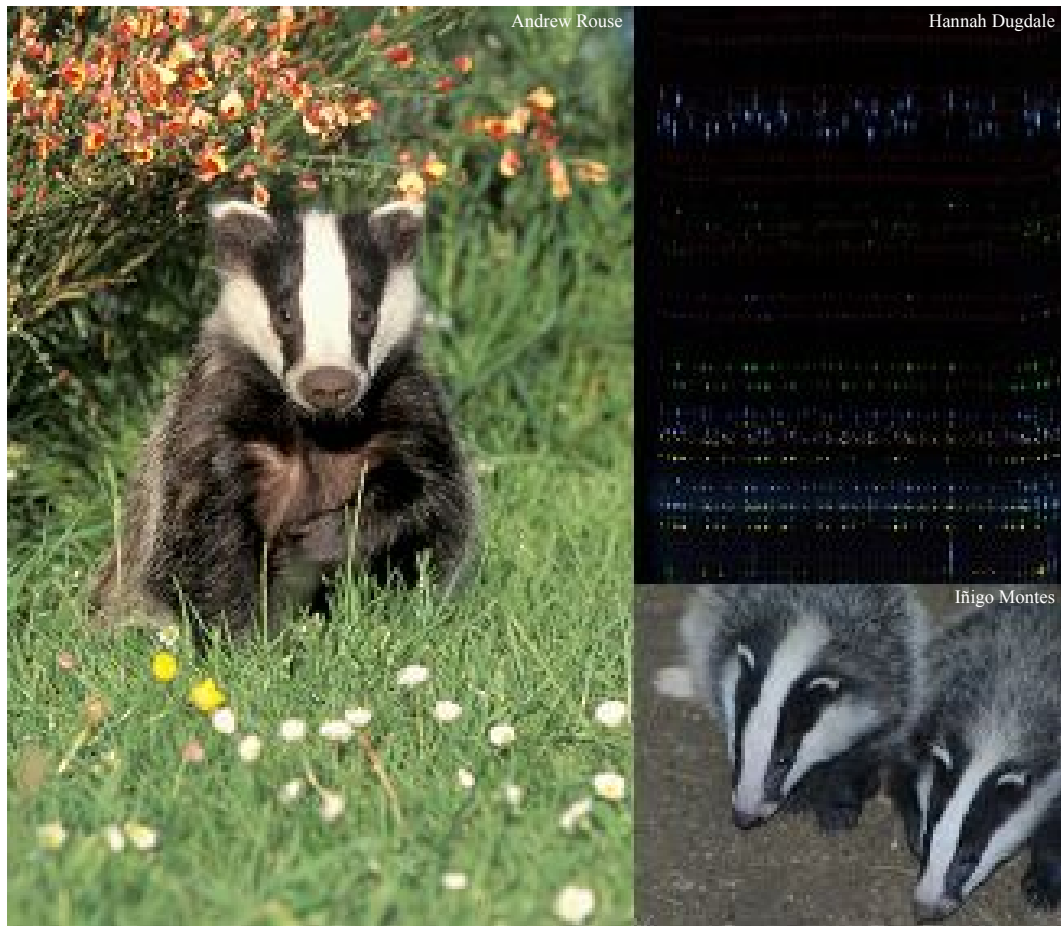


**The evolution of social behaviour: the effect of mating
system and social structure in the European badger
*Meles meles***

A thesis submitted for the degree
Doctor of Philosophy

Hannah Dugdale
Linacre College, University of Oxford
Trinity Term 2007

To Fred, Liz, Dave, and Jonathan



Badger hates Society, and invitations, and dinner, and all that sort of thing...
... Badger, who, though he cared little about Society, was rather fond of children

Kenneth Grahame

The evolution of social behaviour: the effect of mating system and social structure in the European badger *Meles meles*

Abstract

Studies of mating systems and social organisation have been central to understanding of the evolution of social behaviour. The European badger *Meles meles* is a good species in which to study these processes, as its complex social system provides an opportunity to investigate how both natural and kin selection shape the evolution of mating systems and social structure. In this thesis, I use behavioural and genetic data to describe the mating system and social organisation of a high-density badger population and examine the occurrence of cooperative breeding.

I genotyped 915 (85%) badgers trapped in Wytham Woods (1987–2005), 630 of which were cubs, and assigned both parents to 331 cubs with 95% confidence. This revealed a polygynandrous mating system, with up to five mothers and five fathers per social group. Mounting behaviour was also polygynandrous and I show the strongest evidence to date for multiple-paternity litters. I demonstrate, for the first time, that groups consisted of close and distant kin: approximately one third of group members were first-order kin, and overall group members had slightly lower relatedness levels than half-siblings. Within groups, adult and yearling females had higher pairwise relatedness than males, and neighbouring groups contained relatives. These findings result from the high level (42%) of extra-group paternities, 86% of which were assigned to neighbouring males. For the first time I show that females avoided inbreeding by mating with extra-group males; however, incestuous matings did occur. Promiscuous and repeated mountings were observed, which may reduce male–male aggression and infanticide, but may also promote sperm competition, genetic diversity, and / or genetic compatibility.

Just under a third of adult males and females were assigned parentage each year and I quantify, for the first time, reproductive skew within badger groups. Correlations between relatedness, group productivity, and reproductive skew were not consistent with the predictions of incomplete-control models; rather, resource availability may play a role. Older and younger badgers displayed reduced annual breeding success, with male success increasing initially with experience. The Restraint, Constraint, and Selection Hypotheses did not explain the age-related breeding pattern in females. Variance in lifetime breeding success (LBS) was greater for males. Males that only bred within or only outside of their groups had half the LBS of males that did both. Females that were assigned maternity probably bred cooperatively and allonursed non-offspring, which has not been demonstrated previously. No benefit was established, however, in terms of litter size, probability of offspring breeding, or offspring lifetime breeding success, with more mothers in a group.

In conclusion, badger social groups are fostered through kinship ties. Polygynandry and repeated mounting may have evolved originally to reduce male–male aggression and infanticide by males, through paternity masking. Although plural breeding occurs, group living appears to be costly. Motivation to disperse may be reduced through high-levels of extra-group paternities, which may also reduce inbreeding. Cooperative breeding among mothers may represent a low-cost behaviour with indirect benefits due to high levels of relatedness between female group-members. Badger sociality therefore represents an early stage in the evolution of social behaviour.

Statement of contributions

For thesis submitted for the degree of Doctor of Philosophy, Trinity Term 2007

As the principal investigator on all aspects of this thesis, I contributed primary intellectual input and effort into the design of this study, data collection, and data analysis. In addition to my supervisor, David Macdonald, the major contributors in terms of financial, logistical, and / or intellectual input were Terry Burke, Lisa Pope, and Paul Johnson. Terry Burke and Lisa Pope contributed to the scientific input of chapters two and three, and Lisa Pope also provided constructive criticism on the other three data chapters. Additionally, Terry Burke supported, financially and logistically, all of the molecular work and Lisa Pope provided guidance with the genotyping methods. Paul Johnson provided analytical advice and assisted with the relatedness permutation tests. Finally, Eli Geffen kindly ran the rarefaction analysis and Pierre Nouvellet calculated λ_{ind} .

Signed: Date:

Hannah Dugdale

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Introduction

1.1 Sociality

Sociality exists along a continuum and its extent within a species is the result of a complex interaction of factors. These factors can be divided into those that lead to the formation of a group of individuals and those that lead to the evolution of social behaviour once a group has formed (Alexander, 1974). The Resource Dispersion Hypothesis (RDH) proposes that when resources are distributed patchily in time or space, the minimum defensible area that provides adequate resources for a single breeding unit may also accommodate further individuals (Macdonald, 1983; Carr & Macdonald, 1986). A social group will be defined here as a group of conspecifics with overlapping home ranges. Once social groups form, they are expected to be maintained when the benefits of staying within a group are greater than the costs of dispersing (Emlen, 1982). Social groups are formed and sustained either through natal philopatry, or through the immigration of individuals that were born elsewhere and then dispersed. Social groups can therefore consist of unrelated or closely related individuals, or comprise a mixture of related and unrelated individuals. Whatever the group composition, natural selection will act on individuals so that they behave in a way that best promotes their gene contribution to the next generation. Additionally, kin selection may act on individuals to increase their inclusive fitness by helping relatives to breed if the fitness benefit to the recipient (b), weighted by the relatedness between the individuals (r), is greater than the cost to the actor (c), then cooperation can be selected, i.e. when $rb - c > 0$ (Hamilton, 1964).

The way in which individuals pass on their genes will depend upon factors such as the costs and benefits of dispersal, the trade-off between breeding now or later, and the decision of whether or not to alloparent. The dispersal option that is best suited to an

individual will be determined by ecological constraints (Emlen, 1982), benefits of philopatry (Stacey & Ligon, 1991), life-history traits (Arnold & Owens, 1998), or a combination of these factors (Hatchwell & Komdeur, 2000). An individual's decision on whether to breed now or whether to delay breeding will be influenced by competition between relatives (West *et al.*, 2001; West *et al.*, 2002), reproductive conflict (Alexander, 1974; Keller & Reeve, 1994; Reeve *et al.*, 1998), and the fitness costs of breeding with a relative (Charlesworth & Charlesworth, 1987). The decision of whether to alloparent (i.e. provide care for offspring that are not the genetic offspring of an individual) will be influenced by the occurrence of delayed dispersal and reproductive suppression in species in which non-breeders provide alloparental care, and also of care of non-offspring in species that show alloparental care among breeders (Solomon & French, 1997).

In social species, individuals can therefore: remain in their natal group to help relatives breed; they may themselves breed within their natal group; they may disperse and breed; or, they may even disperse and help others to breed before themselves breeding. Overall, the mating strategy that an individual selects should be the one that maximises their lifetime reproductive success (Hamilton, 1964; Williams, 1966), and this will be influenced by the mating system and social organisation, which both play important roles in the evolution of social behaviour.

1.1.1 Mating system

The mating system is characterised by the number of breeding individuals of each sex and the number of mates with which an individual breeds. The number of breeding

individuals in a social group can vary from the extremes of a single pair of breeding individuals, known as high skew which is typical of many small canids, to plural breeding or low skew, typical of the smaller members of the Herpidae (Creel & Macdonald, 1995). Within groups, individuals may be socially monogamous, with paternity gained by the within-group male, as seen in the dik-dik *Madoqua kirkii* (Brotherton *et al.*, 1997). Alternatively females may be promiscuous, with a share of the paternity gained by within-group subordinate males, e.g. African wild dogs *Lycaon pictus* (Girman *et al.*, 1997).

Paternity may be gained by extra-group males as well as within-group males, and genetic technology has shown that this is generally more common than previously suspected. For example, in socially monogamous red foxes *Vulpes vulpes* only 20% of cubs are sired by within-group males (Baker *et al.*, 2004). The degree of such extra-pair paternity is thought to depend upon the association patterns between males and females, as it varies greatly among socially monogamous, polygynous and polyandrous species (Clutton-Brock & Isvaran, 2006). Extra-group paternity may represent a substantial percentage of an individual's lifetime breeding success, and therefore it can be an important reproductive tactic, as demonstrated in meerkats *Suricata suricatta* (Young *et al.*, 2005).

Motivation or selection to disperse may therefore be influenced by both the costs and benefits of group living on direct reproduction. Extra-group paternity may allow genetic dispersal to occur, reducing the direct costs of remaining in a high skew group or of living in a group with both male and female relatives.

1.1.2 Social organisation

Piecing together the jigsaw puzzle of genetic relationships within and between social groups is vital when attempting to unravel the biological importance of social interactions. In many mammalian species males tend to disperse whereas females tend to be philopatric, which may lead to the evolution of cooperative behaviour between these individuals (Greenwood, 1980). Studies of relatedness within social groups of Ethiopian wolves *Canis simensis* revealed high relatedness among within-group males and low relatedness between within-group males and females, probably because of female-biased dispersal (Randall *et al.*, 2007). In comparison, dispersal is male-biased in savannah baboons *Papio cynocephalus*, and within-group relatedness among males was lower than that among females (Altmann *et al.*, 1996). High relatedness among group members does not necessarily mean that cooperation will occur, but it is a requirement for kin selection (Hamilton, 1964). If the relatedness of individuals within groups is greater than that between groups, this further increases the likelihood of cooperation due to kin selection. In both the Ethiopian wolves and savannah baboons, relatedness was greater within groups or matrilineal lines than between them, which may select for the evolution of cooperative behaviour in these species (Altmann *et al.*, 1996; Randall *et al.*, 2007).

1.1.3 Models of reproductive skew

Reproductive skew measures the proportion of individuals that breed within a social group, and is a valuable measure for understanding the evolution and maintenance of sociality. Models of reproductive skew predict the factors that determine reproductive skew within social groups. Empirical data on the mating system and social

organisation of a species can then be used to evaluate these predications. There are three types of reproductive skew models: (1) transactional models, such as concession and restraint models (Emlen, 1982; Vehrencamp, 1983a, 1983b; Reeve & Ratnieks, 1993; Johnstone & Cant, 1999b; Johnstone *et al.*, 1999; Kokko & Johnstone, 1999; Ragsdale, 1999; Reeve & Emlen, 2000); (2) incomplete-control models, such as brood size effect, tug-of-war and infanticide models (Cant, 1998; Reeve *et al.*, 1998; Johnstone & Cant, 1999a); and, (3) synthetic models (Johnstone, 2000; Reeve & Shen, 2006; Nonacs, 2007).

Transactional models assume that individuals limit their own reproduction within the group to ensure group stability. There are many transactional models, and here I introduce two: concession and restraint models. Concession models assume that dominants control the reproductive skew of same-sex group members and group membership. Models of groups with two members predict that reproductive skew should increase with relatedness, as closer relatives can gain greater inclusive fitness benefits from each other and will therefore require fewer concessions to remain in the group (Reeve & Ratnieks, 1993). Adding a third member produces complex predictions, dependent upon the group productivity benefit that the three-member group provides relative to the two-member group, and the relatedness of the subordinates to each other and to the dominant, and can lead to a predicted decrease in reproductive skew with relatedness (Johnstone *et al.*, 1999). Observations compatible with concession models have been made for dwarf mongooses *Helogale parvula* (Creel & Waser, 1991) and social marmots (Allaine, 2000). Restraint models, like concession models, assume that the dominant controls group membership; however, they also assume that subordinates control reproductive skew (Johnstone & Cant,

1999b). Subordinates may restrain from breeding to avoid the dominant evicting them from the group. Restraint models predict a negative relationship between reproductive skew and relatedness. This is because the indirect costs to the dominant of evicting the subordinate are greater when they are close relatives and subordinates have a greater effect on group productivity, so dominants will tolerate more reproduction in these situations. Observations consistent with restraint models have been made for mountain gorillas *Gorilla beringei beringei* (Robbins & Robbins, 2005).

Incomplete-control models, in comparison to transactional models, assume that dominants have only limited control of reproductive skew and that they must compete directly with others to increase their share of the total group reproduction, at the cost of group productivity. Tug-of-war models predict that increasing relatedness has either no significant effect on reproductive skew, when dominance is defined by efficiency, or decreases reproductive skew when dominance is defined by access to resources (Reeve *et al.*, 1998). Reproductive ability is primarily a result of competitive ability, so reproductive skew is affected by the effort of the dominant, which tends to decrease as relatedness increases. Conversely, Cant's (1998) brood size effect model predicts that individuals will adjust their litter size according to the reproductive output of others within the group. Reproductive skew is predicted to increase with relatedness as, for example, subordinates that are highly related to the dominant are less likely to breed if this significantly reduces the indirect fitness benefits that they may otherwise gain (Cant, 1998). Observations compatible with limited-control models have been made for meerkats (Clutton-Brock *et al.*, 2001), spotted hyenas *Crocuta crocuta* (Engh *et al.*, 2002), and certain primates (Widdig *et al.*, 2004; Bradley *et al.*, 2005; Kutsukake & Nunn, 2006).

Transactional and incomplete-control models are not, however, necessarily exclusive and have been conflated as synthetic models (Johnstone, 2000; Reeve & Shen, 2006; Nonacs, 2007). Synthetic models are currently a bit of a ‘hot potato’ and there are ongoing discussions on factors such as the biological applicability of the assumptions of some of these models (Nonacs, 2007). More importantly, synthetic models are misconceived and economic analyses investigating how outside options influence conflict resolution show that current synthetic models are flawed (Michael Cant, pers. comm.).

1.2 European badgers

Meles meles is a medium sized (c. 10 kg) carnivore, distributed from the UK to Japan and from the southern Mediterranean to the Russian Arctic Circle (Kruuk & Parish, 1982; Woodroffe & Macdonald, 1993; Rodríguez *et al.*, 1996; Brøseth *et al.*, 1997; Virgos & Casanovas, 1999; Johnson *et al.*, 2001b; Goszczynski *et al.*, 2005; Kaneko *et al.*, 2006). Researchers refer to *Meles meles* as both the Eurasian and the European badger. Mitochondrial data have revealed four biogeographic groups of *Meles meles*, located in Europe, south-west Asia, north and east Asia, and Japan (Marmi *et al.*, 2006). Craniological data have been used to suggest that *Meles meles* is three distinct species (European, Asian and Japanese badger, Abramov & Puzachenko, 2005), but the current consensus is for one species. I therefore refer to the European badger *Meles meles* throughout this thesis to indicate the biogeographic area from which the data emanate and its group-living status within the studied population, in Wytham Woods, Oxford, UK.

The European badger is unusual among Mustelids, which are primarily solitary (Johnson *et al.*, 2000), as although it is single- or pair-living across most of its range, it also lives in groups in some areas. In south-west England badgers are typically group living (Kruuk, 1978; Cheeseman *et al.*, 1987; Macdonald & Newman, 2002), with up to 29 group members (da Silva *et al.*, 1994). Groups are thought to form passively initially, in accordance with the RDH (Carr & Macdonald, 1986), and are maintained by natal philopatry and restricted dispersal (Cheeseman *et al.*, 1988; da Silva *et al.*, 1994; Pope *et al.*, 2006). Few benefits to group living have been established in badgers (Woodroffe, 1993; Woodroffe & Macdonald, 1995b; Johnson *et al.*, 2004).

Badgers can mate throughout the year, however, there are two peaks in mating activity; the first is post-partum in spring (February–May) and the second is in autumn (August–September, Neal & Cheeseman, 1996). Females exhibit delayed implantation, whereby a fertilised egg ceases development temporarily at the blastocyst stage and remains suspended in the uterus lumen rather than being directly implanted (Fries, 1880). During this period of delayed implantation, superfoetation (conception during pregnancy) may occur (reviewed in Appendix I). Implantation is triggered by a change in photoperiod around the winter solstice (Canivenc & Bonnin, 1981), and individuals may fine tune implantation according to local food availability (Woodroffe, 1995; Dugdale *et al.*, 2003). Females then give birth once a year, around February and cubs remain underground for approximately the first eight weeks. The mean foetal litter size, based on ultrasound data from 1993 to 2005 was 1.9 ± 0.1 (mean with 95% confidence interval, Chapter 2). The mean post-emergence litter size,

based on the number of cubs trapped each year and the number of lactating females (determined by teat size data from 1987–1996), was 1.6 (Macdonald & Newman, 2002). There was no sex difference in the survivorship of badgers, and the greatest mortality was seen in cubs (Table 1.1, Macdonald & Newman, 2002). From the Wytham Woods trapping data, 1987–2005, the mean age of badgers (that were first trapped as a cub, so were of known age) at last capture was 2.6 ± 0.2 years and the two oldest badgers were last trapped at 13 years of age.

Table 1.1 Survivorship (lx) of badgers from Wytham Woods, Oxford, UK, 1987–1996 (Macdonald & Newman, 2002). Based on a starting population of 1000 (nx).

Age (years)	nx	lx
0	1000	1.00
1	749	0.75
2	582	0.58
3	413	0.41
4	301	0.30
5	194	0.19
6	142	0.14
7	71	0.07
8	55	0.05
9	25	0.02
10	5	0.01

1.2.1 Study site and population demography

The data for this study come from long-term work conducted in Wytham Woods, Oxfordshire (01° 19'W, 51° 46'N; UK National Grid Reference SP 46 08). The majority of the badger setts are enclosed by a deer fence encompassing an area of 4 km², which has a varied habitat composition (Table 1.2). Wytham Woods then cover a 6 km² area between 60–165 m altitude, surrounded by permanent pasture and mixed arable crop farmland. This area is enclosed by the river Thames to the north-west, Seacourt stream to the north-east, the A34 to the south-east and the B4044 to the

south-west. These features potentially limit the movement of badgers in and out of the study area (Macdonald & Newman, 2002). The badger population ranged from 60 to 228 adults and 23 to 61 cubs between 1987 and 1996, with the highest density of 44.3 badgers / km² recorded in 1996 (Macdonald & Newman, 2002). The post-emergence cub sex ratio of the population and the adult sex ratio did not differ from 50% (Macdonald & Newman, 2002; Dugdale *et al.*, 2003).

Table 1.2 The habitat type enclosed within the deer fence in Wytham Woods. Data from Ellwood (2006).

Habitat type	Cover in Wytham Woods (%)
Semi-natural high forest	36.5
Semi-natural abandoned coppice	20.5
Plantation	29.0
Improved grassland	7.2
Rough grass	4.1
Arable	2.7

Data included in this thesis were obtained from capture-mark-recapture events of 1,081 badgers that occurred over 19 years, between June 1987 and November 2005. The territory borders of the badger social groups in Wytham Woods were mapped every two years using bait-marking techniques, whereby colour-coded plastic pellets mixed with peanuts and golden syrup were placed at each sett, one colour per sett, for two weeks after which a survey was conducted noting the location of the plastic pellets (Kruuk, 1978; Delahay *et al.*, 2000). Territory fission, the splitting of a social group into two, occurred (da Silva *et al.*, 1993). Based on trapping records, the mean number of social groups in which badgers were present each year was 19 ± 2 (range = 14–26; 1987–2005). The mean numbers of candidate mothers and candidate fathers per social-group-year were 5.6 ± 0.4 (median = 5) and 5.8 ± 0.4 (median = 5), respectively (see Figure 2.2).

Badgers are natally philopatric and dispersal is restricted (da Silva *et al.*, 1994; Pope *et al.*, 2006). In Wytham Woods, 81% of cubs ($n = 216 / 267$ cubs trapped 1987–2005) were natally philopatric (Macdonald *et al.*, submitted). The natal group was defined as the first group in which a cub was trapped. A permanent dispersal event occurred when a badger was trapped twice in a different group, and at least once in the two trapping events prior to that, with a minimum inter-trap interval of 30 days and a minimum trapping event for the four captures of 400 days. Although permanent dispersal was not sex-biased (28 males and 23 females dispersed), males made more temporary excursions than females (Macdonald *et al.*, submitted).

1.2.2 Trapping methodology

Badgers were trapped at least four times a year, over nine days in January, and over twelve days usually in: June, August and November (Tuytens *et al.*, 1999; Macdonald & Newman, 2002). Trapping was suspended between February and May when cubs were dependent upon their mothers. Additional trappings occurred to gain information on focal groups or to ensure capture of cubs if some cubs were too small (< 2 kg) to trap in June. Badgers were trapped in swing-door, galvanised steel, mesh, box-traps (c. $85 \times 37 \times 38$ mm) baited with peanuts. Pre-baiting was abandoned after 1994 as it did not affect trappability. Trappability does not vary between males and females or between adult age classes, but cubs are less likely to be trapped than adults (Tuytens *et al.*, 1999). Ninety-five percent of the inter-trap intervals were within 525 days or 6–7 trapping events ($n = 6,193$).

Badgers were sedated by an intra-muscular injection of approximately 0.2 ml / kg Ketamine hydrochloride (Vetlar; Pharmacia and Upjohns, Crawley, UK). Cubs were first trapped at c. 16 weeks of age (around June) when they were considered independent of their mothers. Cubs judged to weigh ≤ 2 kg were considered too small for sedation and were released from traps after a guard hair sample had been collected. On initial capture, badgers were marked with a unique tattoo number, assigned chronologically, on the inside of their left hind leg (Cheeseman & Harris, 1982). Recaptures were identified by this tattoo. Badgers were classified as being a cub or an adult based upon their size; they were then sexed and weighed to the nearest 100 g using a handheld spring balance (1987–2002) or a digital balance (2002–2005). Head-body length from the tip of the nose to the base of the sacrum was measured on a metre rule, to the nearest 5 mm, with the badger lying flat and ventral side up.

Body condition was graded using a subcutaneous haunch fat scale (one [very thin] – five [very fat]), but this has a subjective element. An index of body condition was therefore estimated using the equation below, originally developed for use on otters (Kruuk *et al.*, 1987), where W is weight (kg), L is length (mm) and k and n are constants:

$$\log W = \log k + n \log L$$

A regression of log transformed body weight against log transformed body length of every adult female trapped between 1995 and 2000 obtained the constants: $k = 1.6649 \times 10^{-7}$ and $n = 2.7044$, which provided an estimate of expected weight (Dugdale *et al.*, 2003). An index of body condition was calculated by dividing observed weight by expected weight (Woodroffe, 1995). Every January from 1993 onwards, with the

exception of 1996 and 2000, pregnancy was diagnosed by ultrasound scanning using an external 5.0 MHz sector probe (Kontron Instruments, Watford, UK). The crown-rump lengths of embryos were then used to estimate implantation date using the formula derived from data in Dunmartin et al. (1989), where a is age (days) and l is length (mm):

$$a = 3.21 + 0.50l - 0.004l^2 + 0.00002l^3$$

The length and width of teats were measured to the nearest mm to assess whether females had ever lactated (Petrides, 1950). Females diagnosed as pregnant through ultrasound in January have been shown to have a teat size of > 5 mm in diameter and > 2 mm in length until August (Macdonald & Newman, 2002), and these criteria were used to assess breeding status through teat size. Tooth wear was graded on a subjective scale of one (no tooth wear: white teeth, pointed canines and unworn ridges on molars) to five (extreme tooth wear: canines broken or missing and molars worn down to the dentine) (da Silva & Macdonald, 1989).

Approximately 3 ml of blood was collected from the jugular vein of each badger using a vacutainer containing EDTA, and mixed immediately. Blood was transferred into two 1.5-ml microcentrifuge tubes and frozen immediately at -4°C . Additionally, from June 2002 onwards, approximately 100 guard hairs were plucked from each badger, and stored at 4°C in 80% ethanol. Ear tissue samples were collected from five road kills.

1.2.3 Molecular studies of the badger

The complex social system of the badger provides an opportunity to investigate how both natural and kin selection shape the evolution of mating systems and social structure. These systems are hard to study in the badger as not only is the badger nocturnal, living underground during the day, but cubs are born and raised underground, and groups contain more than one potential mother and father that are not individually identifiable from natural markings. Molecular studies of the badger have been limited previously due to the low levels of genetic variability that have been identified in allozymes (Evans *et al.*, 1989; da Silva *et al.*, 1994; Pertoldi *et al.*, 2000), mitochondrial cytochrome b gene sequences (Kurose *et al.*, 2001), and single-locus (Burke *et al.*, 1996) and multilocus minisatellites (Pertoldi *et al.*, 2001). These low levels of genetic diversity were not sufficient to identify individuals.

Microsatellites are simple sequence tandem repeats, such as dinucleotide repeats e.g. (GC)_n and tetranucleotide repeats e.g. (ACCG)_n. The number of these repeat units is variable and defines the size of the allele. These alleles are inherited in a codominant, Mendelian fashion enabling parentage to be assigned. Moderate levels of genetic variation were shown in badger populations in the Netherlands and Denmark using seven microsatellites (Bijlsma *et al.*, 2000). Three of these markers and another nine markers (Domingo-Roura, 2000) were tested, and only five were polymorphic in our study population (Wytham Woods, Oxford, Domingo-Roura *et al.*, 2003). These five markers did not provide enough variability to assign parentage. A polymorphic set of 17 microsatellites (Carpenter *et al.*, 2003) was recently used to analyse maternity and paternity in another British population (Woodchester Park, Gloucestershire, Carpenter *et al.*, 2005). I use 22 microsatellite loci (*Mel101–117*, Carpenter *et al.*, 2003; *Mel1*,

Bijlsma *et al.*, 2000; and *Mel10*, *Mel12*, *Mel14* and *Mel15*, Domingo-Roura *et al.*, 2003) to investigate the mating system and social organisation of badgers from a high-density population in Wytham Woods.

1.3 Thesis structure and aims

This thesis is comprised of five inter-linked data chapters that together combine genetic and behavioural data to investigate how both natural and kin selection shape the evolution of mating systems and social structure. Chapter 2 describes the mating system in a high-density badger population and investigates the costs and benefits of natal philopatry. Reproductive skew, relatedness, and group productivity are quantified and correlated in Chapter 3, and predictions of reproductive skew models are evaluated. Chapter 4 investigates how selective forces act on badgers of different ages to influence their breeding success. The evolutionary causes of polygynandry and repeated mounting behaviour are investigated in Chapter 5, including reduction of inbreeding. The evolution of cooperative breeding is then examined in Chapter 6. Finally, Chapter 7 summaries the major findings and discusses potential avenues for future research. The detailed aims of the five data chapters are as follows:

1.3.1 Mating system

The badger's mating system has proved hard to elucidate due to the difficulty in applying conventional ecological methods and the low levels of genetic diversity, as detailed above. One study in Woodchester Park, Gloucestershire used 17 microsatellite loci to describe the badger's mating system (Carpenter *et al.*, 2005). In

Chapter 2, I identify the mating system of a similarly high-density badger population in Wytham Woods, using a larger number of individuals, 22 microsatellite loci, and a different approach that involved both parent-pair assignment and sibship inference. I investigate the costs and benefits of natal philopatry by examining the number of breeders within a group, the occurrence of multiple-paternity litters, and the prevalence of extra-group paternity.

1.3.2 Relatedness and reproductive skew

Badger social groups are thought to consist of related individuals, due to natal philopatry and restricted dispersal (Cheeseman *et al.*, 1988; da Silva *et al.*, 1994; Pope *et al.*, 2006). Relatedness is predicted to be high within badger groups, due to restricted dispersal. Two studies that estimated the Queller and Goodnight (1989) index of relatedness (R) suggest that the relatedness of all within-group badgers is indeed high ($R = 0.15$, Carpenter, 2002; $R = 0.17$, M Huck & TJ Roper, unpublished data); however, these analyses did not include the bias-correction and may therefore underestimate the real values. A recent study of the badger's mating system in south-west England estimated that approximately 30% of the candidate parent population breed each year (Carpenter *et al.*, 2005); however, reproductive skew within social groups has not been estimated. Furthermore, although the literature is rich with models of reproductive skew, there are few empirical datasets available on which to evaluate these models. In Chapter 3, I examine reproductive skew, relatedness and group productivity within badger social groups in Wytham Woods. I then assess the applicability to badger society of the assumptions of two types of reproductive skew models: (1) transactional models (e.g. Cant & Johnstone, 1999); and (2) incomplete-

control models (Cant, 1998; Reeve *et al.*, 1998). Finally, I test the predictions of some of the incomplete-control models and discuss the importance of ecological effects, particularly those of resource availability.

1.3.3 Breeding success

Long-term studies are vital to understand breeding success as they enable quantification of breeding success over the lifespan of individuals (Clutton-Brock, 1988). Knowledge of lifetime breeding success then enables selection on traits to be assessed and their adaptive significance to be evaluated (Arnold & Wade, 1984). In Chapter 4, I provide the first quantification of the lifetime breeding success of both male and female badgers, and I use this to detect selection on the age of first and last breeding in badgers. I also investigate whether annual reproductive success varies with the age of badgers, as has been reported in Woodchester Park (Carpenter *et al.*, 2005). I then use these data to assess, for the first time, the factors that lead to the age-related patterns of breeding success in badgers. I assess whether the Constraint Hypothesis (Curio, 1983), Restraint Hypothesis (Williams, 1966; Pianka, 1976) and Selection Hypothesis (Curio, 1983; Nol & Smith, 1987) explain the initial increase in breeding success with age. I also examine whether senescence in badgers is explained by the Mutation Accumulation Hypothesis (Medawar, 1952) or the Antagonistic Pleiotropy Hypothesis (Williams, 1957).

1.3.4 Mounting behaviour and inbreeding avoidance

Molecular techniques are a vital tool in determining mating systems as it is often the case that behavioural observations of mounting do not correlate with breeding success

(Hughes, 1998; Coltman *et al.*, 1999a); however, there are some cases where they do (e.g. Gilbert *et al.*, 1991; Pemberton *et al.*, 1992). In Chapter 5, I compare behavioural observations of mounting with those of parentage assignments. I also investigate the evolutionary causes of promiscuous mounting behaviour, and I particularly focus upon whether inbreeding is avoided. Inbreeding has been shown to have fitness consequences in wild populations (Foerster *et al.*, 2003; Szulkin *et al.*, 2007), and decreased reproductive success has been observed among inbred mammals (Slate *et al.*, 2000; Charpentier *et al.*, 2005). This study aims to quantify inbreeding in badgers and to investigate whether inbreeding affects the lifetime breeding success of badgers.

1.3.5 Cooperative breeding

Hamilton's (1964) theory of kin selection is fundamental to the study of cooperative breeding. As badgers are natally philopatric and dispersal is restricted (Cheeseman *et al.*, 1988; da Silva *et al.*, 1994; Pope *et al.*, 2006), it is possible that kin selection may have led to the evolution of cooperative breeding in badgers. Indeed, a previous study suggested that badgers may breed cooperatively (Woodroffe, 1993), however, this study was restricted to observations of three individuals at one sett and thus substantiation of alloparental care in badgers is required. In Chapter 6, I analyse behavioural observations from two badger groups, over three years, to quantify potential cooperative breeding behaviours among group members. I then relate these patterns of behaviour to the parentage and relatedness of the group members to evaluate whether cooperative breeding occurs.

**Mating system of social European badgers *Meles meles*:
polygynandry, extra-group paternity
and multiple-paternity litters¹**

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2.1 Abstract

The costs and benefits of natal philopatry are central to the formation and maintenance of social groups. Badger groups, thought to form passively according to the Resource Dispersion Hypothesis (RDH), are maintained through natal philopatry and delayed dispersal; however, there is minimal evidence for the functional benefits of such grouping. We attempted to assign parentage to 630 badger cubs from a high-density population in Wytham Woods, Oxford, born 1988–2005. Our methodological approach was different to previous studies; we used 22 microsatellite loci to assign parent-pairs, which in combination with sibship inference provided a high parentage assignment rate. We assigned both parents to 331 cubs with 95% confidence, revealing a polygynandrous mating system with up to five mothers and five fathers within a social group. We estimated that only 27% of adult males and 31% of adult females bred each year, suggesting a potential cost to group living for both sexes. Any strong motivation or selection to disperse, however, may be reduced because just under half of the paternities were assigned to extra-group males, mainly those from neighbouring groups, with males displaying a mixture of paternity strategies. We provide the strongest evidence to date for multiple-paternity litters, and for the first time show that within-group and extra-group males can sire cubs in the same litter. We investigate the factors that may play a role in determining the degree of delayed dispersal and conclude that the Ecological Constraints Hypothesis, Benefits of Philopatry Hypothesis, and Life History Hypothesis may all play a part, as proposed by the Broad Constraints Hypothesis.

