinate female Meerkats *Suricata suricatta* breed with the group dominant male only when he is unrelated (O'Riain *et al.* 2000, Griffin *et al.* 2003). When subordinate reproduction (or subordinate competition for reproduction) is costly to dominants, dominants may attempt to suppress such behaviour through aggression or mate-guarding, leading to reproductive conflict (Young *et al.* 2006). In summary, it appears that subordinates in many cooperative societies are capable of sexual reproduction but actively avoid inbreeding with close relatives (Cooney and Bennett 2000, O'Riain *et al.* 2000, Chapter 4). Identifying the breeding opportunities that subordinates encounter, and the circumstances under which they breed, provides important information about the amount of reproductive skew observed in groups, the degree of inbreeding avoidance, and why and how subordinate reproduction is suppressed.

Despite extensive study over the last six years, no genetic analyses of Southern Pied Babbler have been conducted. Accurate parentage assignment and description of the breeding system is crucial to understand: (a) the direct fitness benefits of attaining a dominant position, and the direct fitness gained by subordinates through reproduction; (b) the potential indirect fitness benefits of helping as a subordinate group member; (c) the reproductive opportunities for subordinates; and (d) the extent and outcome of reproductive conflict within groups. This information can then be used to understand better the role of kin selection and the origin of the observed reproductive skew in this species. To quantify the breeding system of the babblers, I determined patterns of parentage and measured the mean relatedness within groups using polymorphic microsatellite loci. Life-history data were used to quantify immigration into groups and hence the availability of unrelated breeding partners to subordinate helpers. I asked: (1) who is breeding within each group; (2) how related are group members to one another; and (3) what reproductive opportunities are available to subordinates?

Methods

Study population

Details regarding habituation of groups, blood sampling techniques, and molecular techniques for genotyping can be found in Chapter 2. Many Southern Pied Babbler groups are simple nuclear families, comprising a dominant pair and their retained offspring. Groups of this type are termed simple groups. When a new dominant or subordinate immigrates into the group, such that more than one potential breeding combination of unrelated adults may exist, the group is deemed to be a complex group. Complex groups can contain a potentially breeding subordinate male (and were termed complex-male) or female (complex-female). Complex groups containing potentially breeding male *and* female subordinates are rare and did not produce chicks for parentage analysis, and so are not considered further here.

Parentage analyses

After amplification and genotyping, the program CERVUS v. 3.0.3 (Kalinowski *et al.* 2007) was used to identify parents. Parentage analysis requires information about the loci in the form of the allele frequencies for each locus in the population. However, a common problem when investigating cooperatively breeding species is that the presence of many relatives in the population can bias allele frequencies, causing certain rare alleles to be over-represented. It is important to reduce this bias by attempting to calculate 'true' allele frequencies in the population from a set of unrelated individuals. Therefore an allele frequency file from a sub-set of individuals that were assumed to be unrelated to one another (with a correspondingly lower bias in allele frequencies) was also used to run the analysis. This sub-set included the individuals that were ringed as dominants when first habituating the groups, any subsequent adult immigrants from outside the population that became dominants, and three chicks, each from a group where we failed to habituate or ring any adults (N = 42). Because natal group members do not share dominance (Chapter 4), these individuals are unlikely to be closely related. The parentage analysis was run using first the allele frequency file from the population sub-set described above, and then using the allele frequency file for the entire population. The results were almost identical, so in order to maximize the accuracy of the estimates of rare alleles, the allele frequency file of the entire population was used in the final analysis. For the parentage analysis, only those nestlings that were observed to have hatched into a fully-sampled group with known dominance structure were included. This reduced the sample size to 161 nestlings from 68 broods produced by 12 groups.

For these 161 offspring, the CERVUS program was given no prior information about the social pairing of potential parents. Only adults were included as potential parents. All adult females present in the group at the time that an egg was laid were listed as potential mothers of that chick, and all adult males that may have been present in the population at the time (see below) were listed as potential fathers. Those males that dispersed into the population as adults in later years were also listed as potential fathers, because the true ages of these males were unknown. All natal males that had dispersed or disappeared from the study population were also included, unless there was evidence of their deaths. These data exclude the disappearance of very young fledglings, which have poor motor skills and are completely dependent on older group members for provisioning and hence do not disperse (Raihani and Ridley 2007b).

CERVUS v 3.0.3 first generates an LOD score (LOD = the natural logarithm of the likelihood ratio) for each potential parent-pair with each offspring. This reflects the likelihood, given the allele frequencies, that the genotypes of this pair of potential parents could have led to the genotype of the offspring. Next, it generates the Delta value (Δ) by calculating the difference in the LOD score between the first-choice pair and the second-choice pair. High Δ-values indicate that the first-choice pair is assigned with much higher confidence than the second-choice pair, while lower values of Δ indicate that there is a smaller difference between the two best matches, and thus less confidence of assigning the true parent-pair correctly. To assess whether the first-choice pair can be assigned as the correct pair with high confidence, CERVUS compares the Δ-values with those generated in a simulation. If the observed Δ-value is larger than 95% of all simulated values (strict criterion) or 85% of all simulated values (relaxed criterion), all other pairs except the first choice pair are excluded. CERVUS also indicates best-matches that fail to achieve high Δ-values: these matches are so close in score to the next-best pairings that it is not possible to state which individuals are the true parents.

The simulation mentioned above requires a variety of inputs. The more that these inputs reflect biological reality, the more credible are the confidence levels indicated by CERVUS. These inputs include the number of candidate parents per offspring, the proportion of candidate parents sampled, the proportion of candidate parents that are related to the offspring in question, and by how much they are related. It also includes the allele frequencies of that particular population, the percentage of loci successfully amplified and the estimated genotyping error rate. For Southern Pied Babblers, a simulation of analysis of 100 000 offspring using the analysis option ‘parent pair (sexes known)’ was performed, with the following simulation inputs. Because the babblers are highly territorial, it was assumed that extra-group females would not have opportunities to lay eggs in the group nest: breeding is asynchronous and extra-group individuals are vigorously attacked and chased off whenever they approach a babbler group during the breeding season (Raihani 2008). Therefore, an average of two (range 1 – 5) candidate mothers per offspring, based on the number of adult females present in groups in which chicks were hatched (obtained from continuous life-history records of group composition) was specified for the simulation. The proportion of candidate mothers that were sampled was estimated at 0.95 because although all offspring in this analysis came from fully sampled groups, a margin of error should be included. Because group females may sneak copulations with extra-group males (although this has never been observed), all known adult males present in the population at the time of the offspring's hatching were included as candidate fathers. This number was estimated at 54 (range 29 – 76). The proportion of candidate fathers that were sampled was estimated at 0.80 because most chicks in this analysis were born into groups which were surrounded by other groups with fully sampled adults. CERVUS allows the user to specify what proportion of the candidate parents may be related to the offspring (for example, as aunts, uncles or siblings), and at what level of relatedness. This aids the program in its indication of confidence in the match. The proportion of candidate mothers related to the offspring in question was estimated at 0.90, because immigration of subordinates is very rare and non-immigrant subordinates are likely to be related to at least one of the breeders. These candidate mothers were estimated to be related to the offspring with a mean relatedness of 0.20, approximating half-siblings. The program is designed such that this value does not take the true mother-offspring relatedness ($r = 0.5$) into account. The proportion of candidate fathers that were related to the offspring was estimated at 0.15, based on life-history data on the number of candidate males from the same natal group as one of the parental birds (see below for average estimates of within-group relatedness). Average candidate male relatedness to the offspring was estimated at 0.10. Dispersal distances are short in this species (Chapter 4) and so several of the population's adult males will be distant relatives of these offspring. The proportion of loci typed was set at 0.982 (generated by the program) and the genotyping error rate was set at 0.01.

Relatedness within groups and gene flow between groups

The extent to which the dominant pair monopolises reproduction has a direct effect on levels of intra-group relatedness. High levels of relatedness among group subordinates indicate that a single pair of breeders is monopolising reproduction. Mean values of relatedness within groups and over the population were calculated using the program KINGROUP v2_090218 (Konovalov *et al.* 2004) and an algorithm that is less subject to allele frequency bias (Konovalov and Heg 2008a). Relatedness within groups was compared with the mean relatedness of the population as a whole. Gene flow was quantified between groups using the F-statistic F_{ST} (Wright 1951). F_{ST} is a proportion of the total genetic diversity that separates groups and ranges from 0 to 1: if there is no population sub-structure (i.e. no stable groups), F_{ST} will be close to 0. F_{ST} was calculated using AMOVA (analysis of molecular variation) using the program GenAlEx 6.2 (Peakall and Smouse 2006). The program tests each F-statistic for each year for significance by generating a null distribution of 1000 random permutations of the dataset against which to compare the calculated value. A significant F_{ST} value indicates that there is group-based substructure in the population. Only fully blood-sampled groups were included in this analysis.

Availability of extra-pair breeding partners in the group

Babbler groups are territorial year-round (Ridley and Raihani 2007b). Individuals that attempt to join groups are often initially chased and attacked by group members, especially females (Raihani 2008). Peaceful interactions between babbler groups are very rare (A.R. Ridley, unpublished data), making it difficult for adult subordinates to associate with non-group members while remaining members of their own groups. Although copulation was witnessed between members of the same group (copulation was observed to occur on 53 occasions in 16 groups over 5 years), copulation was never observed between members of different groups. For subordinate adults that delay dispersal, the main route to reproduction lies in immigration of potential breeding partners to their groups. It is thus important to quantify immigration rates of both dominants and subordinates into non-natal groups to determine the availability of reproductive opportunities for philopatric young. Individuals that joined one of the fully sampled study groups during the breeding season and stayed for two weeks or longer were considered to be immigrants (Ridley *et al.* 2008). An individual could immigrate either as a dominant or subordinate. All individuals that were returning former group members, that were an offspring of either of the current dominant pair, or that were never blood-sampled were excluded from the analysis. I measured the proportion of dominants and subordinates each season that were new immigrants in groups, and the proportion of groups each year that became complex groups (with two or more dyads pairs of potentially breeding unrelated adults) due to immigration. From this I could determine the occurrence of reproductive opportunities for subordinates in each year.

Results

Parentage analyses

I was able to determine parentage for 136 offspring from groups with sampled adults (84.5% of 161, Table 3.1). Of the offspring analysed, 102 (63.4%) were determined with 95% confidence, and a further 34 (21.1%) were determined with 85% confidence. All of these matches picked group members as the parents of offspring in the group. I could not allocate parents for 25 offspring. The majority of offspring (94.9%) were the progeny of the dominant male and female of each group (Table 3.1). Some young, however, were the offspring of a subordinate. Subordinate individuals were assigned parentage of only seven offspring (5.1%), and never reproduced in simple groups. Two subordinate females produced a total of five chicks (3.7%). These females lived in complex groups and reproduced with the unrelated dominant male. One subordinate male in a complex group also gained some reproductive success with the unrelated dominant female, fathering two chicks (1.5%). Subordinates were almost always related to one another and there were no instances of subordinates breeding together.

Table 3.1: Summary of parentage analysis showing number of chicks and broods produced by the dominant pair, and by subordinate females and subordinate males.

Group type	Group years	Number of broods	Dominant pair breed	Subordinate female breeds	Subordinate male breeds	Unassigned
Simple	22	39	75 in 39 broods	0	0	21 in 17 broods
Complex - female	6	14	28 in 14 broods	5 in 3 broods	0	0
Complex - male	11	15	28 in 14 broods	0	2 in 1 brood	4 in 4 broods
TOTAL	39	68	129 in 67 broods	5 in 3 broods	2 in 1 brood	25 in 21 broods
Percentage of assigned chicks			94.9%	3.7%	1.5%	

Twenty-five of the offspring could not be assigned parents for a variety of reasons. Twenty matches (12.4% of 161) were best-fit but fell outside the specified significance levels. Although the match scores of each of these 20 parent-pairs were sometimes high, they were not significantly better than those of the second-best matches (low Δ-values). Parents could not be assigned to two further offspring (1.2%) because they were typed at fewer than six loci. Three additional matches (1.9%) were found with 85% confidence but were questionable. One involved a dominant female with a neighbouring dominant male. The dominant female was the only candidate mother. The genotypes of both candidate fathers, when combined with the dominant female, matched to the offspring with no mismatches. The two males were related to one another with R = 0.57 (calculated with KINGROUP v2_090218 – Konovalov *et al.* 2004). Parsimony would indicate that the social father of the offspring (the dominant male of its group) is probably the genetic father and the program chose the neighbouring male because of the high level of relatedness between these two candidate fathers. In addition, the predicted extra-group father was never observed in a non-confrontational association with the predicted mother. However, because the true father could not be discerned with certainty, this offspring could not be assigned parentage. The final two questionable matches involved subordinate females with their fathers. These predicted incestuous matches are discussed in Chapter 4 and are rejected.

What social circumstances surrounded the unassigned offspring? Of the 25 unassigned offspring, four were from complex groups and occurred in four different broods. Two of these offspring could have been the result of a mating between the dominant pair, or between the dominant female and an unrelated adult subordinate male that was a helper in the group, but these two options were indistinguishable. The third unassigned offspring was not genotyped at sufficient loci to be analysed, while the fourth was either the offspring of the dominant pair (first choice) or of the dominant female with a male living several territories away. Twenty-one unassigned offspring from simple groups occurred in 17 broods. Three were rejected at 85%, and one was not genotyped at sufficient loci to be analysed. Of the remaining 17 offspring, the scores received by the first-choice parent-pairs were close enough to the scores received by the next-best parent-pairs to make parentage assignment impossible. Of these, six were first-choice matches involving dominant pairs, four involved incestuous parent-offspring pairings (with a match involving the dominant pair a close second), five involved the dominant female with a first-order relative of the dominant male (father or son) and two involved a subordinate female with a male from a neighbouring group.

Relatedness within groups and gene flow between groups

Mean relatedness within groups was 0.297 ± 0.021 (N = 60 group-years). This intra-group relatedness ranged from –0.231 to 0.516, but was significantly greater than the population mean relatedness of 0.064 (t = 11.31, d.f. = 59, $P < 0.001$), indicating that most groups are highly kin-structured. Each year, F_{ST} values were positive and highly significant (Table 3.2), indicating that groups are separate genetic entities. This pattern was stable and strong for each year, indicating that genetic intermixing between groups is rare.

Table 3.2: F-statistics (F_{ST}) calculated for the Southern Pied Babbler population across five years of study. Values calculated using AMOVA with significance tested with 1000 permutations of the data-set in GENALEX 6.2 (Peakall and Smouse 2006).

Year	N	Groups	F_{ST}	*P*
2003-2004	55	8	0.159	0.001
2004-2005	78	10	0.134	0.001
2005-2006	131	14	0.145	0.001
2006-2007	121	16	0.157	0.001
2007-2008	103	9	0.142	0.001

Availability of extra-pair breeding partners in the group

Sexually mature (> 1 year old post-hatching) subordinates made up 39.7 ± 6.4% (range 24.1 – 62.1%, N = 5 years) of group members. Information is lacking for immigration into groups during the winters of 2004 and 2005 because observers were not present, so data from breeding seasons only (September 1 to May 31) are presented. Individuals that immigrated into groups during winter (June 1 to August 31) and were still present in those groups at the start of the following breeding season were included as immigrants of those breeding seasons. Overall, 47 individuals immigrated into non-natal groups over four years from September 1, 2004 until May 31, 2008 (Table 3.3). Thirty-two adults immigrated into groups where they became the new dominant by overthrowing a current breeder (N = 9) or filling a vacancy left by divorce (when a dominant individual voluntarily disperses to another group) or death (N = 18). For five individuals the transition was not observed, so causality could not be proven. Over the four breeding seasons, 25.3 ± 5.3% of dominants (range = 13.6 – 39.4%) were new immigrants in their groups. Over the same period, 15 subordinates immigrated into non-natal groups. Overall, the rate of subordinate immigration was very low, with only 4.3 ± 0.7% of subordinates present in non-natal groups each year (range = 2.8 – 6.1%, N = 4 years). Each year, 41.1 ± 5.1% of the groups in the study population became complex (range = 31.3 – 50%, N = 4 years) as a result of immigration, creating opportunities for adult subordinates to breed. In general, there were more complex groups than simple groups each year, with 62.1 ± 5.4% of groups containing two or more potential breeding pairs of unrelated adults (some complex groups remained complex for several years but did not experience further immigration events). Totals for this table are not the same as those above in Table 3.2 because this table (3.3) includes all groups for which complete life history was available, but Table 3.2 deals with only those that were fully blood-sampled.

Table 3.3: Summary of immigration into non-natal groups by dominants and subordinates for four breeding seasons. The numbers of subordinates and dominants immigrating, the total population size and the numbers of groups, dominants and subordinates each breeding season is shown. The proportion of subordinates and dominants that were immigrants each year, and the proportion of groups which contained more than one potential breeding pair, are also shown.

Year	N Sub. Imm.	N Dom. Imm.	Pop. size	N Groups	N Sub. total	N Dom. total	Prop. Immig. Sub.	Prop. Immig. Dom.	Complex groups in year *	Complex groups overall
2004	2	6	82	10	57	25	3.5%	24.0%	50.0%	70.0%
2005	3	9	143	16	106	37	2.8%	24.3%	31.3%	56.3%
2006	6	6	142	18	98	44	6.1%	13.6%	33.3%	72.2%
2007	4	11	113	10	85	28	4.7%	39.4%	50.0%	50.0%
Mean	3.8 ± 0.9	8.0 ± 1.2	120	14	87	34	4.3 ± 0.7%	25.3 ± 5.3%	41.1 ± 5.1%	62.1 ± 5.4%

* Groups that became complex due to immigration that season.

Discussion

In many bird species, behavioural observations have under-estimated the frequency of extra-pair parentage (reviewed by Griffith *et al.* 2002). In Southern Pied Babblers, however, extra-pair parentage is very rare, corroborating behavioural observations (Raihani 2008). Dominant pairs within groups are socially monogamous (Raihani 2008) and almost completely sexually monogamous, with rare instances of successful reproduction by subordinates. The monogamy of this breeding system is similar to the Laughing Kookaburra (Legge and Cockburn 2000), the Florida Scrub-Jay *Aphelocoma coerulescens* (Quinn *et al.* 1999) and the Arabian Babbler (Lundy *et al.* 1998). As in these species, there is no confirmed case of extra-group paternity among dominant female babblers. Factors contributing to this may be (1) intense territoriality that limits the opportunities for dominant females to copulate with extra-group males (Quinn *et al.* 1999); (2) dominance positions are rare and dominant females are reluctant to jeopardize their position by cuckolding the dominant male (Legge and Cockburn 2000); or (3) because dominance is fiercely contested, all dominant males may be of high quality and dominant females may do no better by leaving the group to mate (Quinn *et al.* 1999). Because some dominant females have been known to 'divorce' their mates and re-settle with another male (A.R. Ridley, unpublished data), the third option is unlikely: rather, the first and second options seem more likely in this species.

The high levels of monogamy are reflected in the significantly high F_{ST} values, which indicate that groups are genetically distinct entities. The population as a whole is not mating randomly: rather, a few individuals monopolise most breeding opportunities each year. Because reproduction in Southern Pied Babbler groups is skewed so highly towards the dominant pair, dominant positions in groups are extremely valuable because of the considerable direct fitness benefits they confer. In direct contrast, subordinates very rarely acquire direct fitness benefits from reproduction. Mean relatedness values indicate that on average, group members are very closely related to one another, and this is likely due to both the extremely monogamous breeding system and the low immigration rate by subordinates (4.3 ± 0.7%). Similarly low immigration rates occur among cooperatively breeding cichlids *Neolamprologus pulcher*, where only 3.5% of helpers are located in non-natal groups (Dierkes *et al.* 2005), and Red-cockaded Woodpeckers, where 10% of helpers are unrelated to either dominant in their group (Haig *et al.* 1994). The consequence of infrequent immigration and breeding monogamy is that most helpers in Southern Pied Babbler groups are genetically linked to the current brood through at least one of the dominant pair. This creates opportunities for the majority of subordinates to acquire high indirect fitness benefits from helping the offspring of close relatives. As a consequence, kin selection may play a role in the evolution and/or maintenance of cooperative breeding in this species. Even so, subordinate babblers may also help because they can gain direct fitness benefits from membership in a larger group; these possibilities are explored in Chapter 6.

In common with closely related Arabian Babblers (Lundy *et al.* 1998), the few instances of breeding by subordinates were highly predictable in that they occurred only in complex groups where unrelated breeding partners were present. In one group, a subordinate male cuckolded his father by fathering one brood with a newly immigrated (and unrelated) dominant female. In another group, a subordinate female and her mother shared breeding with a new (and unrelated) dominant male for one brood. In a third group, one subordinate female shared reproduction with the unrelated dominant female for two broods in two years with two different (and unrelated) dominant males. However, only a small percentage of subordinates breed successfully each year, despite the fact that apparent breeding opportunities are created in almost half of groups each year. Indeed, in complex groups (where subordinates have a potential breeding partner), only 11.1% of chicks are produced by subordinates. This suggests that, outside of inbreeding avoidance (discussed in Chapter 4), breeding by subordinates may be suppressed by the behaviour of dominants. For example, during the fertile period, dominants increase aggression towards reproductively competitive subordinates, which may suppress subordinate reproduction (Chapter 5). In addition to suppression by dominants, female choice may also constrain reproductive opportunities for subordinate males because dominant females favour breeding with heavier and/or older males (A.R. Ridley, unpublished data), a problem faced by lower-quality males in many bird species (reviewed by Griffith *et al.* 2002). The costs of subordinate competition for reproduction, and the resulting suppression of subordinate reproduction by dominants, are discussed in Chapter 5. Here I have genetically verified that the breeding system is monogamous and that successful subordinate reproduction is very rare, thus setting the stage for investigations of inbreeding and inbreeding avoidance, the factors underlying partitioning of reproduction, and the role of kin selection in helping in Southern Pied Babblers.

Chapter 4

Inbreeding and inbreeding avoidance

Abstract

Breeding with close relatives can cause a decrease in overall fitness and unmasking of deleterious alleles, termed inbreeding depression. In most species, and especially in cooperatively breeding species (where individuals may delay dispersal until long after sexual maturity), inbreeding avoidance mechanisms are expected to have developed. In these species, inbreeding avoidance may play a role in the partitioning of reproduction in social groups, and may also impact patterns of dispersal. Here I use genetic and observational data to show that inbreeding is extremely rare in the cooperatively breeding Southern Pied Babbler. Genetic estimates of inbreeding showed that the population as a whole is slightly outbred. Furthermore, estimates of relatedness between mated pairs are low, indicating that individuals are not breeding with close relatives. Sex-biased dispersal as an inbreeding avoidance mechanism is not well supported for this species. Rather, individuals appear to avoid inbreeding mainly through dispersing away from the natal group, and by means of direct kin recognition, as evidenced by their avoidance of courting relatives. In addition, individuals inherit a dominant position in the natal group only when a suitable, unrelated breeding partner is present. The array of inbreeding avoidance mechanisms found in the Southern Pied Babbler is much like that found in other cooperative breeders, where inbreeding avoidance is well developed at small spatial scales (i.e. within the group). Errors may be possible at the larger spatial scale of the subpopulation, however, resulting in occasional pairings of distant kin.

Introduction

Breeding with close relatives can expose individuals to a variety of problems, including the unmasking of deleterious rare alleles and a decrease in overall fitness, a phenomenon termed inbreeding depression (Pusey 1987, Pusey and Wolf 1996, reviewed by Keller and Waller 2002). It has become clear that inbreeding and inbreeding depression do occur in wild populations, although their effects can vary across taxa, populations and environments (Keller and Waller 2002). In populations where deleterious alleles have been purged, it has been suggested that inbreeding could become beneficial: for example, in the cichlid fish *Pelvicachromis taeniatus*, individuals prefer relatives as mates, possibly a legacy of its isolated river system of only a few kilometers (Thünken *et al.* 2007). In some species, inbreeding is common, though not beneficial: the annual frequency of close inbreeding in a population of

Common Moorhens *Gallinula chloropus* has been estimated at 16%, although inbred offspring have low survival rates (McRae 1996, Koenig and Haydock 2004). Similarly, an insular population of Song Sparrows *Melospiza melodia* has also been shown to inbreed regularly, with high associated inbreeding depression (Keller 1998). In the Naked Mole-Rat *Heterocephalus glaber*, close inbreeding has been observed in captive colonies, and wild populations have extremely high intra-colony relatedness, also implying close inbreeding (Reeve *et al.* 1990, but see Braude 2000, Ciszek 2000). While inbreeding may be very rare in most species, under certain environmental or social conditions, avoidance of inbreeding is not always possible. For example, Grey-Crowned Babblers *Pomatostomus temporalis* are mostly outbred, but male helpers occasionally inherit a breeding vacancy and breed with a closely related dominant female (Blackmore and Heinsohn 2008). Similarly, when groups of Acorn Woodpeckers *Melanerpes formicivorus* lack a breeding male, subordinate males sometimes mate with their mothers or sisters (Haydock *et al.* 2001). Thus, although inbreeding is generally rare, it is not uncommon in certain cooperatively breeding species (Koenig and Haydock 2004).

The advent of molecular tools has enabled increasingly accurate investigation of inbreeding in cooperatively breeding species (Koenig and Haydock 2004). For example, because of incestuous social pairings, the Splendid Fairy-Wren was thought to engage extensively in inbreeding (Rowley *et al.* 1986), but subsequent molecular analyses have shown that the risk of inbreeding is mitigated by a high rate of extra-pair fertilizations (Brooker *et al.* 1990). Similarly, in groups of cooperatively breeding Meerkats, subordinate males sometimes inherit dominance positions alongside their mothers in their natal groups: in these instances, molecular studies, combined with behavioural observations, showed that these dominant females mate with unrelated males from other groups (Griffin *et al.* 2003). Although inbreeding can be difficult to detect using molecular methods because most cooperatively breeding groups consist of close relatives bearing the same set of alleles (McRae and Amos 1999), these genetic techniques have by and large aided an understanding of the extent of inbreeding, and are a necessity for thorough analysis of inbreeding behaviour in cooperatively breeding species.

Cooperatively breeding species may be particularly at risk of inbreeding because of the very nature of their breeding ecology. Some of these species are obligate cooperative breeders (i.e. they require helpers to successfully breed), and breeders and helpers live year-round in relatively small, highly related social groups (Emlen 1997, Koenig and Haydock 2004). Subordinates in these systems are often retained natal offspring that delay dispersal (Ekman *et al.* 2004). In some species both sexes remain in the natal group well beyond reaching sexual maturity, at which point the only available breeding partners in the group may be close relatives (Cockburn 1998, Ekman *et al.* 2004). In addition, many cooperatively breeding species are highly sedentary and disperse over short distances (Zack 1990), resulting in high local densities of close relatives. It is therefore likely to be important for obligate cooperative breeders to be able to identify relatives in the natal group and avoid breeding with them. By the same token, when they do disperse (over short distances) they should continue to avoid pairing with relatives. Inbreeding avoidance may also play an important role in the partitioning of reproduction in cooperatively breeding groups, because adult subordinates in groups formed through philopatry may only rarely encounter unrelated breeding partners (Koenig *et al.* 2009).

Inbreeding avoidance mechanisms are expected to have developed in naturally out-crossing populations or species (Keller and Waller 2002). One such mechanism is for individuals to avoid breeding with those they recognize as kin; such kin recognition can be based on prior association (Hamilton 1964, Komdeur and Hatchwell 1999). Such a mechanism is viable in group-living species in which groups comprise mainly relatives: individuals can then avoid breeding with those that were present in the social group during their dependent period (‘learning by association’ – Komdeur and Hatchwell 1999). Another mechanism for inbreeding avoidance is sex-biased dispersal, such that one sex disperses more frequently or farther than the other (Greenwood 1980). For example, in many bird species, males are mainly philopatric and females disperse from the natal territory to breed (Greenwood 1980). Sex-biased dispersal could function as an inbreeding avoidance mechanism if it results in opposite-sex relatives becoming spatially separated or if relatedness predictably decreases over geographic distance for one sex (Woxvold *et al.* 2006). Although sex-biased dispersal is generally accepted as a mechanism to avoid inbreeding, it has been argued that sex-biased dispersal occurs mainly as a function of sex differences in dispersal costs or the strength of local competition for resources or mates (Greenwood 1980, Perrin and Mazalov 2000), or sex biases in mortality creating more breeding vacancies for one sex than the other (Cockburn *et al.* 2003). Debate continues over the reasons why sex-biased dispersal is widespread (i.e. predominantly male dispersal in mammals and female dispersal in birds – Greenwood 1980, Pusey 1987). Investigating inbreeding avoidance mechanisms could allow a better understanding of observed patterns of mating and dispersal, as these may be driven by forces other than the avoidance of inbreeding (Berg *et al.* 2009).

Inbreeding avoidance mechanisms, such as sex-biased dispersal, have also been investigated using molecular methods. This is extremely useful because in most field studies it is difficult to distinguish between individuals that have dispersed beyond the limits of the study area and those that have died (Koenig *et al.* 1996). Molecular tools can help investigate sex-biased dispersal by examining sex differences in gene flow and relatedness in populations. For example, the correlations between the genetic and geographic distances between individuals have revealed female-biased dispersal in cooperatively breeding White-Breasted Thrashers *Ramphocinclus brachyurus* (Temple *et al.* 2006), Superb Fairy-Wrens *Malurus cyaneus* (Double *et al.* 2005) and Apostlebirds (Woxvold *et al.* 2006). The same techniques demonstrate male-biased dispersal in cichlids (Stiver *et al.* 2007) and sticklebacks *Gasterosteus aculeatus* (Cano *et al.* 1998). In many of these species, dispersal distances may be so short that both sexes appear philopatric, or the nature of the habitat may prevent direct observation of dispersal (e.g. in fish). Overall, molecular techniques can reveal complexities that simple observation may overlook.

In this study I use behavioural observations and genetic data to examine a population of Southern Pied Babblers for evidence both of inbreeding and of inbreeding avoidance mechanisms. Within a group, a large proportion of subordinates are sexually mature adults, both sexes may remain in the natal group for several years, and mating between related group members does not appear to occur (Chapter 3). To investigate inbreeding, I examine the circumstances under which individuals gain dominance (in both natal and non-natal groups), the relatedness between breeding pairs, and the population's inbreeding coefficients (F-statistics). To investigate inbreeding avoidance mechanisms, I first investigate kin recognition through association by examining courting and roving behaviour for evidence of avoidance of natal-familiar breeding partners (i.e. potential partners that were present in the natal group during the dependent stage – Zahavi 1990). I then assess sex-biased dispersal as an inbreeding avoidance mechanism by examining sex differences in the occurrence of dispersal, dispersal distance and immigration into non-natal groups. I also use group genetic and geographic data to investigate population genetic structure for further evidence of sex-biased dispersal.

Methods

Study population and polymorphic microsatellite loci

Habituation techniques, the study site and population, and molecular techniques are described in Chapter 2. The following analyses encompass behavioural observations collected between July 2003 and April 2009, and genetic data from individuals that hatched before May 2008.

Evidence of inbreeding: systematic inbreeding

The propensity for systematic inbreeding can be determined from genetic patterns within the population. I quantified the extent of inbreeding within babbler groups using AMOVA (analysis of molecular variation – Excoffier *et al.* 1992, Huff *et al.* 1993, Peakall *et al.* 1995, Michalakis and Excoffier 1996) to calculate F-statistics (Wright 1951) using the program GenAlEx 6.2 (Peakall and Smouse 2006). The program tests each F-statistic for each year for significance by generating a null distribution of 1000 random permutations of the dataset against which to compare the calculated value. I first measured F_{IS} to investigate the occurrence of inbreeding within the group. F_{IS} is the inbreeding coefficient of individuals relative to the group and compares the observed heterozygosity of individuals to the heterozygosity expected within the group. F_{IS} ranges from -1 to 1. Significant positive values of both F_{IS} and F_{IT} indicate that individuals are more homozygous (or 'inbred') than expected, while significant negative values indicate individuals are more heterozygous (or 'outbred'). I next measured F_{IT}, the inbreeding coefficient of individuals relative to the total population. F_{IT} (which also ranges from -1 to 1) compares the observed heterozygosity of individuals to the heterozygosity expected from the entire population.

I investigated potential inbreeding within mated pairs (the dominant pair in each group) by measuring their relatedness. This population of babblers has been studied since 2003, but because dominance tenures can be long, the origin of many dominant individuals was unknown (because they were already adults when the study began), so I could not use pedigree-calculated values for measurements of relatedness for dominant pairs. I calculated relatedness (R) between pair members using nine polymorphic microsatellite loci and the program KINGROUP v2_090218 (Konovalov *et al.* 2004), and used an algorithm that reduces allele frequency bias (Konovalov and Heg 2008a). I repeated all analyses involving relatedness calculations using the KINSHIP estimator of Goodnight and Queller (1999), but because there was no difference in the results, and because the estimator of Konovalov and Heg (2008a) recaptured known relationships more accurately (Fig. 2.1), I present results from it alone.

To investigate relatedness and mate choice, I compared the relatedness of mated pairs (the dominant male and female) to the relatedness between each mated-pair member and all other opposite-sex adults in the population at the time. If the relatedness of mated pairs is significantly less than that of all other possible pairings for these individuals, it shows that individuals are avoiding closely-related mates. If the relatedness of mated pairs is significantly higher than that of all other possible pairings for these individuals, then individuals are seeking closely-related mates and inbreeding may be common. If the average relatedness between mates does not differ from that of all other possible pairings for these individuals (null hypothesis), then any opposite-sex adult is likely to be chosen as a mate, and the birds are not demonstrating any ability to distinguish degrees of relatedness of kin (Ciszek 2000). Because relatedness estimates are by their nature based on pairs of individuals, analyses ‘sample’ each individual more than once and data are thus non-independent. This non-independence can be controlled for by using a randomization /permutation test, which generates a distribution of mean differences between two groups of values. This distribution is created by randomly assigning the data values into the groups being compared and calculating the mean difference between the simulated groups. This process (permutation) is repeated N times (recommended 1000 or more – Jadwiszczak 2009) to create a distribution of simulated mean differences, against which the observed values can be compared and P-values generated. I used a two-sample randomization /permutation test within the program RUNDOM PRO 3.14 (Jadwiszczak 2009) to compare relatedness of mated pairs to the relatedness between each member of each pair and all other opposite-sex adults in the population at the time, with 100 000 permutations (*sensu* Stiver *et al.* 2008).

In cooperatively breeding species, the presence of highly related older siblings in the pool of potential parents may confound parentage prediction software, leading to false matches (McRae and Amos 1999). Two cases where CERVUS identified inbred pairs (dominant males mating with their subordinate daughters) were investigated by using body mass changes of the predicted mothers. By measuring body mass in the periods leading up to and following egg-laying, it is possible to detect whether the putative mothers could indeed have been the true mothers. First, body mass changes were quantified during egg-laying for confirmed breeding females, and these were compared to the body mass changes of confirmed non-breeding females. Breeding was confirmed from behavioural observations and unambiguous parentage prediction results (Chapter 3). These measures were then used for subsequent investigation of possible inbreeding activity by subordinate females. Females were weighed in each group prior to breeding to monitor their reproductive status. Mean pre-foraging morning body mass (taken at first light) of confirmed breeding and non-breeding adult females were used to compare body mass during the egg-laying period with body mass during the non-egg-laying period. A description of the weighing procedure can be found in Ridley and Raihani (2007a, 2007b). Group size, group identity, rainfall and the presence of dependent fledglings were controlled for by comparing the mass of breeding and non-breeding adult females from the same groups, measured during the same breeding attempts on the same mornings. Body mass differences between the two time periods were compared for breeding and non-breeding females. Egg-laying days were determined from life-history records by backdating 2-3 days from incubation start dates, and non-egg-laying periods were defined as the period two weeks prior to this date.

Inbreeding avoidance: avoiding natal-familiar breeding partners

To determine whether birds avoided familiar relatives as mates, I first investigated the extent to which inbred mated pairs (individuals that were both dominant at their natal group) occurred. For each breeding vacancy that occurred, I recorded the sex of the missing bird, the source of the individual that filled the vacancy (natal or immigrant), and whether the group already contained an unrelated adult subordinate of the appropriate sex to fill the breeding vacancy.

Courtship behaviour in this species occurs primarily as a prelude to copulation and involves either males aerially chasing females, or mutual presentation of nesting material (Raihani 2008). Such behaviour often occurred between group members during the breeding season but was never observed between members of different groups. If courtship behaviour within groups involves relatives this could indicate a lack of avoidance of natal-familiar breeding partners. I examined the occurrence of incestuous courtship by comparing levels of relatedness between individuals observed in courtship behaviour and between those sexually mature birds within the group which undertook no courtship. Relatedness between pairs was calculated using the program KINGROUP v2_090218 (Konovalov *et al.* 2004) and Konovalov and Heg's (2008a) algorithm. I used a two-sample randomization/permutation test using the program RUNDOM PRO 3.14 (Jadwiszczak 2009) with 100 000 permutations (*sensu* Stiver *et al.* 2008) to compare relatedness between pairs of courting birds within groups to relatedness between pairs of non-courting, adult, opposite-sex birds in groups.

I then investigated the avoidance of relatives as mates at the sub-population level by examining roving behaviour. Roving represents an individual's search for a breeding opportunity. This behaviour, which is often a prelude to dispersal (Raihani 2008), occurs when individuals leave their natal group and interact (by fighting, vocalising or displaying) with individuals in non-natal groups. Between such forays, roving individuals often return to their natal group (Raihani 2008). Rovers may visit several different groups, which they may enter either by finding a breeding vacancy or by winning a fight with the existing same-sex dominant. Potentially, roving could serve to help birds gain information about the presence of relatives, available breeding vacancies and partners in non-natal groups. To determine this, I compared levels of relatedness between rovers and opposite-sex dominants (their prospective mates) at the groups they visited, to relatedness between rovers and opposite-sex dominants at the groups they did not visit. Again, relatedness between pairs was calculated using the program KINGROUP v2_090218 (Konovalov *et al.* 2004) and Konovalov and

Heg's (2008a) algorithm. I used a two-sample randomization/ permutation test using the program RUNDOM PRO 3.14 (Jadwiszczak 2009) to compare these measures of relatedness. I also used life-history data to examine whether individuals rove at groups containing natal-familiar prospective mates more or less often than expected by chance, using a GLMM with a binomial distribution of errors and a logit link function. The data-set included every ringed individual that was seen roving per season, all groups present in the population that season and their distances from the home group of the rover. I included in this analysis only those individuals born into fully sampled groups where the life history of all other group members was known. The number of times that an individual was seen roving at a particular group was set as the response variable and the number of times that individual was seen roving at all groups over the season was set as the binomial total. Sex of the rover, size of the 'host' group, distance from home group and natal familiarity (whether the two individuals had lived in the same group during the dependent period of either of them) with the prospective mate (the opposite-sex dominant) were included as explanatory terms, while year and host-group identity were included as random terms. Because the distance of the host group from the rover's home group was highly correlated with whether the host group contained a familiar prospective mate (familiar prospective mates were found closer to the home group), the data-set was restricted to groups within 1.45 km of the home group, roughly the mean of natal dispersal distance (see below). A second correlation was found between host group size and distance from the rover's home group. Thus the data-set was further restricted so that only host groups smaller than eight members were included. Data come from 37 roving episodes by 35 rovers at 12 groups over five years.

Inbreeding avoidance and sex-biased dispersal

Sex-biased dispersal may lead to the physical separation of related potential mates (Greenwood 1980). I investigated whether one sex was traveling farther than the other during dispersal, such that related, opposite-sex individuals were avoiding inbreeding by settling far from one another. Dispersal was considered to have occurred if a ringed individual from the study population left one group, joined another group and stayed there for at least two weeks (Ridley *et al.* 2008). Movements of individuals returning to natal groups from non-natal groups were not considered to be dispersal, nor were movements of individuals that followed their parents when the latter gained new dominance positions in other groups. Following Greenwood (1980), dispersal was categorized as 'natal' if the individual left its natal group to enter another group, while it was categorized as 'breeding' if the individual went from one non-natal group to another. I first examined whether dispersal itself was biased with respect to sex. For every year, I calculated the sex ratio of adults in the population and used a t-test to compare it to the sex bias of dispersing individuals (both natal and breeding) to determine whether biases in dispersal were simply the result of an unequal sex ratio in the population. Additionally, I measured sex bias in immigration by all birds into established study groups. Immigration includes all individuals (both ringed and previously unknown birds) entering previously established groups, and may be a better measure of the consequences of dispersal than movement of individuals out of groups. In the case of the latter, when a bird leaves a group but is not seen again, it is impossible to determine whether this is a dispersal event (to a receiving group outside the study area) or a mortality event (Koenig *et al.* 1996). Immigration occurred when individuals entered established groups in the study population, and stayed at least two weeks. Individuals could join established groups as either subordinates or as dominants.