2.2 Introduction

Mammals exhibit a diverse array of mating systems, characterised by the ecological and behavioural opportunities for individuals to monopolise mates and the way in which mates are acquired (Emlen & Oring, 1977). Breeding system properties determine group composition (Ross, 2001). As individuals are expected to act in a way that maximises their lifetime inclusive fitness (Hamilton, 1964), understanding why individuals live in groups requires knowledge of the costs and benefits of group living. The Resource Dispersion Hypothesis (RDH, Carr & Macdonald, 1986), proposes that when resources are spaced patchily in the environment, the minimum defensible territory that provides sufficient resources for a minimum social unit may also accommodate more individuals. Once social groups form, the factors under which selection will favour natal philopatry (Waser & Jones, 1983) are central to the maintenance of social groups (Macdonald & Carr, 1989). Delayed dispersal may occur due to: ecological constraints on dispersing and breeding elsewhere (Emlen, 1982), benefits of philopatry (Stacey & Ligon, 1991), certain life history traits, such as a low rate of breeder mortality that increases habitat saturation (Arnold & Owens, 1998), or a combination of these factors (the Broad Constraints Hypothesis, Hatchwell & Komdeur, 2000).

Social species that encounter a variety of environmental conditions may exhibit variation in their mating system (Taylor *et al.*, 2000). The European badger *Meles meles* is a promising species for understanding such variation because it has a widespread distribution from the British Isles to Japan and from the southern Mediterranean to the Russian Arctic Circle. It exhibits large variation in social

organisation over this range, living singly, or in pairs and defending territories in parts of mainland Europe, whereas in lowland England badgers are typically group-living (Johnson *et al.*, 2000). Despite their sociality, there is minimal evidence of the functional significance of grouping in badgers (Johnson *et al.*, 2004), whereas there is evidence of an ecological basis of group living that does not invoke cooperation (Johnson *et al.*, 2001a; Johnson *et al.*, 2001c).

Badger social groups have been reported to vary in size from two to 29 individuals (da Silva *et al.*, 1994). One proposal, first advanced for badgers by Kruuk (1978) is that groups form according to the RDH (Macdonald, 1983) and are maintained by natal philopatry (Cheeseman *et al.*, 1988; da Silva *et al.*, 1994). Dispersal is restricted (Pope *et al.*, 2006), with only 20% of the badgers trapped in Wytham Woods at any given time having ‘dispersed’, i.e. resident in more than one group, with residence defined as trapped in the same group in two consecutive trapping events, and at least one of two trapping events prior to that (Macdonald *et al.*, submitted). Dispersal may be costly; females that dispersed failed to produce cubs (da Silva *et al.*, 1993) and reduced fecundity was associated with increased dispersal rates in a culled compared with a control population (Tuytens *et al.*, 2000b). Costs of group living also include low levels of cub productivity (Cheeseman *et al.*, 1987; Cresswell *et al.*, 1992; Woodroffe & Macdonald, 1995b; Rogers *et al.*, 1997; Macdonald & Newman, 2002) and an initial decreased proportion of lactating females in a control versus culled population (Tuytens *et al.*, 2000b). Few benefits to badgers of group living have been identified (Johnson *et al.*, 2004). Although cooperative breeding has been suggested in badgers (Woodroffe, 1993), this has not been confirmed. The number of non-breeding females had a negative effect on mean litter size, after controlling for

territory quality (Woodroffe & Macdonald, 2000); however, longer-term benefits have not been investigated.

Badgers give birth once a year, around February, with cubs remaining underground for the first eight weeks. This, along with the presence of many potential parents, means that it is not possible to identify individuals that have bred successfully using conventional ecological methods. Our study uses microsatellite data to assign parentage in a long-term study in Wytham Woods, Oxford, UK. Microsatellites were also used to determine parentage in Woodchester Park, Gloucestershire, UK, which is a similarly high-density population (Carpenter *et al.*, 2005). Prior to this, multiple maternity and extra-group paternity had been shown (Evans *et al.*, 1989; da Silva *et al.*, 1994; Domingo-Roura *et al.*, 2003), but parentage could not be assigned to individuals. Behavioural observations have previously provided limited evidence for a polygynandrous mating system (Johnson, 2001) and extra-group paternity (Christian, 1995).

Our study combines parentage assignment and sibship inference, a novel approach recommended by Garant and Kruuk (2005), to test whether: (1) badger social groups exhibit plural breeding; (2) females breed with extra-group males; (3) males have different paternity strategies; and (4) multiple-paternity litters occur. We discuss our results in relation to the four proposed hypotheses for the occurrence of delayed dispersal. In particular, we ask: Is group living costly to badgers? Does extra-group paternity reduce inbreeding? Do multiple-paternity litters provide fitness benefits? And, do delayed implantation and the potential for superfoetation (conception during pregnancy, Appendix 1) facilitate multiple-paternity litters?

2.3 Materials and methods

2.3.1 Study site and population demography

Our data come from Wytham Woods, Oxfordshire (01° 19'W, 51° 46'N). This area covers 4 km² and consists of deciduous woodland, surrounded by permanent pasture and mixed arable land (Kruuk, 1978). Enclosing features potentially limit badger movement in and out of the study area (Macdonald & Newman, 2002). From June 1987 to November 2005, trapping events have usually been undertaken at least four times a year, in January, June, August and November (Macdonald & Newman, 2002). The badger population ranged from 60–228 adults and 23–61 cubs (1987–1996) with the highest density being 44 badgers km⁻² (Macdonald & Newman, 2002).

2.3.2 Sample collection

Badgers were sedated by an intra-muscular injection of approximately 0.2 ml / kg ketamine hydrochloride. Cubs were first trapped around 15 weeks of age; those judged to weigh ≤ 2 kg were considered too small for sedation and were released after a hair sample was plucked. On initial capture, badgers were given a unique tattoo number on their inguinal region, through which recaptures were identified. Badgers were classified as cub or adult based upon their size and tooth wear; they were then sexed. Tooth wear was graded on a subjective scale of one (no tooth wear: white teeth, pointed canines and unworn ridges on molars) to five (extreme tooth wear: canines broken or missing and molars worn down to the dentine). Approximately 3 ml of blood was collected from the jugular vein of each badger using a vacutainer containing EDTA, and mixed immediately. Blood was transferred into two 1.5-ml

microcentrifuge tubes and frozen immediately at -4°C. Additionally, from June 2002 onwards, approximately 100 guard hairs were plucked from each badger, and stored at 4°C in 80% ethanol. Ear tissue samples were collected from five road kills.

2.3.3 DNA extraction

DNA was extracted from blood using a GFX genomic blood DNA purification kit (Amersham Biosciences, Little Chalfont, UK) or from a minimum of 20 hairs with visible roots using a Chelex protocol (Walsh *et al.*, 1991). An ammonium acetate protocol (Bruford *et al.*, 1998) was used to extract DNA from 20–50 mg of each road-kill tissue sample.

2.3.4 Genotyping

We genotyped individuals for 22 polymorphic microsatellite loci described by Pope *et al.* (2006). Each 10-μL PCR contained approximately 20 ng of genomic DNA, 0.25 μM of each primer, 0.1 mM of each dNTP, 1.5 or 2.5 (*Mel101*, *Mel104*, *Mel108*, *Mel110*, *Mel112* and *Mel115*) mM MgCl₂ and 0.175 unit of *Taq* DNA polymerase (Thermoprime Plus, ABgene, Epsom, UK), in 1 x PCR buffer containing: 20 mM (NH₄)₂SO₄, 75 mM Tris-HCl pH 8.8, 0.01 % (w / v) Tween. If *Mel101* did not amplify, we performed a 12.5-μL PCR that was identical to the 10-μL PCR, except that the concentrations of genomic DNA and *Taq* DNA polymerase were increased to *ca* 60 ng and 0.2 unit, respectively. *Mel105* and *Mel106* were multiplexed together in a single PCR, as were *Mel103* and *Mel107*, *Mel111* and *Mel113*, *Mel112* and *Mel115*, *Mel114* and *Mel117*, *Mel1* and *Mel12*, and *Mel10* and *Mel14*. Amplification then followed the methods described by Pope *et al.* (2006).

PCR products were separated on either an ABI Prism 377 DNA Sequencer or an ABI Prism 48-capillary 3730 DNA analyser. We analysed data from the 377 sequencer using GENESCAN 3.1 and GENOTYPER 2.5 software, whereas GENEMAPPER 3.5 software (Applied Biosystems, Warrington, UK) was applied to the 3730 data. We ran one set of 95 samples on both sequencers to ensure the compatibility of results. We scored chromatogram data twice and checked for errors using the EXCEL MICROSATELLITE TOOLKIT 3.11 (Parks, 2001). Individuals genotyped at fewer than 16 loci were excluded, except for one cub in 2005 that was genotyped at 11 loci.

We tested for deviations from Hardy-Weinberg equilibrium (HWE), per locus within cohorts, and for linkage equilibrium, between pairs of loci within cohorts, with exact tests (1000 dememorisations, 1000 batches and 1000 iterations) using GENEPOP 3.4 (Raymond & Rousset, 1995). Within each cohort, candidate parents and cubs were tested separately to reduce multigenerational effects. Additionally, we analysed a sample of one male and one female candidate parent from each social group to reduce social structure effects. False discovery rate (FDR) control for type I error corrections was used to generate adjusted p -values, to account for multiple tests (Benjamini & Hochberg, 1995). Candidate parent genotypes in each cohort were used to estimate the amount of power available to distinguish between individuals by generating $PI_{(unbiased)}$ (Paetkau *et al.*, 1998) and $PI_{(sib)}$ (Evetts & Weir, 1998; Waits *et al.*, 2001) using GIMLET 1.3.3 (Valière, 2002).

2.3.5 Parentage analysis

We determined parentage within each cohort through a likelihood based approach using CERVUS 3.0.1.8 (Kalinowski *et al.*, 2007) and COLONY 1.2 (Wang, 2004). CERVUS assigns the most likely parent, whereas exclusion methods may result in more than one individual matching or exclusion of the true parent, if errors are present or relatives are candidate parents (Marshall *et al.*, 1998). Analyses were run systematically according to strict rules (Figure 2.1). Genotypes were first analysed in CERVUS to assign a parent-pair, or if a pair could not be assigned then we assigned either maternity or paternity alone. CERVUS enables the presence of relatives, genotyping error, and the proportion of unsampled individuals to be incorporated; however, only sampled individuals can be assigned as parents. Cubs that were not assigned a mother and / or father using CERVUS were, therefore, included in a sibship inference using COLONY.

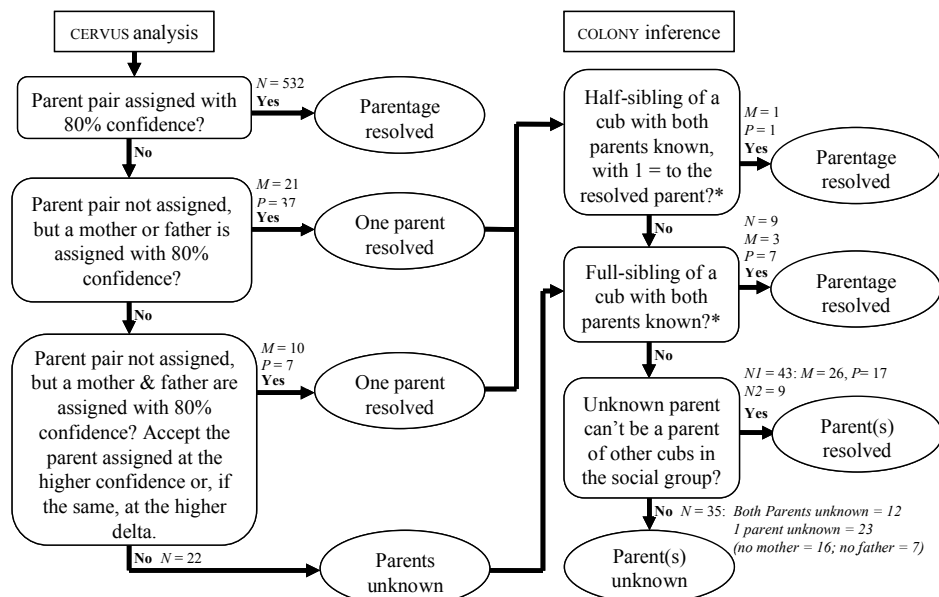


Figure 2.1 Flow chart of the CERVUS parentage assignment and COLONY sibship reconstruction rules. Cubs that were assigned to sibship groups were included in the population parameter estimates with 80% confidence. M = number of cubs assigned a mother; P = number of cubs assigned a father. N = number of cubs that followed that path: $N1$ = with one parent inferred, and $N2$ = with both parents inferred. * = inferred parent only accepted if the inference was logical given the CERVUS assignments and it has ≤ 2 mismatches with the cub and ≤ 4 mismatches with the cub and known parent

2.3.5.1 Selection of candidate parents

Parentage was not known *a priori*, therefore, the power to assign parentage from the whole population was low and we selected candidate parents using mark-recapture data and biological rules, following Carpenter *et al.* (2005). Candidate mothers were reproductive females (aged two or more), present in the cub's social group in the year when the cub was born. Candidate fathers were all males older than one year and present in Wytham Woods in the year before the cub was born, as due to delayed implantation females conceive in the year prior to birth. To minimise error from badgers that were present but were not caught, we included adults and yearlings for an extra two years, rather than three (Carpenter *et al.*, 2005), after their date of last capture, if their death date was unknown. This is because 95% of the inter-trap intervals were within 525 days ($n = 6,193$). Badgers last caught as a cub were only included for one extra year, as the greatest mortality is generally seen in cubs (Macdonald & Newman, 2002). This reduced the possible assignment of a full-sibling instead of the true parent as, on average, full-siblings produce higher log-likelihood values than the true parent (Thompson, 1976).

Candidate fathers assigned to more than one social group within a year were assigned to the group closest to the cub(s) they sired, in the paternity distance analysis. Badgers first trapped as adults with tooth wear of 4–5, were judged to be at least two years old, otherwise they were judged to be at least one year old (da Silva & Macdonald, 1989). The social group in which a cub was trapped was considered its natal group. Cubs trapped in two social groups ($n = 25$) had the candidate mothers from both social groups included in their maternity analysis. The natal groups of nine cubs contained no candidate mothers, according to the trapping data, and the social group of one cub

was not recorded. All candidate mothers in the population that year were included in the maternity analyses for these cubs. Assigned mothers were accepted only if they had not bred in another group that year.

Candidate parents, trapped successively in different social groups, were recorded as resident in both groups for the intervening period (see Carpenter *et al.*, 2005). If a social group split into two groups over this period, or during the extra years that an individual was assumed present, then a candidate parent was assigned to both from the time of the split during those intervening years. If a social group split in year Z, candidate fathers that were present but not trapped in year Z-1 were assigned to both groups in year Z-1 for parentage analysis in year Z. If they were only trapped in year Z-1 at setts belonging to one of the newly formed groups in year Z, then they were assigned to that group in year Z-1 for paternity analysis in year Z. If they were trapped at setts in year Z-1 that were assigned to different groups at the split in year Z, then they were assigned to both groups in year Z-1. When calculating the number of candidate parents per social group as a parameter in the parentage analysis, individuals assigned to more than one group (mean = 22 ± 2 males, and 19 ± 2 females per year) were counted in all of the groups that they were assigned to, to provide a conservative estimate.

2.3.5.2 Parentage simulations

We ran CERVUS simulations for 100,000 cycles, using the allele frequencies of all genotyped badgers (Supplementary Table II.I) and yearly parameters (Table 2.1). We

Table 2.1 Parameters for the CERVUS parentage simulations.

Year	Proportion loci typed ^a	Number of candidate mothers per social group ^b	Number of candidate mothers in population ^b	Proportion candidate mothers sampled ^c	Number of candidate fathers in population ^b	Proportion candidate fathers sampled ^c	Proportion female relatives / social group ^d	Proportion male relatives in population ^d	R^e
1988	0.95	3	47	0.88	33	0.94	0.70	0.11	0.26
1989	0.95	5	62	0.84	57	0.92	0.78	0.07	0.19
1990	0.95	6	73	0.87	79	0.84	0.83	0.09	0.17
1991	0.97	7	78	0.88	76	0.81	0.86	0.07	0.19
1992	0.97	7	77	0.90	82	0.81	0.85	0.08	0.21
1993	0.98	7	95	0.90	90	0.91	0.85	0.06	0.20
1994	0.99	7	100	0.88	106	0.92	0.86	0.06	0.19
1995	0.99	8	119	0.90	137	0.91	0.87	0.05	0.20
1996	0.99	9	136	0.91	161	0.91	0.88	0.05	0.18
1997	0.99	9	154	0.91	178	0.91	0.89	0.05	0.20
1998	0.99	9	156	0.86	180	0.93	0.89	0.05	0.20
1999	0.99	8	154	0.85	170	0.88	0.88	0.05	0.20
2000	0.99	8	143	0.84	157	0.80	0.88	0.05	0.13
2001	0.98	7	141	0.81	137	0.79	0.87	0.05	0.22
2002	0.98	8	155	0.84	124	0.80	0.88	0.05	0.19
2003	0.98	8	161	0.86	135	0.76	0.87	0.04	0.11
2004	0.98	7	146	0.90	130	0.83	0.86	0.04	0.15
2005	0.98	7	139	0.94	124	0.88	0.85	0.04	0.20
Mean	0.98	7	119	0.88	120	0.86	0.85	0.06	0.19

^a Calculated from all of the genotyped badgers that year.

^b Trapping records were estimated to represent 94% of the actual number present each year (the actual number is given in Table 2.1), which we calculated from the percentage of the population in a given year that were candidate parents (Macdonald & Newman, 2002) and a mean 82% trappability over a year.

^c Number of candidate parents genotyped for ≥ 16 loci, as a percentage of the actual number of candidate parents present in the population each year.

^d Mean number of candidate parents in each social group minus one (the parent), divided by the mean number in the population or social group.

^e Estimated using RELATEDNESS 5.0.8 (Queller & Goodnight, 1989). The average relatedness of the candidate mothers to the cub was significantly negatively affected by the number of candidate mothers in the group ($F_{1,16} = 14.91$, $p = 0.001$, $R^2 = 48.2\%$), whereas it was not for the candidate fathers ($F_{1,16} = 0.19$, $p = 0.669$, $R^2 = 1.2\%$). Therefore, we used the average relatedness to the offspring of the candidate fathers in the offspring's social group as the R for both male and female relatives of the offspring.

estimated the proportion of loci that were typed incorrectly by re-genotyping 5% of the population. Of the 823 single-locus genotypes compared, we observed three allelic dropouts each at different loci and one false allele, giving an estimate of 0.005 loci typed incorrectly. PEDANT 1.3 (Johnson & Haydon, 2007) estimated a mean allelic dropout rate per allele of 0.005 and per heterozygote of 0.009, and a mean false allele rate per genotype of < 0.001 . We entered the proportion of loci mistyped as 0.005, in both the simulations and likelihood calculations.

2.3.6 Sibship reconstruction

Cubs that were not assigned a mother and / or father using CERVUS, were included in a full- and half-sibling inference with all other cubs in their social-group-year, using COLONY. We based estimations on the allele frequencies of all genotyped badgers. The allelic dropout rate per allele, estimated using PEDANT, was 0.024 for *Mel101*, 0.073 for *Mel10* and 0.010 for *Mel110*, with the mean being 0.005, which we used for the other loci and for the other typing error rate. COLONY assigns sibships, taking into consideration any assigned parents (from the CERVUS analyses). COLONY assumes that unknown parents are not those that are already known (from the CERVUS analyses), and that one sex is polygamous and the other monogamous. We first tested for siblings of cubs that had one unknown parent. If, say, the mother was unknown and the father was known then we analysed all cubs in that social-group-year with the same father and a known mother specifying the identity of the known father, while entering all mothers as unknown and monogamous. If this did not resolve the mother, or there were no other cubs with the same known parent, then we analysed all the cubs in that social group, specifying all known fathers, and all mothers as unknown

and polygamous. If parentage was still unresolved or for cubs with both parents unknown, we entered all cubs in the social group, specifying all parents as unknown, to test for full-sib assignment to a cub with both parents known. If the unknown parent could not be a parent of any other cubs in the social group then we assigned it as an unknown reconstructed parent (using the COLONY estimated genotype), when calculating multiple-paternity litters and the number of breeders per social group.

2.3.7 Statistical analyses

We used Minitab 14 for the majority of the statistical analyses; however, we used SAS 9.1 to conduct Generalised Linear Mixed Models (GLMMs) with Kenward-Roger denominator degrees of freedom method (Littell *et al.*, 2006). GLMMs allow the fitting of fixed effects, random effects (which model variance between experimental units), and repeated measures (which model covariance between measures on the same experimental unit). Repeated measures recorded closer in time are likely to be more correlated than those further apart in time (Littell *et al.*, 1998). We therefore examined graphically the covariance between pairs of observations on the same experimental unit at different times to determine the most appropriate covariance structure. The GLIMMIX procedure was run with a Poisson error distribution, log link, and social group as a repeated factor with compound symmetry covariance structure, or as both a random factor, and a repeated factor with autoregressive covariance structure (Littell *et al.*, 2006). Where parametric tests were used, we tested differences for normality using the Anderson-Darling test. We tested for homogeneity of group variances using Levene's test. Means are given with the 95% confidence interval (CI), unless otherwise stated.

2.4 Results

We genotyped 915 (85%) badgers trapped and marked in Wytham Woods, Oxford, between 1987 and 2005. One badger's sample did not amplify and 165 were not sampled, the majority of which were only trapped once. Genotyping was 98% complete, with 77% (706) of the badgers genotyped for all 22 microsatellite loci. Of the 735 badgers first trapped as cubs in 1988–2005, 630 (86%) were genotyped. *Mel104* in 1993 and *Mel110* in 1995 showed significant departure from HWE in both the cubs and candidate parents ($m = 22$, $\alpha = 0.05$, adjusted $p = 0.050$ – 0.002), after adjusting for multiple testing by FDR control (Benjamini & Hochberg, 1995). These samples did not depart from HWE when one male and one female adult from each social group, in that cohort, were analysed to reduce the effect of social structure. Deviations from linkage equilibrium for a pair of loci were seen in five out of the 18 cohorts, after FDR control, but these were not the same pair of loci in each cohort. When one male and one female per social group were analysed ($n = 39$ badgers), only *Mel105* and *Mel12* deviated from linkage equilibrium, in 1995. All loci were therefore included in analyses that required HWE and independence of data points, as no pair of loci consistently deviated from linkage equilibrium. $PI_{(\text{unbiased})}$ ($2.43 \times 10^{-13} \pm 1.4 \times 10^{-14}$) and $PI_{(\text{sibs})}$ ($2.94 \times 10^{-6} \pm 8.9 \times 10^{-8}$) indicated a high probability of distinguishing individuals.

Both parents were assigned to 595 (94%) cubs with 80%, and 331 (53%) with 95% confidence. Paternity was assigned to a further 16 and seven cubs, resulting in 611 (97% with 80%) and 338 (54% with 95% confidence) cubs that were assigned a father. Maternity was assigned to an additional seven and five cubs, resulting in a total of 602 (96% with 80%) and 336 (53% with 95% confidence) cubs that were assigned

a mother. A maximum of three mismatches occurred between an assigned parent and cub ($n = 2$); however, 88% had no mismatches. Considering trios, the maximum was four, with 70% having no mismatches with 80%, and 83% with 95% confidence (Table 2.2). Parent-pair assignment rates were generally lower than expected from simulations, when assigning the mother alone, father alone and parent-pairs with 80% ($t_{17} = -5.35, p < 0.001$; $t_{17} = -2.99, p = 0.008$; $t_{17} = -3.04, p = 0.007$) and 95% ($t_{17} = -2.32, p = 0.033$; $t_{17} = -1.34, p = 0.199$; $t_{17} = -4.93, p < 0.001$) confidence, respectively. This may indicate the presence of more unsampled individuals, more relatives, different allele frequencies or a higher error rate than simulated (Marshall *et al.*, 1998; Kalinowski *et al.*, 2007).

Table 2.2 Number of mismatches observed between parents assigned in CERVUS and their offspring.

Number of mismatches	Trio with 80%	Trio with 95%	Mother–cub with 80%	Mother–cub with 95%	Father–cub with 80%	Father–cub with 95%
0	374 ^a	276	29	18	34	18
1	81	38	2	0	9	2
2	55	16	0	0	1	0
3	19	1	0	0	0	0
4	3	0	0	0	0	0
Total	532 ^b	331	31	18	44	20

^a 72 cubs could be placed in more than one trio (i.e. mother–father–cub) with 0 mismatches (max = eight trios) and 60 of these had one trio assigned with 80% confidence.

^b For 75 cubs, trios were not assigned with confidence, but one parent was assigned using CERVUS (see Figure 2.1). Although 595 cubs had both parents assigned with 80% confidence, 63 of these cubs had one or both parents inferred using COLONY.

2.4.1 Distribution of parentage

The mean numbers of candidate mothers and candidate fathers per social-group-year were 5.6 ± 0.4 (median = 5) and 5.8 ± 0.4 (median = 5), respectively (Figure 2.2). The number of candidate mothers or fathers per social-group-year increased as the density

of candidate mothers or fathers increased over the study period (GLMM with Poisson-distributed error and autoregressive covariance structure: $F_{1,124.6} = 19.4$, $p < 0.0001$; $F_{1,303.6} = 29.7$, $p < 0.0001$, respectively).

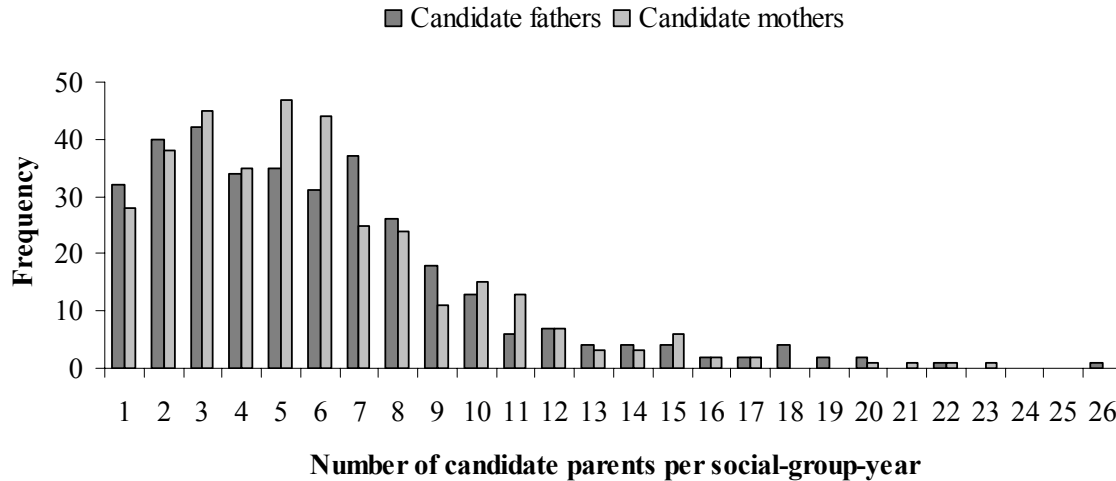
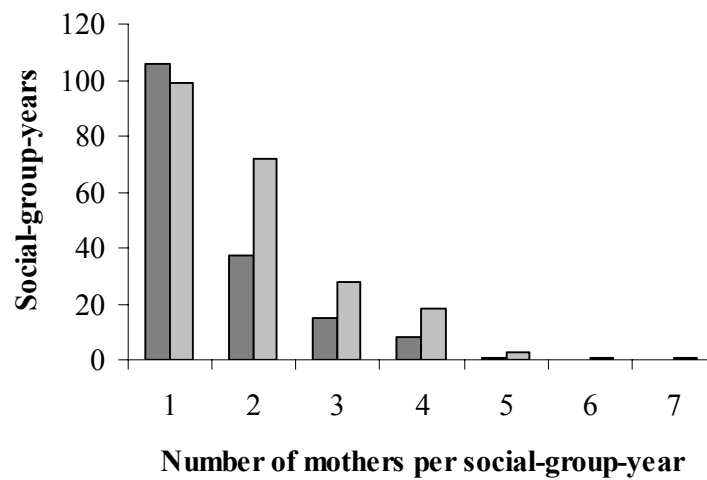


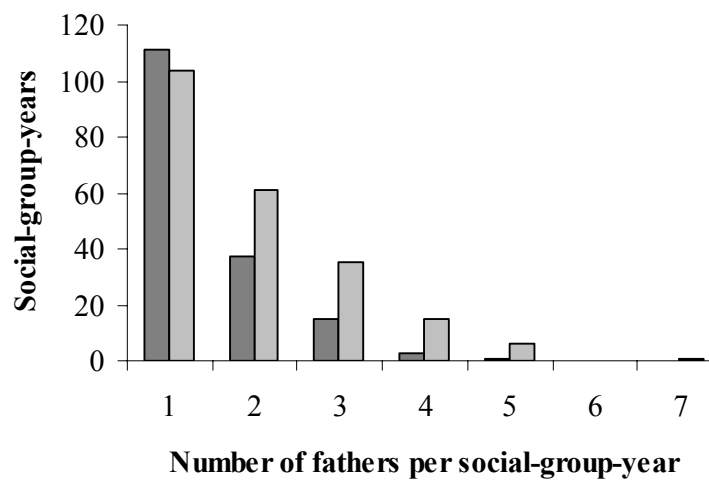
Figure 2.2 Frequency distribution of the number of candidate mothers (light grey bars) and candidate fathers (dark grey bars) per social-group-year (1988–2005). Data include adults for two years after their last capture date. If an individual was trapped in more than one social group within a year, it was split between these, unless it bred, in which case it was assigned to that social-group-year.

The maximum number of mothers and fathers assigned within a social-group-year was seven with 80% and five with 95% confidence (Figure 2.3). The mean number of mothers per social-group-year was 1.9 ± 0.1 ($n = 222$, mode = 1) with 80% and 1.6 ± 0.1 ($n = 167$, mode = 1) with 95% confidence. The mean number of fathers per social-group-year was 1.9 ± 0.1 ($n = 222$, mode = 1) with 80% and 1.5 ± 0.1 ($n = 167$, mode = 1) with 95% confidence. A GLMM with Poisson-distributed error and compound symmetry covariance structure showed no significant difference in the numbers of mothers and fathers per social-group-year using the assignments with 80% ($F_{1,418} = 0.00$, $p = 0.96$) or 95% confidence ($F_{1,311} = 1.03$, $p = 0.31$). Analysing only those social-group-years in which all cubs were assigned a mother or a father, the mean

number of mothers and fathers per social-group-year was 1.9 ± 0.2 and 1.9 ± 0.2 ($n = 156$ & 157 respectively, mode = 1 with 80%), and 1.4 ± 0.2 and 1.3 ± 0.2 ($n = 51$, mode = 1 with 95% confidence). Again, there was no significant difference in the numbers of mothers and fathers within a social-group-year ($F_{1,280} = 0.20$, $p = 0.65$, with 80%; $F_{1,76} = 0.36$, $p = 0.55$, with 95% confidence).



(a)



(b)

Figure 2.3 The number of (a) females and (b) males assigned as parents within each social-group-year with an assigned parent(s). Light grey bars represent parentage assignment with 80% confidence ($n = 222$), and dark grey bars with 95% confidence ($n = 167$). Fewer parents were assigned with 95% confidence, resulting in more social groups with just one mother or father than with the 80% confidence assignments.

The mean percentage of candidate mothers that bred was estimated (from the number of cubs that were assigned mothers and the number of mothers to which the cubs were assigned in comparison to the total number of cubs and candidate mothers in each year) as $28 \pm 5\%$ (range = 8–47%, median = 25%) with 80%, and $31 \pm 6\%$ (range = 8–51%, median = 28%) with 95% confidence (Figure 2.4). In contrast $25 \pm 6\%$ (range = 8–69%, median = 23% with 80%), and $27 \pm 6\%$ (range = 8–60%, median = 24% with 95% confidence) of the candidate fathers bred. The proportions of candidate fathers and candidate mothers estimated to have bred each year were significantly different with both 80% ($Z = -2.7$, $n = 18$, $p = 0.006$) and 95% confidence ($Z = -2.9$, $n = 18$, $p = 0.003$).

The mean post-emergence litter size of females that were assigned at least one cub was 1.4 ± 0.06 (range = 1–4, mode = 1, $n = 427$ litters) with 80% and 1.3 ± 0.06 (range = 1–3, mode = 1, $n = 262$) at 95% confidence. Considering just those social groups where all cubs were assigned a mother, the mean litter size was very similar: 1.4 ± 0.07 (range = 1–4, mode = 1, $n = 293$ with 80%) and 1.3 ± 0.12 (range = 1–3, mode = 1, $n = 71$ with 95% confidence). Males assigned paternity to at least one cub in a given year sired a mean of 1.6 ± 0.11 cubs with 80% (range = 1–9, mode = 1, $n = 377$ paternal litters) and 1.5 ± 0.12 cubs with 95% (range = 1–6, mode = 1, $n = 221$) confidence.