I also examined whether one sex dispersed farther than the other. When an individual dispersed, I measured the distance between the group it left and the group it entered. I used GPS coordinates averaged over the month that the individual was last seen in the group it left, and the month that it was first seen in the new group. Some dispersers entered unhabituated groups outside the study population: in these cases the single GPS point of where the disperser was found was used to calculate distance. I investigated how disperser sex and dispersal type (from a natal or non-natal group) affected dispersal distance. To do this I used a LMM with dispersal distance as the response variable. The sex of the disperser and the type of dispersal (natal or breeding) were included as explanatory terms, and disperser identity was included as a random term. Data include 58 dispersal events by 46 individuals dispersing between 31 groups over six years.

Because recorded dispersal events and distances are usually an underestimate of true dispersal incidences and distances (Koenig *et al.* 1996), genetic data are useful as an alternative measure of sex differences in population structure and dispersal. To determine whether one sex was dispersing more predictably than the other, I examined geographic patterns of relatedness for each sex. In a continuous population, short dispersal distances are expected to result in a pattern of genetic "isolation by distance" (IBD – Wright 1943). I analyzed males and females separately to expose any differences in population structure that could potentially identify differing dispersal strategies (Woxvold *et al.* 2006). IBD can be analyzed by plotting genetic similarity between any two dominant males or females in a population against the geographic distance between these two (Bohonak 2002). Because dominants could move between groups during the year (A.R. Ridley, unpublished data), I used a "snapshot" of the population taken on January 1 of each year from 2004 until 2008. Genetic similarity between pairs of dominant males and pairs of dominant females was estimated by calculating relatedness (R) between pairs using the program KINGROUP v2_090218 (Konovalov *et al.* 2004) and Konovalov and Heg's (2008a) algorithm. Geographic distance between these pairs of dominant males and dominant females was calculated from group GPS coordinates for each month of January from 2004 to 2008 (one coordinate from each day of observation was used to calculate a mean January coordinate for the group: average number of daily coordinates used to calculate the mean coordinate was 7.6 ± 0.6). I used Mantel tests with 10 000 permutations to compare geographic distance (log km) to genetic distance (relatedness – R), using the program IBD v. 1.52 (Bohonak 2002).

Results

Evidence of inbreeding: systematic inbreeding

In each year, both the inbreeding coefficient of the individual to the group (F_{IS}) and the individual to the total population (F_{IT}) were negative but insignificant (Table 4.1), indicating that there is no systematic inbreeding within the population.

Table 4.1: F-statistics (F_{IS} and F_{IT}) for each year of study of the Southern Pied Babbler population. Values were calculated using AMOVA with significance tested with 1000 permutations of the data-set in GENALEX 6.2 (Peakall and Smouse 2006).

Year	N	Groups	F_{IS}	*P*	F_{IT}	*P*
2003-2004	55	8	-0.251	1.000	-0.053	0.957
2004-2005	78	10	-0.225	1.000	-0.061	0.995
2005-2006	131	14	-0.185	1.000	-0.013	0.779
2006-2007	121	16	-0.197	1.000	-0.009	0.672
2007-2008	103	9	-0.191	1.000	-0.022	0.857

In this species, mated (dominant) pairs are almost invariably the pairs that produce offspring (Chapter 3). The mean relatedness of mated pairs was -0.009 ± 0.037 (N = 37 pairs). Pairs that produced offspring together (usually the mated, dominant pair, but very rarely a subordinate with a dominant) had an average relatedness of 0.018 ± 0.038 (N = 31 pairs). There was no difference in the mean relatedness between mated pairs and pairs that reproduced (two sample randomization/ permutation test, *P* = 0.622). While relatedness between mated pairs was low, the mean relatedness of mated pairs was not significantly different from the mean relatedness of the member of each pair with all other opposite-sex adults in the population in any year (unable to reject the null hypothesis: two-sample randomization/ permutation test, Table 4.2). This means that while Southern Pied Babblers do not mate with close relatives within the group, individuals neither seek out nor avoid relatives as mates in the sub-population outside the group: individuals choose mates who are on average neither more nor less related to them than the population mean.

Table 4.2: Relatedness (R) of mated and unmated pairs of opposite-sex adult Southern Pied Babblers per year.

Year	N mated pairs	R mated pairs	N unmated pairs	R unmated pairs	*P*
2003 – 2004	8	0.080 ± 0.072	166	0.103 ± 0.019	0.814
2004 – 2005	13	0.043 ± 0.064	389	0.127 ± 0.011	0.188
2005 – 2006	18	0.059 ± 0.052	716	0.093 ± 0.009	0.551
2006 – 2007	25	0.019 ± 0.041	1455	0.074 ± 0.006	0.245
2007 – 2008	15	-0.010 ± 0.058	333	0.094 ± 0.012	0.622

Investigation of two cases of putative inbreeding predicted by the program CERVUS (Chapter 3) revealed that this was very unlikely to have occurred. These predictions involved two subordinate females breeding with their fathers and received significance scores of over 85%. Breeding females become significantly heavier during the egg-laying period than do non-breeding females (t-test, t = 3.49, d.f. = 22, *P* = 0.002, N = 12 breeding and 12 non-breeding females in 12 groups – Fig. 4.1). The two subordinate females predicted to be breeding with their fathers weighed an average of 78.8 ± 0.8 g and 80.2 ± 0.7 g during the egg-laying period. This was a gain of only 0.3 g and 1.4 g respectively above their mean weights during the non-egg-laying period. These body masses are less than the minimum body mass of any breeding female at egg-laying in the population (Fig. 4.1), and less than the average weight gain of egg-laying females (egg-laying females gained 8.1 ± 1.1 g). These females were most likely identified as mothers because of shared genetic identity through descent, not because they were true mothers. In both cases, CERVUS identified the dominant female and male of the group as the second choice parent-pair. These putative instances of inbreeding can thus be rejected.

Fig. 4.1: The effect of rank and breeding period on body mass (g) of 24 female Southern Pied Babblers in 12 groups. Mean values ± SEM were generated from raw data.

Inbreeding avoidance: avoiding natal-familiar breeding partners

Relatives were never observed to inherit dominance together in the same group. There were 33 breeding vacancies recorded between July 2003 and May 2009 (N = 79 group-years). These vacancies occurred in groups that lost a dominant through divorce or death. There was no sex bias in the availability of breeding vacancies: of the 33 vacancies, 15 were male and 18 were female (Binomial test: P = 0.728). Of these 33 vacancies, 11 were inherited by natal subordinates and 22 filled by unrelated immigrants. There was no sex bias in the likelihood of these outcomes: 6 of 11 subordinate inheritors were male (Binomial test: P = 1.0) and 9 of 22 immigrants were male (Binomial test: P = 0.523). A far better predictor of whether a vacancy would be filled by immigration or by inheritance was the presence in the group of an adult subordinate of the correct sex that was unrelated to the remaining dominant. Of the 33 vacancies, 13 occurred in groups where a subordinate could inherit without inbreeding. Of these 13 vacancies, 11 were inherited by a natal subordinate, and only two were filled by immigrants. The latter two vacancies occurred in the same group (one dominant female left the group and her successor died), and the dominant male in question had immigrated into the group during the dependent period of the adult female subordinate, suggesting that the natal female did not inherit because she identified the dominant male as a close relative. The female subordinate in this case made no effort to resist the immigration of either of the new dominant females. Twenty vacancies occurred in groups with no unrelated adult subordinate of the correct sex. All of these vacancies (100%) were filled by unrelated immigrants. Thus, whether immigration or inheritance occurred depended on the presence in the group of an appropriate (i.e. unrelated) inheritor (N = 2/13, 20/20; Binomial test, $P < 0.001$).

Courtship between related adults was very rare. Courtship was most commonly observed between the dominant pair (85.1% of 242 observed courting interactions, N = 19 groups). In addition, however, courtship occasionally occurred between dominants and subordinates (12.8%), and, very rarely, between subordinates (2.1% of 242 interactions). In the latter case, three of the five such interactions were directed towards fledglings or juveniles by older helpers and may have been dominance-related rather than sexual interactions. Levels of relatedness strongly predicted courtship behaviour independently of social status; adult courtship occurred most commonly between unrelated group members (two-sample randomization /permutation test, $P < 0.001$), with a significant difference in the mean levels of relatedness between courting (N = 46) and non-courting (N = 480) adult opposite-sex dyads (Fig. 4.2). This suggests that, within the group, inbreeding avoidance prevents adult subordinates from courting their relatives.

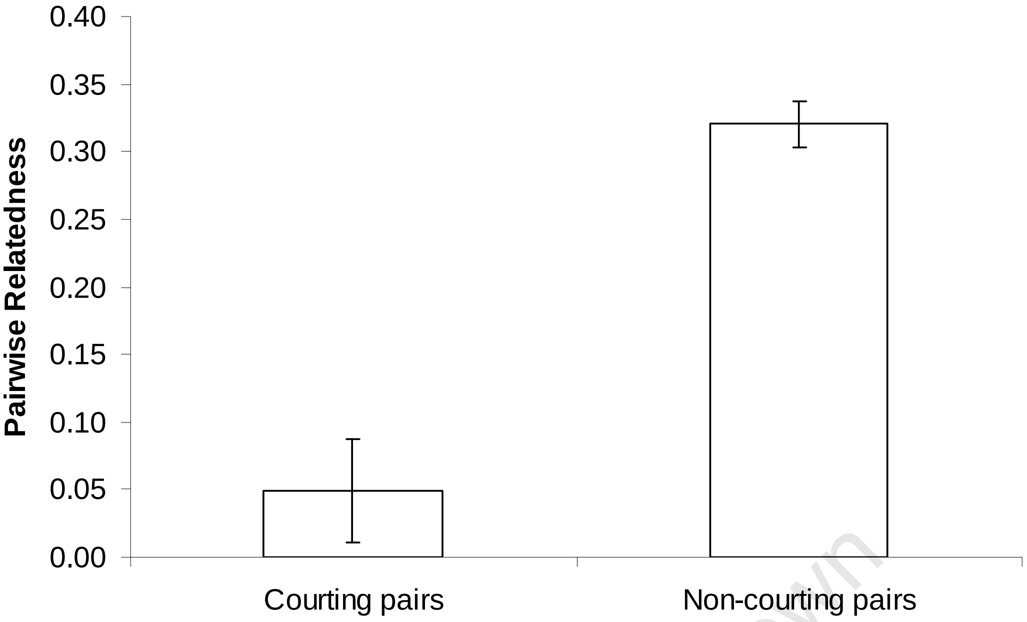

Fig. 4.2: Relationship between the occurrence of courtship behaviour and relatedness between 526 adult heterosexual dyads within 19 groups of Southern Pied Babblers. Means ± SEM are generated from raw data.

Ringed individuals were observed roving on 148 occasions. Most of these incidences (120 of 148) involved females. Levels of relatedness (between the rover and his/her potential mate) could be estimated in 133 of these cases, involving 88 pairs of potential mates. Mean relatedness of rovers to their prospective mates was 0.053 ± 0.021 (N = 88 pairs), not significantly different from the mean relatedness of rovers to opposite-sex dominants in groups where they did not rove (N = 592; two-sample randomization/ permutation test, P = 0.450). This indicates that rovers are visiting groups containing prospective mates that are as related to them as expected by random chance. Although rovers most frequently visited groups close to their home groups, after controlling for this factor, group size, familiarity with prospective mates and the sex of the rover did not affect the frequency with which individuals roved at particular groups (Table 4.3). During these forays, rovers did not avoid visiting groups containing familiar prospective mates, suggesting that they may be gathering information about the locations of known relatives in neighbouring groups.

Table 4.3: GLMM of the terms affecting roving behaviour by Southern Pied Babblers. The number of times that an individual was seen roving at a particular group was set as the response term and the number of roving instances by that individual over the season was set as the binomial total. Data come from 37 roving episodes by 35 rovers at 12 groups over 5 years. Year and identity of host-group at which individuals roved were set as random terms.

Model term	χ^2	***P***
Full model		
Distance	22.09	< 0.001
Group size	0.36	0.550
Familiarity	0.17	0.681
Sex	0.14	0.707
Minimal model	**Effect**	**SE**
Constant	-2.05	0.69
Distance	-3.58	0.76

Inbreeding avoidance and sex-biased dispersal

Sex-biased dispersal can result in inbreeding avoidance through physical separation of opposite-sex relatives. Dispersal was recorded for 36 females and 22 males that moved into non-natal groups and remained there for at least two weeks. Relative to the population sex-ratio, there was no sex bias in overall dispersal or in natal dispersal each year (two-sample t-tests: overall dispersal t = 1.19, d.f. = 5, *P* = 0.29; natal dispersal t = 0.25, d.f. = 5, *P* = 0.81), but there was an (insignificant) trend towards a female sex bias in breeding dispersal (Mann-Whitney U test, U = 46.0, *P* = 0.08, Table 4.4). Immigration, which can potentially provide a better measure of sex differences in movement around the population by removing the ambiguity between death and dispersal, revealed that 44 females (64.7% of all immigrants) and 24 males (35.3%) immigrated into established study groups over six years. When comparing this to the mean sex ratio of the population in the same time period, there was no sex bias in immigration (Binomial test, *P* = 0.272). However, when considering only the immigration of dominants, there was a significant female bias (31 of 43 new dominants were female – Binomial test: *P* = 0.042). Subordinate immigration was not sex biased: 13 of 26 immigrating subordinates were female (Binomial test: *P* = 0.432).

Table 4.4: Sex ratio of the Southern Pied Babbler population, overall dispersal, natal dispersal and breeding dispersal between September 2003 and April 2009.

	Population			Overall dispersal			Natal dispersal			Breeding dispersal		
YEAR	Males	Total	Bias	Males	Total	Bias	Males	Total	Bias	Males	Total	Bias
2003-4	13	32	0.41	0	2	0	0	2	0	0	0	*
2004-5	19	48	0.40	2	6	0.33	2	5	0.40	0	1	0
2005-6	34	65	0.52	3	10	0.30	4	9	0.44	0	1	0
2006-7	48	99	0.48	10	18	0.56	9	11	0.82	1	7	0.14
2007-8	18	46	0.39	6	11	0.55	4	7	0.57	2	4	0.50
2008-9	21	63	0.33	1	11	0.09	1	9	0.11	0	2	0
MEAN RATIO	0.42 ± 0.03			0.30 ± 0.09			0.39 ± 0.12			0.13 ± 0.10		

The type of dispersal (natal *vs* breeding) strongly influenced dispersal distance, although disperser sex had no effect (Table 4.5). Males moved an average of 1.29 ± 0.18 km from their previous group, while females moved an average of 1.78 ± 0.26 km. Natal dispersal averaged 1.58 ± 0.17 km (about two territory widths), compared to 0.89 ± 0.14 km for breeding dispersal (about one territory width). Both sexes dispersed farther from their natal groups than they did from non-natal groups. Overall, although dispersal in Southern Pied Babblers does not physically separate opposite-sex relatives, females successfully disperse as dominants from group to group more often than do males (see above).

Table 4.5: LMM of the terms affecting dispersal distance of Southern Pied Babblers. Dispersal distance was set as the response term. Data come from 58 dispersal events by 46 individuals dispersing between 31 groups over six years. Identity of the group from which individuals dispersed was included as a random term.

Model term		χ^2	***P***
Full model			
Dispersal type		11.24	0.002
Disperser sex		1.16	0.288
Minimal model		**Effect**	**SE**
Constant		-0.30	0.16
Dispersal type	Breeding	0.0	0.0
	Natal	0.57	0.17

Genetic data confirm the above observations. Spatial analysis of the population revealed no general pattern of IBD for males or females. Females in groups that were closer to one another were more related to one another in 2004 (Mantel test: *P* = 0.041, N = 15 group comparisons), but this pattern was not repeated in any other year (Mantel tests: 2005, *P* = 0.294, N = 36; 2006, *P* = 0.398, N = 55; 2007, *P* = 0.201, N

= 120; 2008, *P* = 0.892, N = 28). Males lacked significant fine-scale genetic structure in any year (Mantel tests: 2004, *P* = 0.158, N = 15; 2005, *P* = 0.152, N = 36; 2006, *P* = 0.316, N = 55; 2007, *P* = 0.432, N = 136; 2008, *P* = 0.252, N = 36). The genetic data thus provide no evidence that sex-biased dispersal has shaped different population genetic structures for males and females. If males had a predictable distribution of relatedness over geographic space, females might only need to disperse past the point at which they are likely to encounter relatives. However, because male relatedness is spatially random, females cannot use distance from the home group as a guide to finding unrelated mates.

Discussion

There is no evidence for the occurrence of inbreeding in Southern Pied Babblers. Indeed, mated pairs are generally unrelated to one another, suggesting that some mechanism is operating to ensure inbreeding avoidance. Inbreeding coefficients calculated from genetic data (F_{IS} and F_{IT}) were negative but not significant in any year, indicating that the population is slightly outbred. Finally, although some individual cases of inbreeding were predicted by the parentage prediction program CERVUS, patterns of body mass change of the females involved precluded their being the mothers of the young in question. Parentage prediction programs can be confounded by the genetic similarity between true parents and older full siblings because all may share the same rare alleles, an acknowledged difficulty in investigating parentage in cooperatively breeding systems (Cockburn 1998, McRae and Amos 1999). Overall, there was no confirmed instance of inbreeding in the population, corroborating behavioural observations that suggested incidences of inbreeding are either absent or very rare in this species (Raihani 2008). This indicates that inbreeding avoidance is one limit on subordinate reproduction and plays a key role in the partitioning of reproduction in the Southern Pied Babbler.

The primary way in which Southern Pied Babblers avoid inbreeding appears to be through avoidance of natal-familiar breeding partners. There are several lines of evidence in support of this. First, individuals only inherit dominance in their natal group when the remaining dominant is unrelated to them. When a vacancy arises in a group in which the subordinates are all related to the remaining dominant, a new, immigrating dominant invariably fills the vacancy, regardless of the presence of same-sex subordinates. Second, individuals very rarely engage in sexual behaviour with known relatives: courtship within the group occurs almost entirely between unrelated group members. These patterns of dominance acquisition and sexual behaviour ensure total inbreeding avoidance at the group level. Third, when individuals do disperse, they avoid mating with familiar relatives. Roving may be a means of gathering information about the composition of nearby groups and the breeding opportunities that they do or do not offer, and individuals sometimes rove at groups containing familiar relatives as prospective mates (Young 2003). However, individuals do not permanently join these groups and are never observed to engage in costly fights to gain dominance there. Thus inbreeding is also generally avoided at the sub-population level. I suggest that individual Southern Pied Babblers learn to recognise kin during a period of association during the dependent period (which, in cooperative breeders, is prolonged – Langen 2000), when individuals learn the cues or labels that identify putative kin, and can then use these cues to recognize kin outside of the natal group (the association context) (Komdeur and Hatchwell 1999, Koenig and Haydock 2004). A similar recognition mechanism is used by Long-Tailed Tits *Aegithalos caudatus* (Sharp *et al.* 2005) and Seychelles Warblers (Richardson *et al.* 2003), whereby birds preferentially help individuals that were in their group or fed them during their period of dependence. Because the average Southern Pied Babbler group is highly kin structured (Chapter 3), avoidance of mating with group members known from dependence is likely to prevent first-order ($r = 0.5$) matings between full siblings and between parents and offspring.

A difficulty associated with kin-recognition systems based on associative learning is that unknown relatives may be included in the pool of potential mates (Sherman *et al.* 2004) especially if most dispersal is over short distances (Zahavi 1990). For the Southern Pied Babblers, there is a strong possibility that relatives will be located nearby because mean dispersal distances are short (roughly two territory widths for natal dispersal and one territory width for breeding dispersal, although these distances are bound to be underestimated due to dispersal sampling error – Koenig *et al.* 1996) and lifetimes may be long (some birds were adults in 2003 when the study site was established, and are still alive in 2009). Indeed, 12 of 27 individuals have acquired dominance in a group neighbouring their natal group. This is comparable to Arabian Babblers, where the same figure was 34 of 60 individuals, and although Arabian

Babblers avoid breeding with natal-familiar individuals, they readily mate with distant kin in neighbouring groups (Zahavi 1990). Similarly, female Red-cockaded Woodpeckers disperse within the range in which related males might be found (Daniels and Walters 2000).

Despite the increased risk of breeding with unknown kin, short dispersal distances may be selected for because of the high cost of long dispersal distances (Koenig *et al.* 1998) or floating (when individuals who have no fixed territory and remain alone for extended periods – Ridley *et al.* 2008). Southern Pied Babblers face intense competition for breeding vacancies (A.R. Ridley, unpublished data). Dispersal to faraway groups requires rovers to travel long distances and compete with residents on many territories, which may be costly (Daniels and Walters 2000). Extended periods of roving have been shown to result in chronic elevation of stress hormones in Meerkats (Young and Montfort 2009). Local dispersal allows subordinate Southern Pied Babblers to return to the natal group between roving forays, and maintain body condition, itself an important predictor of successful dispersal (Ridley *et al.* 2008). Furthermore, any costs of breeding with a distant relative may be outweighed by the immense fitness gains available from attaining the dominant position, because dominants monopolise almost all breeding activity (Chapter 3).

Another commonly invoked inbreeding avoidance mechanism is sex-biased dispersal, but this is not supported in this Southern Pied Babbler population. There is no strong spatial evidence for sex-biased dispersal through analysis of IBD or dispersal distances, and no sex differences in the overall propensity to disperse. It is possible that the size of the study site (2800 ha) is too small to detect any sex differences in IBD population structure. On the other hand, a study of cooperatively breeding Apostlebirds made over a similarly sized study area (3100 ha) has found evidence of IBD for males but not females, which provides support for female-biased dispersal and a male tendency towards philopatry (Woxvold *et al.* 2006). Therefore, it is not unreasonable to expect that sex differences in IBD population structure should be detectable in this sample. Sampling over a larger area would provide conclusive evidence for or against the presence of sex differences in genetic population structure (IBD) in Southern Pied Babblers. Although there are no overall sex differences in immigration, when considering only dominants, immigration into established study groups is biased in favour of females. Females aggressively overthrow one another to gain dominance in non-natal groups, which may account for this higher turnover of female dominants (Raihani 2008). Overall, there is no strong evidence that sex-biased dispersal by Southern Pied Babblers functions as an inbreeding avoidance mechanism *sensu stricto*.

Although sex-biased dispersal was not supported as an inbreeding avoidance mechanism in Southern Pied Babblers, dispersal itself is important (Szulkin and Sheldon 2008). A significant difference was found between natal and breeding dispersal distances. For both sexes, dispersal from the natal group is roughly twice as far as dispersal from one non-natal group to another. This pattern is very similar to that seen in the Superb Fairy-Wren, where females disperse farther from natal groups than from non-natal groups, presumably to avoid pairing with related males living near the natal group (Cockburn *et al.* 2003). Whether Southern Pied Babblers disperse farther from their natal groups as a strategy to avoid unknown relatives, or to avoid pairing with known, related potential mates is impossible to discern. Nevertheless, dispersal itself is a primary mechanism whereby both males and females avoid breeding with kin (Szulkin and Sheldon 2008).

Chapter 5

Reproductive conflict and its resolution within groups

Abstract

More than 50% of Southern Pied Babbler groups contain unrelated potential breeding partners for sexually mature subordinates, yet reproductive skew among both males and females is extremely high and successful subordinate reproduction is very rare. I found that only those subordinates with potential breeding partners in the group enter into reproductive competition. The very low number of extra-pair offspring produced is not the result of subordinate passivity, but rather suppression of subordinate reproduction by dominants. Although dominants suppress subordinate reproduction, they cannot prevent subordinates from entering into competition, which is also sometimes costly. Subordinates invest in competition for reproduction far more often than expected, based on the infrequency with which such competition is successful. Female-female competition for reproduction incurs a cost to the dominant female's fitness, but no such cost of competition occurs during male-male competition. When subordinate females compete, a smaller proportion of nests fledge chicks, more nests are abandoned before incubation began and the start of breeding is delayed by over two weeks, compared with groups lacking such a competitor. Both dominant males and females suppress subordinate competition with aggression during the fertile period. Males also exhibit weak mate-guarding behaviour. Subordinate females sometimes respond to suppression with destruction of the eggs of the dominant female. Overall, these observations provide valuable empirical data to examine the key processes underlying the distribution of reproductive success in Southern Pied Babbler groups, and help to further our understanding of reproductive skew and reproductive skew theory.

Introduction

In many species that live in cooperatively breeding groups, only some of the sexually mature individuals in each group breed. The extent to which reproduction is monopolized by certain individuals in each group is defined as the degree of reproductive skew, with greater monopolization resulting in higher skew (reviewed by Magrath *et al.* 2004). Understanding the causes of variation in the way that reproduction is partitioned among members of a social group represents one of the most intriguing problems in the study of social behaviour and animal societies (Keller and Reeve 1994). An extensive theoretical framework ("reproductive skew theory") has been developed to try to predict how reproductive skew varies in response to key social, genetic and ecological factors (Hodge 2009). Reproductive skew theory was first developed by Vehrencamp (1983) and Emlen (1982b), and encompasses a series of models that ask (a) whether control of the division of reproduction is settled by conflict, or if one party has complete control; (b) whether and how “outside options” such as the eviction of a competitor, or of leaving the group to breed elsewhere, affect reproductive skew; and (c) whether and how factors like group productivity or relatedness affect reproductive skew (reviewed by Johnstone and Cant 2009). It has become clear that testing models of reproductive skew empirically is far from easy, because the predictions made by models are impacted by even small changes to the model’s assumptions (Hodge 2009, Koenig *et al.* 2009). All of a model’s assumptions (even those not explicitly stated) must be met by the study system before the model may be empirically tested: the information thus required is extensive, and to measure the required parameters precisely may prove impossible (Magrath *et al.* 2004, Hodge 2009). Another approach to understanding reproductive skew is now appropriate: to conduct empirical research that examines the central factors underlying the partitioning of reproduction in model systems (Hodge 2009). These include the contexts in which subordinates compete for reproduction, the cost of subordinate reproduction (or subordinate competition for reproduction) to dominants, and finally, the reaction of dominants to reproductive competition by subordinates.

First, it is important to understand the factors that influence whether a subordinate enters into competition with a dominant. These can include age, condition and the degree of inbreeding avoidance (Young 2009). In some species, subordinates may be constrained from breeding only by the fact that there are no unrelated breeding partners in the group (Emlen 1997). In Damaraland Mole-Rats, for example, adding unrelated males to colonies stimulates breeding by subordinate females (Cooney and Bennett 2000). Similarly, subordinate female Meerkats are more likely to breed when they live in groups that contain unrelated males (Clutton-Brock *et al.* 2001a). Subordinates may enter reproductive competition more readily, and/ or may be more successful at breeding, when they possess certain characteristics. For example, in Meerkats, subordinate females are more likely to reproduce when they are heavier, closer in age to dominant females, and have been subordinate to dominant females for a shorter time period (Clutton-Brock *et al.* 2001a). Similarly, female Woodland Voles *Microtus pinetorum* are less likely to reproduce in the presence of an older reproductive competitor when the age difference between them is greater, because older females tended to be dominant over younger females (Solomon *et al.* 2006). In these species, subordinates that are "better" competitors and that have access to unrelated mates compete more frequently for reproduction in their groups. Understanding the context of the breeding opportunities available to subordinates allows a fuller understanding of patterns of reproductive skew.

Second, the potential cost of subordinate reproduction or subordinate competition for reproduction must be estimated, because if dominants suffer fitness costs when subordinates reproduce, selection could favour the development of suppression tactics (reviewed by Hodge 2009). In Meerkats, pups born to subordinate females limit the growth of any pups subsequently born to the dominant female because the helper to pup ratio is reduced (Clutton-Brock *et al.* 2001b, S.J. Hodge unpublished data). In addition, as the number of adult female Meerkats in the group increases, survival of litters born to the dominant female decreases, probably due to the increased likelihood of infanticide by pregnant subordinates (Hodge *et al.* 2008). In Alpine Marmots *Marmota marmota*, increased reproductive competition between dominant and subordinate females also decreases the reproductive success of the dominant female (Hackländer *et al.* 2003). In Brown Jays *Cyanocorax morio*, when more than one female in the group breeds, the dominant female experiences a higher total incidence of complete breeding failure than when no other females attempt to breed (Williams 2004). In Galapagos Mockingbirds *Nesomimus parvulus*, dry weather can lower food availability and increase the cost of territory-sharing; dominant pairs are thus less likely to allow subordinate pairs to breed in dry years (Curry 1988). If the observed skew is the result purely of competition between dominants and subordinates (i.e. both are equally capable of breeding), the cost of subordinate reproduction will directly affect the extent of dominant suppression (Hodge 2009).

Third, when reproduction by subordinates is costly to dominant breeders, dominants may attempt to suppress subordinate breeding attempts either physiologically or behaviourally (Young *et al.* 2006). For example, in the Naked Mole-Rat, the single, dominant female physiologically suppresses fertility in both male and female helpers, with the result that more than 99% of Naked Mole-Rats never breed (reviewed by Faulkes and Bennett 2001). Aggression by dominants towards subordinates is another means of suppression. In Meerkats, the reproductive activity of subordinate females is often suppressed by dominant females through aggressive harassment and eviction, which can cause an increase in abortion rates and/or a decrease in subordinate female conception rates (Kutsukake and Clutton-Brock 2006, Young *et al.* 2006). Similarly, in White-fronted Bee-eaters *Merops bullockoides*, older males actively harass and disrupt the breeding attempts of younger males, usually their sons, who then become helpers at the older male's nest (Emlen and Wrege 1992). Aggression by dominant pairs of Galapagos Mockingbirds towards subordinate pairs during laying or incubating causes nest abandonment by the subordinates (Curry 1988). This also occurs in Brown Jays, where aggression directed by primary breeding females towards secondary and tertiary breeding females has been suggested to lower their breeding success (Williams 2004). Queens in many eusocial insect species eat worker-laid eggs or show aggression to egg-laying workers, behaviour that is especially prevalent in small-colony species (reviewed by Ratnieks *et al.* 2006). Rather than aggression, subordinate male reproduction may be suppressed though mate-guarding of the dominant female by the dominant male. In cooperatively breeding species, mate-guarding is expected to increase when there are male competitors in the group, such as in cooperatively breeding Acorn Woodpeckers and Stripe-backed Wrens *Campylorhynchus nuchalis* (Mumme *et al.* 1983a, Piper and Slater 1993). In these ways subordinate reproduction may be constrained by the decisions and behaviour of group dominants.

Empirical research is essential to understand better the forces that lead to observed pattern of reproductive skew in model systems (Hodge 2009). Southern Pied Babblers are a good model system in which to measure these forces because many groups contain subordinates that are unrelated to the opposite-sex dominant and thus can potentially engage in reproductive competition with same-sex group members. However, almost all groups that contain these competitors produce broods that consist of *only* the offspring of the dominant pair (Chapter 3). To determine why reproductive skew is so high in this species, I ask 1) what factors affect the likelihood that subordinates enter into reproductive competition with dominants; 2) what costs of competition for reproduction exist for both dominant and subordinate competitors; and 3) how do dominants suppress the reproductive activities of competing subordinates?

Methods

Reproductive competition and subordinate nest-building behaviour

In any one year, about 50% of Southern Pied Babbler groups contain potential reproductive competitors (subordinates that are unrelated to the opposite-sex dominant): despite this, however, dominants monopolize breeding activity (Chapter 3). To determine whether this was a result of subordinates restraining themselves from entering competition, or dominant superiority during reproductive competition, I investigated whether subordinates with potential breeding partners in the group do indeed compete (albeit unsuccessfully) for reproductive opportunities. Because of the low level of subordinate reproduction (only 5.1% of chicks have a subordinate parent, Chapter 3), subordinate reproductive success itself cannot be used directly to assess the extent of competition for reproduction, necessitating another measure of reproductive competition. This is because the number of subordinate young produced may not be a true reflection of reproductive conflict: subordinates may be competing to breed much more often than the number of young they produce suggests (Koenig *et al.* 2009). In Chapter 4 I show that courtship behaviour often occurs within groups between unrelated opposite-sex adults, but this behaviour may not be easily and reliably observed for every breeding attempt. An alternative measure of reproductive conflict is nest-building behaviour. Nest-building is highly visible and individuals that do and do not participate in this behaviour are readily identifiable. Although nest-building is mainly done by dominant birds (Ridley and Raihani 2008), subordinates occasionally assist in building the nest of the dominant pair. If subordinates that are potential reproductive competitors are more likely to invest in nest-building than subordinates that are not (i.e. those that are related to the opposite-sex dominant), nest-building by subordinates may indicate reproductive competition within groups. I used life-history data to examine this possibility by investigating the relationship between nest-building behaviour and the opportunity to breed. The data-set included the building activity of every subordinate group member that was six months or older at every nest at which data were collected on nest-building. Whether an individual was seen to carry nest material to the nest (at any time during the nest-building period) was set as the response (0 = no nest-building, 1 = nest-building) and the binomial total was set at one. Sex of the subordinate and whether the subordinate was a potential competitor (unrelated to the opposite-sex dominant) were included as potential explanatory terms in a GLMM with a binomial error distribution and a logit link function. Group size was not included as an explanatory factor because it was significantly and negatively correlated with whether the subordinate was a potential competitor (because small groups more readily accept unrelated adults – Nelson-Flower and Ridley, in prep). Nest and individual identity were included as random terms. Data come from nest-building activity of 77 subordinates at 69 nests in 16 groups over five years.

Is reproductive competition predictable in complex groups?

Among subordinates with a potential breeding partner in their current group, what factors influence the likelihood that they will invest in reproductive competition? Specifically, I ask if 1) characteristics of subordinates themselves or 2) differences between subordinates and their reproductive competitors (the same-sex dominant) affect whether these subordinates enter into competition, defined here as contributing to nest-building. I analysed males and females separately because of potential sex-related differences in reproductive constraints or suppression. Only those subordinates that were six months or older and *unrelated to the opposite-sex dominant* were included in the analysis (Koenig *et al.* 2009). Whether an individual was seen to carry nest material to the nest (at any time during the nest-building period) was set as the response (0 = no nest-building, 1 = nest-building) and the binomial total was set at one. Group size, subordinate characteristics (body mass [g] and age [days post-hatching] on the date of nest-building), differences between the subordinate and same-sex dominant (in body mass [g] and age [days post-hatching] on the date of nest-building) and the relatedness of the subordinate to the same-sex dominant were included as potential explanatory terms in a GLMM with a binomial error distribution and a logit link function. When individuals immigrated into the study population with adult plumage (almost invariably as dominants), they were assumed to be at least one year old (this is a minimum estimate, because juveniles attain adult plumage at one year post-hatching – Ridley and Raihani 2007b). For each nest, mean body masses of subordinates and dominants were calculated from the masses measured throughout the nest-building period. Mass differences were set as the differences between the mean masses of the birds involved. For females, age of the subordinate, group size and relatedness between the subordinate and the same-sex dominant were all highly correlated with one another. Therefore, only relatedness was included as an explanatory term in the final analysis because it had the greatest effect out of these three predictor terms. In addition, in females, subordinate body mass and differences between subordinates and same-sex dominants in age and mass were also all significantly correlated with one another. Mass difference had the greatest effect of these three, and so body mass and age difference were not included as potential explanatory terms in the final analysis. For males, differences in age and mass between the subordinate and the dominant were significantly correlated with relatedness, but relatedness had the greatest effect and so age difference and mass difference were not included as potential explanatory terms in the model. In addition, the limited sample size of nests that subordinate males helped build (N = 28) allowed only a few explanatory terms to be tested. I chose to test the age of the subordinate male and his relatedness to his competitor because these had greater effects than body mass or group size. Relatedness was calculated as described in Chapter 2. Subordinate and group identities were included as random terms in both models. For females, data come from the building activity of 20 subordinate females at 39 nests in nine groups over six years. For males, data come from the building activity of 11 subordinate males at 28 nests in eight groups over six years.

Cost of reproductive competition

To determine whether reproductive competition was costly to dominants in terms of reduced reproductive success, I used several measures of potential cost. First, I investigated whether groups containing male and/or female reproductive competitors were less likely progress from one stage of breeding to the next (nest-building to incubation to hatching). Second, I investigated whether groups containing male or female reproductive competitors experienced delays in the onset of breeding. Third, I investigated whether groups containing reproductive competitors produce fewer chicks than simple groups. Although subordinate nest-building can provide a good indication of the occurrence of reproductive competition, the sample size for this behaviour is small, because groups were not always observed while they were building nests. Thus, instead of using observed subordinate nest-building behaviour as an indication of competition, I used the presence of potential male or female competitors in the group (or the absence of competitors in the case of simple groups) as an indicator of the presence or absence of potential competition.

To determine the effect of competition on successfully reaching the next stage of each breeding attempt (stages considered here include incubation and hatching), I used a GLMM with a binomial error distribution and a logit link function. The data-set included every nest built by any group in the study population. I categorized each group as having a male competitor, a female competitor or no competitor. Two models were run: for the first model, whether a completed nest was incubated was set as the response term (0 = nest abandoned, 1 = clutch incubated) and for the second model, hatching of an incubated clutch was set as the response term (0 = nest abandoned, 1 = clutch hatched). Group size and competitor type were correlated, but group size had a smaller effect than competitor type, so group size was not included as a potential explanatory term. For both models, competitor type was set as a potential explanatory term, the binomial total was set at one and year and group identity were included as random terms. The data-set included 262 nests that were built by 20 groups over six breeding seasons, and 188 clutches that were incubated by 19 groups over six breeding seasons.

I also examined the factors affecting the timing of the onset of breeding each year for each group. Only groups that existed at the start of the breeding season were included in this analysis (groups that formed partway through the breeding season were excluded). I used a GLMM with a normal error distribution and an identity-link function. September 1st of each year was set as day one of the breeding season, and every group's date of first incubation was numbered from this date. The number of days delay from the start of the season until the occurrence of incubation was set as the response term. As above, competitor type was included as an explanatory variable. Group size and competitor type (male, female or no competitor) were correlated (only smaller groups contained competitors), so I restricted the data-set to groups that had fewer than six members. This resulted in a data-set that included 43 first-of-the-season incubation dates from six breeding seasons and 17 groups in total. Year and group were included as random terms.

Finally, I investigated the cost of reproductive competition between dominant and subordinate females. I investigated whether the presence of an actively competing subordinate female causes proportionally fewer nests to fledge young: as shown below, only competing females (not males) cause delays in the onset of breeding.

Females were classified as actively competing when they assisted the dominant pair in nest-building at any point during the breeding season. When a group contained such a female, I counted the ratio of nests built to those that produced fledged young in each breeding season, and compared it with the same ratio when competitive females were absent. Some groups were entered twice because group composition changed over the breeding season (for instance when a previously competitive female left the group), but each nest was only ever counted once. The number of nests that fledged chicks was set as the response variable and the number of nests built was set as the binomial total in a GLMM with a binomial error distribution and a logit link function. Group size and the presence of a competitive subordinate female were included as potential explanatory terms and year and group identity were included as random terms. Data include 207 nests built by 19 groups over five breeding seasons.