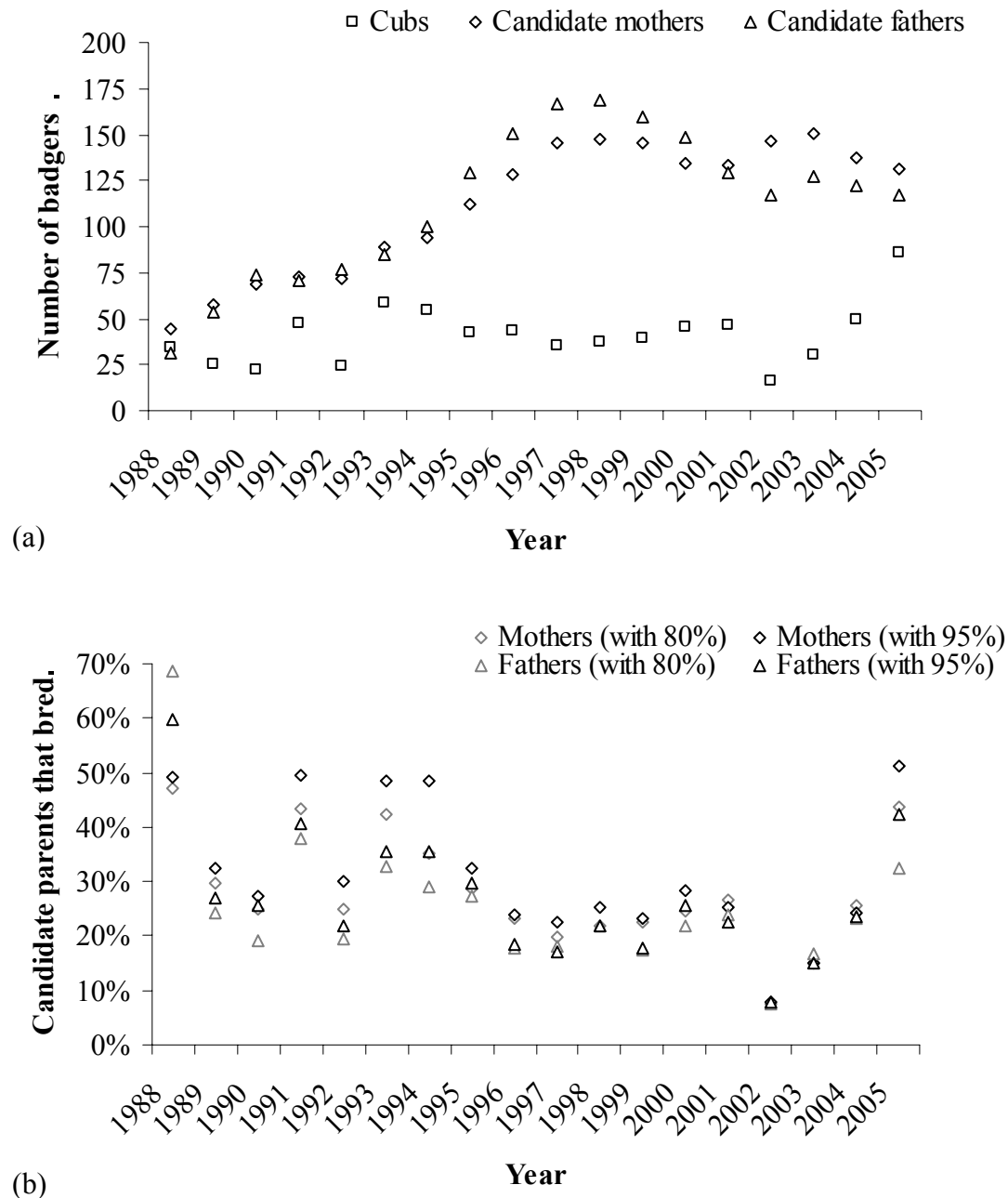


Figure 2.4 The annual (a) number of candidate parents and cubs, and (b) estimated percentage of candidate parents that bred (extrapolating from the number of cubs assigned parents and the number of parents to which they were assigned, in comparison to the total number of cubs and the total number of candidate parents in each year). Although the number of cubs assigned a parent was lower with the 95% than the 80% confidence level, if the mean number of cubs assigned to each parent was lower with the 95% than the 80% confidence this led to a higher estimated proportion of candidate parents that bred at the stricter confidence level. The figure includes individuals that were not genotyped. The dip in cub number in 2002 is potentially a result of the dry conditions during that cub-rearing season.

2.4.2 Multiple-paternity litters

There were 143 litters of more than one cub that had both parents assigned, and 64 (45%) of these were multiple-paternity litters, comprising 49 twins, 11 triplets and four quadruplets. Three litters had more than two fathers: one triplet and one quadruplet had three different fathers, and one quadruplet had four different fathers. Examining the LOD scores of the fathers assigned paternity within these 64 multiple-paternity litters, to the other cubs in the litter, revealed negative LOD scores in 44 litters, indicating that these males were unlikely have sired the whole litter. This, therefore, suggested multiple paternity in 44 / 143 (31%) litters. Multiple paternity was observed in 20 / 65 (31%) litters assigned with 95% confidence, all of which were twins. The LOD scores revealed strong evidence of multiple paternity in 16 / 65 (25%) litters with 95% confidence. Of these multiple-paternity litters, six were sired solely by males from the same social group as the mother, four solely by extra-group males and six had both an extra-group and same-group father.

2.4.3 Patterns of paternity

Fathers from known social groups were assigned to 569 (with 80%) and 331 (with 95% confidence) cubs that had a mother from a known social group in the year of conception, and approximately half of these fathers were extra-group males (50% and 42%, respectively). Neighbouring males gained 74% (37% of all paternities with 80%), and 86% (36% of all paternities with 95% confidence) of the assigned extra-group paternities (Figure 2.5). The mean distance between the main sett of a cub's social group and its father's social group in the year of conception was 214 ± 26 m (range = 0–1,976 m with 80%) and 155 ± 26 m (range = 0–1,657 m with 95%

confidence). Examining only extra-group paternities, the mean distance was 440 ± 40 m with 80% and 376 ± 44 m with 95% confidence. The greatest distance between a cub's social group and its father's social group was 2.0 km (maximum possible = 2.2 km); the father was two years old, and the natal groups of father and cub were located at opposite ends of Wytham Woods. Seventeen further inferred mating sorties were between 1.0 and 2.0 km. The mean distance between the main setts of neighbouring social groups, based on bait-marking (Kruuk, 1978) data from years with the least (1987) and most (2005) social groups, were 486 ± 111 m ($n = 27$ neighbouring pairs) and 373 ± 60 m ($n = 63$), respectively. The natal groups of both parents of 186 cubs (with 80% confidence) were known, and the parents of 31 (17%) of these cubs were born in the same social group as each other. A similarly low number of parents of cubs assigned with 95% confidence had the same natal group (15 / 108; 14%).

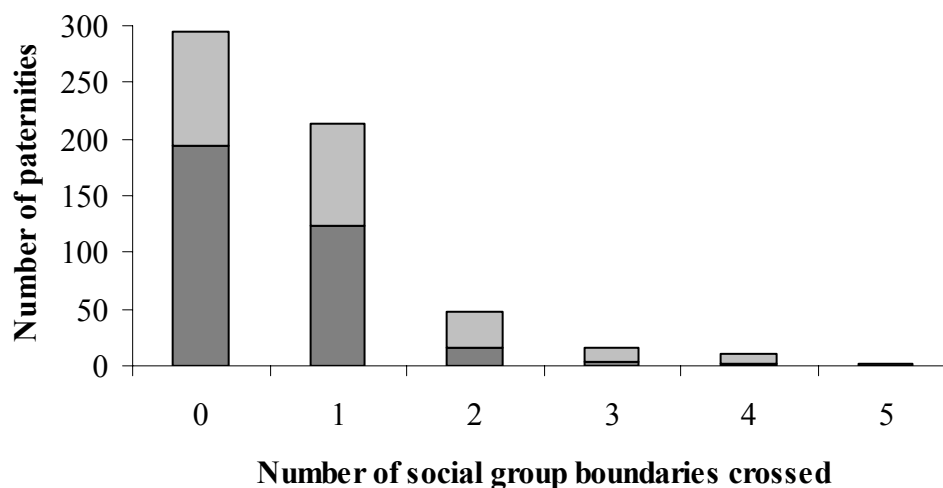


Figure 2.5 Number of paternities assigned according to the number of social-group boundaries that must have been crossed in order to gain paternity with 80% (light grey bars, $n = 585$, includes 95% data) and with 95% (dark grey bars, $n = 338$) confidence.

Of the 134 males assigned paternity to more than one cub in a given year with 80% confidence, 53 sired cubs only in their resident social group, 52 sired cubs only in

other groups, and 29 had a mixed outcome. Similar values were seen with 95% confidence: 35, 20, and 17, respectively.

2.5 Discussion

Our study documents the mating system of the European badger in Wytham Woods, Oxford, 1988–2005, using 22 microsatellite loci. Carpenter *et al.* (2005) also examined the mating system of a similarly high-density badger population in Woodchester Park, Gloucestershire, over a similar time period, 1989–2002. They used 17 microsatellite loci to assign parentage to 425 cubs from ten social groups, with maternity assigned to 185 cubs, paternity to 64 cubs and both parents to 58 (14%) cubs with 95% confidence. In comparison, we attempted to assign parentage to 630 cubs with maternity assigned to 336 cubs, paternity to 338 and both parents to 331 (53%) with 95% confidence. Our study utilised CERVUS 3.0.1.8, which uses revised likelihood equations that correct the way in which the genotyping error rate is interpreted, which had resulted in conservative assignment rates previously (Kalinowski *et al.*, 2007). CERVUS 3.0.1.8 also jointly assigns parent-pairs, which is more powerful and robust than the previous method, in which offspring were first assigned one parent, and then the second parent was assigned dependent upon the first. We also reconstructed sibships of cubs that were not assigned one or both parents using CERVUS. These approaches provided a high rate of parentage assignment, which is notable given that no parents were known *a priori*, there was a large number of candidate parents that included close relatives, and some candidates were not genotyped.

Our discussion focuses upon the results calculated from the more robust parentage assignments with 95% confidence. We provide strong evidence for the occurrence of plural breeding and extra-group paternity, the strongest evidence, to date, of multiple-paternity litters, and the first evidence that cubs in the same litter can have extra-group and within-group fathers. We discuss our results in relation to the four proposed hypotheses for the occurrence of delayed dispersal.

2.5.1 Plural breeding

On average, there were six males and six females of breeding age within a social-group-year, of which a mean of 1.5 males and 1.6 females bred, similar to the estimates in Woodchester Park of 1.2 males and 1.8 females (Carpenter *et al.*, 2005). Plural breeding in Wytham Woods had not been quantified before; previous genetic analyses revealed at least three mothers within one studied social group (da Silva *et al.*, 1994; Domingo-Roura *et al.*, 2003) and field data suggested a mean of two or three (Woodroffe & Macdonald, 1995b; Rogers *et al.*, 1997). Breeding among males has been harder to quantify, and prior to the Woodchester Park study, only Evans *et al.* (1989) demonstrated that more than one male may sire cubs within a group.

The percentage of candidate fathers (or mothers) that bred each year was estimated from the number of cubs that were assigned fathers and the number of fathers to which the cubs were assigned in comparison to the number of cubs and candidate fathers each year (see Figure 2.4). This may lead to overestimates if, for example, one unsampled male was responsible for many unassigned paternities. Estimates based on assignments with 95% rather than 80% confidence may be inflated, because fewer

cubs tended to be assigned to each parent. The 27% of candidate fathers and 31% of candidate mothers estimated to have bred each year were similar to calculations from Woodchester Park (18–31% and 29%, respectively, with 80% confidence, Carpenter *et al.*, 2005) and Wytham field data (29% of candidate mothers, Macdonald & Newman, 2002). This is reduced compared to the estimates in lower-density populations (Table 2.3), suggesting a cost to living in large groups in terms of the proportion of females that breed each year. Post-mortem studies have also reported a decline in per adult productivity with group size (Cresswell *et al.*, 1992). This may be due to female–female competition within social groups, as the number of mothers in a group declines at higher latitudes, where population density is low (Woodroffe & Macdonald, 1995b).

Such a cost would only occur if litter size does not increase with density. Males and females that were assigned parentage had a mean of 1.5 and 1.3 assigned cubs, respectively, similar to Woodchester Park (1.3 for fathers with 95% and 1.5 for mothers with 80% confidence, Carpenter *et al.*, 2005); however, these may be underestimates, as not all cubs were assigned parentage and some cubs may have died before they could be trapped. Teat-size data produced a similar litter size of 1.6 (Macdonald & Newman, 2002). Mean foetal litter size measured using ultrasound in Wytham was 1.9 ± 0.1 (1993–2005, unpublished data). If the trapped females are representative of the population, this may suggest embryo reabsorption (38%, Woodroffe & Macdonald, 1995b; Appendix 1), pre-emergence mortality (up to 25%, Anderson & Trehwella, 1985), and / or that not all of the cubs were caught. Mean litter sizes in lower-density populations were higher (Table 2.3), reinforcing the cost to group living for females at high densities.

Table 2.3 Litter size and percentage of females breeding in lower-density populations. – indicates data not stated.

Study population	Density (badgers km ⁻²)	Females breeding (%)	Mean litter size	Data	Reference
Suburban Bristol, England	4.4–7.5	48%	2.7	Capture-mark-recapture, 1980–1984	Harris & Cresswell (1987), table 4
Central Sweden	2.4–3.2	26–45%	–	26 females	Data in Anderson & Trehwella (1985)
Doñana area, south- west Spain	0.23–0.67	65%	–	Capture-mark-recapture, 1983–1998, 17 females	Revilla <i>et al.</i> (1999)
County Offaly, Ireland	–	35–40%	–	Culling, 1989–1990	Whelan & Hayden (1993)
East Germany	2–4	–	3.3	14 litters	Data in Anderson & Trehwella (1985)
Holland	1	–	2.4	15 litters	Data in Anderson & Trehwella (1985)
Serra de Grândola, south-west Portugal	0.36–0.48	–	4	3 litters	Rosalino <i>et al.</i> (2004)
Bialowieza Primeval Forest, Poland	0.21	–	2.4	16 litters	Kowalczyk <i>et al.</i> (2003)

The percentage of breeding individuals in the population is high compared to other social mammals, such as meerkats *Suricata suricatta* (Griffin *et al.*, 2003) and Ethiopian wolves *Canis simensis* (Randall *et al.*, 2007), where reproductive suppression is thought to occur and the majority of offspring are assigned to the dominant pair. More egalitarian systems, with limited evidence of reproductive suppression, are seen in banded mongooses *Mungos mungo* (71% of females give birth, Cant, 2000) and spotted hyenas *Crocuta crocuta* (all females and two thirds of males were assigned parentage, Engh *et al.*, 2002). Reproductive skew in high-density badger groups may be affected by limited reproductive suppression, although other factors affect this (Chapter 3). Further studies of badger mating systems in lower-density populations are required to enhance our understanding.

2.5.2 Multiple-paternity litters

There is strong potential for the occurrence of multiple-paternity litters in badgers, due to the presence of more than one adult or yearling male per social group and specific features of the badger's reproductive system (delayed implantation and superfoetation, Appendix 1). As badgers can conceive throughout the year, cubs within a litter may be sired by more than one male. We found the strongest evidence, to date, of multiple paternity in 16 / 65 (25%) litters with 95% confidence, compared to 5 / 31 (16% with 80%) and 0 / 7 with 95% confidence in Woodchester Park (Carpenter *et al.*, 2005).

Multiple-paternity litters may provide females with fitness advantages, such as direct benefits like fertilisation assurance, reduced harassment from males or reduced

infanticide risk from males, and genetic benefits such as promoting sperm competition, possibly increasing litter genetic diversity, reducing genetic incompatibilities, compensating for poor quality mates, and obtaining good genes (Jennions, 1997; Jennions & Petrie, 2000; Johnson, 2001; Chapman *et al.*, 2003; Wolff & Macdonald, 2004). Males, conversely, may not benefit from mate-guarding females if females conceive over a long time-period, and indeed, there is no behavioural evidence of mate guarding. As social groups consist of related individuals (Chapter 3), males may not benefit from mate-guarding females from within-group males. Instead, males may seek to prevent extra-group males from mating, through indirect territorial defence and direct aggression around the sett. Given the level of promiscuity and that badgers forage solitarily, males cannot always mate-guard females. Additionally, as females can produce multiple-paternity litters, with a mixture of same-group and extra-group fathers, it may benefit males to make sorties into neighbouring territories to gain extra-group paternities, although exactly where extra-group mating events occur is unknown.

2.5.3 Spatial patterns of paternity and dispersal

Extra-group paternity has been suggested, but not quantified, using allozymes (Evans *et al.*, 1989; da Silva *et al.*, 1994). Extra-group males obtained 42% of the assigned paternities and 86% of these extra-group paternities were by neighbouring males, which is similar to Woodchester Park (45% and 67%, respectively, Carpenter *et al.*, 2005). Neighbouring setts were on average 430 m apart, whereas extra-group paternities occurred over a mean of 376 m. Badgers scent-mark boundaries to advertise their sex and reproductive status (Buesching *et al.*, 2002), which increases

the likelihood of extra-group parentage occurring between neighbouring mates, rather than mates further afield. A maximum of 2 km, or four social groups, was crossed and both assigned parents were trapped in the year of conception. Including badgers not trapped in the year of conception may exaggerate the frequency of extra-group paternities; however, examining only assigned parents trapped in the year of conception produced similar results in Woodchester Park. Furthermore, considering only the paternity success of genotyped males trapped in the population will underestimate gene flow.

Short-term, inter-group movements of badgers of both sexes are common (Macdonald *et al.*, submitted), and may represent a search for mates or a group to disperse to. Extra-group sorties may occur throughout territories, therefore, the actual frequency is likely to be higher than that revealed by trapping data, which only records presence at a sett. Extra-group mountings have been observed (Christian, 1995) and both sexes have been radio-tracked in neighbouring groups (Christian, 1994). Extra-pair paternity may increase the genetic diversity of litters and increase offspring heterozygosity in alpine marmots that live in family groups (Cohas *et al.*, 2007). In Ethiopian wolves, however, where female-biased dispersal may reduce inbreeding, there was no evidence that extra-pair paternity reduced inbreeding (Randall *et al.*, 2007). Badger groups contained relatives (Chapter 3), and they exhibit restricted dispersal and natal philopatry (Macdonald *et al.*, submitted). Motivation or selection for dispersal may be lowered through the potential for extra-group matings, which may have initially evolved as an inbreeding avoidance mechanism, although the fact that delayed dispersal is most likely between neighbouring badger groups (Macdonald *et al.*,

submitted) reduces this likelihood. Further research is required into dispersal and extra-group paternity in lower-density badger populations.

Badger social groups may initially form on a purely ecological basis (Johnson *et al.*, 2001a; Johnson *et al.*, 2001c), as there are few demonstrated benefits to group living (Johnson *et al.*, 2004). Once formed, social groups are fostered through kinship ties; however, group living may be costly, especially to females in terms of a reduced likelihood of direct reproduction and reduced litter size compared to lower-density populations. This may represent a difference in dispersal propensity as, at high density, badgers tend to move to groups with fewer competing individuals of the same sex (Macdonald *et al.*, submitted); however, research is required into dispersal and mating patterns in lower-density populations. If cooperative breeding occurs, which it may (Woodroffe, 1993) or may not (Woodroffe & Macdonald, 2000), then this cost to group living may be reduced. Additionally, there are undoubted costs to dispersing in high-density populations (da Silva *et al.*, 1993; Tuytens *et al.*, 2000b), so any direct or indirect reproduction benefits of natal philopatry may be enhanced by indirect resource benefits, such as access to a known high-quality territory. Any strong selection to disperse may be reduced further through the occurrence of extra-group matings that increase gene flow. That there are potential benefits to natal philopatry (Stacey & Ligon, 1991), as well as ecological constraints on dispersal (Emlen, 1982), plus life-history constraints on dispersal in the form of longevity that increases habitat saturation (Arnold & Owens, 1998), suggests that all of these factors may play a role in determining the degree of delayed dispersal, as proposed by the Broad Constraints Hypothesis (Hatchwell & Komdeur, 2000).

Supplementary Table II.I Number of alleles per locus, allele sizes, and frequencies for each of the 22 microsatellite loci, obtained from the genotypes of all of the 915 genotyped badgers.

Locus	No. alleles	Allele size (bp)	Frequency
<i>Mel01</i>	3	280	0.04
		282	0.96
		286	0.01
<i>Mel10</i>	2	160	0.85
		162	0.15
<i>Mel12</i>	4	153	0.39
		155	0.05
		157	0.11
		159	0.45
<i>Mel14</i>	4	186	0.25
		188	0.18
		194	0.41
		196	0.16
<i>Mel15</i>	7	243	0.01
		247	0.25
		251	0.16
		263	0.37
		267	0.01
		271	0.00
		275	0.20
<i>Mel101</i>	4	122	0.79
		132	0.13
		134	0.01
		136	0.06
<i>Mel102</i>	4	193	0.31
		195	0.52
		197	0.02
		199	0.15
<i>Mel103</i>	5	251	0.04
		255	0.05
		259	0.12
		261	0.45
		263	0.34
<i>Mel104</i>	7	315	0.48
		319	0.03
		321	0.07
		323	0.08
		325	0.10
		327	0.18
		329	0.06

Locus	No. alleles	Allele size (bp)	Frequency
<i>Mel105</i>	8	134	0.00
		136	0.38
		140	0.04
		142	0.14
		146	0.00
		148	0.38
		150	0.06
		152	0.00
<i>Mel106</i>	4	220	0.05
		222	0.77
		224	0.17
		226	0.00
<i>Mel107</i>	4	284	0.34
		286	0.51
		288	0.00
		292	0.14
<i>Mel108</i>	2	322	0.86
		326	0.14
<i>Mel109</i>	5	106	0.00
		116	0.54
		125	0.21
		127	0.24
		129	0.00
<i>Mel110</i>	3	324	0.51
		326	0.25
		328	0.24
<i>Mel111</i>	2	132	0.90
		138	0.10
<i>Mel112</i>	4	418	0.34
		424	0.33
		428	0.21
		430	0.12
<i>Mel113</i>	4	118	0.01
		120	0.73
		122	0.25
		130	0.01
<i>Mel114</i>	2	231	0.00
		233	1.00
<i>Mel115</i>	8	330	0.13
		333	0.01
		335	0.00
		338	0.18
		339	0.09
		343	0.00
		347	0.00
		351	0.59

Locus	No. alleles	Allele size (bp)	Frequency
<i>Mel116</i>	6	113	0.47
		115	0.20
		129	0.13
		131	0.00
		133	0.19
		135	0.01
<i>Mel117</i>	4	174	0.53
		187	0.32
		189	0.15
		193	0.00

**Social organisation of the European badger *Meles meles*:
relatedness and reproductive skew²**

² A modified version of this chapter is in press as: Dugdale HL, Macdonald DW, Pope LC, Johnson PJ, Burke T (2008) Reproductive skew and relatedness in social groups of European badgers *Meles meles*. *Molecular Ecology*.

3.1 Abstract

Reproductive skew is a measure of the proportion of individuals of each sex that breed in a group, and is a valuable measure for understanding the evolution and maintenance of sociality. Here, we provide the first quantification of reproductive skew within social groups of European badgers *Meles meles*, throughout an 18-year study in a high-density population. We use 22 microsatellite loci to analyse within-group relatedness and demonstrate that badger groups contain relatives. Average within-group relatedness was high ($R = 0.20$) and approximately one-third of within-group dyads were more likely to represent first-order kin than unrelated pairs. Adult females within groups had higher pairwise relatedness than adult males, due to the high level of extra-group paternities rather than permanent physical dispersal. Spatial clustering of relatives occurred among neighbouring groups, which we suggest is due to the majority of extra-group paternities being attributable to neighbouring males. Reproductive skew was found among within-group candidate fathers ($B = 0.26$) and candidate mothers ($B = 0.07$), but not among breeding individuals; our power to detect reproductive skew in the latter was low. We use these results to evaluate reproductive skew models. Although badger society best fits the assumptions of the incomplete-control models, our results were not consistent with their predictions. We suggest that this may be due to female control of paternity, female–female reproductive suppression only in years with high food availability resulting in competition over access to breeding sites, extra-group paternity masking the benefits of natal philopatry, and / or the inconsistent occurrence of hierarchies that are linear when established.

3.2 Introduction

Reproductive skew is an index of the proportion of individuals of each sex that breed in groups and this has important consequences on the evolution of sociality (Vehrencamp, 1979). Reproductive skew varies along a continuum from high, when all or most of the group reproduction is by a single male and female, to low skew, when reproduction is more evenly distributed and most individuals breed. High reproductive skew occurs in mammalian species such as meerkats *Suricata suricatta* (Griffin *et al.*, 2003), alpine marmots *Marmota marmota* (Goossens *et al.*, 1998), African wild dogs *Lycaon pictus* (Girman *et al.*, 1997), and Ethiopian wolves *Canis simensis* (Randall *et al.*, 2007). Examples of egalitarian systems are seen in the banded mongoose *Mungos mungo* (Cant, 2000; de Luca & Ginsberg, 2001), spotted hyenas *Crocota crocuta* (Engh *et al.*, 2002; van Horn *et al.*, 2004), African lions *Panthera leo* (Packer *et al.*, 2001) and farm cats *Felis catus* (Macdonald *et al.*, 1987).

One important factor affecting reproductive skew is relatedness. Hamilton's (1964) classical work on kin selection demonstrated how cooperative behaviour may evolve such that individuals may enhance their indirect fitness benefits by cooperating with relatives, even if this incurs direct fitness costs. Social groups that consist of unrelated individuals are therefore less likely to cooperate because of kin selection. Alternatively, when groups consist of relatives and relatedness between groups is lower than within groups, kin selection may promote cooperation between group members. Vehrencamp (1983a; 1983b) developed the first reproductive skew models. These models incorporate kin selection and predict that relatedness, dispersal costs, and the relative benefits of group living versus solitary living may have a major

impact on the evolution of reproductive skew. These models predict how varying these three factors alters the magnitude of the reproductive opportunities that a dominant individual may offer a subordinate group-member as an incentive to stay in the group. These predictions can then be tested; however, such models are based on specific assumptions, and it is important to check that these assumptions apply to the study species (Johnstone, 2000).

In this chapter, we investigate the applicability of reproductive skew models to the European badger *Meles meles* and we assess patterns of relatedness and reproductive skew in a high-density population. Badgers represent a good species in which to study relatedness and reproductive skew due to their complex social system. The social organisation of badgers varies over their range, which extends from the British Isles to Japan and from the southern Mediterranean to the Russian Arctic Circle. In south-west England badgers are typically group-living (Rogers *et al.*, 1997; Macdonald & Newman, 2002), with up to 29 members (da Silva *et al.*, 1994). Badgers give birth once a year and at high density they breed plurally (Carpenter *et al.*, 2005; Chapter 2); however, few functional benefits to group living have been established (Woodroffe, 1993; Woodroffe & Macdonald, 1995b; Johnson *et al.*, 2004; Dugdale, 2007). Groups are thought to initially form passively, in accordance with the Resource Dispersion Hypothesis (RDH, Carr & Macdonald, 1986), and are maintained by natal philopatry and restricted dispersal (Cheeseman *et al.*, 1988; da Silva *et al.*, 1994; Pope *et al.*, 2006). The degree of reproductive skew within badger groups has not been quantified. At a population level only approximately 30% annually of the potentially reproductive population, in each of two high-density populations, produced cubs that survived to

independence (Carpenter *et al.*, 2005; Chapter 2), suggesting that some reproductive skew must occur.

There are several possible explanations for the disposition to positive or high reproductive skew in badgers; first, if group-living females gain higher fitness than solitary females; second, if dispersing individuals suffer reduced fitness; or third, if groups contain relatives (Keller & Reeve, 1994). Several studies suggest that the first of these explanations, that group-living females gain higher fitness than solitary females, is unlikely. Breeding female badgers may provide alloparental care, however, a higher number of mothers within a group was not found to increase the mean litter size within the group, the probability of a cub breeding, or the lifetime breeding success of a cub (Chapter 6). Furthermore, there was no relationship between the number of non-breeding females in a group and the number of yearlings produced by that group, once territory quality was controlled for (Woodroffe & Macdonald, 2000). The addition of an extra female to a group, therefore, does not appear to increase cub productivity, although the possibility of other benefits cannot be ruled out. Current evidence indicates that the second explanation, involving dispersal, may indeed influence reproductive skew. This is because several studies indicate that dispersal may be costly: (1) dispersal is restricted (Pope *et al.*, 2006); (2) females that dispersed did not produce cubs (da Silva *et al.*, 1993); (3) females endured higher rates of bite-wounding during recolonisation (Delahay *et al.*, 2006b); and (4) increased dispersal rates have been associated initially with a reduced fecundity (Tuytens *et al.*, 2000b). Finally, relatedness is predicted to be high within badger groups, due to restricted dispersal. Two studies that estimated the Queller and Goodnight (1989) index of relatedness (R) suggest that the relatedness of all within-

group badgers is indeed high ($R = 0.15$, Carpenter, 2002; $R = 0.17$, M Huck & TJ Roper, unpublished data). These analyses, however, did not include the bias correction that removes all potential relatives of the individual whose relatedness is being calculated (Queller & Goodnight, 1989) and may therefore underestimate the real values.

Our study aimed to assess whether relatedness is a predictor of reproductive skew in badger groups. Using microsatellite genotypes, in combination with extensive field records, we quantify levels of relatedness and reproductive skew in badger social groups, in Wytham Woods, Oxford, UK. Specifically, we assess whether: (1) badger groups contain relatives; (2) significant reproductive skew occurs for both sexes within social groups; and (3) reproductive skew varies with relatedness and group productivity. We then assess these results in the light of current theories on reproductive skew.

3.3 Materials and methods

We present data from a high-density population of badgers from Wytham Woods, Oxfordshire ($01^{\circ} 19'W$, $51^{\circ} 46'N$), an area that covers 4 km^2 and consists primarily of deciduous woodland, surrounded by permanent pasture and mixed arable land. Its highest reported badger density is $44 \text{ individuals km}^{-2}$ and both the annual post-emergence cub sex-ratio of the population (1987–2001, Dugdale *et al.*, 2003) and the annual adult sex-ratio (1987–1996, Macdonald & Newman, 2002) did not differ from 50%. Based on trapping records, the mean number of social groups in which badgers were present each year was 19 ± 2 (range = 14–26). The territory borders of social

groups were mapped every two years using bait-marking techniques (Kruuk, 1978; Delahay *et al.*, 2000). Capture-mark-recapture events were usually undertaken four times a year, over one week in January, and in two weeks in each of June, August and November (Macdonald & Newman, 2002), from June 1987 to November 2005. Ninety-five percent of the inter-trap intervals were within 525 days ($n = 6,193$); further information on trapping efficiency is provided by Macdonald & Newman (2002). An intra-muscular injection of approximately 0.2 ml / kg ketamine hydrochloride was used for sedation. Cubs are born below ground and do not emerge until around eight weeks, but for welfare reasons we do not catch the cubs until independence at around 15 weeks. Cubs weighing ≤ 2 kg were considered too small to sedate and were released after a hair sample had been plucked. Badgers were identified through a unique tattoo number, classified as cub or adult based upon their size and then sexed. Badgers first trapped as adults were aged according to tooth wear, graded on a one (none) to five (extreme) scale. We judged those with tooth wear of four or five to be at least two years old, otherwise we judged them to be at least one year old (Chapter 2). We collected a blood or hair sample for genetic analysis. The extraction, amplification and genotyping methods are described in Chapter 2. In total, 915 badgers (85% of the trapped population), of which 630 were cubs born in 1988–2005, were genotyped for 22 microsatellite loci (*Mel101–117*, Carpenter *et al.*, 2003; *Mel1*, Bijlsma *et al.*, 2000; and *Mel10*, *Mel12*, *Mel14* and *Mel15*, Domingo-Roura *et al.*, 2003). Genotyping was 98% complete, re-genotyping provided an estimate of 0.5% of loci typed incorrectly and PEDANT 1.3 (Johnson & Haydon, 2007) estimated a mean allelic dropout rate per allele of 0.005 and a mean false allele rate per genotype of < 0.001 . Observed heterozygosity was 0.50 and there were a mean of 4.4 alleles per

locus. No locus, or pair of loci, consistently deviated from Hardy-Weinberg equilibrium or linkage equilibrium (Chapter 2).

3.3.1 Parentage analysis

We employed a likelihood based approach to assign parent-pairs, using CERVUS 3.0.1.8 (Kalinowski *et al.*, 2007), and to infer sibships, with COLONY 1.2 (Wang, 2004). The proportion of loci typed, presence of relatives, proportion of unsampled individuals, and genotyping error were incorporated into the CERVUS analyses, and the genotyping error was also incorporated into the COLONY analyses (for further details see Chapter 2). Neither parent was known *a priori*, therefore, we selected candidate parents using mark-recapture data and biological rules. Candidate mothers were females aged two years or older, present in the cub's social group in the year that the cub was born. Candidate fathers were yearling males or older, present in Wytham Woods the year before the cub was born, as females conceive up to 11 months before implantation (Appendix 1). Based on recapture and survival rates, we included adults and yearlings as candidate parents for two years after their last date of capture, and cubs for one year (Chapter 2). Excluding individuals after their date of last capture, there were a mean of 5.6 ± 0.4 (range = 1–23) candidate mothers and 5.8 ± 0.4 (range = 1–26) candidate fathers per social-group-year. Both parents were assigned to 595 (94%) cubs, maternity to 602 (96%) and paternity to 611 (97%), with 80% confidence (Chapter 2). As we cannot capture cubs before independence, breeding individuals are those individuals that successfully raised cubs to independence.

3.3.2 *Relatedness and kinship*

We estimated both the average and pairwise Queller and Goodnight (1989) index of relatedness (R) using RELATEDNESS 5.0.8. We calculated allele frequencies using the genotypes of all 915 badgers. We included a bias-correction by removing all potential relatives of the individual whose relatedness was being calculated; otherwise, the analysis underestimates relatedness, given a small sample or few groups of relatives (Queller & Goodnight, 1989). We therefore assigned each of the 915 badgers to the social group assumed to contain the majority of their potential relatives, based on where they were trapped. Badgers are natally philopatric (Cheeseman *et al.*, 1988; da Silva *et al.*, 1994), dispersal is restricted (Pope *et al.*, 2006) and there is no evidence of sex-biased dispersal in our study population (Macdonald *et al.*, submitted). The group in which a badger was first trapped as a cub was assumed to contain most of its potential relatives. For a badger that was first trapped as an adult, the group in which an individual was most frequently trapped was assumed to contain most of its potential relatives, as temporary inter-group movements do occur (Christian, 1994; Macdonald *et al.*, submitted) or, in the case of ties ($n = 12$), the first group in which it was trapped. Territory fission, the splitting of a social group into two, occurred (da Silva *et al.*, 1993), therefore, we assigned groups according to the to the earliest classification in 1987. We used a rarefaction analysis to calculate the number of loci required to generate reliable relatedness estimates (Girman *et al.*, 1997). We selected one locus randomly and calculated the pairwise R for dyads drawn from all of the candidate parents in 2005. We then added a second locus without replacement and recalculated R . We continued until all 22 loci were included, repeated the procedure 1,000 times in total, and calculated the mean difference in R between consecutive samplings, which we then plotted as a function of the total number of loci drawn.

We conducted two randomisations, each with 1,000 simulations, to ask if badgers in the same group were significantly more related to (a) each other, or (b) neighbouring badgers, than to other badgers in the 2004 population. For (a), we randomly allocated badgers to groups, preserving the age and sex composition of each group (Girman *et al.*, 1997). The mean within-group pairwise R was averaged across groups for each simulation. For (b), we randomly allocated the same number and type (age / sex) of neighbour to each group, as observed. Each simulation generated the mean, across groups, of the mean pairwise R between group members and their neighbours. We selected 2004 for the same reasons we selected 2005 for the rarefaction analysis: high-quality samples, a large proportion of sampled badgers and stable groups. Temporary inter-group movements occur in badger groups (Christian, 1994; Macdonald *et al.*, submitted), which may result in badgers being assigned to more than one social group, although they are not resident in this group. As females must be resident in the group in which they breed, females assigned to more than one group were only assigned to the group in which they bred. We excluded badgers assigned to more than two groups, resulting in a sample size of 250 individuals.

To enable the classification of dyads of unknown relationship we calculated the average R between first-degree relatives (1°: mother–offspring, father–offspring and full-siblings) and some second-degree relatives (2°: maternal and paternal half-siblings) identified using parentage results with 95% confidence (Chapter 2). We estimated the average R of unrelated dyads using 100 randomly selected dyads of female cubs, drawn from different and non-neighbouring groups that had a different father assigned with 95% confidence. Female cubs, from non-neighbouring groups,

are generally expected to be unrelated, as badgers are natally philopatric (Cheeseman *et al.*, 1988; da Silva *et al.*, 1994), dispersal is restricted (Pope *et al.*, 2006) and most extra-group matings occur between neighbouring groups (Carpenter *et al.*, 2005; Chapter 2). We assessed whether within-group adults and yearlings could be confidently assigned as being unrelated, 2° kin or 1° kin using KINSHIP 1.3.1 (Goodnight & Queller, 1999). To determine the power that we had to do this we simulated the genotypes of 10,000 pairs of individuals of a given relatedness and determined the proportion of these that were wrongly rejected as not belonging to that relationship versus a null hypothesis.

3.3.3 Reproductive Skew

Skew in reproduction within social groups may occur by chance, especially when group size and productivity are low. We therefore quantified the degree of reproductive skew within groups by calculating the binomial skew index (B) using SKEW CALCULATOR 2003 1.2 (Nonacs, 2000). B calculates the observed variance in reproductive skew and then subtracts the expected variance if all individuals had equal chance of gaining any given group benefit (i.e. parentage of one cub), adjusted for group size, group productivity, and residency times. B can range from minus one to two; it is positive when reproductive skew is greater than expected, zero when randomly distributed and negative when more evenly distributed than expected. A power analysis, based on 10,000 simulations, inputting the mean number of candidates and assigned cubs per social-group-year, and the probability of each candidate gaining one benefit, based on mean litter size, was used to estimate the sample size required to discriminate skewed reproduction from random distributions.

Per-capita group productivity was defined as the number of cubs in each social-group-year that were assigned a mother or a within-group father, divided by the number of candidate mothers or the number of within-group candidate fathers, respectively.

We calculated B for both male and female candidate parents and breeders, for each cohort and for all 18 cohorts pooled within each group. Only groups with at least two cubs assigned a parent were analysed, as SKEW CALCULATOR 2003 cannot detect significant reproductive skew in groups with just one cub, unless there is a large discrepancy in residency times. Significance levels were estimated using 10,000 simulations. Mothers trapped in more than one group in a given year, most probably due to temporary inter-group movements (Christian, 1994; Macdonald *et al.*, submitted), were only assigned to the group in which they bred, whereas fathers were included in all of the groups in which they were trapped and assigned parentage.

3.3.4 Statistical analyses

In order to assess the relationships between reproductive skew, relatedness and group productivity, we used SAS 9.1 to conduct General Linear and General Linear Mixed Models (GLMs & GLMMs). The MIXED procedure was run with social group as a repeated factor, generally with an auto-regressive heterogeneous covariance structure, and the Kenward-Roger denominator degrees of freedom method. The most appropriate covariance structure was selected through graphical examination. The response variable relatedness was log-transformed whilst B was either square-root or log-transformed, unless otherwise stated, by first adding a constant to move the minimum value to one (Osborne, 2002). We also used SAS 9.1 to conduct

permutations to compare the relatedness of dyads of individuals within and between social groups. Normality was examined by the Anderson-Darling test and by examining the histogram of residuals. Means are given with the 95% confidence interval (CI), unless otherwise stated.

3.4 Results

3.4.1 *Relatedness and kinship*

Rarefaction analysis indicated that 22 loci were sufficient to obtain reliable relatedness estimates (Figure 3.1), with a mean pairwise R difference of less than 0.04 with ≥ 16 loci or less than 0.03 with ≥ 21 loci. Dyads of 1° and 2° relatives assigned with 95% confidence had slightly higher average relatedness than the theoretical values of a half and a quarter (Figure 3.2a). Dyads of within-group members in 2004 had slightly lower average R than assigned half-siblings, but greater than unrelated individuals (Figure 3.2a compared with Figure 3.2b). The average relatedness of within-group females was higher than that of within-group males: adult and yearling females 0.241 ± 0.046 ; adult and yearling males 0.162 ± 0.039 ; adult females 0.228 ± 0.046 ; and, adult males 0.158 ± 0.041 (Figure 3.2b). Considering all within-group adults and yearlings, the average relatedness of females to all within-group members ($R = 0.204 \pm 0.036$) was greater than that of males to all within-group members ($R = 0.161 \pm 0.031$).

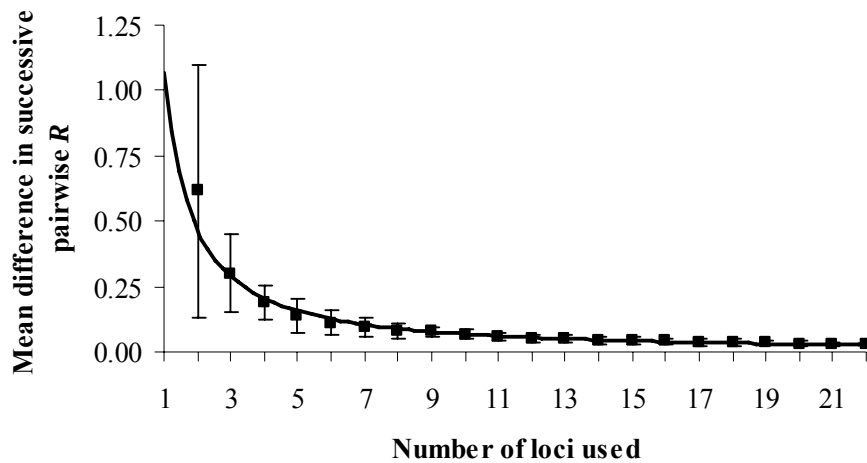
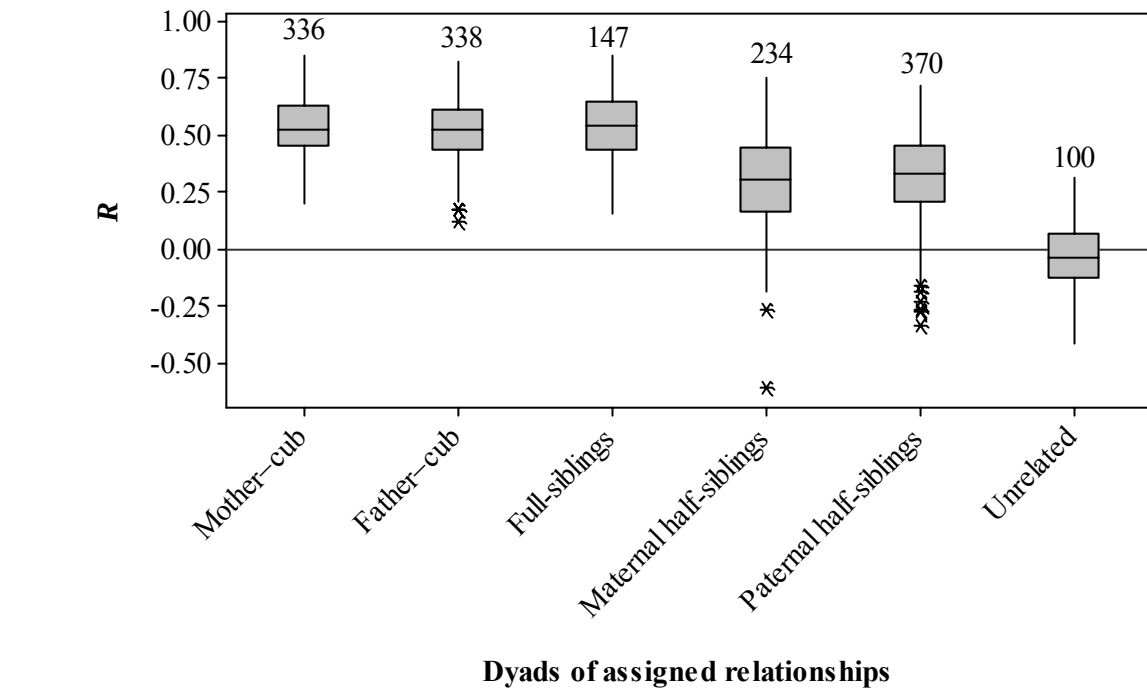
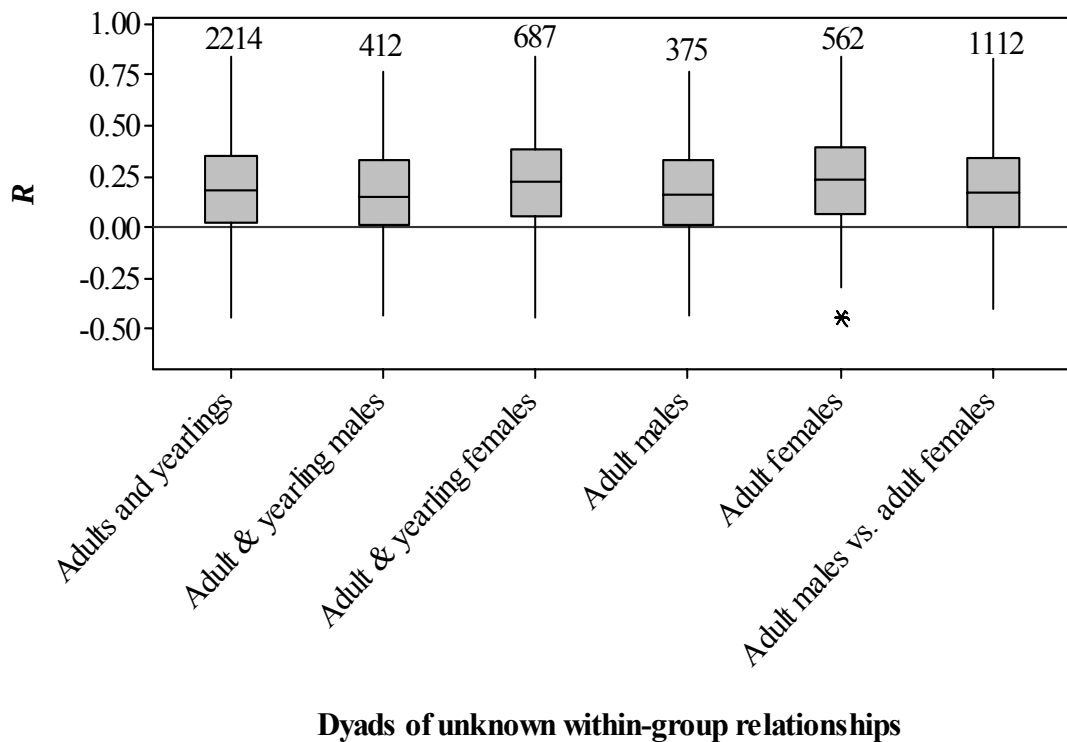


Figure 3.1 The mean difference in successive pairwise relatedness (R) of all possible dyads of candidate parents in 2005. R was calculated using one locus, and then recalculated by consecutively adding one more locus, selected at random, without replacement. This was repeated 1,000 times and the mean difference in R between consecutive sampling is expressed as a function of the total number of loci drawn, which is described by the curve $y = 1.067x^{-1.193}$, $r^2 = 0.99$. Error bars display $\pm$ one standard deviation.

Badgers within a group had significantly higher pairwise relatedness than expected from randomisations (Table 3.1). Within-group females had significantly higher pairwise relatedness than males, using all possible same-sex within-group dyads of adults and yearlings in 2004 (mean observed difference in $R = 0.102 \pm 0.121$, $n = 22$ social groups; randomised 2.5–97.5% range = -0.019 – 0.039 , $p < 0.001$). Analysing non-transformed pairwise relatedness in social groups in 2004, male–male pairwise relatedness ($F_{1,23} = 0.07$, $r^2 = 0.00$, $p = 0.79$; Fig. 3a) and female–female pairwise relatedness ($F_{1,20} = 2.16$, $r^2 = 0.10$, $p = 0.16$; Figure 3.3b) did not vary with the number of same-sex group members. One group strongly affected the female regression (Figure 3.3b) and when this was removed females had significantly higher pairwise relatedness in groups with fewer females ($F_{1,19} = 10.06$, $r^2 = 0.35$, $p = 0.0050$). Badgers from neighbouring groups had significantly higher pairwise relatedness than expected from randomisations (Table 3.1).

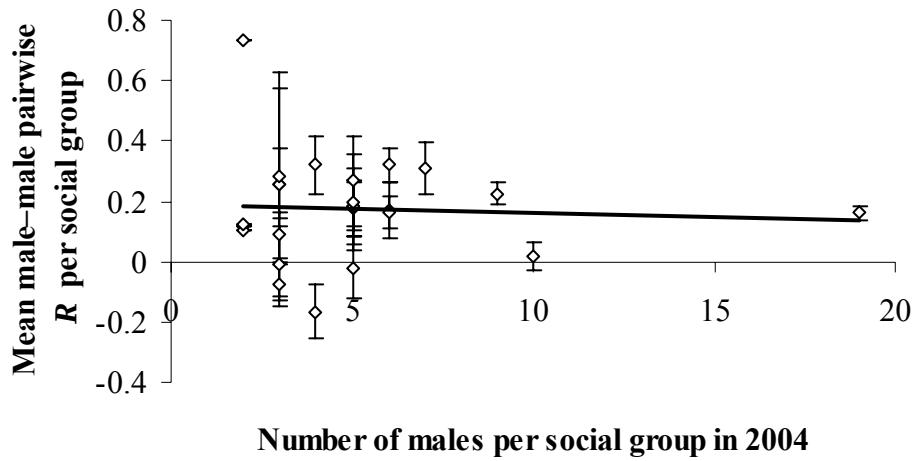


(a)

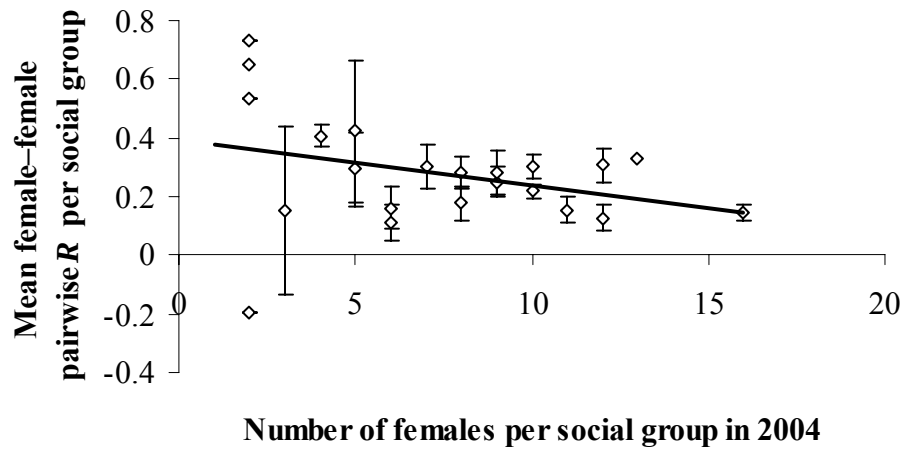


(b)

Figure 3.2 Average relatedness (R) of dyads of: (a) relationships assigned with 95% confidence and (b) individuals within the same social groups in 2004. Data labels display the number of dyads. The top and bottom of the boxes represent the inter-quartile range and the middle line the median. Each whisker extends to 1.5 x the inter-quartile range, and * represents outliers.



(a)



(b)

Figure 3.3 Number of (a) males and (b) females in each social group in 2004 as a predictor of their mean pairwise relatedness (R). Error bars display the 95% confidence intervals. The equations for the trend lines are: a) $y = 0.190 - 0.003x$, and b) $y = 0.390 - 0.015x$.

Table 3.1 Mean observed (with 95% CI) and randomised pairwise relatedness (R), and the randomised 2.5% to 97.5% percentile points for R , for categories of badgers living in the same or neighbouring social groups in 2004. Randomised values were generated by randomly allocating yearlings and / or adults to a group of the same sex and size structure.

		Adults & yearlings	Adult males	Adult females
Within social groups	n social groups	25	23	21
	Observed R	0.198 ± 0.039	0.164 ± 0.082	0.245 ± 0.077
	Randomised R	0.009	0.005	0.012
	Percentile points	-0.019–0.039	-0.041–0.055	-0.029–0.058
	p	< 0.001	< 0.001	< 0.001
Between neighbouring social groups	n social groups	26	25	24
	Observed R	0.088 ± 0.027	0.103 ± 0.038	0.090 ± 0.029
	Randomised R	-0.003	-0.006	-0.005
	Percentile points	-0.015–0.008	-0.028–0.014	-0.027–0.017
	p	< 0.001	< 0.001	< 0.001

Simulations revealed that the power to distinguish between the pairwise relatedness of 1° and 2° kin (0.60), and 2° and unrelated individuals (0.52) was in each case low; however, that between 1° kin and unrelated individuals was high (0.95; Figure 3.4). Of the male–male dyads, 26% ($n = 107 / 412$) were significantly ($p < 0.05$) more likely to represent a dyad of 1° kin than unrelated individuals, as were 36% ($n = 252 / 687$) of the female–female dyads, and 30% ($n = 338 / 1115$) of the male–female dyads. Twenty-nine dyads contained individuals that were assigned the same parents and all were classified as 1° kin.

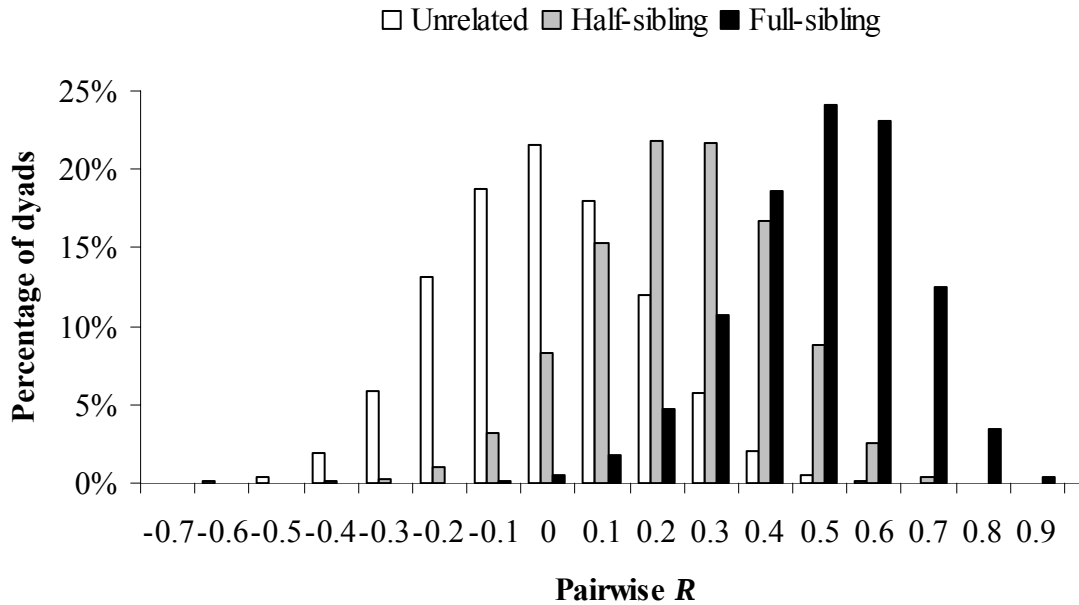


Figure 3.4 Pooled relatedness distribution of pairs of unrelated individuals (white bars), half-siblings (grey bars) and full-siblings (black bars) generated in 10,000 simulations using KINSHIP 1.3.1 (Goodnight & Queller, 1999), based on the population allele frequencies.

3.4.2 Reproductive skew

Female badgers breed once a year. In our study population the mean number of candidate mothers within a social group was six, and the mean number of cubs that were assigned a mother per social-group-year was 2.7 ± 0.2 ($n = 222$). To assess the power that we had to detect reproductive skew, we conducted a power analysis based on a typical group of six candidate mothers and three cubs, with a mean litter size of 1.4. We therefore entered two females as having a probability of gaining maternity of one cub of 0.4, and four females of 0.05, suggesting that six groups are required to detect significant reproductive skew. For a group with two mothers and three cubs, with probabilities of gaining maternity of one cub of 0.66 and 0.33, nine groups are required to detect a significant difference. If each mother has an equal chance of being assigned one cub, however, it is not possible to detect a significant difference.

Mean reproductive skew across all social-group-years was significantly greater than expected by chance for both candidate mothers and within-group candidate fathers, but it was not significantly different from random for mothers and all within-group fathers (Table 3.2). Similar results were observed when all badgers were excluded from analyses after their year of last capture (Table 3.2). A GLMM was run with B for within-group candidate fathers and candidate mothers as the response, sex as the predictor, social group as a repeated factor and separate covariance patterns for each sex. Within-group candidate fathers had a significantly higher B than the candidate mothers of cubs in the same social-group-year ($F_{1,16} = 45.04$, $p < 0.0001$; excluded after last year of capture, $F_{1,7.09} = 21.36$, $p = 0.0023$). When values were pooled for each group over all 18 years, to increase the sample size, candidate mothers and within-group candidate fathers showed reproductive skew that was significantly different from random, but mothers, and within-group fathers did not (Table 3.2). Excluding individuals after their last year of capture produced similar results, except for the analysis of within-group candidate mothers that was no longer significant (Table 3.2). Overall, there was significant positive reproductive skew among same-sex group members; however, among within-group breeders reproductive skew was randomly distributed.

A GLMM with social group entered as a repeated measure and B as the response, revealed that the average relatedness between all candidate mothers ($F_{1,49.9} = 0.08$, $p = 0.79$; excluded after last year of capture, $F_{1,9.22} = 4.19$, $p = 0.07$) and within-group candidate fathers ($F_{1,25.5} = 0.66$, $p = 0.42$; excluded after last year of capture, with heterogeneous compound-symmetry covariance structure, $F_{1,5.8} = 0.01$, $p = 0.91$) in a social-group-year did not predict the degree of reproductive skew.

Table 3.2 Reproductive skew among badgers in social groups in Wytham Woods from 1988–2005. Data were analysed per social-group-year, or per social group with all years pooled. Candidate parents or parents were either included for two years after their date of last capture, or were excluded after their date of last capture. Analyses where the skew was significantly positive are indicated by probabilities shown in bold type.

Class	Grouping	Included for 2 years after last capture					Excluded after last capture				
		<i>n</i>	Mean <i>B</i>	Min <i>B</i>	Max <i>B</i>	<i>p</i>	<i>n</i>	Mean <i>B</i>	Min <i>B</i>	Max <i>B</i>	<i>p</i>
Candidate mothers	social-group-year	147	0.068	-0.250	0.590	< 0.0001	133	0.042	-0.250	0.561	0.0089
Mothers	social-group-year	112	-0.146	-0.250	0.080	1.0000	104	-0.145	-0.250	0.056	1.0000
Within-group candidate fathers	social-group-year	69	0.261	-0.127	0.775	< 0.0001	54	0.241	-0.161	0.711	< 0.0001
Within-group fathers	social-group-year	37	-0.090	-0.250	0.247	1.0000	24	-0.096	-0.250	0.264	0.9994
Candidate mothers	social group (pooled)	24	0.039	-0.031	0.178	0.0009	23	0.017	-0.125	0.202	0.1435
Mothers	social group (pooled)	24	0.007	-0.084	0.143	0.3903	23	-0.006	-0.125	0.210	0.8196
Within-group candidate fathers	social group (pooled)	25	0.178	-0.062	0.632	< 0.0001	18	0.261	-0.037	0.684	< 0.0001
Within-group fathers	social group (pooled)	19	-0.054	-0.250	0.060	0.9996	11	0.019	-0.120	0.156	0.2469

Per-capita group productivity, of cubs assigned within-group parents, did not predict the degree of reproductive skew between candidate mothers ($F_{1,19.8} = 0.00$, $p = 0.97$; excluded after last year of capture, $F_{1,11.8} = 0.68$, $p = 0.42$) or within-group candidate fathers ($F_{1,10.4} = 1.03$, $p = 0.33$; excluded after last year of capture, $F_{1,5.52} = 1.97$, $p = 0.21$). Group productivity of all cubs in a social-group-year, with compound symmetry covariance pattern for the repeated factor, was not significantly related to average relatedness for candidate mothers ($F_{12,291} = 0.38$, $p = 0.97$; excluded after last year of capture, $F_{12,256} = 0.44$, $p = 0.95$) or within-group candidate fathers ($F_{7,284} = 1.53$, $p = 0.16$; excluded after last year of capture, $F_{6,237} = 1.42$, $p = 0.21$).

3.5 Discussion

3.5.1 Within-group relatedness

Relatedness between group members can shape the evolution of social behaviours (Hamilton, 1964), and relatedness may (Vehrencamp, 1983a, 1983b) or may not (Reeve *et al.*, 1998) affect the degree of reproductive skew. In badger social groups dispersal is restricted (Pope *et al.*, 2006) and groups are maintained by natal philopatry (Cheeseman *et al.*, 1988; da Silva *et al.*, 1994), so group members are expected to be related. The precision and accuracy of relatedness estimates will vary, depending on the allele frequency distribution and the distribution of true relatives in the population (van de Castele *et al.*, 2001; Wang, 2002; Blouin, 2003; Csilléry *et al.*, 2006). Average relatedness between dyads of assigned 1° relatives was slightly higher than 0.5 (mother–offspring $R = 0.54 \pm 0.03$, father–offspring $R = 0.52 \pm 0.02$, full-siblings $R = 0.54 \pm 0.05$), and for assigned half-siblings this was slightly greater

than 0.25 (maternal half-siblings $R = 0.30 \pm 0.05$; paternal half-siblings $R = 0.31 \pm 0.03$). This is comparable to the relatedness distributions of 1° or 2° relatives being skewed to the right (Csilléry *et al.*, 2006). We demonstrate that adult and yearling group members are related ($R = 0.19 \pm 0.03$) by slightly lower levels than assigned half-siblings and higher levels than expected from random ($R = -0.03 \pm 0.06$), suggesting that social cohesion may be maintained by high relatedness levels. Average relatedness between within-group adults and yearlings was higher than relatedness in groups of long-tailed macaques *Macaca fascicularis* ($R = 0.07$, de Ruiter & Geffen, 1998), and lower than that in meerkat groups ($R = 0.28$, A Griffin pers. comm.) and Ethiopian wolf groups ($R = 0.30$, Randall *et al.*, 2007).

Our KINSHIP analyses revealed that we had the power to distinguish between 1° kin and unrelated individuals, but not between 2° kin and either unrelated or 1° kin. This is not surprising, given that power to distinguish between 2° kin and either unrelated or 1° kin is estimated to require 50 loci, whereas 15–20 are required for 1° kin and unrelated individuals (Blouin, 2003). Approximately a third of the within-group dyads represented 1° kin, suggesting that badger groups consist of closely related and distantly related kin. The occurrence of multiple maternity, multiple-paternity litters and extra-group paternity within badger social groups (Carpenter *et al.*, 2005; Chapter 2) will facilitate the presence of 1° kin and more distantly related kin within groups.

Within badger groups, females had higher pairwise relatedness than males, based on both adults and yearlings ($R = 0.241 \pm 0.046$ v 0.162 ± 0.039 ; $n = 687$ v 412) and adults only ($R = 0.228 \pm 0.046$ v 0.158 ± 0.041 ; $n = 562$ v 375 respectively). Sex-biased dispersal may result in one sex being more related than the other within groups,

as seen in long-tailed macaques that have male-biased dispersal (adult male–male $R = -0.10$, adult female–female $R = 0.14$, de Ruiter & Geffen, 1998). Sex-biased dispersal may occur in either physical or genetic terms. The similarity of relatedness estimates when yearlings are included raises the possibility that the sex differences in within-group relatedness may arise in cubs. The likelihood of sex-biased dispersal occurring primarily due to extra-group mating rather than permanent physical terms is strengthened by the lack of evidence of sex-biased dispersal from trapping studies. Three studies suggested that permanent physical dispersal is slightly more common in female than male badgers (Christian, 1994; da Silva *et al.*, 1994; Woodroffe *et al.*, 1995). These studies involved small sample sizes, the inclusion of a greater proportion of females than males, and were not analysed statistically. When the data from these studies were analysed statistically, no sex-biased dispersal was found (Fisher's exact test: Christian, 1994, $F = 7$, $n = 13$, $p = 0.07$; da Silva *et al.*, 1994, $F = 64$, $n = 140$, $p = 0.77$; Woodroffe *et al.*, 1995, $F = 48$, $n = 21$, $p = 0.73$). Nonetheless, two studies based on larger sample sizes have shown male-biased dispersal (Cheeseman *et al.*, 1988; Rogers *et al.*, 1998), whereas two others, including one from our study population, detected no sex difference (Pope *et al.*, 2006; Macdonald *et al.*, submitted).

Sex-biased physical permanent dispersal is unlikely to explain why females have higher pairwise relatedness than males within small groups, at least in our study population in which 81% of cubs (216 / 267 trapped 1987–2005) were natally philopatric and in which no sex-biased permanent dispersal from the natal group occurred (28 males and 23 females dispersed). The natal group was the group in which a badger was first trapped as a cub. Dispersing badgers were those that were

then trapped twice in the same non-natal social group and at least once out of the previous two trapping events before that in the same non-natal group, with a minimum inter-trap interval of 30 days and a minimum of 400 days between the four trapping events (Macdonald *et al.*, submitted). The difference, instead, is likely to arise from males siring offspring in multiple groups; approximately 50% of paternities are assigned to extra-group males (Carpenter *et al.*, 2005; Chapter 2). Given that there is no sex-biased permanent dispersal and reproductive skew is higher for within-group candidate fathers than for within-group candidate mothers, females are therefore more likely than males to breed within their natal group. More offspring will therefore have their mother present in their natal group than their father, resulting in fewer group-members that are highly related to males than females. This is shown in the lower pairwise relatedness of males in small groups, compared to females, and the lower average relatedness of males to other adult and yearling group members, compared to females. Males tend to make more temporary inter-group movements (i.e. trapped in a different group to its previous trapping location) than females: for example, by two years of age twice as many males as females had been trapped in more than one group (DW Macdonald, C Newman, CD Buesching & PJ Johnson, unpublished data). This suggests that temporary inter-group movements by males may facilitate extra-group paternity, leading to the higher within-group relatedness of female–female dyads than of male–male dyads.

3.5.2 Neighbouring-group relatedness

In addition to social groups containing relatives, members of neighbouring groups had higher relatedness than expected. Although dispersal is restricted (Pope *et al.*, 2006),

when it occurred in our study population it tended to be to neighbouring groups (Macdonald *et al.*, submitted), but more importantly, approximately 50% of paternities are assigned to extra-group males, the majority of which are neighbouring males (Carpenter *et al.*, 2005; Chapter 2). The high level of extra-group paternity, especially between neighbouring groups, rather than physical permanent dispersal to new groups, is therefore the primary driving factor that results in the clustering of relatives within neighbouring social groups. Extra-group paternity may reduce the motivation for permanent physical dispersal, as both males and females may mate with extra-group badgers, thereby reducing the likelihood of inbreeding and reducing costs that may be imposed through permanent physical dispersal.

3.5.3 Quantifying reproductive skew

The degree of reproductive skew among all females and all within-group males of breeding age per social-group-year was positive and significantly greater than random, when considering only cubs that were assigned a parent; however, the mean B was low, especially for candidate mothers. Few studies have quantified paternity skew among within-group males in mammalian species. Those that have, using B , have shown higher (mean $B = 0.32$, data from mountain gorillas *Gorilla beringei beringei* pooled over 6–14 years, Bradley *et al.*, 2005) or lower values (mean $B = 0.08$ per cohort of rhesus macaques *Macaca mulatta*, Widdig *et al.*, 2004; mean $B = 0.03$ per cohort of greater horseshoe bats *Rhinolophus ferrumequinum*, or 0.02 pooled over 10 years, Rossiter *et al.*, 2006) than observed here (mean $B = 0.26$ per cohort, or 0.18 pooled over 18 years). The degree of reproductive skew among mothers and within-group fathers in a social-group-year was not significantly different from random.

Small litter sizes, few mothers per group, and a high proportion of cubs sired by extra-group males (Carpenter *et al.*, 2005; Chapter 2) will restrict the power of detecting significant reproductive skew over that which occurs by chance. Excluding individuals after their date of last capture did not affect the results, with the exception that skew was no longer significant for candidate mothers pooled over the years; however, B was similar and this was the weakest result when individuals were included for the additional two-years after last capture.

3.5.4 Assessing the applicability of reproductive skew models to badger society

Models of reproductive skew make many predictions, such that any pattern of reproduction will probably fit one of the models. It is therefore important to check the applicability of the assumptions of the models to the study species (Johnstone, 2000). Synthetic models are one type of reproductive skew model; however, there is ongoing discussion on factors such as the biological applicability of the assumptions of these models (Nonacs, 2007) and indeed whether current approaches may be flawed (M Cant, pers. comm.); thus, we have not tested these. Two other types of reproductive skew models, the transactional (Vehrencamp, 1983a, 1983b; Cant & Johnstone, 1999) and incomplete-control (Cant, 1998; Reeve *et al.*, 1998) models, differ in their assumptions on whether dominants have full control over reproduction. Furthermore, transactional models assume that the addition of an extra female to a group increases group productivity. Woodroffe (1993) suggested that non-breeding female badgers may provide alloparental care; however, although there was a positive relationship between the number of non-breeding females in a group and the number of yearlings produced, this relationship disappeared when territory quality was incorporated

(Woodroffe & Macdonald, 2000). Further investigation revealed that non-breeding females did not alloparent, but that breeding females babysat and allonursed more cubs than the number to which they were assigned maternity (Chapter 6). The number of females assigned maternity in a group, however, was not a predictor of the mean litter size in the group, the probability of a cub breeding or the lifetime breeding success of a cub (Chapter 6). Overall, it therefore looks as though an increase in the number of females in a badger group does not increase cub productivity; hence, the assumptions of the transactional models are not applicable to badger society.

3.5.4.1 Incomplete control and resource availability

Reproduction in female badgers may be controlled by two factors: first, individual adaptation to local food availability, and second, female–female competition (Woodroffe & Macdonald, 1995b). Incomplete-control models assume that, in both sexes, no one individual completely controls the reproduction of same-sex group members and that individuals compete over reproduction (Reeve *et al.*, 1998). Alternatively, resource availability may explain variation in reproductive skew in both sexes, through access to resources such as breeding sites, food and even mates. Incomplete control of reproductive skew within social groups can be difficult to demonstrate in wild populations. In badgers, female–female competition over reproduction may first occur over access to breeding sites (Cresswell *et al.*, 1992). Although sett sites are not thought to be limiting (Macdonald *et al.*, 2004c), breeding sites may vary in quality. Female scent-marking behaviour around sett entrances is highest between January and March (Buesching *et al.*, 2003) and females show an increase in hinterland latrine usage in spring (Roper *et al.*, 1993), which may advertise

ownership of breeding areas or reproductive status. Competition through aggressive interactions between females at sett entrances may explain the high ranking of females within the dominance hierarchies established in three out of six social groups (Appendix 2). Bite wounds peak around gestation in some populations (Cresswell *et al.*, 1992; Delahay *et al.*, 2006b), but not in others (Macdonald *et al.*, 2004b). These aggressive interactions may lead to embryo reabsorption, which has been observed (Woodroffe & Macdonald, 1995b; Appendix 1).