Reproductive suppression of subordinate competition

Where possible, dominants should attempt to limit the likelihood that a subordinate reproduces successfully within the group because this may reduce the dominant's share of parentage. Males may attempt to mate-guard the dominant female, and subordinates that appear to enter into reproductive competition may experience higher levels of aggression directed at them by dominants. To determine whether this was the case, I examined the existence and extent of mate-guarding by dominant males, and aggression by dominant males and females towards same-sex competitive subordinates. First, I investigated whether dominant males spent more time guarding the dominant female during the presumed fertile period (up to seven days before incubation begins) than during the non-fertile period, and whether the presence of male competitors in the group had an effect on the amount of dominant male mate-guarding that occurred. Only groups that contained a subordinate male were included in the analysis. At every group during the breeding season, I collected mate-guarding focal watches in which I followed the dominant female for 20 minutes, recording how long the dominant male remained within 1.5 m of her. I conducted these focal watches only when the dominant female was foraging, not on guard as a sentinel or grooming (because during these activities she was often highly visible, making sneaky copulations from other males almost impossible). To analyse these data, I used a GLMM with a binomial error distribution and a logit link function, with the number of seconds that the dominant male spent close to the dominant female as the response term, and the binomial total set as the length of the focal watch (in seconds). Because the presence of male competitors was highly and negatively correlated with group size (large groups did not contain male competitors, only sons of the dominant female), I performed two analyses. In the first, I used groups that were small (five members or fewer) to test the effect of the presence of male competitors on mate-guarding by dominant males. Group size, presence of a male competitor and fertile status were included as potential explanatory terms, and dominant male identity was included as a random term. This analysis included 30 watches of the mate-guarding activity of seven dominant males at six groups over two breeding seasons. In the second analysis, I used groups with no competitors to test the effects of group size. Group size and fertile status were included as potential explanatory variables, and dominant male identity was included as a random term. This analysis included 33 watches of the mate-guarding activity of eight dominant males at seven groups over two breeding seasons.

I further tested whether adult subordinates are more likely to receive aggressive attacks from same-sex dominants during the fertile *vs* non-fertile periods. Aggression was defined as any physical attack (pecking or jumping on top of the subordinate) or dominance displays such as charging at subordinates or splaying feathers (feathers held erect and wings held out). All aggressive interactions were recorded *ad libitum* (Altmann 1974) on handheld data loggers, noting both the aggressor and recipient identities. The number of times that a subordinate was the focus of aggression by the same-sex dominant in the group was summed and divided by the total hours of observation for that subordinate to obtain the rate of aggressive acts per hour for the fertile and non-fertile periods. Subordinates were classified as ‘competitive’ if they were unrelated to the opposite-sex dominant and ‘non-competitive’ if they were related. No individual or group was included in the analysis more than once. Because of limited sample size, male and female subordinates were analysed together using a paired t-test comparing aggression towards these subordinates from the same-sex dominant in the fertile *vs* non-fertile periods. Data were collected over two breeding seasons from eight subordinates that were potential reproductive competitors in eight groups, and nine subordinates that were not potential reproductive competitors in nine groups.

Results

Reproductive competition and subordinate nest-building behaviour

The presence of a potential breeding partner within the group affected a subordinate's investment in nest-building. Subordinates that were potential reproductive competitors (with access to a potential breeding partner) were significantly more likely to participate in nest-building (7.3 ± 1.7% of nest-building visits – Table 5.1) than were subordinates with no access to breeding opportunities (0.5 ± 0.2% of nest-building visits). In addition, although the sample size of subordinates that reproduced successfully is small (N = 4 broods), these subordinates regularly engaged in nest-building (20.1% of all nest-building visits), indicating that nest-building by subordinates is a useful measure of the occurrence of reproductive competition.

Table 5.1: GLMM investigating the effect of reproductive opportunities on the occurrence of nest-building by subordinate Southern Pied Babblers. Data come from observations of 77 subordinates at 69 nests in 16 groups over five years. Nest and individual identity were set as random terms in the model.

Model term		χ^2	***P***
Full model			
Subordinate is potential competitor		34.84	< 0.001
Sex		0.86	0.358
Minimal model		**Effect**	**SE**
Constant		-3.05	0.32
Subordinate is potential competitor			
	No	0.0	0.0
	Yes	2.46	0.42

Is reproductive competition predictable in complex groups?

Dominants were never observed to prevent subordinates from participating in nest-building. Overall, 28.9% of all breeding attempts occurred in groups containing a subordinate female with potential reproductive opportunities, and these subordinate females participated in nest-building in 48.7% of breeding attempts by these groups. This occurrence of within-group reproductive competition among females was weakly affected by the relatedness of the subordinate female to her competitor (Table 5.2). When a subordinate female was less closely related to the dominant female, she was more likely to enter into reproductive competition, that is, the intensity of reproductive competition was inversely linked to the relatedness of the two birds involved. Mean relatedness between competing females was 0.101 ± 0.045, while between non-competing females, relatedness was 0.301 ± 0.037.

Table 5.2: Result of a GLMM investigating terms affecting nest-building by subordinate female Southern Pied Babblers that are unrelated to dominant males in their group. Data come from 56 records of nest-building by 20 subordinate females in 39 nests at nine groups over six years. Random terms included are identities of groups and subordinate females.

Model term	χ^2	***P***
Full model		
Relatedness to competitor	5.53	0.034
Mass difference with competitor	2.99	0.084
Minimal model	**Effect**	**SE**
Constant	-0.30	0.45
Relatedness to competitor	-4.62	1.97

Subordinate males that were unrelated to dominant females also occasionally entered into reproductive competition with the group dominant male: overall, 20.7% of all breeding attempts occurred in groups containing subordinate males that were unrelated to the breeding female, and in these groups subordinate males participated in nest-building in 35.7% of breeding attempts. However, none of the explanatory terms tested significantly predicted whether subordinate males contributed to nest-building (Table 5.3).

Table 5.3: Result of a GLMM investigating terms affecting nest-building by subordinate male Southern Pied Babblers that were unrelated to the dominant females in their groups. Data come from 28 records of nest-building by 11 subordinates at 28 nests in eight groups over six years. Random terms included are group identity: subordinate male identity was bound.

Model term	χ^2	***P***
Full model		
Age	0.57	0.448
Relatedness to competitor	0.51	0.477
Minimal model	**Effect**	**SE**
Constant	-0.38	0.49

Cost of reproductive competition

The type of competitor present in the group affected whether completed nests were abandoned before incubation, but did not affect whether incubated clutches hatched. Groups with female competitors were more likely to abandon nests before incubation than were any other group type (Table 5.4, Fig. 5.1). In groups with a competitive female, 44.6% of built nests were abandoned before incubation, compared to only 19.6% and 20.7% of nests built in groups with a competitive male and no competitor, respectively. Part of this nest failure comes from overt reproductive competition: in one group I was able to confirm the occurrence of egg-eating by a female reproductive competitor over two consecutive days (Fig. 5.2).

Table 5.4: Results of GLMMs investigating the terms affecting whether (a) a completed nest was incubated and (b) whether an incubated clutch hatched. Year and group identity were included as random terms in both models, but for model (b) year was bound. The data-set included 262 built nests from 20 groups over six breeding seasons, and 188 incubated clutches from 19 groups over six breeding seasons.

Response term	**Explanatory terms**	χ^2	***P***
a) Incubation	**Full model**		
N = *262 built nests*	Competitor type	14.26	0.001
	Minimal model	**Effect**	**SE**
	Constant	0.21	0.29
	Competitor type		
	Female	0.0	0.0
	Male	1.15	0.42
	None	1.16	0.38
b) Hatching	**Full model**	χ^2	**P**
N = *188 incubated clutches*	Competitor type	2.19	0.337
	Minimal model	**Effect**	**SE**
	Constant	1.19	0.38

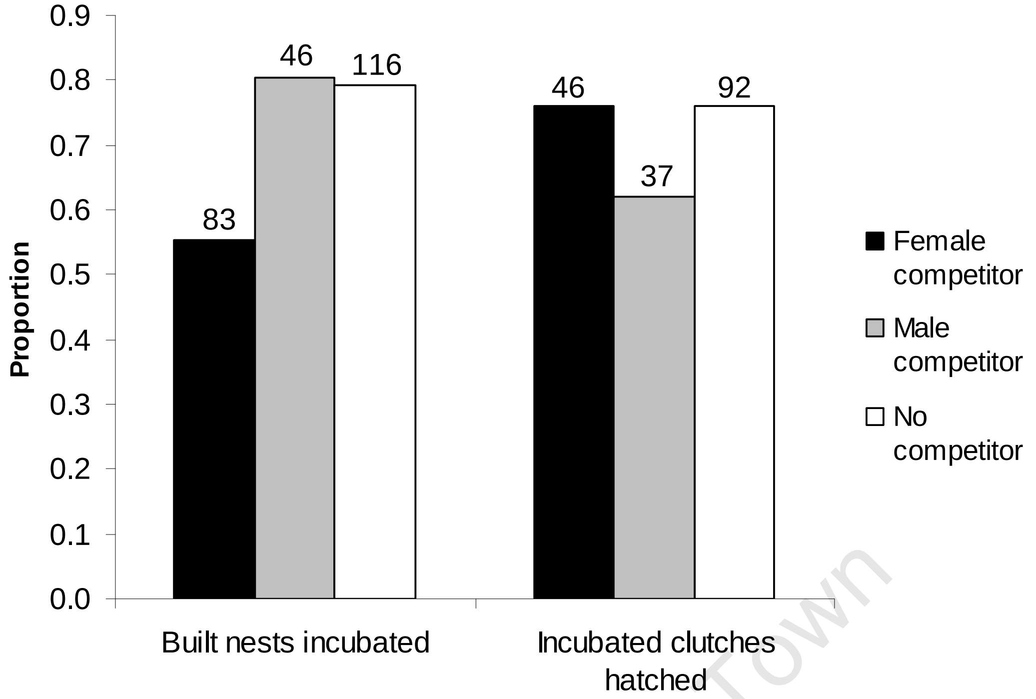

Fig. 5.1: Proportion of nests in which clutches were incubated and the proportion of incubated clutches that hatched as a function of competitor type. Proportions are raw data. Sample sizes as shown.

Fig. 5.2: Image from a nest camera of a competing subordinate female destroying and eating the egg laid moments before by the group's dominant female.

Groups containing a female competitor were more likely to experience a delay of over two weeks in the onset of incubation of the first clutch, compared with groups containing no competitor or a male competitor (Table 5.5, Fig. 5.3). This is likely to be due to the repeated building and then abandonment of nests (Table 5.4).

Table 5.5: Result of a GLMM investigating terms affecting the timing of the onset of incubation by Southern Pied Babbler groups. Data come from 17 groups over six breeding seasons with 43 first incubation dates. Year and group identity were included as random terms.

Full model		χ^2	***P***
Competitor type		9.49	0.015
Group size		2.78	0.104
Minimal model		**Effect**	**SE**
Constant		55.91	5.28
Competitor type			
	Female	0.0	0.0
	Male	-14.71	5.97
	None	-15.41	5.55

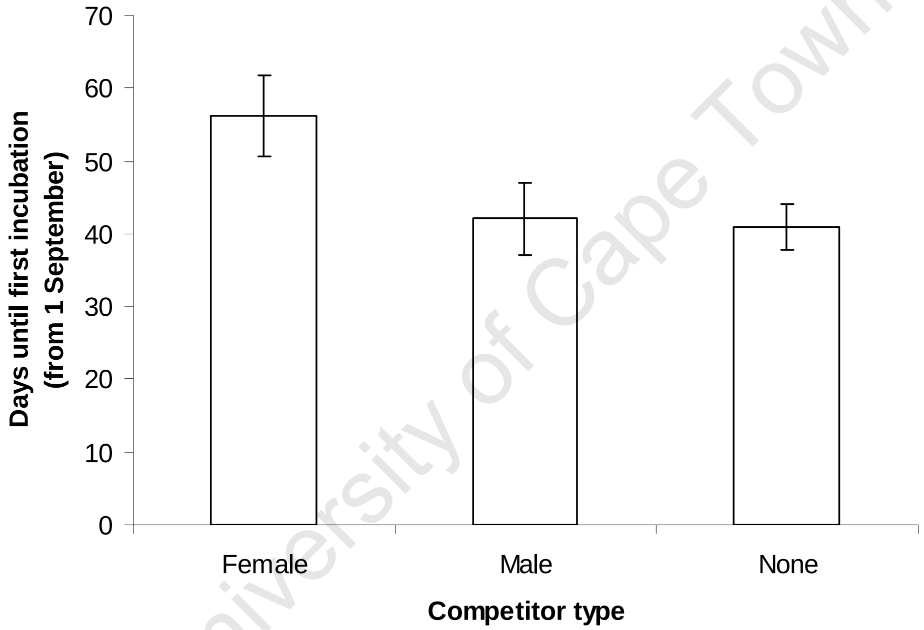

Fig. 5.3: Average delay in the onset of incubation each season as a function of competitor type. Means ± SEM are generated from raw data. Data come from 14 first-incubation dates at groups containing a female competitor, 10 first-incubation dates at groups containing a male competitor and 19 first-incubation dates at groups lacking a competitor.

After controlling for group size, groups with actively competing females fledged chicks from a smaller proportion of completed nests than those groups that lacked such females (Table 5.6, Fig. 5.4).

Table 5.6: Result of a GLMM investigating terms affecting the proportion of completed nests from which chicks were fledged. Group identity and year were included as random terms. Data come from 207 nests in 19 groups over five breeding seasons.

Full model		χ^2	***P***
Actively competing female		5.93	0.019
Group size		4.75	0.033
Minimal model		**Effect**	**SE**
Constant		-0.25	0.25
Actively competing female			
	Absent	0.0	0.0
	Present	-0.85	0.39
Group size		0.21	0.10

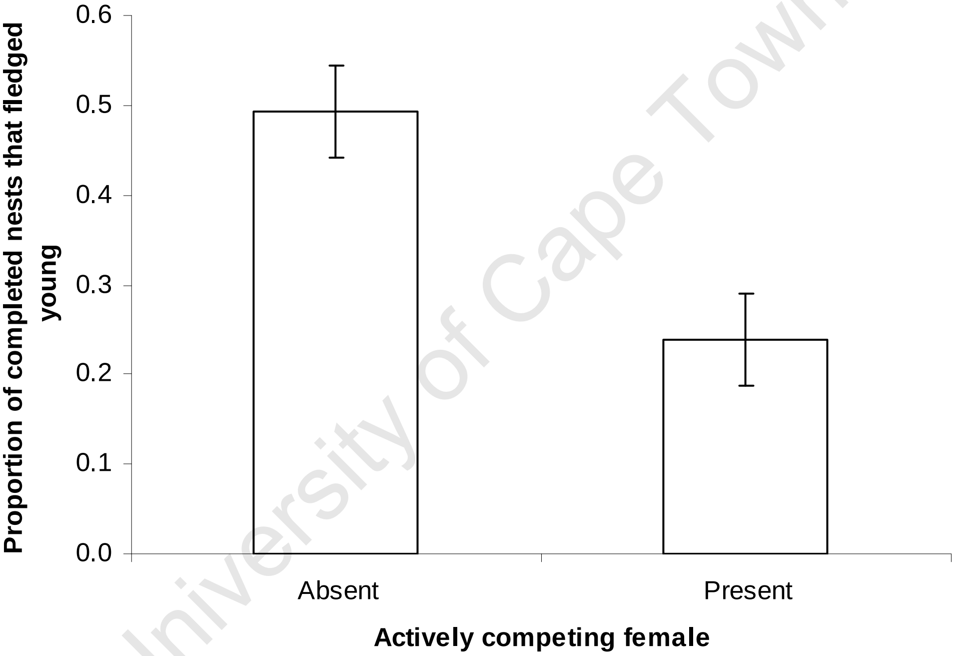

Fig. 5.4: The mean proportion of completed Southern Pied Babbler nests that successfully fledged young per breeding season in groups with and without an actively competing subordinate female. Means ± SEM calculated from raw data. Data include 48 proportions from groups without an actively competing female and 15 proportions from groups with an actively competing female.

Reproductive suppression of subordinate competition

The presence of competitors in the group did not have a significant effect on mate-guarding behaviour by the dominant male. In small groups containing competitors, mate-guarding behaviour by the dominant male was also unaffected by the fertile period; i.e. males did not respond to the likely increased risk posed by cuckoldry during this period (Table 5.7 – model a). In groups that contained no competitors, however, after controlling for the effect of group size, dominant males spent more time close to the dominant female during her fertile period than during the non-fertile period (Table 5.7 – model b). Mate-guarding is easier to detect when groups are large and more spread out than small groups, but the extent of mate-guarding is not a good indicator of reproductive conflict in this species.

Table 5.7: Result of a GLMM investigating mate-guarding by dominant male Southern Pied Babblers. In model (a), to test the effect of male competitors in the group, data come from seven dominant males at six groups over two breeding seasons. In model (b), to test the effect of group size, data come from eight dominant males at seven groups over two breeding seasons. Male identity was included as a random term in both models.

(a) Small groups (< 6 members)	**Full model**		χ^2	***P***
	Group size		2.28	0.138
	Fertile status		2.24	0.141
	Presence of competitor		0.66	0.433
	Minimal model		**Effect**	**SE**
	Constant		-0.35	0.16
(b) Groups with no competitor	**Full model**		χ^2	**P**
	Group size		8.56	0.017
	Fertile status		7.86	0.009
	Minimal model		**Effect**	**SE**
	Constant		-1.15	0.25
	Group size		-0.23	0.08
	Fertile status	Non-fertile	0.0	0.0
		Fertile	0.85	0.30

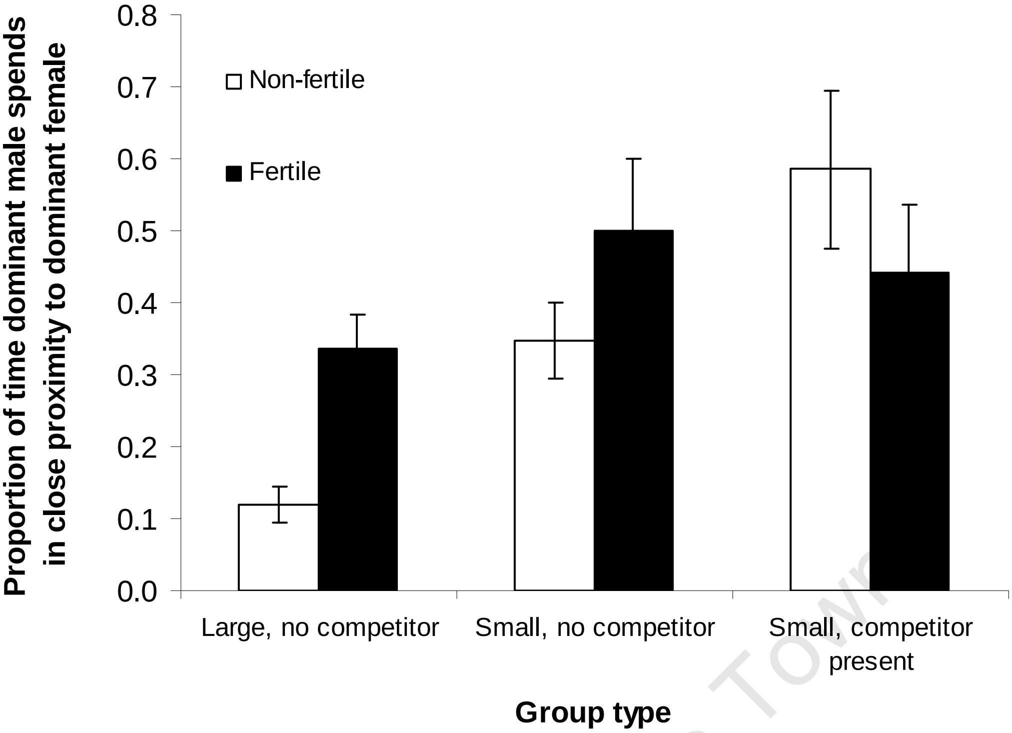

Fig. 5.5: Mean (± SEM) proportion of time dominant male Southern Pied Babblers accompanied dominant females during the fertile and non-fertile periods in different group types. Data come from 11 males at ten groups over two breeding seasons.

Subordinates that were potential reproductive competitors were subjected to more aggression from same-sex dominants during the fertile period than during the non-fertile period (paired t-test, t = 2.68, d.f. = 7, $P = 0.031$, Fig. 5.6). However, same-sex dominants did not elevate aggression levels towards non-competitive subordinates during the fertile *vs* non-fertile period (paired t-test, t = 1.09, d.f. = 8, $P = 0.307$).