Although the majority of females in their third year or older are pregnant by early summer (Appendix 1), a high proportion of females fail to lactate (70%, Cresswell *et al.*, 1992; 58%, da Silva *et al.*, 1994; 58–90%, Rogers *et al.*, 1997; 71%, Macdonald & Newman, 2002). Reproductive failure has been linked to female body condition (Cresswell *et al.*, 1992), especially after dry summers when only females in good condition breed (Woodroffe & Macdonald, 1995b). In years when food availability is high, reproductive failure has been linked to assumed social status (Woodroffe & Macdonald, 1995b). After parturition, 35% (Cresswell *et al.*, 1992) to 42% (Page *et al.*, 1994) of females ceased lactation early, indicating the loss of complete litters, and there is circumstantial evidence of infanticide by females (Cresswell *et al.*, 1992). In Wytham Woods there has been an increase in the number of annex and outlier setts over the study period (Macdonald *et al.*, 2004c). Increased reproductive productivity in young females correlates with the use of annex or outlier setts and may reduce female–female competition (Cresswell *et al.*, 1992). Reproductive skew in females may therefore be controlled through two factors: resource availability and incomplete reproductive suppression.

Post-mortem and field studies (summarised in Appendix 1) have shown that throughout the year there are always males capable of reproduction, with testicular activity highest in late winter to summer. Competition for reproductive status among males has been implied through behavioural studies. Roper *et al.* (1993) showed that males visited boundary latrines more often than females, which they hypothesised was to deter extra-group males from entering their territory for mating purposes. Expansion of one male's territory after removal of a neighbouring male was suggested to be a response to reduced competition over access to females (Revilla & Palomares, 1999). Males showed a significant tendency to scent-mark within-group females (Buesching *et al.*, 2003) and one study reported resident males chasing away extra-group males (Christian, 1994). Bite-wounding is more common among males (Delahay *et al.*, 2006b), increasing with the number of badgers in adjoining territories (Macdonald *et al.*, 2004b), which may reflect competition over mating access. These studies therefore suggest that males defend their territory and that subtle forms of mate guarding may occur. As badgers forage solitarily and can mate year-round, it would seem implausible for males to mate-guard females all of the time, as illustrated by the high rates of extra-group paternity (Carpenter *et al.*, 2005; Chapter 2). Furthermore, the proliferation of outlier setts (Macdonald *et al.*, 2004c) away from main setts further restricts the opportunity for males to mate-guard within-group females. Additionally, male–male aggression between group members is limited and there is little evidence of active mate guarding, with males from the same group mating with the same female in succession (Johnson, 2001; Chapter 5). Limited control of reproduction is therefore likely between within-group males, but, additionally, resource availability may also influence reproductive skew.

3.5.5 Effect of relatedness and group productivity on reproductive skew

Incomplete-control models assume that dominants have only limited control of reproductive skew and that they must compete directly with others to increase their share of the total group reproduction, at the cost of group productivity. Reeve *et al.*'s (1998) incomplete-control model predicts that relatedness has either no significant effect on reproductive skew, when dominance is defined by efficiency, or that there is a negative relationship when dominance is defined by access to resources. Reproductive ability is primarily a result of competitive ability, so skew is affected by the effort of the dominant that either shows no relationship or a decrease with relatedness (Reeve *et al.*, 1998). Cant's (1998) incomplete-control model, on the other hand, predicts that individuals will adjust their litter size according to the reproductive output of others within the group. Reproductive skew is predicted to increase with relatedness; possible reasons include subordinates that are highly related to the dominant being less likely to breed if this significantly reduces the indirect fitness benefits that they may otherwise gain (Cant, 1998). Observations compatible with limited-control models have been made for meerkats (Clutton-Brock *et al.*, 2001), spotted hyenas (Engh *et al.*, 2002) and certain primates (Widdig *et al.*, 2004; Bradley *et al.*, 2005; Kutsukake & Nunn, 2006).

Skew in the paternity of within-group candidate fathers was higher, but varied over a greater range, than that of the skew in maternity of candidate mothers within social-group-years. Adult females had higher pairwise relatedness than adult males within groups, and incomplete-control models predict no relationship or a negative relationship between reproductive skew and relatedness (Reeve *et al.*, 1998), or a positive relationship (Cant, 1998). We found no relationship between the average

relatedness of candidate mothers or within-group candidate fathers and the observed degree of reproductive skew within social-group-years. This is inline with the predictions of Reeve *et al.*'s (1998) incomplete-control model, but it is not consistent with Cant's model (1998). Our results of no relationship between reproductive skew and per-capita group productivity are consistent with the predictions of Reeve *et al.*'s (1998) incomplete-control model. Reeve *et al.* (1998) state that:

“both the dominant and subordinate will exert decreasing effort (e.g. aggression) to enhance their shares of group reproduction, as relatedness increases”.

There is some evidence that badgers direct less aggression towards closer relatives during the cub-rearing season; however, this was only observed in half of the six social-group-years studied (Appendix 2), consistent with Reeve *et al.*'s limited-control model (1998). No correlation was found between average relatedness and group productivity, however, whereas the incomplete-control model of Reeve *et al.* (1998) predicts a positive relationship. Overall, our results are not consistent with the predictions of incomplete-control models, although it is worth noting that relatedness estimators may have low explanatory power, especially when there is low variance in relatedness across the population (Csilléry *et al.*, 2006). Resource availability may play a large role in determining the degree of reproductive skew in badger social groups and investigation of resource availability in relation to reproductive skew may enhance our understanding.

3.5.5.1 Limitations

Reproductive skew models generally assume that a dominance hierarchy exists and that individuals are either dominant or subordinate. Within badger social groups,

however, dominance hierarchies have only been established in three out of six filmed groups (Appendix 2), and when present the hierarchies were linear, such that a second-ranking individual was subordinate to the top-ranking individual, but dominant to the third-ranking member. We examined the average relatedness between all possible within-group dyads, rather than relatedness between dominants and subordinates. We feel this is justified given that in multi-member groups reproductive skew may depend on the relatedness between subordinates, and of each to the dominant (Johnstone *et al.*, 1999). It is also worth noting that for males we only considered paternity of within-group candidate fathers, even though approximately 50% of cubs are assigned to extra-group males (Carpenter *et al.*, 2005; Chapter 2). This is because we could not determine accurately which males had access to females within a group, and inclusion of a large number of males will inflate reproductive skew. In species without paternal care, such as badgers (Chapter 6), models of reproductive skew only consider skew between group members; thus, we only investigated skew between within-group candidate fathers. Given the occurrence of multiple mating in badgers (Carpenter *et al.*, 2005; Chapter 2), females may control the paternity of their litters. Reproductive skew models, however, assume that subordinates have perfect knowledge of their benefits if they stay in the group, which is not always the case, especially when extra-group paternity or multiple-paternity litters occur (Cant & Reeve, 2002; Kokko, 2003). Problems in detecting kin and the benefits of following a simple rule of thumb may play a role in the development of reproductive skew (Nonacs, 2006). Recent papers have suggested that skew studies should now focus on the mechanisms of how conflict over reproduction is exhibited and resolved (Cant, 2006; Nonacs, 2006, 2007).

3.5.6 Conclusions

Our study demonstrates that badger social groups correspond to groups of relatives, with spatial clustering of relatives among neighbouring groups, and provides the first quantification of the degree of reproductive skew in badger groups. Within social groups, adult and yearling females showed higher levels of relatedness than adult and yearling males. This is unlikely to be sex-biased physical permanent dispersal as badgers are natally philopatric (Cheeseman *et al.*, 1988; da Silva *et al.*, 1994) and exhibit restricted dispersal (Pope *et al.*, 2006) that is not sex-biased in our study population (Macdonald *et al.*, submitted). Approximately 50% of paternities are assigned to extra-group males in our study population (Chapter 2), suggesting that the sex difference in within-group relatedness is due to genetic dispersal. Badger society best fits the assumptions of limited-control models; however, overall our results did not match the predictions of any limited-control model. This may be due to female control of paternity, extra-group paternity masking the benefits of natal philopatry, and / or the inconsistent occurrence of hierarchies that are linear when established. Furthermore, as female–female reproductive suppression only occurs in years with high food availability, when only females of higher assumed social status breed (Woodroffe & Macdonald, 1995b), resource availability in the form of access to breeding sites may play a major role in defining the degree of reproductive skew within badger social groups and further investigation into this is required.

Reproductive constraint and senescence in the European badger *Meles meles*³

³ A modified version of this chapter is currently being prepared as: Dugdale HL, Nouvellet P, Pope LC, Newman C, Burke T, Macdonald DW (in prep-a) Reproductive constraint and senescence in the European badger *Meles meles*.

4.1 Abstract

The Restraint, Constraint, and Selection Hypotheses predict that breeding success should increase with age, whereas the Antagonistic Pleiotropy and Mutation Accumulation Hypotheses examine the reasons for senescence. We examine these hypotheses and provide the first estimates of lifetime breeding success (LBS) of European badgers *Meles meles*, using microsatellite data from 915 badgers collected over 18 years in a high-density population. Badgers assigned parentage in one year, versus those that were not, had a greater probability of breeding the following year. Males and females had similar LBS, variance was greater for males, and a mixed paternity strategy increased LBS compared with siring cubs only within or only outside the resident group. Age of first breeding (α) and age of last breeding (ω) were under strong selection, independently, and in opposite directions when measured by LBS, but not by λ_{ind} (the population growth rate of an individual). In a declining population, selection will favour an older α . As approximately 50% of badgers were assigned only one offspring this evened out selection measured by λ_{ind} . α and ω were positively correlated phenotypically, and younger and older badgers did experience lower annual breeding success. Despite this, we did not detect additive genetic variance or heritable components for α and ω ; however, we estimate that our power to do so was limited. As no heritable component could be demonstrated, we were unable to test hypotheses for the occurrence of senescence. Breeding success increased initially for males that were previously assigned as a father, consistent with the Constraint Hypothesis, however, overall our results were not consistent with either the Selection or Constraint Hypotheses.

4.2 Introduction

Lifetime breeding success (LBS) and the age at which individuals commence and cease breeding are key components of life-history evolution (Clutton-Brock, 1988). An initial increase in breeding success with age has been related to factors such as maturation (Krüger, 2005), breeding experience (Bowen *et al.*, 2006), physical condition (Robbins *et al.*, 2006), dominance position (Cote & Festa-Bianchet, 2001) and number of alloparents (Russell *et al.*, 2007). Three hypotheses, that are not mutually exclusive, propose why breeding success should increase with age, the: Constraint, Restraint and Selection Hypotheses (reviewed in Forslund & Pärt, 1995).

The Constraint Hypothesis (Curio, 1983) suggests that individuals gain experience from breeding or foraging, such that over time they improve their skills, which increases their later breeding performance. It predicts that individuals with breeding experience should perform better than inexperienced individuals of the same age, within the same breeding attempt (Nol & Smith, 1987), as observed in grey seals *Halichoerus grypus* (Bowen *et al.*, 2006) and captive chimpanzees *Pan troglodytes* (Fessler *et al.*, 2005).

The Restraint Hypothesis (Williams, 1966; Pianka, 1976), proposes that reproductive effort varies according to an individual's residual reproductive value. Younger individuals will refrain from breeding or reduce their breeding effort, in order to improve their chances of survival and thus future reproduction, whereas older individuals should invest relatively more in reproduction due to their diminished chances of surviving to breed again. This hypothesis predicts that inexperienced

individuals will increase their reproductive expenditure with age (Forslund & Pärt, 1995), and this was considered as weak evidence for the Restraint Hypothesis in female goshawks *Accipiter gentilis* (Krüger, 2005). Results consistent with both the Constraint and Restraint Hypothesis have been presented for Columbian ground squirrels *Spermophilus columbianus* (Broussard *et al.*, 2003).

The Selection Hypothesis (Curio, 1983; Nol & Smith, 1987) predicts differential survival of individuals based on their phenotype, such that a cohort should consist of proportionally more good-quality individuals (i.e. fitter individuals) over time. The loss of individuals of lower-fitness phenotype results in the initial increase in breeding success with age. This increase should, therefore, not be apparent when examining only individuals of higher-fitness phenotypes that live to an old age (Forslund & Pärt, 1995). New Zealand stitchbirds *Notiomystis cincta* show evidence of the loss of lower-fitness females with age (Low *et al.*, 2007), and a similar loss under severe environmental conditions has been reported in Soay sheep *Ovis aries* (Tavecchia *et al.*, 2005).

Senescence is the decrease in somatic or reproductive investment with age, which has been documented in some wild mammalian populations (Broussard *et al.*, 2003; Broussard *et al.*, 2005; Bowen *et al.*, 2006; Nussey *et al.*, 2006; Robbins *et al.*, 2006). There are two main theories for senescence, both of which rely on purifying selection being stronger on genes that act earlier rather than later in life (Hamilton, 1966). The Mutation Accumulation Hypothesis (Medawar, 1952) proposes that the gene pool accumulates weakly deleterious mutations that act late in life. These genes therefore accumulate neutrally. The Antagonistic Pleiotropy Hypothesis (Williams, 1957)

suggests that traits, selected to improve reproduction early in life, may have deleterious effects later in life when selection is weaker. There is, therefore, positive selection for these genes despite their negative effects late in life. A specific case of William's (1957) Antagonistic Pleiotropy Hypothesis is the Disposable-Soma Hypothesis (Kirkwood, 1981), which predicts that reproductive investment is at the cost of somatic repair, which affects longevity. Individuals that have been selected for an early age of first breeding (α) will have a stronger rate of senescence, which will be seen in an earlier age of last breeding (ω). This prediction is not shared by the Mutation Accumulation Hypothesis (Charmantier *et al.*, 2006).

To examine such theories of age-related breeding success, long-term data on individuals are ideally required (Clutton-Brock, 1988). We quantify, for the first time, LBS of both male and female European badgers *Meles meles* from a high-density population in Wytham Woods, Oxford, 1987–2005. Female badgers are generally first capable of conceiving as yearlings, however, due to delayed implantation (Fischer, 1931) females give birth once a year, around February, hence cubs are generally not born until a parent's second year. We examine whether annual breeding success varies with age; in particular, we investigate whether there is an initial increase and later decrease in breeding success with age, as seen in the Woodchester Park badger population (Carpenter *et al.*, 2005). We investigate whether initial relationships of breeding success and age can be explained by the Constraint (Curio, 1983), Restraint (Williams, 1966; Pianka, 1976), or Selection (Curio, 1983; Nol & Smith, 1987) Hypotheses. We then measure selection on α and ω , test whether phenotypic variation in either α or ω is heritable, and investigate whether there is genetic covariance between these two traits.

4.3 Materials and methods

Our study was conducted in Wytham Woods, Oxford, UK (01° 19'W, 51° 46'N), a 4 km² area of deciduous woodland surrounded by mixed arable and permanent pasture (Hofer, 1988). We trapped badgers usually at least four times a year, over one week in January and two weeks in June, August and November. Badgers were sedated by an intra-muscular injection of approximately 0.2 ml / kg ketamine hydrochloride, identified through a tattoo, sexed, and their tooth wear graded on a five-point scale. Teat lengths and diameters of females were measured to the nearest millimetre. A blood or guard hair sample was collected from each of 915 badgers, 1987–2005 and these were extracted, amplified and genotyped for 22 microsatellite loci using the methods described in Chapter 2. We selected candidate mothers and fathers for parentage analyses according to biological rules and trapping data. Candidate mothers were reproductive females (aged two or more), present in the cub's social group in the year when the cub was born. Candidate fathers were all males older than one year and present in Wytham Woods in the year before the cub was born. As badgers may be present but not trapped we included adults and yearlings for two years after their last date of capture, and cubs for one year. We used CERVUS 3.0.1.8 (Kalinowski *et al.*, 2007) and COLONY 1.2 (Wang, 2004) to attempt to assign parent-pairs and infer sibship of 630 genotyped cubs, from a total of 735 cubs born 1988–2005 (Chapter 2).

Both parents were assigned to 595 cubs (94% of those genotyped and 81% of those trapped), maternity to 602 (96% or 82%) and paternity to 611 (97% or 83%) cubs with 80% confidence (Chapter 2). Breeding badgers were those that were assigned offspring with at least 80% confidence, with the offspring having survived to independence at 12–15 weeks of age. Reproductive failure occurs at all stages of

pregnancy in badgers and cub mortality also occurs pre-independence (discussed in Chapter 3 and Appendix 1); we therefore only consider successful breeding attempts at the end of parental investment, which is the first point at which we can currently count breeding attempts throughout our study population. We calculated α for badgers that were first trapped as cubs and ω for badgers that were also of known age, but additionally had not been trapped for at least two years by the end of 2005. Similarly, LBS was calculated for badgers that were of known age, survived to breeding age and were considered dead by the end of 2005. The number of grand-offspring was counted for each badger whose LBS was calculated and whose offspring were considered dead by the end of 2005. In growing populations, as has generally been the case in the Wytham badger population (Chapter 2), the rate-sensitive fitness measure λ_{ind} (McGraw & Caswell, 1996) may provide better fitness estimates than LBS, as it takes into account the timing of breeding events as well as their outcome (Brommer *et al.*, 2002). In growing populations ($\lambda_{\text{ind}} > 1$), early reproduction is favoured as this will increase the population growth of an individual, whereas in declining populations ($\lambda_{\text{ind}} < 1$) late reproduction is favoured (Brommer *et al.*, 2002). λ_{ind} has been used alongside LBS in several studies (Oli *et al.*, 2002; Brommer *et al.*, 2004; Krüger, 2005). λ_{ind} is the dominant eigenvalue of a population projection matrix consisting of age-specific fertility (offspring production scaled by the genetic contribution of 0.5) and survival (McGraw & Caswell, 1996). The opportunity for selection (I) was calculated as the standardised variance (σ^2 / mean^2) in LBS of males and females (Wade & Arnold, 1980). We estimated the Queller and Goodnight (1989) index of relatedness (R) and quantified the degree of reproductive skew within age categories of badgers by calculating the binomial skew index, B (Nonacs, 2000) using methods described in Chapter 3.

4.3.1 Pedigree

We built a pedigree, using all of the parentage assignments with 80% confidence, and sibship inferences from breeding events in Wytham Woods, 1988–2005, and estimated inbreeding coefficients (f) using PEDIGREE VIEWER 5.5 (Kinghorn, 1994). If a sibship was inferred, and one or both parents of the siblings were unknown, then we assigned each parent a unique identification number, specific to that breeding event, to enable identification of siblings. The pedigree includes 843 badgers; 618 of these badgers are offspring and 56 are inferred and unknown parents. The pedigree contains 191 assigned fathers and 25 inferred fathers, and 196 assigned mothers and 31 inferred mothers. It has a mean depth of three generations, a median of three, and a maximum of eight. The inbreeding coefficient (f) for an individual is the probability that two homologous genes are identical by descent i.e. derived from the same gene in a common ancestor. The founder population and all immigrants are considered to be unrelated, and individuals are then assumed to be inbred if their parents share ancestors (see Keller & Waller, 2002). Inbreeding was seen in one individual at $f = 0.3125$, 15 individuals at $f = 0.25$ (i.e. from a mating between the equivalent of full-siblings; 2.5% of offspring with both parents known, Marshall *et al.*, 2002), three individuals at $f = 0.1875$, eight at $f = 0.125$ (i.e. from a mating between the equivalent of half-siblings; 4.9% of offspring with all four grandparents known, Marshall *et al.*, 2002) and 22 at $0.125 < f > 0$.

4.3.2 Statistical analyses

In order to investigate the effect on breeding success of covariates such as age, and previous assignment as a parent (successful breeding experience), we used SAS 9.1 to

conduct General or Generalized Linear Mixed Models (GLMMs) with the Kenward-Roger denominator degrees of freedom method (Littell *et al.*, 2006). When the response was count data a GLIMMIX procedure was run with a Poisson error distribution and a log link. For continuous responses, the MIXED procedure was run with normal error distribution and the GENMOD procedure when a log transformation was required. Random effects were specified to control for variance between experimental units, and repeated effects were specified to control for covariance within experimental units. Modelling the covariance structure between repeated measures is important given that repeated measures, taken on the same experimental unit, that are closer together in time are more likely to be highly correlated (Littell *et al.*, 1998). We selected the best fitting model based on the Akaike information criterion. We also ran Generalized Linear Models (GLMs) in the GLIMMIX procedure with negative binomial or Poisson error distribution and a log link, or binomial error distribution and logit link.

In the GLIMMIX procedure, the default scale parameter is one for Poisson or binomial distributions. If the data are over-dispersed, however, setting the scale parameter to one can result in inflated test statistics, whereas under-dispersion leads to conservative estimates. We therefore compared the ratio of the generalized χ^2 statistic and its degrees of freedom, to the Pearson statistic for the conditional distribution (calculated as the variance of the Pearson-type residuals using the best linear unbiased predictors). When the ratio was greater than the Pearson-type residual we included an overdispersion parameter through an R-side residual effect (Littell *et al.*, 2006); when using a Poisson distribution we first checked whether a negative binomial distribution was better suited. We estimated the scale parameter for the negative binomial

distribution. Further details of the precise models used in each analysis follow. We tested for equality of variance in t-tests using the folded form F statistic. Where parametric tests were used, normality was examined using the Anderson-Darling test and by examining the histogram of residuals. Means are provided with the 95% confidence interval (CI), unless otherwise stated.

4.3.2.1 Age-related effects on breeding success

We modelled the number of cubs assigned each year to candidate mothers and fathers of known age, separately for each sex, in a GLMM with Poisson-distributed error. We included linear and quadratic age terms as fixed effects, along with year as a covariate. When badger identity was entered as a repeated measure the models did not converge; however, plotting time lag between pairs of observations against covariance, showed that covariance between pairs of repeated measures generally did not change over time and was around zero. Badger identity was entered as a random factor to model the variance between individuals and thereby account for repeated measures. As badgers are included as candidate parents for two years after their last capture (Chapter 2) we also ran this only including candidate parents up until their year of last capture. Badger identity was included as a repeated measure in these models with compound symmetry covariance structure. We then restricted the data set, ages 2–5, and also ages five or older to test whether breeding success initially increased and then decreased with age. The analysis was the same, except that age^2 was not included as a fixed effect. For females age five or older, badger identity was entered as a random factor. We tested the Selection Hypothesis by examining the same relationship for a subset of badgers that were all trapped after five years of age.

The Constraint Hypothesis was tested by examining the breeding success of badgers of the same age, at ages three, four and five, with the additional fixed effect of inexperienced or experienced. Experienced refers to individuals that have been assigned as a parent before, whereas inexperienced individuals have not been assigned as a parent previously. As badgers give birth and raise cubs underground we were unable to assess rates of breeding failure prior to cub independence at approximately 15 weeks. We ran a GLMM with Poisson error distribution, badger identity as a repeated measure with autoregressive covariance structure and as a random effect, and year, age and experience as covariates, for each sex separately. We also analysed breeding success for females in terms of the number of male cubs divided by litter size, as reproductive investment may vary with offspring sex ratio in badgers (Dugdale *et al.*, 2003). We used a binomial error structure; year, age and a binary code, to indicate whether an individual had previously been assigned as a parent, as fixed effects; and, female identity as a repeated factor with compound symmetry covariance structure. The Restraint Hypothesis was tested by examining the breeding success of inexperienced breeders (that have not been assigned as parents previously) at different ages in a GLM with Poisson error distribution and year as a covariate. We also ran a similar analysis, just for females, with the response as the number of male cubs divided by the number of cubs, binomial error distribution, year as a covariate, and age as a fixed effect.

4.3.2.2 *Probability of breeding*

Badgers were scored (0 or 1) on whether they were assigned parentage in a given year and in the following year. We ran a GLMM, separately for each sex, with binomial

error distribution, age and year as covariates, parentage score in given year as a fixed effect and parentage score in following year as the response with one as the denominator. Badger identity was included as a repeated factor with compound symmetry covariance structure. Data were not included for years after the date of last capture.

4.3.2.3 *Paternity strategy*

We ran a GLIMMIX procedure with negative binomial error structure, lifetime breeding success as the response, and two covariates for the number of years a male was a candidate father and a code for paternity strategy (code = only sired cubs within their own group, only sired cubs outside of their group, or had a mixed strategy).

4.3.2.4 *Natural selection estimations*

We estimated selection acting on two traits, α and ω , using standard regression techniques (Lande & Arnold, 1983; Arnold & Wade, 1984). We included individuals of known age, that were assumed dead by the end of the study period and that reproduced at least once ($n = 154$). We standardised α and ω (mean zero, unit variance) by subtracting the mean and dividing by the standard deviation (Hereford *et al.*, 2004). We calculated relative fitness as LBS or λ_{ind} standardised to a mean of one.

We estimated standardised directional (S') and disruptive (c') selection differentials by regressing relative fitness on each trait separately, and on each trait and its squared value, respectively. We ran GLMs with Poisson–distributed error when standardised LBS was the response, and normal error with log link in GENMOD procedure when

standardised λ_{ind} was the response. A selection differential estimates total selection on the mean value of a trait within one generation and represents both direct selection and selection resulting from correlations with other traits subject to selection. We then estimated the standardised directional (β') and quadratic (γ') selection gradients by multiple regression of both traits, so as to estimate the selection acting on each trait independent of correlations with the other trait, and multiple regression of both traits, both traits², and the cross products of the two traits, respectively. Inclusion of the cross product estimates the selection acting on the covariance between α and ω (the standardised correlational selection gradient, γ'_{ij}).

4.3.2.5 Inbreeding bias

To check whether inbred individuals were potentially biasing results, we also ran the natural selection analyses excluding individuals with $f > 0$. We ran GLMMs with Poisson-error distribution, and birth year, sex and natal social group fitted as random effects. Random effects were dropped from the α model as they explained none of the variance.

4.3.2.6 Estimating heritability and components of variance

We estimated variance components and the heritability of α and ω using bivariate animal models (Kruuk, 2004), which we implemented in WOMBAT 1.0 (Meyer, 2006). We used our pedigree to fit an animal model that partitioned the phenotypic variance of α and ω into their additive genetic values, fixed effects, and random effects:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e}$$

where $\mathbf{y}$ is a vector of phenotypic traits, $\boldsymbol{\beta}$ is a vector of fixed effects with $\mathbf{X}$ design matrix, $\mathbf{u}$ is a vector of random effects with $\mathbf{Z}$ design matrix and $\mathbf{e}$ is a vector of residual values. We tested whether natal group, sex and birth year had an effect on ω or α . We included year of birth as a random effect as this had a significant effect on ω (GLMM with Poisson error: $F_{1,152} = 19.02$, $p < 0.0001$). Total phenotypic variance of each trait (V_P) was therefore partitioned into additive genetic variance (V_A), variance attributed to different years of birth (V_{YOB}) and residual variance (V_R): $V_P = V_A + V_{YOB} + V_R$. Narrow-sense heritability (h^2), the degree of resemblance between relatives, was estimated as the ratio of additive genetic variance (V_A) to phenotypic variance (V_P): $h^2 = V_A / V_P$ (Falconer & Mackay, 1996). We also calculated the coefficient of additive genetic variance (CV_A), the additive genetic variance scaled by the trait mean ($\bar{X}$): $CV_A = 100 \times \sqrt{V_A} / \bar{X}$.

4.3.2.7 *Breeding status and teat size*

Lactating females have been assessed as those with teats > 5 mm long and > 2 mm in diameter before August, by measuring the teats of females diagnosed as pregnant in January by ultrasound (Macdonald & Newman, 2002). The accuracy of this, however, has not been evaluated, which is important given that reproductive failure is common throughout the cycle (Appendix 1). We compared the teat lengths and diameters, recorded May–July, of females assigned maternity with 95% confidence, to all adult females not assigned maternity and only assigned to social groups in which all cubs were assigned a mother with 95% confidence. We ran a MIXED procedure with either teat diameter or log-transformed teat length as the response, breeding status as the predictor, month and teat position as covariates, badger identity as a random factor, to

account for variation between individuals, and trapping record as a repeated factor, to account for covariance between each female's teat measurements at the same trapping event, with a compound symmetry covariance structure. We ran a paired t-test to test whether the mean teat size of females differed in years when they were not assigned maternity and were assigned to groups where all cubs were assigned maternity with 95% confidence, compared to an earlier year when they were assigned maternity with 95% confidence.

4.4 Results

4.4.1 Age Structure

An age-related effect on annual breeding success was suggested by reproductive skew of candidate mothers ($B = 0.007$, $p = 0.0196$, ages = 2–11) and candidate fathers ($B = 0.024$, $p < 0.0001$, ages = 2–13) pooled by age. Variation in breeding success within age classes among both candidate fathers and candidate mothers, however, indicated that factors other than age also influence breeding success (Table 4.1). The age of a candidate father ($F_{1,1224} = 38$, $p < 0.0001$) and candidate mother ($F_{1,1093} = 13$, $p = 0.0003$) had a significant quadratic effect, with the number of cubs produced increasing in the young and decreasing in the old (Figure 4.1). The same quadratic result was observed when data from the two years after last capture were excluded for both candidate fathers ($F_{1,636} = 22$, $p < 0.0001$) and candidate mothers ($F_{1,664} = 5.2$, $p = 0.0235$).

Reproductive output over the ages of 2–5 increased from a mean of 0.11–0.48 cubs for males, but less so for females, from 0.26–0.34 cubs. This increase was significant

for males ($F_{1,769} = 22$, $p < 0.0001$; Figure 4.2a), but not for females ($F_{1,588} = 2.5$, $p = 0.11$; Figure 4.2b). This effect was not due to selection weeding out low-fitness individuals as removal of males that were not trapped after five years of age produced a similar significant increase ($F_{1,244.3} = 19$, $p < 0.0001$; Figure 4.2c). For females, the increase in breeding success from ages 2–5 was significant when such poor-quality individuals were removed ($F_{1,304.1} = 4.9$, $p = 0.0284$; Figure 4.2d).

Table 4.1 Reproductive skew (B) in the annual (within age categories) and lifetime breeding success of badgers. Individuals included in the analysis were of known age, and were assumed dead by the end of 2005. Age was analysed up to nine years, as there were too few breeders in the older categories to enable analysis.

Age	Candidate mothers		Candidate fathers	
	B	p	B	p
2	0.010	0.0071	0.004	0.1831
3	0.011	0.0064	0.017	< 0.0001
4	0.006	0.0665	0.015	< 0.0001
5	0.009	0.0249	0.036	< 0.0001
6	0.029	0.0037	0.030	< 0.0001
7	0.051	0.0412	0.032	0.0389
8	0.012	0.1144	0.086	0.0006
9	0.035	0.0397	0.022	0.2009
Mean	0.020	0.0037	0.030	< 0.0001
LBS	0.010	< 0.0001	0.011	< 0.0001

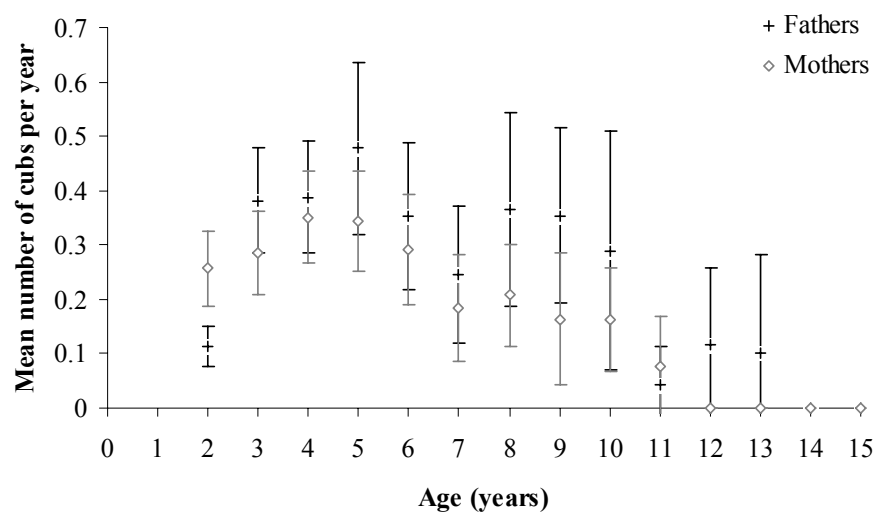


Figure 4.1 The mean annual number of cubs sired by mothers and fathers of different ages and assigned with 80% confidence, based on 375 cubs whose fathers were of known age and 285 cubs whose mother's were of known age. Error bars display the 95% confidence interval.

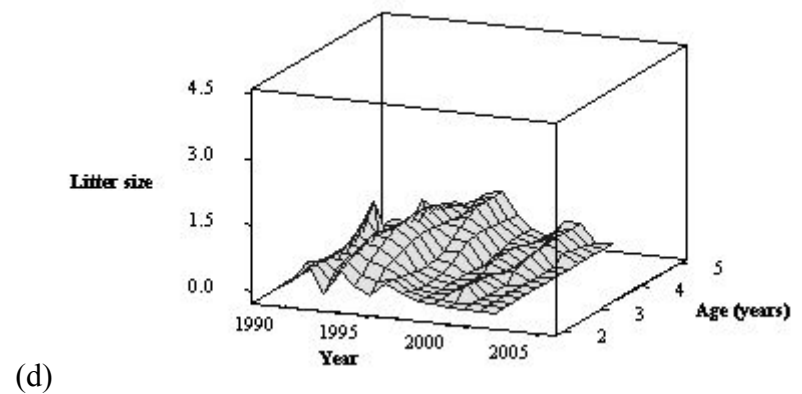
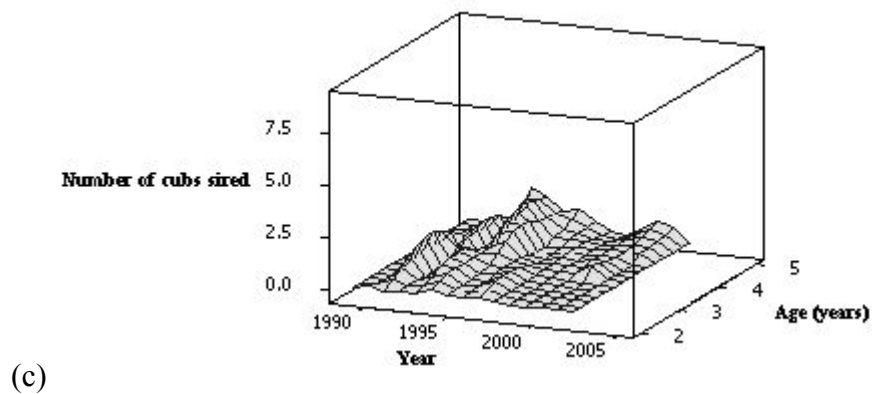
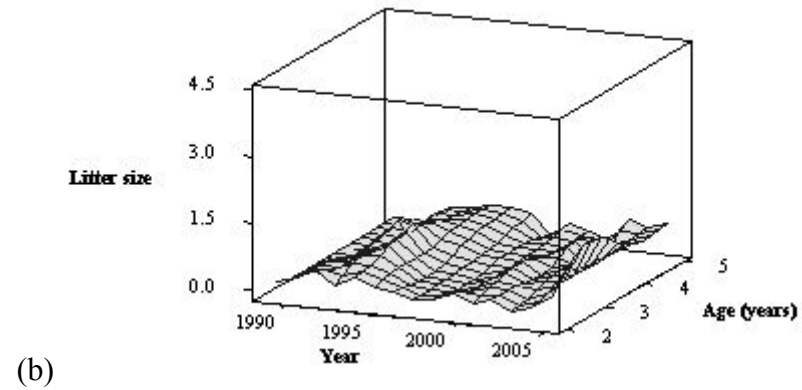
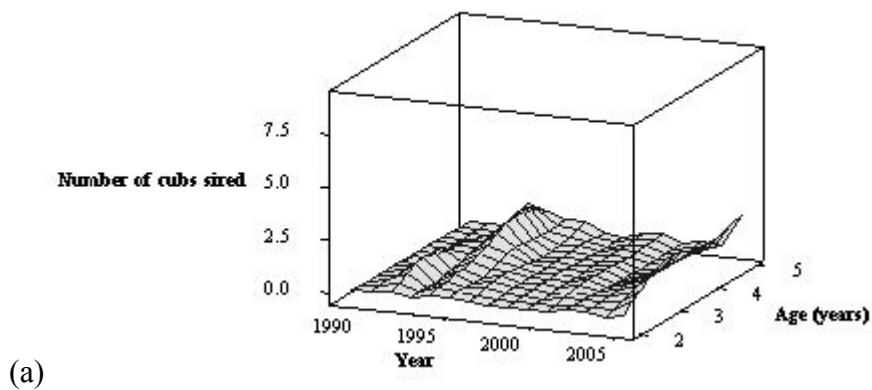


Figure 4.2 The number of cubs produced from ages two to five, by all (a) male and (b) female badgers of known age, and all (c) male and (d) female badgers of known age that survived to at least six years of age, against year and age. Based on parentage assignments with 80% confidence.