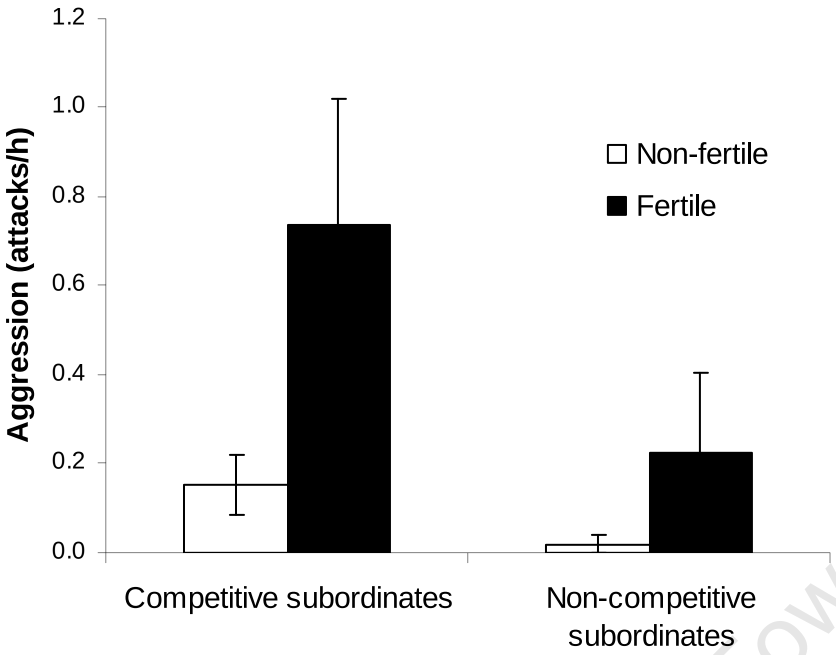

Fig. 5.6: Aggression rates (interactions per hour; means ± SEM) by dominant Southern Pied Babblers towards same-sex adult subordinates during the fertile and non-fertile periods. Data come from 17 subordinates from 17 groups over two breeding seasons.

Discussion

To understand variation in reproductive skew in social groups, it is essential to understand the contexts in which subordinates compete for reproduction, the cost of subordinate reproduction (or subordinate competition for reproduction) to dominants, and the reaction of dominants to reproductive competition by subordinates. Without such detailed information, it is impossible to determine which reproductive skew model to test (Hodge 2009). Even so, the empirical data required to unambiguously test the assumptions of a model may be extremely difficult (or even impossible) to acquire (Magrath *et al.* 2004, Koenig *et al.* 2009). In Southern Pied Babblers, inbreeding is avoided (Chapter 4), and extra-group fertilizations do not occur (Chapter 3), so subordinates in simple groups have no opportunities to breed. These subordinates have no unrelated breeding partners, do not engage in courtship behaviour (Chapter 4), rarely invest in nest building and do not compete for reproduction, probably due to the high cost of breeding with close relatives (Pusey 1987, Pusey and Wolf 1996). Subordinates enter reproductive competition only when there are unrelated, potential breeding partners present in their group. When subordinates lived in complex groups with potential breeding partners, subordinate females competed in 48.7% and subordinates males in 37.5% of breeding attempts in their groups. There is no evidence that age and body mass play a role in the likelihood of a subordinate attempting to breed with a dominant, although female subordinates are less likely to compete when the dominant female is a close relative. This may provide support for the role of indirect benefits in the decision of a subordinate female to enter reproductive competition (see Chapter 7).

When subordinates successfully compete for reproduction, dominants pay considerable costs. For dominant males, the cost of losing the competition for reproduction is high, because this may result in an entire brood being fathered by another male, as is common in Splendid Fairy-Wrens (Brooker *et al.* 1990). This complete cuckoldry occurred in the only case of subordinate male reproduction documented in this population of Southern Pied Babblers (Chapter 3). Losing the competition for reproduction may carry high costs for dominant females as well, although these costs are slightly different. Unlike dominant males, dominant females do not completely lose parentage of a brood, always being the mother of at least one of the chicks hatched in a mixed-parentage brood (Chapter 3). In cases where subordinate females do reproduce (by egg-dumping in the nest of the dominant female), dominant females face costs that attend a larger-than-average brood size. The average size of mixed broods is 4, almost double the population mean brood size of 2.03 ± 0.14 (Ridley and Raihani 2007b). Enlarged broods may decrease survival or future reproductive output of breeders, as in Common Kestrels *Falco tinnunculus* (Daan *et al.* 1996). A larger brood also affects the ratio of adults to fledglings in the group. In Southern Pied Babblers, a low adult:fledgling ratio negatively affects the physical condition of the dominant female and reduces the likelihood that she will breed again that season (Ridley and Raihani 2008). The adult:fledgling ratio also strongly affects offspring quality: chicks in groups with low adult:fledgling ratios weigh less, are less competent at foraging when they reach independence, and are less successful at dispersing and breeding when they reach adulthood (Ridley and Raihani 2007b). Thus, both the dominant female and her offspring incur costs when a subordinate female reproduces alongside her, supporting the observations made in other species such as Meerkats (Clutton-Brock *et al.* 2001b, S.J. Hodge unpublished data).

Dominant females face further potential costs of competition from a subordinate, namely those of the competition itself. For dominant females, competition for reproduction with a subordinate female appears to be expensive. Overall, nests that are built in groups that contain an actively competing female are less likely to fledge chicks than nests in groups lacking such competition. This is likely due to delays at the incubation phase. In some cases of subordinate female competition, body mass data showed that dominant females had laid eggs, but these were not incubated and, when later checked, nests were empty. These nests were then abandoned and a new nest started. On average, this nest abandonment caused incubation to be delayed for over two weeks. A shorter breeding season is costly to the dominant female because she may not be able to fit as many breeding attempts into the season (Ridley and Raihani 2008). In some groups, this cycle of nest-building and abandonment continued for the entire breeding season, and no chicks were ever hatched. My evidence of a subordinate female eating a dominant female's eggs (Fig. 5.2) suggests that this repeated nest abandonment could (at least in some cases) be caused by the subordinate female destroying the eggs of the dominant female. Similar destruction of eggs by co-breeding females has been reported in Acorn Woodpeckers (Mumme *et al.* 1983b), Great Reed Warblers *Acrocephalus arundinaceus* (Hansson *et al.* 1997) and Groove-Billed Anis *Crotophaga sulcirostris* (Vehrencamp 1977). In these species, competing females remove eggs until they themselves are ready to lay (Koenig *et al.* 1995). Destruction of the dominant female's eggs represents a large cost to her (Monaghan and Nager 1997). Alternatively, the repeated abandonment of nests could be due to predation which could disproportionately affect groups that contain competitors, because these groups tend to be small and small groups tend to experience higher predation (Raihani and Ridley 2007a). Whilst an egg-eating predator cannot be totally discounted as the cause of egg loss and subsequent nest abandonment, it seems very unlikely that such a predator should preferentially eat the eggs of those groups with competitive subordinate females and not the eggs of those groups containing competitive subordinate males.

Subordinate reproduction and competition for reproduction is costly to males due to lost paternity, and to females due to overly large broods, delayed onset of breeding, and lost eggs. Selection therefore should favour those dominants that suppress subordinate breeding activity (Hodge 2009). Dominants suppress the reproductive activity of potentially competitive subordinates through aggression. In complex groups containing unrelated potential competitors, aggression towards competitive subordinates significantly increases during the fertile period of the dominant female. In nuclear family groups, where all group members are closely related, there is no such significant increase in aggression. Aggression may result in high stress levels which do decrease reproductive success in mammals (Sheriff *et al.* 2009) and can play an important role in reproductive suppression during critical life-history stages, such as the fertile period (Young *et al.* 2006). Potentially, therefore, stress makes subordinate Southern Pied Babblers less likely to lay eggs or to compete successfully for fertilizations during the fertile period of the dominant female. Increased aggression by conspecifics disrupts breeding attempts in other cooperatively breeding birds, including White-fronted Bee-eaters (Emlen and Wrege 1992), Brown Jays (Williams 2004), Galapagos Mockingbirds (Curry 1988) and Stripe-backed Wrens (Piper and Slater 1993). In order to counter this, subordinates may be selected to use aggressive tactics of their own to evade suppression by dominants (Hodge 2009): this could well explain the destruction of the dominant female's eggs by a subordinate female.

Another means of reproductive suppression of potentially competitive male subordinates is mate-guarding. Although the evidence for mate-guarding by dominant males was strong only in large groups (Fig. 5.5), this may be an artifact of the observational protocol, which recorded the time that the dominant male was within 1.5 m of the dominant female. Foraging birds regulate their spacing from one another (Radford and Ridley 2008). It is likely that in small groups this results in the dominant male and female often being in closer proximity than in larger groups, whether mate-guarding is occurring or not, simply because in larger groups there is a greater choice of individuals with whom to associate. Although mate-guarding was not very strong in Southern Pied Babblers, reproductive skew among males was high: subordinate males very rarely gained paternity. Paternity of the brood is the result of not only a struggle between males, but also the mate choice of the female (Kokko and Morrell 2005). Kokko and Morrell (2005) suggested that low levels of mate-guarding imply high levels of female faithfulness. Females in many species prefer to mate with the oldest, heaviest, largest, most dominant or best-ornamented male (reviewed by Griffith *et al.* 2002, but see Haydock and Koenig 2002). In Southern Pied Babblers, the dominant female may prefer the dominant male over any subordinate male in the group because of his higher quality, (because the dominant male is likely to be the oldest and heaviest male in the group – A.R. Ridley, unpublished data). Whether dominant males suffer increased paternity loss when there are attractive competitors in the group can be further tested as more data accumulate regarding the role of physical condition in determining subordinate male reproductive success. This will help to explain the relative importance of mate-guarding and female mate choice in male reproductive skew.

A final method of reproductive suppression of subordinates is simple eviction from the group by the same-sex dominant. This happens in both Meerkats (Clutton-Brock *et al.* 1998) and cichlids (Dierkes *et al.* 1999), and there is some evidence it also occurs in Southern Pied Babblers (A.R. Ridley, unpublished data). Although the sample size for this behaviour is still too small for analysis (N = 5 cases), when dominants evict same-sex subordinates, it may be because these subordinates are reproductive competitors. In Southern Pied Babblers, eviction carries a very high cost because floaters typically lose body condition (Ridley *et al.* 2008) and are likely to be more exposed to predation risk due to the loss of group vigilance benefits.

Although over 50% of groups every year contain a subordinate reproductive competitor, these individuals rarely reproduce, resulting in a breeding system that is almost entirely monogamous (Chapter 3). The very small number of extra-pair offspring produced is not a consequence of subordinate passivity, but rather resulted from dominant suppression of subordinate reproduction. Based on the infrequency with which such competition is successful, subordinates invest in competition for reproduction far more often than expected. This result highlights the importance of using behavioural observations, as well as genetic data, when investigating pre-reproductive sexual conflict. Using genetic data alone strongly underestimates subordinate investment in reproductive competition.

In summary, the Southern Pied Babbler provides valuable empirical evidence of how reproduction is partitioned in social groups in a cooperatively breeding bird. This evidence offers critical insights into the application of reproductive skew theory to this species. First, not all subordinates are equally capable of reproduction in their groups: inbreeding avoidance and (for female subordinates) relatedness to the dominant female can decrease the likelihood of subordinate reproduction or competition for reproduction. This immediately violates one assumption of most skew models that the distribution of reproductive success is determined solely through competition between dominants and subordinates (Hodge 2009). Individuals that cannot breed in the group (e.g. due to inbreeding avoidance) should be excluded from the pool of potential breeders (Koenig *et al.* 2009). Second, reproduction and competition for reproduction by subordinates imposes a cost on dominants, and although dominants generally do prevent subordinate reproduction, they cannot prevent subordinate competition. This violates the assumption of the models called "transactional models" that either dominants or subordinates have full control over the respective shares of reproduction in the group (reviewed by Johnstone 2000). The difficulties of testing models of skew are well documented (reviewed by Magrath *et al.* 2004), but this and further empirical data from the Southern Pied Babblers may help in gaining a better understanding of the nature of the social interactions by which reproductive conflicts within groups are negotiated.

Chapter 6

Kin selection and helping by subordinates

Abstract

In cooperatively breeding societies, some individuals do not breed and instead, help to raise young that are not their own. Kin selection has been postulated as an explanation for helping behaviour and states that individuals help to raise the offspring of relatives because indirect fitness benefits can be gained by furthering the spread of those genes shared between relatives. In recent years, however, the importance of kin selection and indirect fitness benefits in explaining the evolution and maintenance of cooperative breeding systems has been called into question, and direct benefits (from, for example, group augmentation) have been postulated as the primary source of fitness for helpers. It is therefore important to continue investigations of the relationship between helping and relatedness in cooperatively breeding species to better understand the role of indirect benefits (kin selection) and direct benefits in the evolution and maintenance of helping behaviour. Here I show that the genetic relationship between helpers and young does not affect helper provisioning rates to either nestlings or fledglings. In addition, helpers did not change their provisioning rates to broods of full-siblings (observed just before the immigration of a new dominant to the group) *vs* half-siblings (observed just after such an event). Kin selection cannot be discounted as a factor in promoting cooperation in this species, because helpers increase the fitness of both breeders and young, to whom helpers are almost always closely related. Helpers thus do gain indirect benefits. These data demonstrate, however, that helpers do not discriminate between young on the basis of their relatedness, and as such provide no evidence of kin selection *per se*.

Introduction

In cooperatively breeding societies, some individuals do not breed and instead, help to raise young that are not their own (Brown 1987, Koenig *et al.* 1992, Dickinson and Hatchwell 2004). The apparent altruism of individuals that help to raise the offspring of others, rather than pursuing their own independent breeding opportunities or not helping at all, presents a challenge to evolutionary theory. Hamilton’s (1964) theory of kin selection has been partially successful in explaining the existence of helping behaviour and states that individuals will help to raise the offspring of relatives instead of breeding independently because fitness can be gained by furthering the spread of the genes shared between relatives. Kin selection specifically predicts that individuals will help when the cost of help (C) to the donor is less than the benefit of help (B) to the receiver, weighted by the relatedness between the donor and receiver (r), such that $rB > C$. In other words, the high indirect fitness benefits gained by those individuals helping their relatives to breed could drive the evolution and maintenance of cooperative breeding (Hamilton 1964). This does not imply that individuals will give up all opportunities to breed, because in many cooperatively breeding vertebrates, dispersal is delayed because of ecological constraints (Emlen 1982a). Individuals may thus help to rear the offspring of relatives to gain indirect benefits while awaiting their own breeding opportunities (Emlen 1991). The fitness benefits available to those helpers that are related to breeders include indirect fitness benefits (described above) and future indirect fitness benefits (when the help provided increases the breeder's survival and future reproductive fitness) (Mumme *et al.* 1989).

The magnitude of the indirect benefits that are available to helpers varies with the relatedness between helpers and recipients, according to Hamilton's (1964) equation. Helpers are often closely related to recipients because they are the offspring of one or both of the breeding pair (Emlen 1995), such as occur in Arabian Babblers (Zahavi 1990) and Meerkats (Griffin *et al.* 2003). Similarly, in social insects such as honeybees *Apis mellifera*, helpers (workers) are the offspring of the dominant female (the queen), while in paper wasps *Polistes* spp, helpers are the cofoundresses of the colony or are the offspring of the queen and her cofoundresses (reviewed by Reeve 1991). In the case of *P. bellicosus*, cofoundresses are full sisters (Field *et al.* 1998). In many species, helpers may be individuals that have failed in their own breeding attempts and help close relatives (full siblings, offspring or parents) in order to "make the best of a bad job," such as occurs in Long-tailed Tits (Russell and Hatchwell 2001). However, in some cases, such as Pied Kingfishers *Ceryle rudis*, helpers are unrelated to the breeders (Reyer 1990). Unrelated helpers are also sometimes found in species in which relatedness is the norm, such as in Meerkats and Southern Pied Babblers (Griffin *et al.* 2003, Chapter 3).

In recent years, the importance of kin selection and indirect fitness benefits in the evolution and maintenance of cooperative breeding systems has been called into question, and direct benefits have been postulated as a main source of fitness for helpers (Clutton-Brock 2002). Direct benefits are those that increase an individual's own immediate or future chances of survival and/or reproduction (reviewed by

Dickinson and Hatchwell 2004). These possible direct fitness benefits of helping include: (1) an increase in "experience" or "skills" such that the helper is eventually more successful as a breeder (Skutch 1961, Komdeur 1996); (2) improved future access to mates (Reyer 1990, Zahavi 1995); or (3) an increase in group size ('group augmentation' – Brown 1987, Kokko *et al.* 2001). Increases in group size have several potential benefits including increased future help from larger numbers of current recipients ('delayed reciprocity', Kokko *et al.* 2001, Clutton-Brock 2002), increased chances of survival and reproduction due to better vigilance and larger coalition sizes at dispersal (reviewed by Heinsohn and Legge 1999, Russell 2004), and decreased chances of group extinction (Courchamp *et al.* 1999, Heg *et al.* 2005). Importantly, direct benefits and indirect benefits are not mutually exclusive (Clutton-Brock 2002), and both types of benefits, as long as they outweigh the costs of helping, may promote helping in cooperatively breeding groups (Kokko *et al.* 2001).

The debate continues over the role of kin selection in the evolution and maintenance of cooperative breeding. A recent meta-analysis found a broad correlation across species between the degree of relatedness and the amount of help provided by subordinates (Griffin and West 2003), and it is generally accepted that kin selection does play a role in maintaining cooperative behaviour between close kin (Clutton-Brock 2009). Indeed, in many species, kin selection has been supported as a cause of cooperation: for example, this has been found in eusocial insects such as all termites and ants, and many bees and wasps (reviewed by Keller and Chapuisat 1999), Brown Hyenas *Hyaena brunnea* (Owens and Owens 1984), African Lions *Panthera leo* (Pusey and Packer 1994), White-fronted Bee-eaters (Emlen and Wrege 1988), Galapagos Mockingbirds (Curry 1988), Bell Miners *Manorina melanophrys* (Clarke 1984), Long-tailed Tits (Russell and Hatchwell 2001), and Carrion Crows *Corvus corone corone* (Baglione *et al.* 2003). However in other species, kin selection has not been supported as a significant factor in cooperation: for example, this is the case in Mexican Jays (Brown and Brown 1990), Green Woodhoopoes *Phoeniculus purpureus* (du Plessis 1993), Superb Fairy-Wrens (Dunn *et al.* 1995), Arabian Babblers (Wright *et al.* 1999) and Meerkats (Clutton-Brock *et al.* 2001c). Kin selection also cannot provide an explanation for cooperative behaviour performed by unrelated helpers, and such unrelated helpers do occur in many species, including Southern Pied Babblers (Chapter 3).

To determine whether kin selection contributes to the evolution of helping behaviour, it must be shown that help leads to inclusive fitness benefits. This can happen by increasing (a) the fitness of the related recipient (the nestling or fledgling) and/or (b) the fitness of the related breeder (reviewed by Dickinson and Hatchwell 2004). However, because increases in the fitness of the recipient or the breeder may also result in direct benefits to the individual (through group augmentation etc.), a further test of the importance of kin selection is whether subordinates vary the amount of help they provide depending upon their relatedness to the recipient (Griffin and West 2003). Southern Pied Babblers provide a good opportunity to test the occurrence of kin selection, because they typically live in groups comprising a dominant pair and their offspring and group members are generally closely related to one another (Chapter 3). In many groups, however, some helpers are unrelated to either one or both breeders. In this species, the presence of helpers benefits both offspring and breeders (Ridley and Raihani 2007b, 2008). Therefore, because subordinates are very often helping close relatives, high indirect fitness benefits are available to them, supporting kin selection as a likely factor leading to cooperation. Dominants can be replaced by immigrants, however, and helpers can recognize when an unrelated immigrant enters the group (courting often occurs between helpers and unrelated immigrant dominants – Chapter 4). Helpers may thus be able to recognize that their parents are no longer the breeding pair and then may decrease the amount of food they provide to a brood of half-siblings produced after such an immigration event. In this chapter, I investigate whether decreases in relatedness between the donors and recipients of help result in decreased amounts of help. I ask whether subordinate helpers vary the amount of help (as measured by contributions to chick feeding) that is given to (1) nestlings and (2) fledglings in response to changes in relatedness. I estimate the relatedness between each adult subordinate and nestling or fledgling from a combination of parentage analysis, pedigrees and group life histories. I also compare provisioning rates for focal subordinates before and after one parent (a dominant breeder) is replaced in the group. I ask whether the resultant decrease in relatedness (from full siblings to half siblings) between the focal subordinate helper and the brood they help to raise affects the helper's provisioning rate.

Methods

Does relatedness affect nestling provisioning rates?

I first investigated whether individuals bring food less often to nestlings that are less related to them. I measured provisioning rate by observing nests containing nestlings and recording the identity of the feeder and the size of the food item brought for all nest feeds that occurred. Food items were assigned a size category based on a comparison with bill length, following the definitions of Raihani and Ridley (2007b): these categories were tiny, small, medium, large and extra large. Each size category was assigned an average biomass value based on the wet mass of 50 representative items for each category (Raihani and Ridley 2007b). I first calculated the average biomass delivered to the nest per hour by each adult subordinate in the group during each observation session. All observations sessions were at least one hour long. This value was then divided by the number of nestlings in the brood to calculate biomass delivery/nestling/hour as a measure of 'helping effort' per nestling. Group subordinates that were less than nine months old were not included in the analysis. These individuals rarely feed young because of their own poor foraging skills (Ridley and Raihani 2008) and accounted for only 4.4% of the total helping effort.

I assessed the relatedness between each subordinate and each brood of nestlings based on a combination of parentage analysis, pedigrees and group life histories (*sensu* Clarke 1984, Owens and Owens 1984, Curry 1988, Emlen and Wrege 1988, du Plessis 1993, Clutton-Brock *et al.* 2000, Russell and Hatchwell 2001, Pemberton 2008). One subordinate gained maternity in one brood, and her contributions to feeding the subsequent brood was therefore excluded from the analysis. Relatedness could be estimated at 0.5 (full siblings), 0.25 (half siblings) 0.125 (mixed brood of half siblings and unrelated nestlings) or 0 (unrelated). Due to sample size restrictions, I categorized these relationships into those that were full siblings and those that were not full siblings. This analysis is biologically realistic, because the relatedness between subordinates and the recipients of their help is directly affected by whether one (or both) of the dominant breeders in their group has been replaced, and subordinates appear to recognize that these replacements are unrelated to them (Chapter 4). The number of adults (potential helpers) in the group, rainfall (see below), nestling age (days post-hatching) and subordinate age (months post-hatching) and body mass (measured at the end of each observation session) on the day of observation, and the average relatedness of the subordinate to the brood were included as potential explanatory terms. Rainfall was used as a proxy for food availability: generally, there is a protracted period between rainfall and subsequent increased insect abundance (Cumming and Bernard 1997). Rainfall was calculated as the number of millimetres that fell in the two months prior to the date that the brood hatched. However, group size (number of adult feeders) was subsequently found to be highly correlated with rainfall and because group size had a larger effect, rainfall was excluded as a potential explanatory term. Data were analysed using a GLMM with a normal distribution of errors and an identity link function, with feeding rate (biomass/nestling/hour) as the response variable, which was square-root transformed to achieve normality. The nest and subordinate identity were included as random terms in the model. This analysis includes 73 daily feeding rate measurements involving 24 subordinates at 13 nests over two breeding seasons.

Does relatedness affect fledgling provisioning rates?

I next investigated whether relatedness affected the amount of biomass delivered by subordinates to young during the extended post-fledging dependency period. I observed groups with fledglings from the day that all nestlings were observed to have fledged until one month after this date. Fledglings from groups in which a subsequent brood of nestlings was hatched less than one month post-fledging were not included in this analysis, because brood overlap affects provisioning patterns (Ridley and Raihani 2008). As for the previous analysis, during each feeding event I recorded the identity of the adult and the size of the food item being delivered (Raihani and Ridley 2007b), and the number of hours that each subordinate was present in the group on the dates observed. In this analysis, fledgling identity was also recorded because feeds to individual fledglings were easily observed, which was not possible when the subordinates fed the brood in the nest. I could then calculate the feeding rate (food biomass delivered/hour) for every subordinate to every fledgling in every group.

As above, I estimated the relatedness between each adult and fledgling based on a combination of parentage analysis, pedigrees, and group life histories. I again classified the relatedness between subordinates and fledglings as full siblings or less than full siblings. The potential explanatory terms included in the model were group size (number of adults), rainfall in the two months prior to fledging, subordinate body mass, subordinate age (months post-hatching), fledgling age (days post-hatching), and the estimated relatedness between subordinates and fledglings. In the first field season in which I collected data, a drought, combined with heavy depredation of nestlings and extremely early cessation of breeding, resulted in very few groups fledging chicks. Consequently, only data from the second season are included in these analyses. All terms were calculated as described above. Adult age was highly correlated with group size, and because adult age had the smaller effect of the two, it was removed as a potential explanatory term. Also, adult body mass was highly correlated with relatedness to fledglings, and because adult body mass had the smaller effect, it too was removed as a potential explanatory term. Biomass/hour was square-root transformed to achieve normality and then used as the response variable in a GLMM with a normal distribution of errors and an identity link function. Random factors included in the models were subordinate and fledgling identity. This analysis includes 156 daily feeding rate measurements involving 14 subordinates to 29 fledglings over one breeding season.

Paired comparison of relatedness and provisioning rate

Detailed provisioning data were collected for eight subordinates that helped to provision at the nests of their parents (containing their full siblings), and helped to provision a new brood of nestlings that were half-siblings after the immigration of a new dominant. The provisioning rate for each subordinate at one nest before and one nest after the immigration event was calculated as the average biomass of food brought per hour to the nest. These rates were compared using a paired t-test.

Results

Does relatedness affect nestling provisioning rates?

Relatedness of subordinates to the brood had no effect on the amount of food brought to the nest after controlling for nestling age and group size (Table 6.1, Fig. 6.1).

Table 6.1: Result of a GLMM investigating feeding rates by subordinate Southern Pied Babblers to nestlings, using estimated values of relatedness. Nest and individual identity were included as random terms in the model. Analysis includes data from 73 daily feeding rate measurements involving 24 subordinates at 13 nests over two breeding seasons.

Model term	χ^2	***P***
Full model		
Nestling age	23.84	< 0.001
Group size (adults)	13.38	0.004
Relatedness to nestlings	0.74	0.410
Subordinate body mass	0.56	0.459
Subordinate age	0.12	0.733
Minimal model	**Effect**	**SE**
Constant	0.506	0.054
Nestling age	0.036	0.007
Group size (adults)	-0.125	0.034

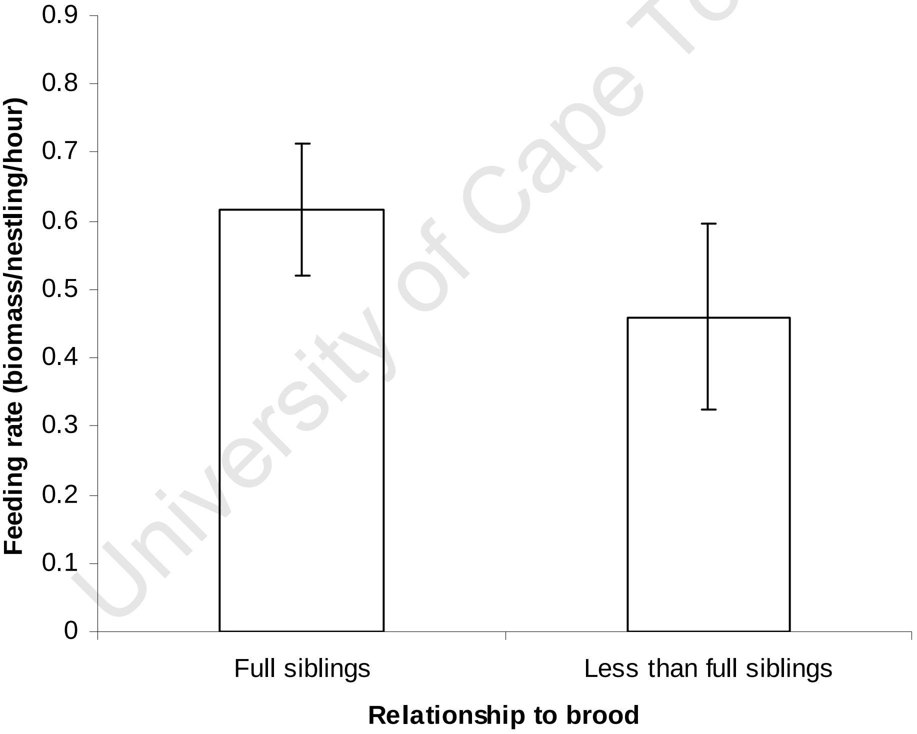

Fig. 6.1: The feeding rate (biomass/nestling/hour) of subordinates as a function of their relationship to the brood. Mean values ± SEM were generated from the predictions of the GLMM presented in Table 6.1. N = 73 daily feeding rate measurements involving 24 subordinates at 13 nests over two breeding seasons

Does relatedness affect fledgling provisioning rates?

Relatedness also had no effect on the amount of food fed to fledglings by subordinates, after controlling for the effects of rainfall and group size (Table 6.2, Fig. 6.2).

Table 6.2: Result of a GLMM investigating feeding rates by subordinate Southern Pied Babblers to fledglings, using estimated values of relatedness. Subordinate and fledgling identities were included as random terms. Analysis includes 156 feeding rate measurements involving 14 subordinates to 29 fledglings over one breeding season.

Model term	χ^2	***P***
Full model		
Rainfall	12.75	< 0.001
Group size	3.28	0.086
Fledgling age	0.96	0.330
Relatedness to fledgling	0.01	0.906
Minimal model	**Effect**	**SE**
Constant	0.651	0.080
Rainfall	-0.006	0.002

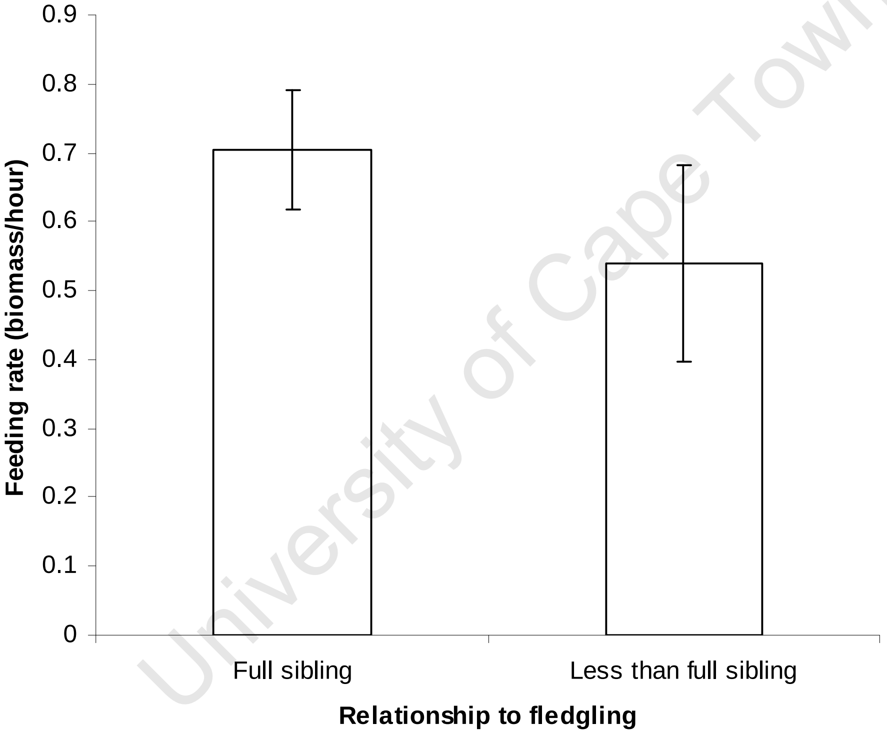

Fig. 6.2: The helping effort of subordinates (biomass/hour) in relation to their estimated relatedness to the fledgling. Mean values ± SEM were generated from the predictions of the GLMM presented in Table 6.2. N = 156 feeding rate measurements involving 14 subordinates to 29 fledglings over one breeding season.

Paired comparison of relatedness and provisioning rate

There was no difference in the provisioning rates of eight subordinates that helped to feed first their full siblings, then their half siblings (paired t-test, t = -0.51, *P* = 0.627 – Fig. 6.3).

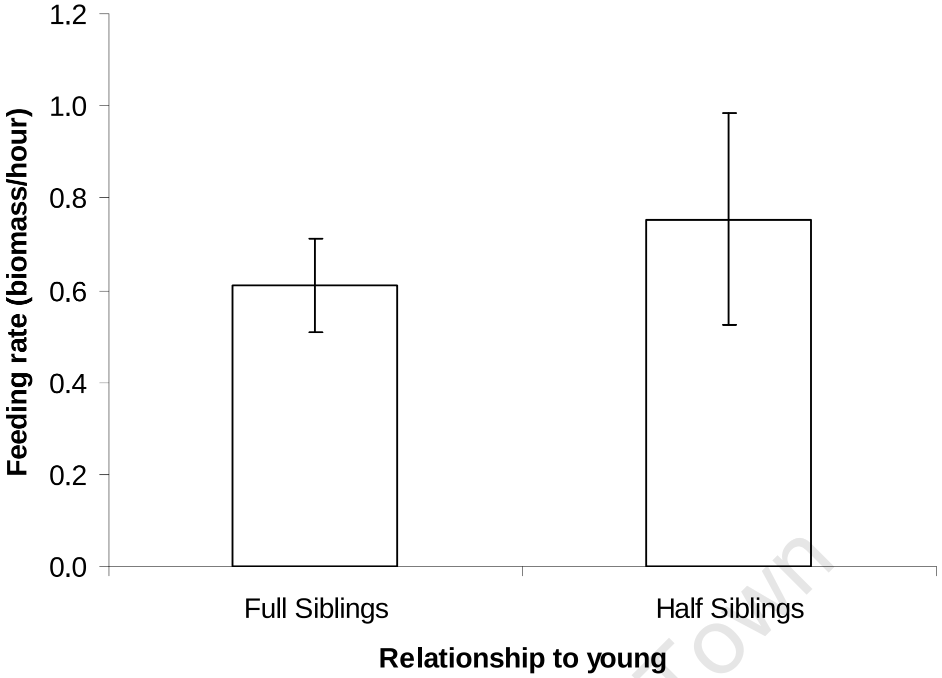

Fig. 6.3: Provisioning rate of eight subordinate Southern Pied Babblers to full- or half-sibling broods hatched before and after immigration of a new dominant, respectively. Means ± SEM are generated from raw data.

Discussion

Provisioning rates by subordinate Southern Pied Babblers to nestlings and fledglings were unaffected by relatedness. This is similar to situations described for Superb Fairy-Wrens (Dunn *et al.* 1995), Arabian Babblers (Wright *et al.* 1999) and Meerkats (Clutton-Brock *et al.* 2001c). These results provide no concrete support for kin selection as the basis for cooperation, but neither do they constitute evidence against it (Clutton-Brock *et al.* 2001c, Russell 2004). In most cooperatively breeding vertebrates (including Southern Pied Babblers – Chapter 3), groups are formed mostly by natal philopatry: this increases the likelihood that groups will comprise closely related individuals and that helping will be kin-biased, even if no preferences for close kin exist (Dickinson and Hatchwell 2004). This creates a difficulty when testing the importance of kin selection by investigating the linkage between relatedness and helping behaviour in these species simply because philopatric helpers may have little opportunity but to help kin (Russell 2004). In addition, when individuals live in closely related groups, the cost of development and employment of mechanisms to discern close from more distant kin may be very high (Keller 1997).

Most Southern Pied Babbler helpers are related to one or both of the dominant breeders, creating opportunities for them to accrue indirect fitness benefits from helping (Chapter 3). Some helpers are completely unrelated to breeders, however, and may be present in groups as immigrant subordinates or as philopatric subordinates who have experienced replacement of both dominants. The presence of these helpers requires explanation if kin selection is to be considered the primary factor driving the occurrence of helping behaviour. Although helpers that were unrelated to both dominants were uncommon, they did occur in almost every year of study of this population. Helping data are more difficult to obtain from complex groups containing unrelated helpers because breeding attempts in such groups often fail at a very early stage, due mostly to reproductive competition (Chapter 5). I suggest there are two potential explanations for the occurrence of unrelated helpers: first, that helping may promote direct rather than indirect benefits, and thus helpers are essentially indiscriminate in who they help, or second, that individuals are making the ‘best of a bad job’ by joining or remaining in a group as an unrelated subordinate, because the costs of living alone are very high (Ridley *et al.* 2008).

While subordinates do not help closely related chicks more than they help distantly related chicks, subordinate Southern Pied Babblers do accrue indirect benefits. For example, an increased adult:fledgling ratio benefits fledglings: at six months of age, relative to fledglings from groups with smaller adult:fledgling ratios, these fledglings are heavier, more proficient at foraging and more likely to disperse successfully (Ridley and Raihani 2007b). Increased adult:fledgling ratios also benefit breeders because intervals between broods decrease and annual productivity increases (Ridley and Raihani 2008). Because the vast majority of helpers are closely related to breeders and thus to chicks in their groups (Chapter 3), these helpers are accruing both indirect benefits, because their help improves the fitness of the recipients, and future indirect benefits, because their help allows the dominant breeders (at least one of whom is usually a parent) to re-nest more quickly and more often (Ridley and Raihani 2007b, 2008). Consequently, subordinates are gaining indirect benefits from helping, though this does not necessarily mean that kin selection is the main cause of helping by subordinates in this species.

Although subordinate Southern Pied Babblers do accrue indirect benefits, the current and future direct benefits of helping may be equally if not more important in driving cooperation (Kokko *et al.* 2001). In Southern Pied Babblers, increased help eventually increases group size (because they rear more young – Ridley and Raihani 2007b, 2008), which is likely to directly benefit the helper (Kokko *et al.* 2001). Group anti-predator vigilance levels increase with group size (Radford and Ridley 2007) resulting in higher food intake rates (Hollén *et al.* 2008). It is therefore likely that survival is highest in large groups (Hollén *et al.* 2008). In addition, the cost of helping is lowered in larger groups because individual contributions to nestling feeding are decreased (this chapter). Subordinates may also gain future direct benefits if the chicks they feed subsequently become dispersal coalition partners, which are more successful than single prospectors in evicting resident dominant females (Raihani 2008). Another such benefit is the acquisition of better skills or experience through helping (Skutch 1961, Komdeur 1996, but see Khan and Walters 1997). Overall, in addition to indirect benefits, current and future direct benefits are also being accrued by helpful subordinates. Because subordinate Southern Pied Babblers derive both direct and indirect benefits from helping to feed chicks, it is impossible at this point to quantify the relative importance of kin selection and direct benefits in driving the evolution and maintenance of help in Southern Pied Babblers. Future accumulation of life history data may ameliorate this situation.

Although helping to feed the offspring of dominant breeders may bring both direct and indirect fitness benefits to subordinate Southern Pied Babblers, in most species these benefits are unlikely to compensate helpers for failing to breed independently (Dickinson and Hatchwell 2004). In Southern Pied Babblers, the direct fitness payoffs of acquiring dominance are very high (Chapter 3), so subordinates might be expected to disperse rather than help. Offspring are expected to remain in the natal group when the benefits of living there exceed the benefits of floating or attempted dispersal (Dickinson and Hatchwell 2004). Indeed, it has been suggested that floating, rather than independent breeding, should be regarded as an alternative to delayed dispersal (Koenig *et al.* 1992). Floating and attempted dispersal are very costly to Southern Pied Babblers: older and heavier individuals are the most successful dispersers, but attaining a breeding position in a non-natal group may take several years (Raihani 2008, Ridley *et al.* 2008). Subordinates may choose to stay in the natal group as a safe haven while they build body condition (Kokko and Ekman 2002), make roving forays into nearby territories (Chapter 4) and perhaps acquire helping experience (Komdeur 1996). Helping therefore may be a strategy to garner extra (indirect) fitness benefits while staying in the natal group for current and future direct fitness benefits (Emlen 1991). Additionally, if there is an unrelated breeding partner for a subordinate in the group, potential direct fitness benefits of breeding in the natal group may be an important reason to postpone dispersal (Chapter 5).