Female breeding success did not increase initially because of previous assignment as a mother (i.e. successful breeding experience), contrary to the Constraint Hypothesis. Females, up to the age of five that had been assigned as mothers before, did not have a different litter size to females that had not been assigned as mothers previously ($F_{1,106.4} = 1.2, p = 0.28$). Neither did breeding success increase for females when the response was entered as the number of male cubs divided by litter size, rather than purely the number of cubs ($F_{1,105.1} = 0.3, p = 0.62$). For males, however, the number of cubs sired, from ages 2–5, was significantly greater for those that had previously been assigned as a father ($F_{1,124} = 4.3, p = 0.0395$), as predicted by the Constraint Hypothesis. Breeding success did not increase with age for first time mothers, as predicted by the Restraint Hypothesis ($F_{1,104} = 0.1, p = 0.80$). Litter sex-ratio, of females that had not been assigned as mothers previously, did not differ with age ($F_{1,104} = 0.4, p = 0.55$). Breeding success did increase for males, however this was not significant ($F_{1,123} = 0.6, p = 0.43$). One male that first bred at age 11 appeared to influence the regression, however, removal of this point did not influence the regression significantly ($F_{1,122} = 1.3, p = 0.26$). Reproductive output decreased from age five for both males ($F_{1,256.6} = 4.9, p = 0.0278$) and females ($F_{1,560} = 19.1, p < 0.0001$).

The mean age of male and female badgers when their first offspring were born did not differ significantly (mean $\alpha_{(\text{male})} = 4 \pm 0.2$, mode = 3, range = 2–11, $n = 126$; mean $\alpha_{(\text{female})} = 4 \pm 0.4$, mode = 2, range = 2–10, $n = 107$; $U = 6350, p = 0.43$; Figure 4.3a). ω did not differ between the sexes (mean $\omega_{(\text{male})} = 5 \pm 0.5$, mode = 4, range = 2–13, $n = 90$; mean $\omega_{(\text{female})} = 5 \pm 0.6$, mode = 5, range = 2–11, $n = 64$; $U = 2439, p = 0.10$; Figure 4.3b).

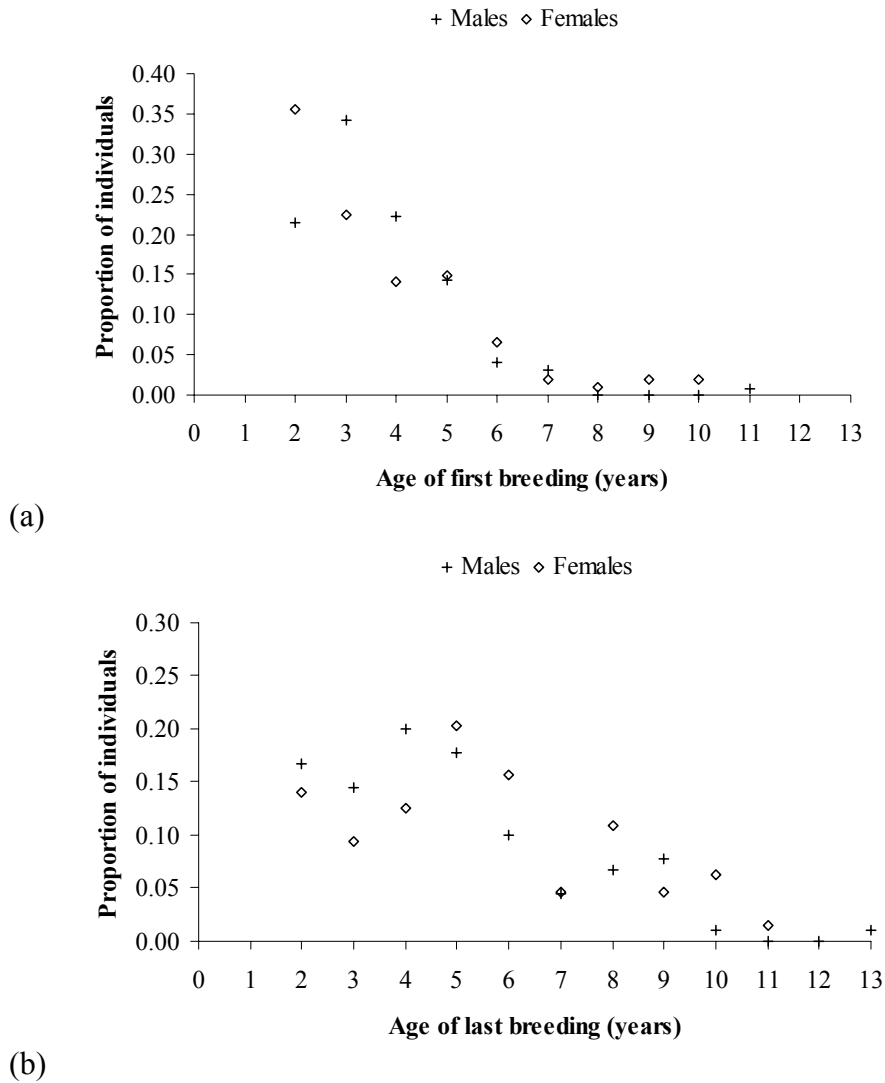


Figure 4.3 Distribution of (a) the age of first breeding [α] of badgers of known age, and (b) the age of last breeding [ω] of badgers of known age that were considered dead by the end of 2005. Based on parentage assignments with 80% confidence.

4.4.2 Probability of breeding

Badgers that were assigned parentage in a given year were more likely to be assigned parentage in the following year (females: $F_{1,629.4} = 19$, $p < 0.0001$; males: $F_{1,555.9} = 15$, $p < 0.0001$).

4.4.3 *Lifetime breeding success*

Significant skew in LBS occurred for candidate fathers and candidate mothers (Table 4.1), suggesting that some individuals gained greater than the mean annual breeding success over their lifetime. LBS varied from 0–11 for females (mean = 1.5 ± 0.4 , mode = 0, $n = 124$) and 0–18 for males (mean = 1.2 ± 0.3 , mode = 0, $n = 213$). The greater number of males compared to females is due to the inclusion of yearling males that can potentially sire offspring in the following year, due to delayed implantation, even if they do not survive. Fifty-seven yearling males were only ever trapped as cubs, and of these six were assigned one offspring and one was assigned two offspring. Excluding these 57 males, male LBS ranged from 0–18 (mean = 1.6 ± 0.3 , mode = 0, $n = 156$). Badgers with a LBS greater than zero, were assigned parentage in $36 \pm 4\%$ ($n = 90$ males) and $29 \pm 4\%$ ($n = 64$ females) of the years in which they were included as candidate parents (range = 1–6 breeding years and 1–14 potentially reproductive years for both sexes). Of the individuals that bred in more than one year, from their first year of breeding to their last, females were assigned parentage $74 \pm 7\%$ ($n = 35$) and males $75 \pm 8\%$ ($n = 38$) of the time. The maximum number of consecutive years over which an individual was assigned parentage was five for females ($n = 2$) and six for males ($n = 1$).

Males had greater variation in LBS than females ($\sigma^2 = 5.27$ and 4.85 , respectively); however, this was not statistically significant ($F_L = 1.4$, $p = 0.24$). The opportunity for selection was greater in males than in females ($I_m = 3.80$; $I_f = 2.18$; $I_m / I_f = 1.74$). Excluding the 57 males only ever trapped as cubs, males had greater variance in LBS ($\sigma^2 = 6.61$) than females, but this too was not significant ($F_L = 0.0$, $p = 0.89$). Excluding the 57 males, $I_m = 2.72$ ($I_m / I_f = 1.25$). There was no significant difference

in the LBS of males and females (median = 0 & 1 respectively, $U = 11773$, $n_1 = 213$, $n_2 = 124$, $p = 0.068$); however, the p -value was bordering on significance. Excluding the 57 yearling males, there was no significant difference in the LBS of males and females (median = 1 & 1 respectively, $U = 9597$, $n_1 = 156$, $n_2 = 124$, $p = 0.905$).

The mean number of grand-offspring produced did not differ significantly between males (2.1 ± 1.0 , $n = 55$, range = 0–20) and females (1.9 ± 0.8 , $n = 39$, range = 0–9; $t_{92} = 0.19$, $p = 0.85$). GLMs with negative binomial error distribution revealed that the number of grand-offspring was positively correlated with both LBS ($F_{1,92} = 21$, $p < 0.0001$) and λ_{ind} ($F_{1,92} = 34$, $p < 0.0001$). λ_{ind} was a near significant factor when included in the model after LBS using type one significance ($F_{1,91} = 3.9$, $p = 0.0508$), but LBS was not significant when included after λ_{ind} ($F_{1,91} = 0.9$, $p = 0.36$).

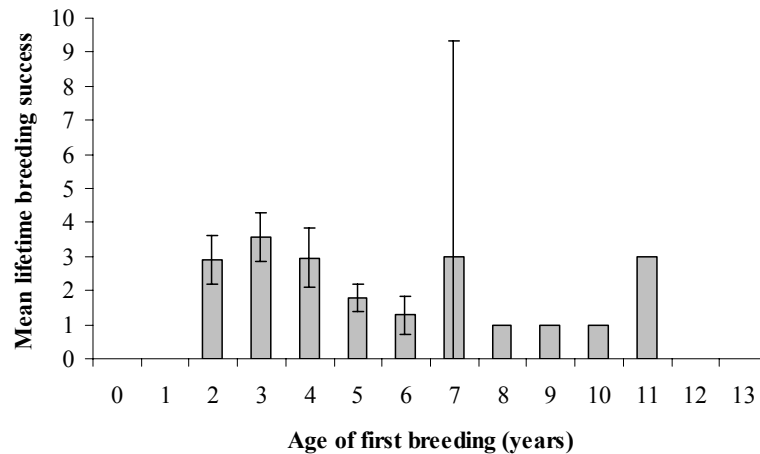
4.4.3.1 Paternity strategy

Males that sired cubs both within and outside of their social group had a greater LBS (mean = 4.8 ± 1.5 , median = 4, $n = 25$) than males that only sired cubs within their resident group (mean = 2.1 ± 0.9 , median = 1, $n = 28$) or only outside of their group (mean = 1.9 ± 0.5 , median = 1, $n = 37$), controlling for the number of years included as a candidate father ($F_{2,86} = 9.0$, $p = 0.0003$).

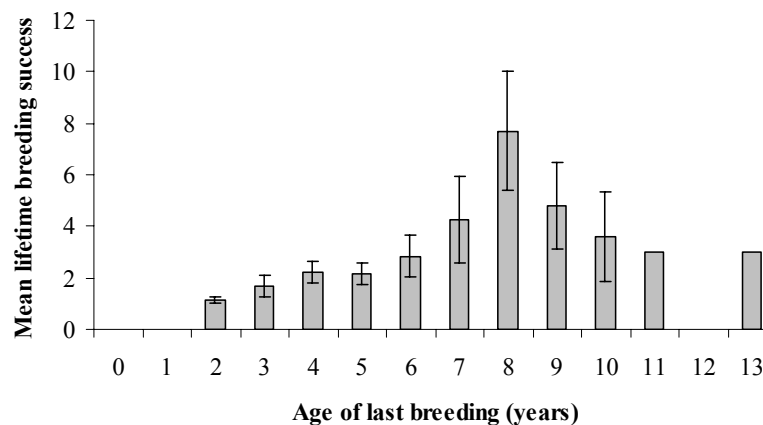
4.4.4 Selection

Although not always significant, S' was bordering on significance ($p = 0.054$) whereas β' was highly significant, and linear selection coefficients were always negative for α ; thus, selection generally favoured breeding to commence at an early

age (Table 4.2 and Figure 4.4a). Linear directional selection on ω was strong and positive (Table 4.2 and Figure 4.4b), favouring termination of breeding at an older age. Quadratic (non-linear) selection coefficients were negative and significant for ω , indicating that stabilising selection was acting on ω ; these were not significant for α . There was no significant correlational selection between α and ω when fitness was measured as LBS (Table 4.2), suggesting that selection on breeding at an early age was independent of the ω . When fitness was measured as λ_{ind} no directional selection was detected on α , and there was correlational selection between α and ω (Table 4.2).



(a)



(b)

Figure 4.4 Mean LBS of badgers of known age, assumed dead by 2005, that reproduced at least once, in relation to (a) age of first breeding [α], and (b) age of last breeding [ω]. Results are based on parentage assignments with 80% confidence. Error bars display the 95% confidence interval.

Table 4.2 Selection acting on age of first breeding (α) and age of last breeding (ω) in a population of badgers.

Trait	Fitness	S'			c'			β'			γ'			γ'_{ij}		
		Coeff	$\pm$ SE	p	Coeff	$\pm$ SE	p	Coeff	$\pm$ SE	p	Coeff	SE	p	Coeff	$\pm$ SE	P
<i>All f</i>																
α	LBS	-0.181	0.093	0.0539	-0.006	0.058	0.9136	-0.419	0.095	< 0.0000	-0.076	0.086	0.3793	0.253	0.148	0.0895
α	λ_{ind}	-0.017	0.017	0.3175	-0.002	0.010	0.8282	-0.092	0.013	< 0.0000	0.001	0.011	0.9563	0.046	0.020	0.0180
ω	LBS	0.424	0.073	< 0.0000	-0.169	0.071	0.0194	0.576	0.078	< 0.0000	-0.261	0.098	0.0083			
ω	λ_{ind}	0.110	0.013	< 0.0000	-0.057	0.011	< 0.0000	0.151	0.012	< 0.0000	-0.093	0.013	< 0.0000			
<i>$f = 0$</i>																
α	LBS	-0.176	0.096	0.0676	-0.011	0.060	0.8491	-0.418	0.098	< 0.0000	-0.073	0.089	0.4094	0.254	0.154	0.1012
α	λ_{ind}	-0.016	0.018	0.3714	-0.004	0.011	0.7288	-0.092	0.014	< 0.0000	0.002	0.012	0.8793	0.046	0.020	0.0244
ω	LBS	0.425	0.075	< 0.0000	-0.183	0.076	0.0174	0.580	0.080	< 0.0000	-0.276	0.103	0.0085			
ω	λ_{ind}	0.111	0.013	< 0.0000	-0.061	0.012	< 0.0000	0.152	0.013	< 0.0000	-0.098	0.014	< 0.0000			

Standardised directional (S') and disruptive (c') selection differentials and standardised directional (β'), quadratic (γ') and correlational selection gradient (γ'_{ij}), with standard errors and p -values. γ is negative when selection is stabilising and positive when disruptive. Parameters were calculated for badgers of known age that reproduced at least once and were dead by the end of the study, and for the same badgers excluding those with $f > 0$ ($n = 8$). Parameters were calculated in a GLM with a log link; they are therefore not directly comparable with estimates that are not log transformed.

4.4.5 Inbreeding bias

Exclusion of individuals with $f > 0$ from our selection analyses ($n = 8$; Table 4.2) did not alter our findings. The inbreeding coefficient showed no effect on α ($F_{1,231} = 0.02$, $p = 0.90$) or ω ($F_{1,152} = 0.31$, $p = 0.58$).

4.4.6 Variance components and heritability

There was no significant additive genetic variance or heritability of α or ω (Table 4.3), and the residuals of the animal model were not normally distributed ($A^2 = 5.5$, $p < 0.005$). There was a significant phenotypic correlation between α and ω (0.493 ± 0.063 , $t = 7.8$, n badgers with offspring = 343, n badgers with offspring and both traits = 154, $p < 0.0001$). Small pedigrees lead to inflated standard errors (Charmantier & Reale, 2005; Quinn *et al.*, 2006), therefore we probably did not have enough power to estimate the genetic correlation. Univariate analyses of α and ω resulted in non-significant estimates of additive genetic variance and heritability (α , $V_A = 0.00 \pm 0.59$, $t = 0.0$, $p > 0.05$, $h^2 = 0.0 \pm 0.22$; ω , $V_A = 1.30 \pm 1.87$, $t = 0.7$, $p > 0.05$, $h^2 = 0.20 \pm 0.29$).

Table 4.3 Variance components and heritabilities (h^2) of the age of first breeding (α) and the age of last breeding (ω).

	α	ω
n	233	154
$V_P \pm \text{se}$	2.696 ± 0.262	5.628 ± 0.645
$V_A \pm \text{se}$	0.136 ± 0.285	0.635 ± 0.863
$V_{YOB} \pm \text{se}$	0.168 ± 0.136	0.047 ± 0.217
$V_R \pm \text{se}$	$2.392 \pm 0.346^{***}$	$4.946 \pm 0.936^{***}$
h^2	0.051 ± 0.105	0.113 ± 0.151
Trait mean $\pm$ StDev	3.622 ± 1.630	5.097 ± 2.378
CV_A	10.20	15.63

Significance was assessed by one-tailed t-tests. *** $p < 0.0001$

4.4.7 Breeding status and teat size

Breeding status was a significant predictor of both teat length (breeder mean = 6.5 ± 0.2 , non-breeder mean = 3.0 ± 0.2 , $F_{1,256} = 151$, $p < 0.0001$; Figure 4.5a) and diameter (breeder mean = 4.6 ± 0.4 , non-breeder mean = 2.0 ± 0.2 , $F_{1,239} = 62$, $p < 0.0001$; Figure 4.5b). Females assigned maternity in one year and then not assigned in a later year, had significantly shorter (mean = 6.1 ± 1.1 then 3.8 ± 1.7 , $t = 2.92$, $n = 11$, $p = 0.015$) and thinner teats (mean = 4.2 ± 1.5 then 2.5 ± 0.7 , $t = 2.64$, $n = 11$, $p = 0.025$) when unassigned.

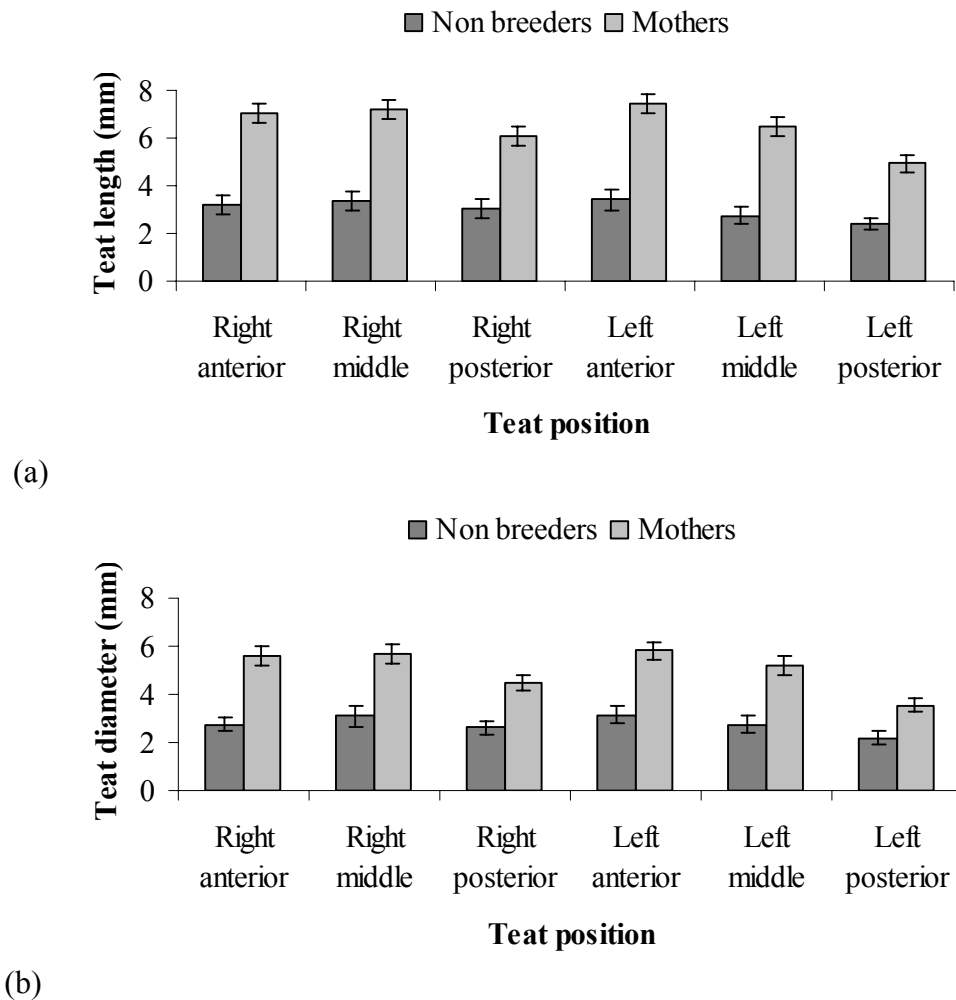


Figure 4.5 The (a) teat lengths [mm] and (b) teat diameters [mm] of adult females that were assigned maternity with 95% confidence, and adult females that were not assigned maternity, but all cubs in their social group were assigned a mother with 95% confidence. Error bars display the 95% confidence interval.

4.5 Discussion

Long-term studies are vital to understand reproductive success as they enable quantification of reproductive success over the lifespan of individuals (Clutton-Brock, 1988). Knowledge of lifetime breeding success then enables selection on traits to be assessed and their adaptive significance to be evaluated (Arnold & Wade, 1984). In this chapter, we provide the first quantification of LBS, in both male and female badgers, the first examination of α and ω in badgers, and strong evidence of age-related effects on breeding success.

4.5.1 *Age-related annual breeding success*

Reproduction may be reduced in young individuals due to reproductive restraint, reproductive inferiority, and selection for superior reproductive performance (Williams, 1966; Curio, 1983; Forslund & Pärt, 1995), or in old animals due to senescence (Medawar, 1952; Williams, 1957). An age-related effect on annual breeding success was suggested by significant reproductive skew of candidate mothers and candidate fathers pooled by age. Previous pooling of reproduction in a social group over 18 years, decreased B for both within-group candidate fathers (0.261 to 0.178) and candidate mothers (0.068 to 0.039, Chapter 3). This also suggests an age-related effect on annual breeding success, with less skew over a longer period compared to a yearly snapshot. Indeed, the age of a father or mother had a significant effect on the number of cubs that they produced, with lower reproductive rates observed in younger and older individuals, as seen in the Woodchester badger population (Carpenter *et al.*, 2005) and in other species (reviewed in Clutton-Brock, 1988). The annual breeding success of both sexes increased initially with age, but to a

lesser extent for females. Contrary to the Selection Hypothesis (Curio, 1983; Nol & Smith, 1987) this was not due to selection removing individuals of lower fitness; restriction of the data set to individuals that survived to at least six years of age showed a similar increase of breeding success with age in males and this increase was actually significant in females. The initial increase in breeding success in females is weaker than that in males, and therefore any age-related pattern in females is more likely to be obscured by random events such as road fatalities.

The Constraint Hypothesis predicts that this initial increase in breeding success with age is due to the acquisition of skills such as breeding experience (Curio, 1983). This appeared to be the case for males; males that had not been assigned as fathers previously had lower success than males of the same age that had been assigned paternity before. Younger badgers may be poor reproductive competitors compared to older badgers; hence, the breeding success of young males, up to and including five years of age, may be constrained. The breeding success of young females increases with the presence of annex setts in the social group, which may enable them to breed away from the aggression of older females (Cresswell *et al.*, 1992). A high proportion of females that are old enough to produce a litter fail to lactate and this proportion tends to be higher in large groups (da Silva *et al.*, 1994; Woodroffe & Macdonald, 1995b). For females, however, parity did not influence breeding success. In Wytham Woods there has been an increase in the number of annex and outlier setts (Macdonald *et al.*, 2004c), which might increase the number of breeding sites, thereby reducing female–female competition and infanticide rates, although the quality of these sites may vary. Additionally, rather than breeding experience, age may be correlated with other factors such as dominance (Berdoy *et al.*, 1995) which may play

a role in female breeding success, although no relationship has so far been established between dominance and age in badgers (Appendix 2).

The Restraint Hypothesis predicts that individuals may vary their reproductive effort according to their residual reproductive value (Williams, 1966; Pianka, 1976), such that older females should invest relatively more in reproduction. Breeding is costly for female badgers; those that lactate are in worse condition the following summer and delay moulting until autumn when food availability is high (Stewart & Macdonald, 1997). The slightly lower proportion of two-year-old badgers assigned parentage may be due to the high cost of producing offspring at a young age; lactating two-year-old females displayed a higher mortality rate than two-year-old females that did not lactate (Woodroffe & Macdonald, 1995a). More two-year-old females than those three years or older fail to lactate (da Silva *et al.*, 1994). This pattern has also been reported in field studies in other populations (Cheeseman *et al.*, 1987; Harris & Cresswell, 1987; Revilla *et al.*, 1999; Carpenter *et al.*, 2005), post-mortem studies (Ahnlund, 1980; Anderson & Trehwella, 1985; Whelan & Hayden, 1993), or both (Cresswell *et al.*, 1992). There was no significant increase in breeding success with α in either sex, contrary to the prediction of the Restraint Hypothesis. Reproductive investment may not just vary with number of offspring, but it may also vary with litter sex-ratio in badgers (Dugdale *et al.*, 2003); however, this did not differ significantly between females, of different ages, that had not been assigned as mothers before. Additionally, the breeding success, of individuals that had not been assigned as parents previously, was similar in females and lower in males than that of breeders that had been assigned as parents before, and that were of the same sex and age, whereas the Restraint Hypothesis would predict a greater success.

Overall, our results suggest that there is a strong effect of age on initial breeding success in males, with males that had previously been assigned as fathers gaining greater success than males that had not been assigned as fathers before, as predicted by the Constraint Hypothesis. What causes this increased success with previous assignment as a father is not apparent immediately, but it is likely to be a surrogate for some related aspect of male quality. Further studies are required to investigate whether factors such as body weight, head-body length, or condition vary between these individuals, or whether factors such as infanticide play a role. The initial increase in breeding success of females was much weaker, and our results for females were not consistent with the predictions of the Constraint, Restraint, or Selection Hypotheses. A significant quadratic effect between annual breeding success and age was observed for both males and females, suggesting that senescence occurs in badgers.

4.5.2 *Lifetime breeding success*

Overall, a high percentage of the badgers (53%) that were estimated to have survived to breeding age and were dead by the end of the study, were not assigned any parentage. As some cubs were not assigned a parent, this will lead to an overestimate. Equally though, as not all badgers were trapped each year, we included badgers for extra years after last capture unless they were known to have died (Chapter 2). Excluding badgers that were last trapped before reaching breeding age, which consisted of males last trapped as cubs and females last trapped as yearlings, 43% of badgers were not assigned an offspring. Although badgers that were not assigned parentage may increase their somatic rather than reproductive investment, which may

increase longevity, given that non-breeding badgers do not alloparent (Chapter 6) the adaptive significance of this tactic is unclear. Badgers that bred at least once were only assigned parentage in approximately a third of the years in which they were included as candidate parents. This increased to three quarters when considering individuals that bred at least twice. Badgers that were assigned parentage in a given year, versus those that were not, were significantly more likely to be assigned parentage in the following year. A similar trend has been reported for female badgers in Woodchester Park using teat size data (Delahay *et al.*, 2006a). Breeding success of females has been linked to relatively good body condition (Woodroffe & Macdonald, 1995b; Delahay *et al.*, 2006a) and social status (Woodroffe & Macdonald, 1995b). Females that bred were in worse body condition at the end of lactation, but this difference disappeared by autumn (Woodroffe & Macdonald, 1995a). Although the costs of breeding may be high for females, it appears as though females in better body condition or of better social status are more likely to breed, and those that breed in one year are more likely to breed in the following year. Overall, it therefore appears as though there are high costs to reproduction in both sexes.

Males that were assigned offspring both within and outside of their resident group had greater LBS than males that were assigned paternity only within or only outside of their resident group. The Anti-Kleptogamy Hypothesis (Roper *et al.*, 1986) suggests that male badgers defend their territory in order to prevent extra-territorial males from breeding with within-group females. Only breeding with within-group females, or only breeding with extra-group females, were the worst paternity strategies. Instead males with a mixed paternity strategy have the greatest LBS, contrary to the predictions of the Anti-Kleptogamy Hypothesis (Roper *et al.*, 1986).

In terms of LBS, our long-term measures of the opportunity for selection represent an upper limit to the strength of directional sexual selection. These were lower ($I_m = 3.80$, excluding the 57 males $I_m = 2.72$, $I_f = 2.18$) than those seen in species with highly male biased size dimorphism (21.8 in male northern elephant seals *Mirounga angustirostris*, Le Boeuf & Reiter, 1988; 3.46 in Soay rams *Ovis aries*, Coltman *et al.*, 1999; 4.52 in bighorn rams *Ovis canadensis*, Coltman *et al.*, 2002). Males had greater variance in LBS than females, although this was not significantly greater. In sexually dimorphic non-flying mammalian species, the larger of the sexes is usually expected to show greater variation in breeding success, especially when they display negligible parental investment (Trivers, 1972). As badgers show slight sexual dimorphism (Johnson & Macdonald, 2001) and no paternal care (Chapter 6), males were expected to show greater variation in breeding success. As females can mate throughout the year and they are solitary foragers, this restricts the ability of males to use their marginally larger body size to control the reproduction of females. The mean ages at which males and females first and last bred were not significantly different. Overall, there was no significant difference in LBS between the sexes.

4.5.3 Selection

Our regression results show that α and ω in badgers are under selection, in opposite directions. A review of published field studies reported median absolute gradients of 0.16 for linear and 0.10 for quadratic selection (Kingsolver *et al.*, 2001). Our results are not directly comparable with these values, as they require back transforming given the use of a log link; however, except for the directional selection gradient of ω , they are comparable to those reported from a similar analysis in mute swans *Cygnus olor*

(Charmantier *et al.*, 2006), indicating strong selection overall. Although natal philopatry (da Silva *et al.*, 1994) and restricted dispersal (Pope *et al.*, 2006) occur, high levels of extra-group paternity may reduce inbreeding (Chapter 2) and exclusion of inbred individuals indicates that they do not alter our conclusions. The finding of selection on α when fitness is measured as LBS but not when it is measured as λ_{ind} is interesting given contrasting results in other species. Significant linear selection has been recorded on both of these measures in mute swans (Charmantier *et al.*, 2006) and goshawks *Accipiter gentilis* (Krüger, 2005), whereas only linear selection on λ_{ind} has been presented in wood ducks *Aix sponsa* (Oli *et al.*, 2002), and in both European sparrowhawks *Accipiter nisus* and blue tits *Parus caeruleus* (McGraw & Caswell, 1996). λ_{ind} not only incorporates breeding success but also the timing of breeding, and this can therefore result in the observed differences in these studies. For example, LBS was twice as great for Ural owls *Strix uralensis* that first breed in years with increasing rather than peak prey abundance, where prey are voles that have a three-year cycle of low, increase and peak phases. No relationship was, however, seen when using λ_{ind} , as females that initiated breeding in the increase phase were older than those that commenced in peak phase, and this evened out λ_{ind} across the prey-abundance phases (Brommer *et al.*, 1998). In our study, 41% of the badgers were only assigned one offspring in their lifetime, which represents a declining rate of the individual population growth, and as such, λ_{ind} is greater for individuals that breed when older. In comparison, an increasing rate of individual population growth was seen for 40% of badgers that were assigned more than two offspring, and in these individuals λ_{ind} is greater for those that bred at an early age. The large number of individuals assigned one offspring for whom λ_{ind} is larger when α is later, balances out the similar number of individuals assigned more than two offspring where early α is

favoured, and no directional selection on α was therefore observed with λ_{ind} . Brommer *et al.* (2002) highlighted the importance of the sensitivity of λ_{ind} to the timing of reproduction, and we provide an example in this study highlighting the need to be aware of the sensitivity of λ_{ind} to the overall number of offspring produced in a lifetime.

Oli and Dobson (2003) suggested that the proportional sensitivity (a measure of selection pressure) of λ_{ind} to changes in α depends on the magnitude of breeding relative to α , measured simply as m / α (where m = average litter size of all females of breeding age and α = the first age class, in years, in which individuals bred). When $m / \alpha \geq 0.75$, age at sexual maturity was shown to exert a large effect on λ_{ind} and thus strong selection pressure, whereas when it was ≤ 0.25 survival rates, rather than α , greatly influenced λ_{ind} . For badgers, including females that did not breed, the average litter size of females of known reproductive age was 0.25 ± 1.16 , or 0.31 ± 1.23 excluding females after their date of last capture. As the first age at which breeding occurred was two, m / α was between 0.13 and 0.15, suggesting that λ_{ind} may be more sensitive to survival rate than age at maturity in our study population, which may further explain the lack of a selection pressure on α when we used λ_{ind} . The near significant selection that we have observed on α using LBS is indicative of the benefits of early α when senescence occurs, when demographics are not taken into consideration (Charmantier *et al.*, 2006).

LBS and λ_{ind} only measure fitness in terms of the next generation, and to investigate how well these measures quantify selection Brommer *et al.* (2002) advocate correlating these measures with the number of grand-offspring. We found both LBS

and λ_{ind} to be good fitness measures, as both were correlated significantly and positively with the number of grand-offspring assigned. Concepts of r^2 are not well developed for generalised models (Littell *et al.*, 2006), therefore we investigated whether one measure significantly improved the goodness of fit, when added as a fixed effect into the model after the other fitness measure. Neither did, suggesting that both perform equally well as fitness measures in terms of the number of grand-offspring assigned.

4.5.4 Heritability and variance components

We did not detect any significant additive genetic variance or heritability in either α or ω . The residuals from this model were not normally distributed, however, animal models are generally thought to be relatively robust to deviations from normality (Kruuk, 2004). It is important that maternal or other environmental effects be taken into consideration in animal models (Kruuk & Hadfield, 2007); however, our models did not converge when maternal identity or natal group were included. Although bivariate analyses are vital in trade-off situations, as each trait provides information on the other, they require more power than univariate analyses, as not only are the variance components estimated but so to is the correlation between the two traits. We, therefore, ran a univariate animal model for both α and ω , identical in every other way to the bivariate analysis, however, neither trait showed significant additive genetic variance or heritability. Previous studies investigating α and ω (e.g. Charmantier *et al.*, 2006) have used a much larger sample size of individuals expressing these traits and a more detailed pedigree. Using a highly heritable trait, and a detailed pedigree Quinn *et al.* (2006) suggest that a minimum of 100 individuals in each of three years

are required to obtain confidence in genetic variances and heritability estimates. Using few individuals or few years resulted in fairly consistent estimates of genetic variance, but the standard errors were inflated (Quinn *et al.*, 2006). Inflated standard errors due to a small pedigree (less than 100 broods of five offspring) were also reported by Charmantier & Reale (2005). The standard errors that we obtained were very large compared to the variance estimates for both ω and α , and as our sample sizes were less than 300 it may be that we did not have enough power to detect significant heritability. α and ω showed positive phenotypic correlation; however, this may be a result of autocorrelation and we did not have enough power to estimate the genetic correlation. We were, therefore, unable to assess whether the Mutation Accumulation or the Antagonistic Pleiotropy Hypotheses best explain the decrease in annual breeding success that we observed in older badgers.

Although we did not detect heritability of α or ω , probably due to the small pedigree, it is worth considering the effects that misassigned paternity or maternity may have on the animal model results. A rate of less than 20% extra-pair paternity, as is common in birds, is not thought to influence estimates of heritability when using a social rather than a genetic pedigree (Charmantier & Reale, 2005). Underestimates are larger with higher levels of heritability or extra-pair paternity, such that with an extra-pair paternity rate of 40% and a heritability of 0.4, heritability is underestimated by approximately 20% (Charmantier & Reale, 2005). If a pedigree is small, as it is in our study, and if heritability is low, as it potentially is in the two traits that we examined, then misassigned paternities may overestimate heritability (Charmantier & Reale, 2005). In the Wytham Woods badger population, the number of offspring per parent (mean litter size = 1.4; mean annual number of offspring sired per male = 1.6, Chapter

2) is smaller than the brood size of five that was included in the simulated pedigrees by Charmantier & Reale (Charmantier & Reale, 2005). Our smaller number of offspring per brood may therefore exacerbate any effect of misassigned paternities, or indeed maternities. Effects of misassigned maternity may be lower than effects of misassigned paternity, given that: (1) candidate mothers were those present in the cub's social group and therefore were subject to the same environmental effects; (2) female–female relatedness within groups is high (mean pairwise $R = 0.245 \pm 0.077$, Chapter 3); and (3) a misassigned mother is likely to be a full-sibling of the offspring as, on average, full-siblings produce higher log-likelihood values than the true parent, if the other parent is unsampled (Thompson, 1976). Nevertheless, the combination of both paternity and maternity assignment errors may affect heritability estimates. Software that runs power and sensitivity analyses (PEDANTICS) has recently been made available, which would facilitate investigation of the effect of pedigree errors, pedigree paucity and genetic architecture on estimates of quantitative genetic parameters (Morrissey *et al.*, 2007).

The very high proportion of individuals that are ignored in our selection analyses and animal models, due to their non-reproduction, are noteworthy. Cooperative breeding may (Woodroffe, 1993) or may not (Woodroffe & Macdonald, 2000) occur in badgers and further work is required to determine whether it does occur (see Chapter 6). Measuring the indirect fitness of non-reproducing individuals, both in terms of the next generation and the next plus one, may shed light on the seemingly genetically futile life-history strategy of non-reproduction.

In conclusion, unravelling the relationship between age and breeding success has enhanced our understanding of badger population dynamics. We provide strong evidence of age-related effects on breeding success, and show that the LBS of males and females are similar. The initial increase in male breeding success with age is best explained by the Constraint Hypothesis. Neither the Constraint, Restraint, nor Selection Hypotheses explained the initial increase in breeding success in females with age; however, this was a weak relationship. Badgers were selected for an early α and a late ω . Both sexes experienced senescence. We did not find additive genetic variance of α or ω , but this may be due to the relatively small number of badgers expressing these traits, or paucity of our pedigree. Finally, our results have important practical application in the field, confirming that data on teat width and diameter can be confidently applied to determine whether female badgers have produced young in a given year.

Promiscuous and repeated mounting behaviour in European badgers *Meles meles*: promoting sperm competition, genetic diversity, and genetic compatibility, and reducing infanticide and male–male aggression⁴

⁴ A modified version of this chapter is currently being prepared as: Dugdale HL, Isherwood A, Pope LC, Burke T, Johnson PJ, Macdonald DW (in prep-b) Promiscuous mounting behaviour and inbreeding avoidance in the European badger *Meles meles*.

5.1 Abstract

We provide strong evidence for the occurrence of polygynandrous and repeated mounting behaviour in the European badger *Meles meles*. We discuss the evolutionary causes of these behaviours. We analysed the mounting behaviour of group members during the post-partum mating season in early spring, in three years, at two neighbouring social groups each year, in a high-density badger population. We also analysed the occurrence of inbreeding using microsatellite data from 915 badgers to attempt to assign parentage to 630 cubs and assess the relatedness of assigned parents. Male mounting success was not related to dominance rank, or to the frequency of self-grooming. Males with more subcutaneous haunch fat, however, were observed mounting more often and this might function as a signal of relative male fitness. In terms of mate choice, females did not appear to trade mountings for allogrooming from males. Promiscuous and repeated mounting of female badgers does not appear to devalue the previous male's sperm; however, it is possible that females promote sperm competition by being mounted promiscuously. Females may reduce the risk of inbreeding by mating with extra-group males. Although potentially incestuous matings occurred, these were more likely to occur with within-group than extra-group males. Promiscuous mounting of female badgers may therefore promote genetic diversity or reduce genetic incompatibility. Finally, promiscuous and repeated mounting of female badgers may have evolved originally to reduce male–male aggression and reduce the likelihood of infanticide from males by masking paternity.

5.2 Introduction

Theory predicts that males should mate with as many females as possible as they produce considerably more, smaller gametes, whereas females, which produce fewer larger gametes and often invest more in parental care, should be choosier (Trivers, 1972). The observation that females may be mounted by multiple males, which is commonly seen in mammals (Møller & Birkhead, 1989; Wolff & Macdonald, 2004), has therefore received much attention and many hypotheses have been advanced to explain this (Halliday & Arnold, 1987; reviewed in Birkhead & Møller, 1992; Jennions, 1997; Jennions & Petrie, 2000; Wolff & Macdonald, 2004). Moreover, in some species such as the thirteen-lined ground squirrels *Spermophilus tridecemlineatus*, females be mounted repeatedly by the same male (Schwagmeyer & Parker, 1994). Since mountings may be expected to be costly (Daly, 1976), hypotheses have been developed to explain the evolution of such repeated mounting behaviour (reviewed in Hunter *et al.*, 1993).

In species where females mate multiply, sexual selection studies have focused on the characteristics of males that obtain mountings and genetic paternity. This enables the identification of traits associated with successful mountings. Some such traits include self-grooming (Wiepkema, 1979) and allogrooming (Stopka *et al.*, 2001). Self-grooming spreads scent, such as that present in saliva and inter-digital gland secretion, over the body. Self-grooming may be sexually selected and may function as an advert for individual identity, reproductive condition and sexual attractiveness (Wiepkema, 1979), as observed in prairie voles *Microtus ochrogaster* (Wolff *et al.*, 2002). Additionally, allogrooming may be a commodity that is exchanged in a biological

market for mountings (Stopka *et al.*, 2001), as demonstrated in the wood mouse *Apodemus sylvaticus* (Stopka & Macdonald, 1999).

To understand the breeding system of a species, knowledge is required of both the social and genetic mating system. The European badger *Meles meles* is group-living in southern England, with up to 29 individuals resident within a social group (da Silva *et al.*, 1994). It is a good species in which to study social behaviour as, although badgers may live in groups, there is little evidence that they gain cooperative benefits from this (Woodroffe & Macdonald, 2000; Johnson *et al.*, 2004; Chapter 6). Badger social groups contained relatives, and relatives were also clustered in neighbouring groups (Chapter 3). Parentage analyses using microsatellite data have established that plural breeding, multiple-paternity litters and extra-group paternities occur in high-density populations (Carpenter *et al.*, 2005; Chapter 2). Up to five males and five females breed within groups in the Wytham Woods population and approximately half of the paternities are assigned to extra-group males, primarily from neighbouring groups (Chapter 2). Within social groups reproductive skew occurs, but this is not as high as that seen in species where one pair dominates the breeding (Chapter 3).

Males show peaks in plasma testosterone (Maurel *et al.*, 1977) and spermatozoa levels (Page *et al.*, 1994) in February, declining to a minimum in October or November, when testes may ascend into the body cavity. Females also show a peak in the proportion carrying large follicles in February, and another around August (Cresswell *et al.*, 1992). Due to delayed implantation (Fischer, 1931), females do not implant ova until triggered by a change in the photoperiod around December (Canivenc *et al.*, 1985) and they give birth once a year around February. During delayed implantation

females may exhibit ovarian cycles of 28 days (Service *et al.*, 2002). It is possible that badgers are induced ovulators and that they may exhibit superfoetation (conception during pregnancy; reviewed in Appendix 1). These factors, together with behavioural observations (Neal & Cheeseman, 1996), show that although copulations may occur throughout the year, there is one post-partum peak in early spring and a smaller peak from July to September. The fact that the main peak in mounting behaviour is around February (Neal & Cheeseman, 1996) and that blastocysts from this period represent the majority of those present pre-implantation (Cresswell *et al.*, 1992), suggests that this is the most important mating period.

Despite the large number of studies on the European badger, very few published studies have investigated its behavioural mating system. This is primarily because badgers are nocturnal, living underground during the day, with the main mating period occurring in the colder months. Additionally, badgers can live in large groups but are not individually identifiable from natural markings, and although they socialise around sett entrances, they forage solitarily. Opportunities to observe mounting behaviour are therefore limited without infrared illumination and marking of individuals. Nevertheless, a few studies have observed badgers over several years and noted that extra-group mountings may occur (Paget & Middleton, 1974; Christian, 1994, 1995). Aggression has been reported between neighbouring and resident badgers (Kruuk, 1978; Roper *et al.*, 1986), and resident males have been observed to chase away extra-group males (Christian, 1994, 1995). Mountings appear to vary greatly in duration lasting from less than a minute to several hours (Neal & Harrison, 1958; Paget & Middleton, 1974; Christian, 1995). Females have been observed to be mounted by more than one male over a few days, which is assumed to

represent an oestrous period (Christian, 1995), and within the same night (Neal & Harrison, 1958; Paget & Middleton, 1974). The most detailed study observed marked badgers over two years, although the majority of mounting observations were drawn from the post-partum mating season in one year (Johnson, 2001); this study revealed a polygynandrous mounting system, with repeated mountings by the same male and limited aggression between males. The badger is therefore a useful species in which to investigate why females mount multiply and repeatedly, and also whether females exhibit mate choice.

In this study, we use three years of behavioural observations from two social groups, in combination with parentage data, to ask: Is mounting promiscuous and repeated, and which hypotheses best explain the evolutionary occurrence of these behaviours? What traits are associated with males that mount more frequently? Are all mounting events of similar duration and does duration relate to parentage success? Do females trade mountings for social services, such as allogrooming? Does inbreeding occur and can females avoid inbreeding by mating with extra-group males?

5.3 Materials and methods

5.3.1 Study population and study site

We filmed at two neighbouring social groups in Wytham Woods, Oxfordshire (01° 19'W, 51° 46'N) between 1st February and 31st May in 1995, 2004 and 2005. The woods are enclosed by a deer fence that contains most of the badger setts and encompasses an area of 3.9 km² (Ellwood, 2006). The area consists primarily of deciduous woodland, surrounded by permanent pasture and mixed arable land (Kruuk,

1978). The highest density, from 1987–1996, was 44.3 badgers km⁻² (Macdonald & Newman, 2002).

5.3.2 Sample collection and genetic analyses

We trapped badgers at least four times a year, for one week in January and two weeks in each of June, August and November (Tuytens *et al.*, 1999). Badgers were trapped in box-traps baited with peanuts, sedated by an intra-muscular injection of approximately 0.2 ml / kg ketamine hydrochloride, and identified through a unique tattoo on the inguinal area (Dugdale *et al.*, 2003). In particular, we graded body condition on a subjective five point scale of subcutaneous haunch fat, and estimated an index of body condition that we calculated as observed body weight divided by expected weight, obtained from a regression of weight and head-body length (Dugdale *et al.*, 2003). Body condition score was taken as the mean over the period May–August after the observed mating period. Blood or guard hair samples were collected for genetic analyses; additionally, ear tissue samples were collected from five road kills.

We genotyped 915 badgers for 16–22 microsatellite loci (*Mel101–117*, Carpenter *et al.*, 2003; *Mel1*, Bijlsma *et al.*, 2000; and *Mel10*, *Mel12*, *Mel14* and *Mel15*, Domingo-Roura *et al.*, 2003). We amplified samples, analysed microsatellites, assigned parentage and calculated relatedness using the methods described previously in Chapters 2 & 3. We attempted to assign parentage to 630 genotyped cubs with 80% and 95% confidence, through a likelihood based approach using CERVUS 3.0.1.8 (Kalinowski *et al.*, 2007) and COLONY 1.2 (Wang, 2004). We based analyses in this

chapter solely on assigned parents whose social group was known. Both parents were assigned to 542 (86% of the genotyped cubs) cubs with 80%, and 331 (53%) with 95% confidence. Paternity was assigned to 585 (93% with 80%) and 338 (54% with 95% confidence) cubs and maternity was assigned to 566 (90% with 80%) and 336 (53% with 95% confidence) cubs. Due to delayed implantation, females give birth in the year following conception (reviewed in Appendix 1). We assigned a parent-pair to all 14 cubs that resulted from the mountings in the filmed social groups in 1995 and 2004; the study ended in 2005 so the cubs born in 2006 were not genotyped (Table 5.1). We estimated the Queller and Goodnight index of relatedness (R) using RELATEDNESS 5.0.8 (Queller & Goodnight, 1989). We built a pedigree, using all of the parentage assignments and sibship inferences from breeding events in Wytham Woods, 1988–2005, and estimated inbreeding coefficients (f) using PEDIGREE VIEWER 5.5 (Kinghorn, 1994); the methods are described in Chapter 4. Lifetime breeding success (LBS) was calculated, separately for each sex, for badgers that were of known age, survived to breeding age (yearling males or two-year-old females, as due to delayed implantation females conceive in the year before birth), and were considered dead by the end of the study in 2005.

5.3.3 Behavioural analyses

We used three infrared-sensitive remote video surveillance systems (Stewart *et al.*, 1997) at Pasticks (P) social group in all three years, and one at the neighbouring groups of Pasticks Outlier (PO) in 2004 and 2005 or Sunday's Hill (SH) in 1995. Infrared lighting was used to observe nocturnal behaviour and minimise disturbance of the subjects (Lythgoe, 1979). Cameras were focused on active sett entrances and

filming was conducted continuously; nights without footage due to equipment failure were minimal. We could not film all mountings given that the fixed field of views limited the observation area. Our behavioural data therefore provides a snapshot view of aboveground mounting behaviour around sett entrances during the post-partum mating period in early spring. We analysed 960 videotapes, corresponding to 319 calendar nights (totalling 11,230 hours; Table 5.1). Adult and yearling badgers were identified through clip marks (Stewart & Macdonald, 1997); two symbols were clipped into the guard hairs on the flank of the badger, creating a mirror image on each side. We also recorded, in separate categories, unmarked badgers, unmarked cubs and the occasional clip-marked badger that we could not identify confidently. The social group compositions are detailed in Table 5.1.

We recorded each incidence of mounting behaviour (ejaculation cannot be detected through observation) and the duration of each incidence. Mounting events commenced when the mounting badger grabbed the mounted badger by the scruff of the neck and finished when the neck hold was released, after which the badger dismounted. Occasionally the neck hold was released during the mounting event in which case the end time was when the male dismounted. If the male dismounted only briefly this was still classified as a separate mounting event. We recorded failed mounting events when: (1) the male was not directly aligned with the female (and thus genital contact was not possible); (2) the mounting badger was female or a cub; or (3) the mounted badger was male or a cub (cubs are not sexually mature generally until they are yearlings, Neal & Harrison, 1958; Ahnland, 1980). We also recorded the identity of badgers that were present during mounting events and whether they interacted with the mounting badgers.

Table 5.1 The composition of each of the six filmed social-group-years, along with the number of hours of footage analysed, the number of mountings observed and the number of parents assigned to the cubs from these mating seasons.

Year	1995		2004		2005	
Social group	P	SH	P	PO	P	PO
Adult & yearling females	7	4 ^{^^}	4 ^{**}	4	7	4
Adult & yearling males	4 [^]	10	3 [*]	3 [*]	2 [*]	2
Unmarked [†]	5	4	2	1	2	3
Badgers known to be unmarked [‡]	8 ^a	4 ^b	1 ^c	1 ^d	0	3 ^e
Group size (excl. cubs & incl. known unmarked badgers)	19	18	8	8	9	9
Total observation time (hours)	1383	1242	2444	798	3872	1491
Total number mountings	72	15	52	15	34	15
Mountings of identifiable females	18	2	25	13	34	10
Resulting cubs	2	1	6	5	3	6
No. of assigned mothers	1	1	4	3	?	?
No. of within-group fathers	1	1	1	1	?	?
No. of extra-group fathers	0	0	3	2	?	?

* One additional badger was not present for all of the study period, either because they were found dead ($n = 2$) or presumed dead as they were only seen for a maximum of 3 days and then were not seen again ($n = 3$). We did not observe any of these individuals mounting, therefore, they were excluded from the analyses and the group size estimates.

[^] One more marked badger was present in this category, but it was not seen on screen, so they were excluded from the analyses and the group size estimate.

[†] Estimated by the maximum number of unmarked badgers seen on screen at any one time.

[‡] Resident adults and yearlings known to be unmarked for all or the majority (2 males & 1 female at P & SH in 1995 were not clipped until May) of the study, from trapping records are: ^a 2 males & 6 females; ^b 3 males & 1 female; ^c 1 female; ^d 1 male; and, ^e 2 males & 1 female.

? Parentage was not resolved for the cubs born in 2006, as the study ended in 2005. All of the other cubs trapped the following year in the filmed social groups had both parents assigned.

We recorded each incidence of directed aggression, whereby one badger initiated aggression and the other received it without reciprocating. We also recorded escalated aggression whereby both badgers initiated and received aggression in the same incidence. Each incidence between the same dyad was considered finished when the individuals moved two body lengths apart and there was a minimum of twenty seconds without further aggression. We also recorded each allogrooming event. Finally, for mate choice analyses, we recorded the number of bouts of activity in

which males were observed self-grooming, along with the number of times that males allogroomed, sequentially allomarked (Buesching *et al.*, 2003) and directed aggression at females. A bout of activity began when the first badger was seen on screen and ended when there was one minute without a badger on screen.

5.3.4 Statistical analyses

We used SAS 9.1 for all of our statistical analyses and we ran Generalised Linear Mixed Models (GLMMs) using the MIXED and GLIMMIX procedures (Littell *et al.*, 2006). Where parametric tests were used, normality was examined with the Anderson-Darling test and by examining the histogram of residuals. When transformations were required, we used a log transformation, first adding a constant so that the minimum value was one, when necessary (Osborne, 2002). Means are provided with the 95% confidence interval (CI), unless otherwise stated.

5.3.4.1 Correlates of male mounting frequency

We ran the GLIMMIX procedure with Poisson error structure, log link, badger identity and social group as random factors and Kenward-Roger denominator degrees of freedom method (Littell *et al.*, 2006). We entered the number of mountings observed by males as the response, and the body condition score or body condition index (the mean over the period May–August after the observed mating season) as the predictor, along with a measure of dominance rank (controlled for group size; Appendix 2), and the number of times that the male was observed self-grooming. Social group was removed as a random factor from the model including body condition score as it had a negative estimate of variance. The number of bouts of

activity in which the male was observed was included as a continuous covariate effect to control for the likelihood of observing the male. This is because the fixed field of view may result in some badgers being observed rarely, although they are nearby.

5.3.4.2 Female mounting partner choice

We calculated Kendall's partial rowwise matrix correlation ($\tau_{rw;XY.Z}$, de Vries, 1993). We ran 10,000 permutations, permuting rows and columns independently, to assess the significance of $\tau_{rw;XY.Z}$ using the software MATMAN 1.1 (de Vries *et al.*, 1993). We created matrices with females in the rows and males in the columns for each social-group-year, including the one extra-group male who was observed mounting in the 2004 PO matrix. We tested the rowwise conjecture that the number of times each female was mounted by each male was correlated with their pairwise relatedness or the number of times each male initiated aggression at, sequentially allomarked or allogroomed each female. We controlled for the number of bouts of activity in which each pair were present. False discovery rate (FDR) control was used to generate adjusted p -values, to account for multiple tests (Benjamini & Hochberg, 1995).

5.3.4.3 Inbreeding

We assessed whether parent-pairs could be confidently assigned as being first-order relatives (1°: full-siblings or parent-offspring) versus unrelated using KINSHIP 1.3.1 (Goodnight & Queller, 1999). KINSHIP takes into account the pairwise relatedness of two individuals, their genotypes, and the population allele frequencies to calculate the likelihood that their genotypes could have been produced by direct descent by a specified relationship; the significance was then assessed through simulations.

Previous analyses revealed that the power to distinguish between 1° kin and unrelated individuals was high, whereas that between 1° and 2° (the equivalent of half-siblings) kin, and 2° kin and unrelated individuals was low (Chapter 3). We ran a χ^2 test to see whether the number of within-group and extra-group parent-pairs assigned at both confidence levels, of unrelated or 1° kinship, differed from a null hypothesis that the kinship distribution of within- and extra-group mates did not differ.

We tested whether females that were assigned maternity and that had multiple-paternity litters, by at least one within-group and one extra-group father assigned with 80% and 95% confidence, were more related to the within-group than to the extra-group mates, using a paired t-test. Two females with 80% confidence (0 with 95%) had more than one extra-group father within a litter, so the mean pairwise R was taken. Three females had more than one multiple-paternity litter with 80% confidence and one female with 95%, so the mean pairwise R for within-group and extra-group males was obtained. We also used all of the parent-pair assignments to test whether females that were assigned maternity were more related to within-group than to extra-group mates. We used the MIXED procedure with female and male identity as random factors (to control for males or females mating with more than one mate) and one entry per parent-pair, excluding parent-pairs that were assigned to both within-group and extra-group categories due to dispersal ($n = 7$ with 80% and $n = 3$ with 95% confidence). We included a code for within-group or extra-group sire as the predictor in the model, with the log-transformed response of pairwise relatedness.

To test whether extra-group paternities may enable breeding females to avoid inbreeding, we conducted randomisations to ask if females that were assigned

maternity were significantly more related to within-group candidate fathers than to candidate fathers in the rest of the population, i.e. a null model of random mating. We calculated the observed mean pairwise R among within-group breeding females and within-group candidate fathers. We then randomly allocated all of the candidate fathers in the population to groups, preserving the size and sex composition of each group (Girman *et al.*, 1997). We calculated, for each simulation, the mean pairwise R , for each group, between all dyads of within-group assigned mothers and permuted within-group candidate fathers, and we took the average among groups. We also compared this permuted value to the mean pairwise relatedness of assigned parents in 2005, among groups, to see if this was less than expected under random mating (evidence of inbreeding avoidance) or whether it was greater than expected (evidence of outbreeding avoidance). As the majority of extra-group paternities are assigned to neighbouring males (Chapter 2) and neighbouring-groups contain relatives (Chapter 3), we also conducted a randomisation to see whether females that were assigned maternity were significantly more related to neighbouring candidate fathers than to extra-group candidate fathers. This was exactly as above, except that the observed mean pairwise R was based on neighbouring candidate fathers rather than within-group candidate fathers. Candidate fathers were all yearling and adult males that were present in the year of conception ($n = 117$). Breeding females were those females that were assigned maternity with 80% confidence. Randomisations were run with 1,000 simulations. We used females assigned as mothers in 2005 (i.e. conceived in 2004) as this year had the highest number of assigned maternities (48 mothers in 22 social groups with 80% confidence).

5.3.4.4 Inbreeding and lifetime breeding success

We ran a χ^2 test on a two by two contingency table with the number of badgers that had an inbreeding value (f) of $f \geq 0.25$ or $f = 0$, and the number of these badgers that did or did not breed in their lifetime.

5.4 Results

We observed 198 mounting events (Table 5.1) on a total of 90 calendar nights over the four-month filming periods in three years; 38% of these events were observed in February (1 every 31 hours), 23% in March (1 every 74 hours), 20% in April (1 every 69 hours) and 19% in May (1 every 74 hours). In 89 of these events, both mounting partners were identified and these occurred on 50 calendar nights with a similar distribution over the months (27%, 39%, 21% and 12%, or 1 mounting event every 99, 98, 142 and 249 hours, respectively). Six, out of the 16 marked females that were observed mounted (four of which were observed in two years), were mounted in more than one four-week period in the same year. We observed one mounting between an extra-group male and a resident female in PO 2004; no other male was present in this bout of activity. The remainder of observed mountings were between group members.

5.4.1 Egalitarian mounting

All 11 males from the filmed social groups were observed mounting (three were observed in two years and were observed mounting in both), except in SH in 1995 where only the three yearling males and two out of seven adults were observed mounting. Six (55%), five (63%) and 10 (91%) females were observed mounted in

1995, 2004 and 2005, respectively. One female was not observed mounted in either 2004 or 2005, and two females were not observed mounted in 2004, but were in 2005.

5.4.2 Repeated mounting

Over the four-month study, analysing mounting behaviour where both participants were identified individually, females were mounted (by any combination of males) a mean of 4.5 ± 2.2 times (range = 1–20, median = 2.5, $n = 20$) and males performed 5.6 ± 3.9 mounts (range = 1–31, median = 3.5, $n = 16$). On a given night on which mounting was observed, mounted females were observed mounted a mean of 1.5 ± 0.4 times (range = 1–9, median = 1, $n = 58$), by any combination of males. On 78% of these occasions, females were only observed mounted once. Considering nights when females were observed mounted more than once, by any combination of males, the mean number of mounts was 3.4 ± 1.4 (range = 2–9, median = 2, $n = 13$). On a given night males were observed mounting any combination of females a mean of 1.5 ± 0.3 times (range = 1–7, median = 1, $n = 58$). On 74% of these occasions males were observed mounting only once; when mounting occurred more than once, with any combination of females in a night, the mean was 3.1 ± 0.8 mounts (range = 2–7, median = 2, $n = 15$). Identified pairs were observed mounting a mean of 2.8 ± 1.2 times (range = 1–19, median = 2, $n = 32$), or on the same night a mean of 1.4 ± 0.3 times (range = 1–7, median = 1, $n = 63$).

5.4.3 Promiscuous mounting

Analysing only mounting between identified pairs, males were observed mounting a mean of 2.0 ± 0.8 females (range = 1–6, median = 1.5, $n = 16$) and females were

observed mounted by 1.6 ± 0.3 males (range = 1–3, median = 1.5, $n = 20$). Nine out of 16 females (four females were present in two years) were observed mounted by more than one male. Four of the nine females that were observed mounted by more than one male, were mounted again by the first male after they were mounted by a second male. There were five observations of males mounting more than one female on the same night ($n =$ four males). There were five observations of a female being mounted by more than one male on the same night ($n =$ four females). The mean time between two males mounting the same female was $13\text{min } 36\text{s} \pm 27\text{min } 47\text{s}$ (range = 6s–53min 6s, median = 2min 56, $n = 5$). On three occasions, the second male was present while the first male was mounting and interacted with the mounting pair. The second male was observed, once, grabbing the female by the scruff of the neck while the first male was mounted. The second male directed aggression at the mounted pair on three occasions and this terminated the mounting on two occasions, after which the second male mounted the same female. In the third case the second male directed aggression at the mounted male 12 times, the mounted male directed aggression at the second male once and they both directed aggression at each other twice. However, the second male also allogroomed the mounted male six times and the female five times, and sequentially allomarked the mounted male 10 times and the female 11 times. This mounting was terminated by the female turning on her side. The first male was present on two of the three occasions when the second male mounted, but was never observed interacting with the mounting badgers.

5.4.4 Male–male behaviour around mounting

On 29 (33%) mounting events, which occurred in 11 bouts of activity, a second male was present while one male was mounting. The male that was present interacted with the mounting pair during 21 (72%) mounting events and aggression was observed between the mounted and second male in eight (73%) bouts. Incidences of aggression that were directed from the mounted male to the second male, without the second male reciprocating, were observed in four (37%) of these bouts, and *vice versa* in four (37%) bouts. Incidences of escalated aggression were observed in six (55%) bouts. Allogrooming was observed between these males in six out of the eight bouts in which aggression was observed, or in seven out of the 11.

5.4.5 Correlates of male mounting frequency

Males that were observed mounting the least, including mounts of unidentified individuals, had a lower mean body condition score between May and August after mounting than those that were observed mounting most ($F_{3,9} = 5.79, p = 0.0174$), but mounting frequency was not related to rank ($F_{1,9} = 0.07, p = 0.80$) or self-grooming frequency ($F_{1,9} = 0.03, p = 0.86$). In this model (containing the three predictors body condition score, rank and self-grooming frequency) the parameter estimates ($\pm$ SE) for the number of mounts by badgers with a body condition score of one was 0.3 ± 0.7 , a score of two was 1.8 ± 1.1 , and three was 1.5 ± 0.7 . Mounting frequency was not related to the index of body condition (body weight relative to body length: $F_{1,9,4} = 0.59, p = 0.46$).

5.4.6 Mounting duration

The 198 observed mounts had a mean duration of 3min 50s $\pm$ 1min 35s (median = 34s; Figure 5.1). Of these 198 events, 127 were short-duration mountings (< 1 min: range = 1–58s, mean = 20 $\pm$ 3s, median = 16s), 43 were medium duration ($1\text{min} \leq t < 5\text{min}$: range = 1min 1s–4min 56s, mean = 2min 21s $\pm$ 21s, median = 1min 46s) and 28 were long duration ($\geq 5\text{min}$: range = 5min 21s–82min 29s, mean = 22min 59s $\pm$ 8min 55s, median = 12min 44s).

Excluding all observations with unknown mounted ($n = 101$) and mounting ($n = 49$) badgers, the mean duration was 3min 55s $\pm$ 2min 31s ($n = 89$, median = 33s; Figure 5.1). Sixty-two of these events were less than 1min (range = 1–58s, mean = 22 $\pm$ 4s, median = 18s), 16 were between 1min and 4min 59s (range = 1min 1s–3min 35s, mean = 2min 10s $\pm$ 28s, median = 2min 2s) and 11 were five minutes or longer (range = 6min 55s–82min 29s, mean = 26min 31s $\pm$ 16min 35s, median = 19min 49s).

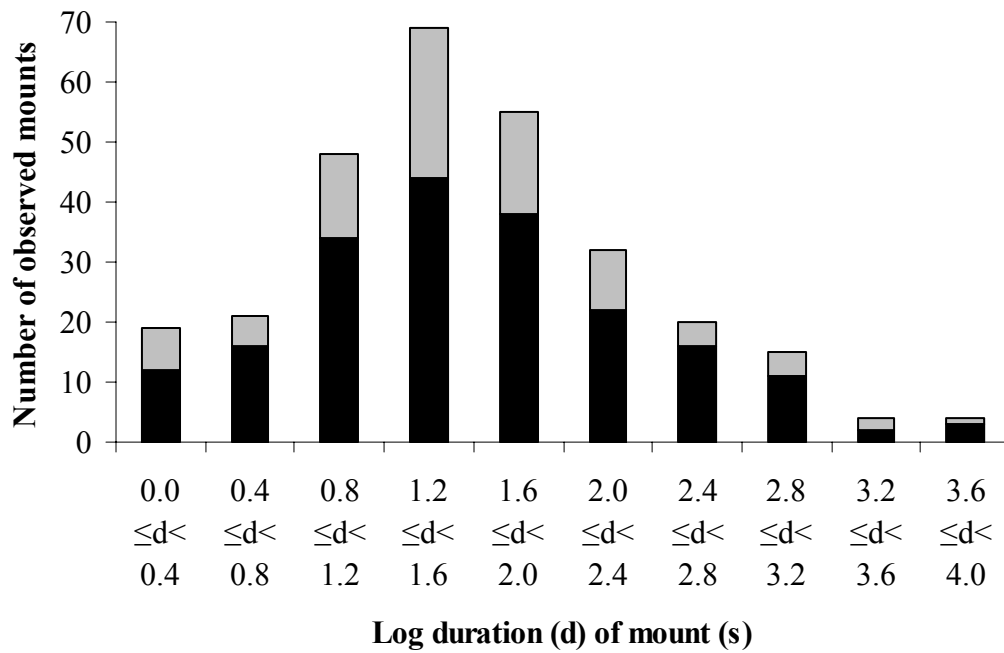


Figure 5.1 Duration of observed mounting events in seconds, log transformed, against the number of observed mounting events for all six social-group-years combined. Grey bars include unmarked or unidentifiable individuals. Black bars represent identified badgers only. d = log duration.

Long-duration mountings were observed in 1995 and 2004; however, only those observed in 2004 were between identified mounting-pairs and these involved three females. The first female was mounted by two different males with a gap of two days; the second was mounted by two different males, each for a long-duration, with 53min 6s between the two males. The third female was mounted by two different males, each for a long-duration, with an interval of 6s. On the following evening, the same female was mounted twice for a long-duration by the same first male and then once by the same second male after 2min 12s. Females were therefore observed in long-duration mountings within a 1–3 day period.

In seven of these 11 long-duration mountings, between a known male and female, the male and female were observed in mounting events with other partners previously that

evening. In the four long-duration mountings where the male or female was not observed in a mounting event earlier in that evening, the female was observed mounted up to two nights previously on two occasions, and the male was observed mounting 3–15 days previously on two occasions.

5.4.7 *Parentage success of mounting badgers*

We did not observed any of the 12 assigned parent-pairs (of the cubs born the following year) mounting together. Of the two within-group males that were assigned paternity of the cubs born in the filmed groups in 1996, one was not observed mounting and the other was observed mounting in one of the 13 mountings observed in their group in the previous year. The two within-group males that were assigned paternity to a cub born in their social group in 2005 were observed mounting in nine out of 49 mountings, and eight out of 15 mountings.

Of the three identified females that were observed mounted for a long-duration by identified males, only one was assigned maternity, to one cub, in the following year and an extra-group father was assigned. The father was only observed once on camera at that social group and was not observed interacting with any of the residents. Of the five identified males that were observed mounting for a long duration with identified females, three were assigned paternity in the following year. One male was assigned three within-group paternities (with two mothers) and one extra-group paternity in the other filmed group. The second male was assigned two within-group paternities and the third male was assigned one extra-group paternity in the other filmed group.