In most cooperatively breeding species, groups are year-round residents and are comprised of a dominant pair with their philopatric offspring that have delayed dispersal (Arnold and Owens 1999, Dickinson and Hatchwell 2004). In these species (including Southern Pied Babblers) it is difficult to investigate the evolution of helping because the direct and indirect benefits of help cannot be considered in isolation. Here I failed to provide evidence of variation in help with relatedness, which may be due to the minimal benefits of doing so in groups that are, in the main, very closely related. Nevertheless, where the indirect or direct benefits of help vary independently of each other, we can determine whether they are having a definite effect. Species in which helpers must choose between related and unrelated recipients such as Western Bluebirds *Sialia mexicana* and Long-tailed Tits are thus more appropriate for investigating the importance of kin selection, and it has indeed been easier to conclude that kin selection is at work in these species (Dickinson *et al.* 1996, Russell and Hatchwell 2001). Similarly, investigation of how help varies in species in which philopatric offspring do not accrue indirect benefits (such as Siberian Jays – Ekman *et al.* 1994), will indicate the importance of direct benefits in the evolution and maintenance of helping in cooperatively breeding species.

Chapter 7

Discussion

Overview

Understanding how reproduction is partitioned among individuals within social groups is pivotal in understanding the evolution and maintenance of helping behaviour in cooperatively breeding species. Knowing which individuals succeed in breeding allows assessment of the costs and benefits of breeding and helping for both dominant and subordinate group members. It can also lend insights into observed behavioural patterns of dispersal and competition for reproduction. The partitioning of reproduction also affects the patterns of relatedness within groups: when only one pair consistently breeds, the resulting young are highly related to one another. This may affect the extent to which kin-selected benefits drive cooperation, because subordinates that are closely related to young may gain higher indirect benefits from helping them than do helpers in species with more distantly related helpers. Molecular techniques have revolutionized understanding of the ecology and evolution of cooperatively breeding species (Koenig and Dickinson 2004). True family relationships within and between groups and populations can now be determined through molecular techniques allowing for parentage analyses and pedigree construction. When pedigrees are not possible, molecular analyses allow relatedness between two individuals to be calculated, in turn making it possible to test kin associations statistically.

In this thesis, I used field observations and molecular techniques to determine how reproduction is partitioned within groups of Southern Pied Babblers and examine the consequences of kinship on dispersal decisions and inbreeding, intra-group conflict between breeders and helpers, and levels of investment in young by helpers. I aimed to answer the following questions:

1. How is reproduction partitioned among individuals in social groups and what fitness benefits do non-breeding subordinates accrue?
2. Do individuals avoid inbreeding and does this affect dispersal patterns?
3. In what contexts do subordinates compete for reproduction, and with what implications for dominants? Do dominants suppress subordinate reproduction?
4. Do subordinates vary the amount of help they give as a function of their relatedness to recipients? In other words, is there evidence for kin selection to drive the evolution and maintenance of cooperative breeding in this species?

Here, I discuss the findings of this thesis with regard to these questions and consider the broader implications of this study.

Kinship – are helpers breeding?

In order to address fundamental questions regarding the nature of the costs and benefits of cooperative breeding behaviour, it is important to verify which individuals in social groups are gaining parentage. This allows better understanding of the benefits of helping by subordinates, and provides answers to the outcomes of any observed reproductive conflicts between individuals in the group. Together, molecular techniques and field observations are powerful tools that can be used to answer these questions. Since the 'molecular revolution', the breeding systems of cooperatively breeding bird species have proved to be unexpectedly diverse (Cockburn 2004). They range from highly monogamous, to polygynandrous (within-group breeding with several co-breeders of both sexes, e.g. Acorn Woodpeckers – Haydock *et al.* 2001), to the extremely high levels of extra-group paternity found in Splendid and Superb Fairy-Wrens (Brooker *et al.* 1990, Mulder *et al.* 1994). The breeding system of Southern Pied Babblers is equally extreme, in that extra-pair parentage is very rare and extra-group parentage is entirely absent. Dominant pairs are socially monogamous and almost completely sexually monogamous, with very infrequent instances of successful reproduction by subordinates (Chapter 3). Only a few other cooperatively breeding bird species that live in year-round groups exhibit similar or greater levels of monogamy. These include Red-cockaded Woodpeckers (Haig *et al.* 1994), Arabian Babblers (Lundy *et al.* 1998), Florida Scrub-Jays (Quinn *et al.* 1999), and Laughing Kookaburras (Legge and Cockburn 2000).

Most members of Southern Pied Babbler groups are closely related to one another and are either parents and offspring, or full or half siblings: mean relatedness within groups is high and groups themselves are discernable as separate genetic entities (Chapter 3). Immigration rates of subordinates into non-natal groups are very low, similar to Red-cockaded Woodpeckers (Walters *et al.* 1992, Haig *et al.* 1994), and Laughing Kookaburras (Legge and Cockburn 2000). The combination of monogamy and infrequent subordinate immigration causes groups to be highly kin-structured. Although immigration of subordinates is rare, immigration of dominants into non-natal groups is common, and even though group members are generally closely related, many groups each year contain unrelated potential breeding partners for subordinates (Chapter 3). Despite this, subordinates in Southern Pied Babbler groups generally receive no share of parentage (Chapter 3), raising the question of whether these individuals restrain themselves from breeding or are prevented from doing so (Chapter 5). Because dominants are the parents of virtually all group offspring, dominance positions confer very high direct fitness benefits. Although subordinates helpers receive almost no direct fitness benefits from reproduction, high indirect benefits are available to them because all helpers are generally closely related (full- or half-siblings) to the young they provision. Because of the indirect benefits available to helpful subordinates, kin selection may play a role in the evolution or maintenance of cooperative behaviour in Southern Pied Babbler groups (Chapter 6).

Why should rates of subordinate immigration be so low? Two reasons are likely to account for this. First, dominants may repel the immigration of same-sex foreign subordinates because of the possibility of reproductive conflict or aggressive overthrow, although the possible benefits of additional help seem to overcome this resistance when groups are very small (Nelson-Flower and Ridley in prep.). Second, the benefits of being an immigrant subordinate (as opposed to a philopatric subordinate) are likely to be low. Immigrant subordinates are accepted only into small groups (Chapter 5). If the natal group of the immigrant is large, moving to a smaller group will reduce their direct benefits of group membership. Additionally, no indirect fitness benefits are available in non-natal groups. However, despite the lack of indirect fitness benefits available, subordinates may have no choice but to immigrate to non-natal groups if the natal group has become extinct (as occurred to several groups during the harsh winter of 2008), or if they have been evicted from the natal group (as has occurred to several young males). Floating is very costly for such individuals (Ridley *et al.* 2008); it is likely that joining any group is preferable to living alone, simply because of elevated survivorship. It is possible that some individuals voluntarily enter non-natal groups as subordinates because even though the short-term benefits in the new group may be lower than those that could be accrued at home, inheritance of the dominance position in a non-natal group is a possible longer-term benefit (Chapter 4). In addition, some subordinates do gain parentage when an unrelated breeding partner is present (Chapter 3), and the possibility remains, however remote, that direct fitness benefits from reproduction may be accrued.

Consequences of kinship – inbreeding and inbreeding avoidance

It is important to identify the extent of inbreeding and inbreeding avoidance in cooperatively breeding species to better understand the role of inbreeding in the partitioning of reproduction, and the role of inbreeding avoidance in influencing dispersal patterns. Although inbreeding depression (the negative effects of inbreeding) was once doubted to be a selective force in wild populations, these doubts have been dispelled (Keller and Waller 2002). Subordinates in cooperatively breeding societies are often philopatric offspring that live in groups of relatives and lack access to extra-group mates, thus setting the stage for inbreeding avoidance (Emlen 1997). Inbreeding avoidance may therefore be a major factor constraining the reproduction of subordinates in many cooperatively breeding species (Koenig *et al.* 2009). In Southern Pied Babblers, there is no question that inbreeding avoidance plays an important role in limiting subordinate reproduction (Chapter 4). Inbreeding may occur very rarely, as has been seen in some (outbreeding) species observed by long-running research projects for which deep pedigrees exist (Woolfenden and Fitzpatrick 1984, Szulkin and Sheldon 2008). As yet, however, Southern Pied Babblers have not been recorded as breeding with closely-related kin, whether familiar or unfamiliar. Further evidence to support or refute the idea of inbreeding avoidance through recognition of familiar, known kin is required: this may be provided by future data, including whether individuals pair with unfamiliar, closely related kin, and whether avoidance of familiar kin is strict.

Although kin recognition by associative learning was suggested as the mechanism by which individuals avoid inbreeding (Chapter 4), this possibility must be investigated experimentally. Kin recognition has been elegantly investigated in some cooperatively breeding species (e.g. Sharp *et al.* 2005), but in general this area remains poorly researched. I have suggested here that Southern Pied Babblers come to know and recognize other group members after a period of association, and that if this period of association occurs during the dependent period of either party, these individuals perceive one another as kin (Chapter 4). This type of kin recognition has been suggested to occur in the closely-related Arabian Babblers (Zahavi 1990) and was first proposed by Hamilton (1964) and later explored more thoroughly by Komdeur and Hatchwell (1999). I attempted to use cross-fostering of nestlings to investigate this question (data not shown in previous chapters), but due to drought and a lack of synchronous breeding, was successful in only three instances. There was no evidence in any of these episodes that Southern Pied Babblers recognized the existence of a foreign nestling (indeed, several cuckoo chicks have been observed with babbler groups – A.R. Ridley, unpublished data). Unfortunately, each of these cross-fostered chicks was depredated before reaching adulthood. To determine unequivocally if kin recognition by associative learning is occurring in Southern Pied Babblers or similar species, a series of presentation and cross-fostering experiments is required. Presentation experiments involving a choice between two individuals in an aviary could be used to determine whether recognition of unfamiliar kin is possible, and probe the mechanism of that recognition (vocal *vs* visual). Experiments that attempt to cross-foster fledglings of various ages (from newly fledged to independent) could investigate the recognition learning period, by observing the age at which fledglings recognize (or are recognized as) non-group members. Together, these might inform us as to the nature of the kin recognition and the timing and length of the period of association required to develop such recognition in Southern Pied Babblers.

Inbreeding avoidance impacts dramatically on dispersal dynamics in Southern Pied Babblers, because subordinates never inherit dominance in the natal group when the opposite-sex dominant is their parent (or other relative), and do not pair with known relatives when they disperse (Chapter 4). This is very different from species such as Splendid Fairy-Wrens, Meerkats, and Grey-Crowned Babblers where close relatives may hold dominant positions together (Rowley *et al.* 1986, Griffin *et al.* 2003, Blackmore and Heinsohn 2008). In these species, individuals that are paired with close relatives may avoid inbreeding depression through extra-pair and extra-group reproduction (Brooker *et al.* 1990, Griffin *et al.* 2003, Blackmore and Heinsohn 2008). However, the Southern Pied Babbler breeding system is highly monogamous (Chapter 3), and extra-group copulation has never been observed, nor can it be inferred from genetic data. Southern Pied Babbler individuals should therefore avoid inheriting dominance in a group where the prospective mate is a close relative, because there are few routes to successful reproduction other than with the social partner (Chapter 3, Chapter 5).

Consequences of kinship – reproductive conflict and skew

Although many subordinates do not attempt reproduction in their natal groups because of inbreeding avoidance, nearly 50% of groups contain breeding opportunities for them because of the relatively high turnover of dominants (Chapter 3). Nevertheless, subordinates very rarely reproduce, even when potential breeding partners are present. This is not because these subordinates are restraining themselves from attempting to breed. Rather, in these situations subordinates commonly enter into competition. This is evidenced by courtship between subordinates and unrelated dominants (Chapter 4), increased participation in nest-building by subordinates when there are potential breeding partners in the group (Chapter 5) and destruction of the dominant female's eggs by subordinate females (Chapter 5). These subordinates fail to breed because of a combination of aggressive suppression by dominants and mate choice by the dominant female (Chapter 5, A.R. Ridley, unpublished data). Thus, conflict can be both expressed and resolved very early in the breeding cycle – an aspect of cooperative breeding that has often been overlooked. Only through the use of both field observations and molecular tools can such a situation be truly understood, because the conflict between dominants and subordinates may not be reflected in the genetic outcome. This is particularly the case in Southern Pied Babblers, where the number of young produced by subordinates is far lower than the frequency of subordinate competition for reproduction.

A female subordinate in a group containing a potential mate is less likely to compete when the dominant female is closely related to her. The explanation for this may lie in the cost of female-female competition. Such competition causes delays in the onset of reproduction, increased nest abandonment, and shorter breeding seasons which are likely to represent fitness costs to the dominant female. In addition, when subordinate females do (rarely) succeed in hatching eggs, the resultant increase in brood size can impose costs on both the dominant female and her offspring. Subordinate females that are highly related to dominant females may benefit more from refraining from competition and investing in helping, because in this way they gain increased direct benefits from being in a larger group (such as increases in vigilance, foraging success and survivorship) as well as accruing indirect benefits from assisting close relatives (Raihani and Ridley 2007a, Ridley and Raihani 2007a, 2007b, Radford and Ridley

2007, Hollén *et al.* 2008). These observations correspond to some of the predictions of Cant (1998), who hypothesized that the costs of sub-optimal brood size could work to prevent related subordinate females from breeding. Alternatively, female subordinates that are highly related to dominant females may refrain from competition because of extreme dominance asymmetries developed over the subordinate's lifetime, which could reduce the subordinate's competitive ability (Clutton-Brock 1998).

Reproductive competition between females limits the reproductive success of dominant females (Chapter 5), but the presence of potentially competitive subordinates in babbler groups may sometimes benefit dominants due to the increased direct benefits that dominants receive from the presence of helpers. First, dominants that have helpers during the nestling and fledgling periods generally have increased reproductive success and can re-nest more quickly (Ridley and Raihani 2007b, 2008). Second, as part of a larger group, dominants may experience increased survival due to increased group vigilance (especially when foraging) (Hollén *et al.* 2008). Small Southern Pied Babbler groups have poor reproductive success (Ridley and Raihani 2008) and dominants in these groups are much more likely to accept an unrelated subordinate (and thus potential reproductive competitor) than dominants in large groups, which invariably repel immigrant subordinates (A.R. Ridley, unpublished data). A similar effect is seen in fish, in which a territory-holding male may tolerate competitors when their presence benefits the male in terms of territory defense, brood care, and attraction of mates, although he may lose some direct fitness if these competitive males obtain sneak fertilizations of eggs (reviewed by Taborsky 2001). When the benefits of tolerating the reproductive competitor outweigh the fitness increase (assured paternity or maternity, or increased reproductive success) of its absence, dominants should allow competitors to stay (Gaston 1978), as is observed in Southern Pied Babblers, where very small groups tolerate the presence of reproductive competitors (Nelson-Flower and Ridley in prep.).

Reproductive skew theory addresses the struggle between dominants and subordinates for reproduction, and encompasses a series of models (reviewed by Johnstone and Cant 2009). Some models of reproductive skew ('transactional' models) assume that one party has complete control over the other's reproductive share. For instance, some such models assume that dominants completely control subordinate reproductive activity, suppressing it as much as possible without causing subordinates to leave. Other such models assume that subordinates completely control their own reproductive share, but maintain their reproductive effort at levels low enough to ensure that they are not evicted from the group (reviewed by Johnstone 2000). Thus these models assume there is no intra-group conflict, which may not reflect biological reality. Other reproductive skew models (‘compromise’ or ‘tug-of-war’ models), assume that neither dominants nor subordinates have total control over the division of reproduction and that the competition between them will negatively impact group productivity (reviewed by Johnstone 2000). In these models, intra-group conflict leads to reproductive compromise, and group stability is not addressed, which also fails to reflect biological reality. Recently, models have been developed that draw assumptions from both transactional and compromise models (reviewed by Magrath *et al.* 2004, Johnstone and Cant 2009). These synthetic models assume that individuals concede shares of reproduction to one another without conflict, but then a tug-of-war (conflict) takes place over the remaining fraction (Johnstone 2000, Reeve 2000, Reeve and Shen 2006, Cant and Johnstone 2009). They assume that conflict can exist between individuals (and neither side has complete control over the division of breeding), but that subordinates may voluntarily depart or dominants may forcibly evict subordinates (reviewed by Magrath *et al.* 2004). These models address both intra-group conflict and its effect on group stability, and thus reflect biological reality better than do transactional and compromise models.

The Southern Pied Babbler breeding system fits the assumptions of the synthetic models better than those of either the transactional or compromise models. The reason for this is that these synthetic models incorporate ‘outside options’ (such as leaving the group, or evicting the competitor) as potential solutions to or consequences of reproductive conflict. In Southern Pied Babbler groups, reproductive suppression clearly *is* occurring, and obvious conflict occurs between competitive subordinates and dominants such that neither is wholly in control of the subordinate’s reproduction (Chapter 5). In addition, potentially competitive subordinates are very rarely found in large babbler groups, suggesting that when groups become large, these subordinates are either evicted by dominants, or repelled when they attempt to immigrate (Chapter 5). I suggest that only in small groups do the benefits of admitting an unrelated subordinate justify the potentially high costs of competition or loss of parentage. Within these small groups, a “zone of conflict” or “window of selfishness” exists (Reeve 2000), leading to conflict between dominants and subordinates.

Consequences of kinship – kin selection

In some species, such as White-browed Scrubwrens *Sericornis frontalis* and Seychelles Warblers, subordinates get a share of parentage in the brood, which may explain why they help to raise it (Magrath and Whittingham 1997, Whittingham *et al.* 1997, Richardson *et al.* 2002). This is not the case for subordinates in Southern Pied Babbler groups (Chapter 3). Rather, these subordinates are helping for (1) direct benefits, possibly including group augmentation (Kokko *et al.* 2001), increased breeding experience (Skutch 1961, Komdeur 1996) and improved future access to mates (Reyer 1990, Zahavi 1995); and (2) for high indirect benefits (Hamilton 1964). Chapter 3 shows that these high indirect benefits are available to helpers because all helpers are generally closely related to the young they provision, most being either full or half siblings. This is due to both the monogamous breeding system and the low immigration rate of subordinates into non-natal groups. An increased number of helpers positively influences the physical condition of chicks and breeders: subordinates that help are thus gaining indirect fitness benefits (Ridley and Raihani 2007b, 2008). However, helpers do not adjust their provisioning rates to account for their relatedness to the recipient young (Chapter 6). As such, this provides no concrete support for the hypothesis that kin selection drives helping by subordinates, although neither does it refute it (Russell 2004). Because Southern Pied Babbler groups are formed by natal philopatry, any help given in them will be kin-biased, even when no preferences for close kin exist (Dickinson and Hatchwell 2004); there is no strong selective pressure that requires subordinates to be able to distinguish (within the group) between relatives that are close and those that are more distant or are unrelated. Thus, philopatric helpers may have little opportunity but to help kin (Russell 2004).

Although kin-selection is likely to have a role (albeit possibly minor) in the evolution and maintenance of helping in Southern Pied Babblers, the importance of kin selection in cooperative breeding species overall is still unclear. For example, even in species with very similar breeding systems to Southern Pied Babblers, such as

Laughing Kookaburras and Arabian Babblers, evidence for the role of kin selection in driving cooperation varies widely. In Laughing Kookaburras, an increase in helper number has no effect on reproductive success, although it was suggested that breeders may gain an energetic advantage from the presence of helpers (Legge 2000a). In Arabian Babblers, however, similar to Southern Pied Babblers, increased numbers of helpers do increase reproductive success (Wright 1998). In Arabian Babblers and Laughing Kookaburras, there was no relationship between the provisioning rate and relatedness of helpers to the brood (Wright *et al.* 1999, Legge 2000b), paralleling the results of this study (Chapter 6). Although this provides no concrete support for kin selection as the basis for cooperation, neither is it evidence against it (Clutton-Brock *et al.* 2001c, Russell 2004). Across species, the importance of kin selection may vary with the benefits of helping, but a broad correlation does exists between the degree of relatedness to recipients and the amount of help provided by subordinates (Griffin and West 2003). Kin selection favours helping behaviour in cooperatively breeding vertebrates (Griffin and West 2003), but the magnitude of the importance of indirect benefits when compared to that of direct benefits is still unclear, and the debate continues.

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