Johnson (2001) observed mounting behaviour in two social groups (P and SH) in 1996. One group had no cubs born the following year and only one marked female was observed mounted in this group. In the second group, 10 marked females were observed mounted. Three cubs were born the following year and three females were assigned maternity, two of which were observed mounted, but neither female was observed mounted for a long duration. One female was observed mounted four times by one male who did not obtain paternity, and once for a short-duration by a second male who was assigned paternity. The second female was observed mounted on six occasions by two marked males and an unmarked badger; these marked badgers were not assigned paternity. Three within-group males obtained paternity. One of these males was unmarked and the other two males were marked and were observed mounting. Aside from unmarked badgers, these were the two most frequently mounting badgers, each obtaining 20 mounts, out of the 72 observed.

5.4.8 Failed mounting events

We recorded 592 failed mountings. In 141 events the mounting badger (male, $n = 34$; unmarked $n = 107$) was not directly aligned with the mounted badger (female, $n = 59$; unmarked, $n = 82$). Cubs were observed mounting in 22 events, and the mounted badger was a cub ($n = 21$) and an adult female ($n = 1$). Eight females were observed mounting 78 times, with the mounted badger being a: cub ($n = 43$), female ($n = 18$), male ($n = 8$), unmarked ($n = 8$) and unidentifiable ($n = 1$). Eighteen males were mounted on 142 occasions, with the mounting badger being a: male ($n = 117$), unmarked ($n = 16$), female ($n = 8$) and unidentifiable ($n = 1$). The mounted badger

was a cub in 281 events, with the mounting badger being a: male ($n = 152$), unmarked ($n = 63$), female ($n = 43$), cub ($n = 21$) and unidentifiable ($n = 2$).

5.4.9 Mounting partner choice

There was a positive correlation between the number of times that a female was observed mounted by a male and the number of times that the male allogroomed the female in two social-group-years (Table 5.2). The relationship was not significant in the other three social-group-years, in which the correlation was negative. On average, females within a social-group-year were allogroomed $44 \pm 18\%$ of the time by males. There was no consistent relationship between the number of times that females were observed mounted by males and the number of times that males directed aggression at them or allomarked them (Table 5.2). Females, in Pasticks in 2004, were observed mounted significantly more by males that they were less related to; however, this relationship was not significant after FDR control, nor was it significant for the other social-group-years (Table 5.2).

Table 5.2 Partial Kendall rowwise correlation ($\tau_{rw;XY.Z}$) of the number of times that females, in each social-group-year, were observed mounted by within-group or extra-group males and the number of times that the males allogroomed, initiated aggression at or allomarked the females, or their pairwise relatedness. The number of bouts of activity in which pairs of badgers were observed was included as a control.

	1995 P		1995 SH [^]		2004 P		2004 PO		2005 P		2005 PO	
	$\tau_{rw;XY.Z}$	<i>p</i>	$\tau_{rw;XY.Z}$	<i>p</i>	$\tau_{rw;XY.Z}$	<i>p</i>	$\tau_{rw;XY.Z}$	<i>p</i>	$\tau_{rw;XY.Z}$	<i>p</i>	$\tau_{rw;XY.Z}$	<i>p</i>
Allogrooming	0.46	0.0128	0.29	0.0008	0.37	0.08	-0.18	0.24	-0.17	0.50	-0.33	0.51
Aggression	0.40	0.0388	-0.01	0.42	-0.13	0.37	-0.09	0.36	0.17	0.29	1.00	0.13
Allomarking	0.07	0.38	0.28	0.0300	0.00	0.51	-0.15	0.25	undefined*		-0.52	< 0.0001
Relatedness	-0.16	0.22	0.05	0.40	-0.55	0.0400	-0.39	0.07	0.35	0.21	0.33	0.49

[^] Only one female was observed mounted in this group.

* Six females were mounted by two males and the male that mounted them the most was the male that allomarked each female the most and was observed the most with each female. $\tau_{rw;XZ}=1$ and therefore the partial correlation was undefined, as it resulted in division by zero.

p-values in bold represent significant results after false discovery control for multiple tests.

5.4.10 Inbreeding

Potentially incestuous matings were observed between 24% of the parent-pairs assigned with 80% and 18% with 95% confidence (Table 5.3). This resulted in 132 (24%) cubs with 80% and 65 (20%) cubs with 95% confidence (Table 5.3). A higher percentage of the potentially incestuous parent-pair dyads, assigned with 80% confidence were within-group (53%) than extra-group pairs (47%); the corresponding values were 69% and 31% with 95% confidence (Table 5.3). The observed number of within-group and extra-group parent-pairs, assigned with 80% confidence, with unrelated or 1° kinship was not significantly different from expected, under the null hypothesis that the kinship distribution (unrelated or 1°) of within- and extra-group mates did not differ ($\chi^2 = 3.4$, $df = 1$, $p = 0.0672$), although the p -value was marginal. Significantly more potentially incestuous parent-pairs were within-group pairs with 95% confidence ($\chi^2 = 5.6$, $df = 1$, $p = 0.0182$).

Table 5.3 The number of parent-pairs (n) that were significantly more likely to represent unrelated dyads or dyads of 1° kin (full-siblings or parent–offspring), using CERVUS assignments with 80% and 95% confidence, and the number of resulting cubs. Parent-pairs that were classified as within-group mates in one year and extra-group mates in another year (due to dispersal) were excluded from the within-group and extra-group analyses ($n = 7$ with 80%, and $n = 3$ with 95% confidence).

Parent-pair confidence	Parent-pair kinship	All				Within-group				Extra-group			
		Min R	Max R	n	No. of cubs	Min R	Max R	n	No. of cubs	Min R	Max R	n	No. of cubs
80%	Unrelated	-0.46	0.47	286	410	-0.30	0.44	118	185	-0.46	0.47	161	202
80%	1° kin	0.04	0.76	90	132	0.04	0.71	48	79	0.17	0.76	42	53
95%	Unrelated	-0.46	0.46	194	266	-0.26	0.38	94	142	-0.46	0.46	98	117
95%	1° kin	0.04	0.71	43	65	0.04	0.71	29	44	0.18	0.58	13	19

Table 5.4 The number of parent-pairs, assigned with 80% or 95% confidence, that were inbred and the number of cubs to which they were assigned parentage. The relatives of the parent-pairs that were required to detect the inbreeding are stated and these relationships were assigned at the same confidence level as that of the parent-pair. f = inbreeding coefficient.

Parent-pair relationship	f	Relatives of parent-pair required		80% confidence				95% confidence			
		Female	Male	n inbred	Out of	%	n cubs	n inbred	Out of	%	n cubs
mother–son	≥ 0.25	–	mother	3	244	1.2	3	0	0	–	0
father–daughter	≥ 0.25	father	–	7	191	3.7	10	2	51	3.9	3
full-siblings	≥ 0.25	both parents	both parents	3	122	2.5	3	1	18	5.6	1
maternal half-siblings*	≥ 0.125	mother	mother	4	124	3.2	5	0	0	–	0
paternal half-siblings*	≥ 0.125	father	father	4	123	3.3	6	1	19	5.3	1

* excludes the three full-sibling parent-pairs.

Similar analyses using the pedigree showed lower levels of close inbreeding, with 13 out of 244 (5%) parent-pairs assigned as 1° relatives (Table 5.4). Of the assigned parent-pairs for which the natal groups were known, 31 cubs (17%) and 15 cubs (14%) assigned with 80% and 95% confidence, respectively, had parents that were born in the same social group (Chapter 2); similar results of 42 (22%) and 17 (31%), respectively, were reported in Woodchester Park (Carpenter *et al.*, 2005). The values in Wytham Woods corresponded to 30 and 15 parent-pairs with 80% and 95% confidence, respectively. Of the 30 parent-pairs, twenty-one parent-pairs were resident in the same group in the conception year, eight were in neighbouring groups and one was two groups away with 80% confidence (R range = -0.234–0.683); the values with 95% confidence were 10, four and one (R range = -0.111–0.683). Of the 30 parent-pairs, one pair did not have any parents that could be compared, one had one of their parents known but they did not match, and of the remaining 28 parent-pairs for whom both parents were assigned, three were full-siblings and five half-siblings. With 95% confidence, one parent-pair had one of their parents known but they did not match, and for the 14 parent-pairs for whom both parents were assigned, one represented full-siblings and three were half-siblings.

Breeding females with multiple-paternity litters containing cubs sired by within-group and extra-group males, assigned with 80% confidence, were on average less related to extra-group ($R = 0.101 \pm 0.061$, $n = 15$) than within-group sires ($R = 0.228 \pm 0.060$, $n = 15$; $t_{14} = 2.3$, $p = 0.0357$). The relationship was in the same direction using parentage assignments with 95% confidence, but this was not significant (extra-group $R = 0.010 \pm 0.040$, within-group $R = 0.163 \pm 0.120$, $t_5 = 1.4$, $p = 0.23$), although the sample size was small ($n = 6$ females). Overall, breeding females were significantly

more related to within-group than extra-group sires with 80% ($R = 0.157 \pm 0.020$ and 0.109 ± 0.017 respectively, $F_{1,359} = 6.98$, $p = 0.0086$) and with 95% confidence ($R = 0.134 \pm 0.022$ and 0.061 ± 0.020 respectively, $F_{1,189} = 5.79$ and $p = 0.0170$).

Females that were assigned maternity had significantly higher pairwise relatedness with the candidate fathers in the social group that they resided in, in the year that they conceived (mean $R = 0.181 \pm 0.074$ [$\pm 95\%$ CI], $n = 19$ social groups containing both assigned mothers and candidate fathers) than expected from random, using all candidate fathers in the population that year (mean $R = 0.003$, randomised range = -0.087 – 0.109 , $p < 0.001$). These permuted relatedness values were lower than the mean pairwise relatedness of assigned mates ($R = 0.167 \pm 0.107$, $n = 17$ social groups, $p < 0.001$), assigned within-group mates ($R = 0.259 \pm 0.190$, $n = 9$, $p < 0.001$) and assigned extra-group mates ($R = 0.111 \pm 0.140$, $n = 14$, $p < 0.001$), averaged across groups in 2005. Females that were assigned maternity had significantly higher pairwise relatedness with the candidate fathers in neighbouring social groups, in the year that they conceived (mean $R = 0.103 \pm 0.038$), than expected from random, using candidate fathers from the population that year (mean $R = 0.015$, randomised range = -0.071 – 0.099 , $p < 0.001$).

5.4.10.1 *Inbreeding and lifetime breeding success*

Lifetime breeding success was known for 12 of the 16 cubs with $f \geq 0.25$ (Table 5.4). Eight had no assigned offspring, two had one, one had three and one had four (mean = 0.75 ± 0.86). Of the eight badgers with $0.125 \geq f < 0.25$, one was assigned two cubs, one was assigned one cub and the rest were assigned none (mean = 0.38 ± 0.62). This

compared to a mean lifetime breeding success of 1.41 ± 0.27 ($n = 299$) for badgers with $f = 0$, or 0.46 ± 0.27 ($n = 41$) for those that also had all four grandparents known. Considering only badgers with all four grandparents known, breeding success did not differ for potentially inbred and for non-inbred badgers (Figure 5.2). A similar percentage of potentially inbred badgers ($f \geq 0.125$) produced offspring (30%), compared to badgers with $f = 0$ (49%: $\chi^2 = 2.7$, $df = 1$, $p = 0.10$). The percentage of potentially inbred badgers did not differ when the presence of all four grandparents was controlled for ($f \geq 0.125$, 25%; $f = 0$, 29%: $\chi^2 = 0.1$, $df = 1$, $p = 0.75$).

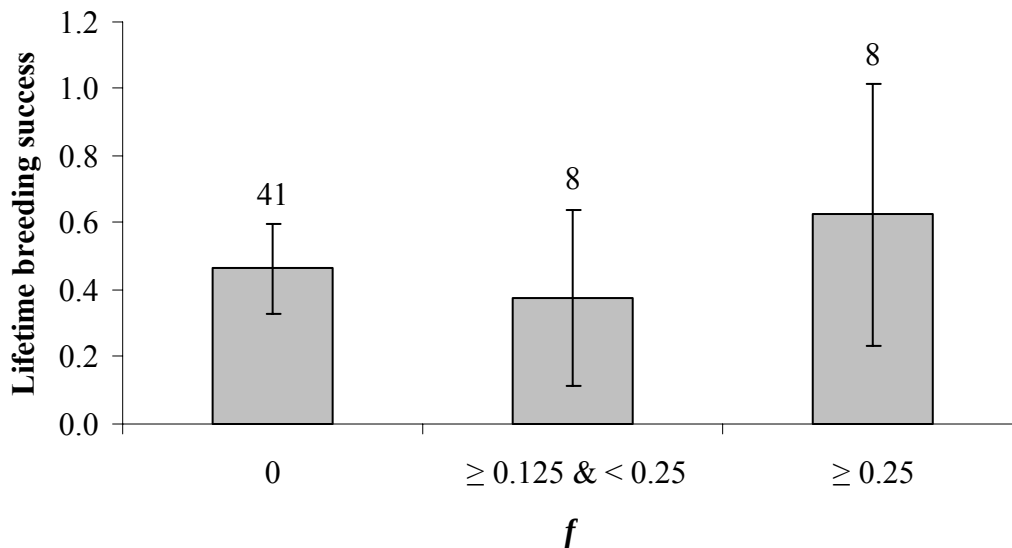


Figure 5.2 Mean lifetime breeding success of badgers with different inbreeding values (f) that had all four grandparents known. Error bars display $\pm$ one standard error and data labels display the sample size.

5.5 Discussion

We provide strong evidence for the occurrence of both polygynandrous and repeated mounting behaviour in the badger. Mounting was egalitarian, but durations were very variable and ejaculation may potentially only occur in the long-duration mountings. Observed long-duration mountings, however, did not result in assigned parentage in

the following year, reinforcing the findings that mounting observations do not always correlate with genetic success (Hughes, 1998; Coltman *et al.*, 1999a).

5.5.1 Egalitarian mounting behaviour

Nearly all males in the filmed social groups were observed mounting and on average 70% of the females seen each year were observed mounted. As filming was restricted to the areas around a limited number of active sett entrances, it is probable that individuals also mounted underground, outside the camera's field of view, or away from the sett. Vocalisations associated with mounting behaviour (Neal & Harrison, 1958; Wong *et al.*, 1999) have been heard from within setts (Paget & Middleton, 1974). Additionally, mounting has been heard (Neal & Harrison, 1958) and observed (Neal & Cheeseman, 1996) in the territory away from the main sett. Furthermore, radio-tracking has shown that both males and females enter neighbouring territories (Christian, 1994) and mounting has been observed in neighbouring territories (Paget & Middleton, 1974; Christian, 1995). Females varied in their observed mounting behaviour between the two years for which repeated observations were made, with some observed mounted in one year but not in the next. Most of the eleven yearlings took part in mounting behaviour; only one female yearling was not observed mounted. This suggests that the majority of group members of reproductive age take part in mounting, which corresponds to post-mortem studies in which the majority of females conceive (reviewed in Appendix 1).

5.5.2 Female mate choice

We observed 59 failed mountings of identified females in which genital contact was not made. Females have been observed to refuse mountings by flattening their body on the ground (Neal & Harrison, 1958) or, as we observed in this study, by turning on their side or backing into sett entrances. It is therefore possible that females select the males with which they mount. There was no relationship between the number of times males were observed self-grooming and mounting, suggesting that self-grooming is not used as an indicator of sexual attractiveness (Wiepkema, 1979) in badgers. Males of higher rank were not observed mounting more than males of lower rank; however, there is no evidence of an effect of rank on reproductive success (Appendix 2). Males with higher subcutaneous haunch fat scores were observed mounting more often. There was no relation between an index of body condition and mounting success, but this index reflects the observed weight of a badger compared to its expected weight (based on its weight and body length). Subcutaneous haunch fat may therefore be an index of male quality. We did not analyse whether the mean duration of observed mounting events also varied for these males, however, the few males that we observed in long-duration mountings were not assigned paternity from these mounting events.

Females may trade mountings for a social service such as allogrooming (Stopka *et al.*, 2001). Although three social groups showed positive correlations between the number of observed mountings and allogrooming events of male–female dyads, the relationship was not significant and more importantly, although not significant, it was negative in the other three groups. If male body condition score is an index of quality, females may prefer such males and may not require them to ‘pay’ as much to mount (Stopka *et al.*, 2001). Given that females are allogroomed more frequently by females

than by males, the biological market concept of trading allogrooming for mountings does not seem applicable. No consistent relationship was observed between the amount of aggression that a male directed at a female, or the number of times that they allomarked a female, and the number of mountings observed between them. Dominance hierarchies have been detected in some badger social groups around the same period, but not in all groups (Appendix 2). It is possible that groups vary in their social interactions, thus producing the different patterns that we observed between groups.

5.5.3 *Promiscuous and repeated mounting behaviour*

Males mounted more than one female and females were mounted by more than one male during the post-partum mating period in early spring, and on the same night. Furthermore, in approximately a quarter of the nights when females were observed mounted, females were mounted repeatedly by the same male. Fourteen hypotheses, reviewed by Johnson (2001), may explain either the observations of promiscuous mounting and / or repeated mountings of female badgers. Johnson (2001) surmises that four of these hypotheses are more likely to apply to badgers: promoting genetic diversity (Williams, 1975), reducing socially disruptive male–male competition and the risk of infanticide from males (Bertram, 1975; Hrdy, 1979; Ebensperger, 1998; Wolff & Macdonald, 2004), promoting sperm competition (Møller & Birkhead, 1989) and devaluing the previous male's sperm (Walker, 1980; McKinney *et al.*, 1983). We discuss evidence for these hypotheses and for the Genetic Incompatibility Hypothesis (Zeh & Zeh, 1994). We include the latter hypothesis as although it was thought unlikely to apply to badgers, there were no data to base this on (Johnson, 2001).

5.5.3.1 *Devaluing the previous male's sperm*

Females that are mounted multiply may also be mounted repeatedly by the most recent male in order to devalue the previous male's sperm (Walker, 1980; McKinney *et al.*, 1983). This hypothesis predicts that when copulation is not forced females should not allow the first male to mount again, after the female has been mounted by a second male. This hypothesis is not consistent with copulations outside of the oestrous period (Hunter *et al.*, 1993). We observed females being mounted by more than one male and being mounted repeatedly by some males. We also observed for the first time, however, female badgers being mounted by one male, then by a different male, and later being mounted by the first male again. As mounting duration varies, the later mounting by the first male may have occurred outside of the oestrous period or may not represent a successful mounting. One female, however, was observed being mounted, for a long-duration, by two males on the same night and then by the first male on the next night, again for a long duration. This suggests that females are not devaluing the previous male's sperm.

5.5.3.2 *Promoting sperm competition*

Sperm competition provides an arena in which the highest quality males should gain paternity, and if male ability to bias paternity post-copulation is heritable females will gain genetic benefits from sons that can bias paternity (Birkhead & Møller, 1992). To incite post-copulatory sperm competition, females may advertise their reproductive status to attract mates either directly, for example through sexual swellings or vocalisations (O'Connell & Cowlshaw, 1994; but see Maestripieri *et al.*, 2005), or

indirectly through scent-marking behaviour. Female badgers vocalise during mounting (Paget & Middleton, 1974; Wong *et al.*, 1999), which may serve to attract other potential mates and promote sperm competition. Additionally, the vulva of the female badger may swell and turn pink (Neal & Cheeseman, 1996), which may advertise reproductive status, although whether this can be detected by males is unproven. We observed males sniffing the genital area of females before mounting and also while another male was mounting. Urine carries information on sex (Service *et al.*, 2001), and subcaudal gland secretion carries information on sex, and reproductive status (Buesching *et al.*, 2002). Males may therefore detect reproductive status by sniffing females, or indirectly through their scent-marks. Increased latrine use, corresponding to peaks in mounting behaviour, have been reported (Kruuk, 1978; Roper *et al.*, 1986; Revilla & Palomares, 2002). Use of boundary latrines (Pigozzi, 1990) and hinterland latrines (Roper *et al.*, 1993), specifically by females, increases around the peaks in mounting behaviour. Female object-marking behaviour around sett entrances (Buesching & Macdonald, 2004) also increases around the post-partum mating season in early spring. Female scent-marking behaviour has been described as a form of resource defence of breeding areas from other females and to a lesser extent as a method of conveying information on reproductive status to males (Buesching & Macdonald, 2004). We suggest that female object-marks may attract mates, thereby promoting promiscuity and sperm competition. Sperm competition may therefore play a role in female promiscuity in badgers, but it does not explain why mountings occur outside of the oestrous period nor does it explain repeated mountings.

5.5.3.3 Promoting genetic diversity and genetic compatibility

The Genetic Diversity and the Genetic Incompatibility Hypotheses propose that females will allow more than one male to mount them in order to mate with extra-group males that are dissimilar to both themselves and their social mates (Williams, 1975; Zeh & Zeh, 1994). Our kinship and pedigree analyses revealed that potentially incestuous matings did occur and that these were more likely between within-group than extra-group males. Kinship analyses suggested that 24% of the badger parent-pairs assigned with 80% were 1° kin and this corresponds to 27% (4 / 15) of the parent-pairs in Ethiopian wolves *Canis simensis* (Randall *et al.*, 2007). Potentially incestuous matings occurred between all classes of 1° kin (father–daughter, mother–son and full-siblings) at a rate of approximately 5%. This is greater than the 0.5% of matings that occurred between 1° kin in Soay sheep *Ovis aries* and 1% in red deer *Cervus elaphus* (Marshall *et al.*, 2002) and also slightly higher than the 3% in African elephants *Loxodonta africana* (Archie *et al.*, 2007), but lower than the 10% observed in the Arabian oryx *Oryx leucoryx* (Marshall *et al.*, 2002). As we did not measure inbreeding between all categories of 2° kin we do not compare these values to those in other species, although it is worth noting that these may produce different results. Females that were assigned maternity were less related to extra-group fathers than within-group fathers, both within multiple-paternity litters and overall. Moreover, inbred individuals had a lower lifetime breeding success than non-inbred individuals, suggesting a cost to inbreeding; however, this did not differ when only badgers for which all four grandparents were known, were analysed. It may therefore benefit females to avoid inbreeding. Permutation analyses showed that females that were assigned maternity were less related to both extra-group and neighbouring-group candidate fathers than they were to within-group candidate fathers. We only found a

significant relationship between the number of times that a female was mounted by a male and their pairwise relatedness in one social-group-year; however, this was not significant after FDR control and as not all mountings result in paternity this may obscure any relationship. By mating with extra-group males, females may therefore avoid inbreeding and this may promote genetic diversity and reduce genetic incompatibility.

Establishing the occurrence of inbreeding avoidance through mate choice patterns, requires determination of mating patterns that deviate from null models, such as random mating (Part, 1996). Evidence of inbreeding avoidance through mate choice has not been demonstrated in collared flycatchers *Ficedula albicollis* (Part, 1996), song sparrows *Melospiza melodia* (Keller & Arcese, 1998), or great reed warblers *Acrocephalus arundinacues* (Hansson *et al.*, 2007). In this study, the mean pairwise relatedness of assigned mates was greater than that of random candidate parents and assigned mothers in 2005, averaged across social groups, suggesting outbreeding avoidance. Several factors complicate the comparison of relatedness values from null models to observed relatedness, however, such as knowledge of which mates were available during mate choice (Part, 1996). As 94% of all paternities assigned with 95% confidence are to within-group or neighbouring-group males (Chapter 2), a more appropriate null model would only consider candidate fathers in the same or neighbouring group as the assigned mothers. This is particularly important given the clustering of relatives within and between neighbouring groups (Chapter 3).

The Genetic Diversity Hypothesis proposes that promiscuous mating should increase genetic diversity at the level of the litter (Williams, 1975), whereas with the Genetic

Incompatibility Hypothesis the proposed increase is at the level of the individual (Zeh & Zeh, 1994; Jennions, 1997; Jennions & Petrie, 2000). Analyses therefore need to be run at the level of the relatedness of the offspring to the mother and of the litter to the mother, to distinguish between these hypotheses (Cohas *et al.*, 2007). These hypotheses do not explain the occurrence of repeated mountings or mountings outside of the oestrous period.

5.5.3.4 Reducing male–male aggression and the risk of infanticide from males

One hypothesis may best explain the paradoxical mating behaviours of promiscuous and repeated mountings of female badgers and both the cooperative allogrooming behaviour observed between males and the low levels of male–male aggression. This hypothesis is that as all males mount this reduces the need for male–male aggression, and in return paternity confusion reduces the risk of infanticide from males (Bertram, 1975; Hrdy, 1979; Wolff & Macdonald, 2004). During bouts of activity in which mountings were observed and more than one male was present, it was common to observe aggression between the two males, but equally the two males allogroomed each other in a similar number of bouts. Males did not appear to mate-guard females from within-group males; when more than one within-group male mounted the same female on the same night, the male that mounted first was not observed to interact with the second male when he mounted; however, interactions may have occurred before or after the observed behaviour, outside of the field of view. Long-duration mountings may be a form of mate-guarding; however, this seems unlikely as when a female was mounted by a male for a long duration this male did not interact with a second male that mounted with the female soon after. Alternatively, males may

continue mounting well after ejaculation, as a form of mate guarding, in an attempt to maximise their likelihood of paternity. As ejaculation could not be detected, and mating order effects are unknown in badgers, this cannot be ruled out. As the majority of males were observed mounting, this may reduce the need for male–male aggression. Additionally, as males within social groups are related (Chapter 3), this may further reduce the need for male–male competition over access to mates.

Promiscuous mounting of females may therefore reduce the need for competition between within-group males; however, it is likely that competition does exist between within-group and extra-group males. Bite-wounding occurs in badgers and bite-wounds are more commonly seen in males than females (Macdonald *et al.*, 2004b; Delahay *et al.*, 2006b). The incidence of bite-wounds generally peaks around the post-partum mating period (Cresswell *et al.*, 1992; Delahay *et al.*, 2006b), although one study found no seasonal trend (Macdonald *et al.*, 2004b). Resident males were not present during the only observed mounting between a resident female and an extra-group male. Christian (1995) reported a resident male chasing an extra-group male from his territory, after which the resident male object-marked around his territory border. Object marking (Buesching & Macdonald, 2004), sequential allomarking (Buesching *et al.*, 2003) and the use of boundary latrines by males (Roper *et al.*, 1993) increase around the post-partum mating season in early spring, which may be a subtle form of mate-guarding within-group females from extra-group males. Additionally, extra-territorial ranging (Roper & Lüps, 1993) and expansion of a neighbouring male's territory (Revilla & Palomares, 1999) upon the deaths of resident males, have been inferred as attempts to gain access to females for mating. Overall, promiscuous

mounting of females may reduce male–male aggression, although subtle forms of mate-guarding, especially from extra-group males may occur.

Although infanticide has been suggested in the badger (Kruuk, 1989; Lüps & Roper, 1990; Cresswell *et al.*, 1992; Woodroffe & Macdonald, 1995b; Dugdale *et al.*, 2003) the evidence is circumstantial. The hypotheses for the occurrence of infanticide in mammals have been summarised by Ebensperger (1998). Infanticide may be a form of predation; Lüps & Roper (1990) report a cub in the stomach of a female road kill, however, the circumstances surrounding the cub's death were unknown. If male badgers do commit infanticide, which is feasible given the altricial state of cubs at birth, infanticide is unlikely to be an attempt to reduce paternal care, such as babysitting, as this does not occur in badgers (Chapter 6). Infanticide is unlikely to reduce the inter-birth interval as females give birth once a year, and although females do not always breed every year, females that were assigned maternity one year were significantly more likely to be assigned maternity in the following year (Chapter 4). A similar result was reported in Woodchester Park using teat data (Delahay *et al.*, 2006a). In years of low food availability, however, infanticide may decrease competition for food resources. If males do commit infanticide, females that are mounted promiscuously will obscure the paternity of their litters and may reduce the risk of infanticide from males (Bertram, 1975; Wolff & Macdonald, 2004). Oestradiol levels in urine (Service *et al.*, 2002) and the clustering of long-duration mountings within a three-day period, suggest that female badgers may have an oestrous period. By being mounted by males outside of this period (if males are unable to detect correctly the oestrous period) or being mounted by males for a short duration within

this period (if short-duration mountings are less likely to be successful), females may reduce the risk of infanticide from males, while masking paternity.

These hypotheses are not mutually exclusive and it is likely that more than one may play a role in the promiscuous and repeated mounting of female badgers. Genetic diversity and compatibility, along with sperm competition, may have played a part in the evolution of promiscuous mounting of females, but they do not explain the occurrence of repeated mountings and / or mountings outside of the oestrous period. Promiscuous mounting of female badgers is likely to have evolved originally as a strategy to reduce male–male aggression and infanticide from males. Once this strategy evolved, sperm competition and benefits from increased genetic diversity or genetic compatibility may be a factor in the occurrence of promiscuous mounting of females.

Cooperative breeding: alloparental care among breeding female badgers⁵

⁵ A modified version of this chapter is currently being prepared as: Dugdale HL, Ellwood SA, Pope LC, Burke T, Macdonald DW (in prep-c) Cooperative breeding: alloparental care among breeding female badgers *Meles meles*.

6.1 Abstract

Cooperative breeding occurs when individuals care for offspring of which they are not the genetic parents. We analysed the alloparental behaviours of group members during the cub-rearing period over three years, at two social groups each year, in a high-density population of the European badger *Meles meles*. We assigned both parents to all of the cubs in the filmed social groups, using 22 microsatellite loci. We provide the first evidence that female badgers that are assigned maternity may breed cooperatively. Breeding females babysat, allogroomed cubs without reciprocation, and allomarked cubs more than any other group-member did. When a breeding female was the only babysitter, 28% of their time was spent babysitting more cubs than their assigned litter size. Furthermore, two breeding females probably allonursed, as they suckled more cubs than the number to which they were assigned maternity. Breeding individuals spent less time digging at sett entrances than did other group-members, but contribution to bedding collection did not differ from that of other group-members. Mothers within a badger group therefore seem to breed cooperatively; however, no benefit was found in terms of litter size, probability of offspring breeding, or offspring lifetime breeding success, in groups with a higher proportion of mothers. If badger groups establish passively according to the Resource Dispersion Hypothesis, additional non-breeders may remain in the group without cost to breeding individuals. Cooperative breeding by mothers in badger social groups may represent a low-cost behaviour with indirect benefits due to high levels of relatedness between female group-members.

6.2 Introduction

Cooperative breeding refers to a social system where members of a group that are not the genetic parents provide care for offspring (Brown, 1987; Solomon & French, 1997). Alloparental care can come from breeding individuals caring for non-offspring, or from non-breeders. Where non-breeding individuals provide care, cooperative breeding requires the occurrence of delayed dispersal and reproductive suppression (Solomon & French, 1997) or failure of the non-breeder's reproductive attempt (Hatchwell *et al.*, 2004). Alloparental behaviour consists generally of direct interactions with offspring (König, 1997), such as: babysitting (banded mongoose *Mungos mungo*, Cant, 2003), grooming (red foxes *Vulpes vulpes*, Macdonald, 1979), playing (meerkats *Suricata suricatta*, Gilchrist, 2004), allosuckling (dwarf mongoose *Helogale parvula*, Creel *et al.*, 1991), infant carrying (moustached tamarins *Saguinus mystax*, Huck *et al.*, 2004), food provisioning (African wild dogs *Lycaon pictus*, Malcolm & Marten, 1982) and thermoregulatory assistance (monogamous marmots, Allaine, 2000). Additionally, care may be indirect in the form of maintenance of the breeding area, such as digging and bedding arrangement (pine voles *Microtus pinetorum*, Powell & Fried, 1992). Some of these behaviours, such as allosuckling, have obvious energetic costs, whereas others such as playing with cubs will have costs in terms of reduced time for other tasks such as foraging.

Cooperative breeding raises the question of why non-breeding individuals provide alloparental care rather than breeding themselves. Studies of cooperative breeding have been largely restricted to social groups that exhibit high reproductive skew, where generally only the dominant pair breeds, with fewer examples of low skew

societies in which reproduction is more evenly distributed (Macdonald *et al.*, 1987; Lewis & Pusey, 1997; de Luca & Ginsberg, 2001; Gilchrist, 2006). In reproductively skewed mammalian societies several hypotheses have been put forward to explain why non-breeding individuals provide alloparental care (summarised in: Riedman, 1982; Jennions & Macdonald, 1994; König, 1997). The proposed benefits include indirect fitness benefits (Hamilton, 1964), and direct benefits such as breeding experience (Brown, 1987; Komdeur, 1996), increased future probability of breeding (Kokko & Johnstone, 1999; Ragsdale, 1999), enhanced survival (Emlen *et al.*, 1991) or mutual benefits (König, 1997). Conversely, it has been argued that alloparental behaviour may be an unselected, stimulus driven response to the presence of young (Jamieson & Craig, 1987), but this is more likely to explain how alloparental behaviour first arose than account for its persistence in many mammalian populations (Emlen *et al.*, 1991). Functional cooperative behaviour has been reported in meerkats where parents rarely babysit young, but alloparents often do at considerable cost through reduced foraging (Clutton-Brock *et al.*, 1998), resulting in a positive long-term effect on the reproductive success of young (Russell *et al.*, 2007). Studies of other species, however, have not detected any benefit (Macdonald *et al.*, 2004a; Ebensperger *et al.*, 2007).

Our study investigates the contribution of social-group members to potentially cooperative breeding behaviours in a high-density population of the European badger *Meles meles*. In southern England, badgers breed once a year around February, social groups exhibit plural breeding and females have a mean litter size of 1.3–1.5 (Carpenter *et al.*, 2005; Chapter 2). Cubs are born underground, where they usually remain for their first eight weeks of life, and they are independent by around 15 weeks

(Neal & Cheeseman, 1996). Dispersal is restricted (Rogers *et al.*, 1998; Pope *et al.*, 2006) and social groups are maintained by natal philopatry (Cheeseman *et al.*, 1988; da Silva *et al.*, 1994; Macdonald *et al.*, submitted). Adults and yearlings within social groups are related (average $R = 0.2$) by less than assigned half-siblings ($R = 0.3$), but significantly more than unrelated individuals ($R = 0.0$, Chapter 3). Social groups exhibit a medium degree of reproductive skew, and skew in females is likely to be controlled by individual adaptation to local food availability and reproductive suppression through female–female aggression (Chapter 3). Badgers have a polygynandrous mating system, and approximately half of the cubs are sired by extra-group males (Carpenter *et al.*, 2005; Chapter 2). Linear dominance hierarchies have been established through analysis of aggressive interactions at sett entrances in three out of six studied social groups, with breeding females ranked highest (Appendix 2); however, no hierarchies were found at artificial feeding sites (Macdonald *et al.*, 2002). Cooperative breeding in badgers has been examined by Woodroffe (1993) who observed three females at one sett for ten hours. Two non-breeding females were observed grooming cubs and babysitting when the breeding female was not present, suggesting cooperative breeding (Woodroffe, 1993). Further observations are required, over longer periods, and at several social groups, which we aimed to provide in our study.

Throughout this chapter we use the term cooperative rather than communal breeding, as communal breeding implies shared parentage (Solomon & French, 1997) and although more than one female may breed within a badger social group (Carpenter *et al.*, 2005; Chapter 2) we do not know whether cubs are raised in the same nest

chamber. Additionally, we use the term alloparent rather than helper, to avoid fitness implications (Solomon & French, 1997).

For behaviour to be classified as helpful, it must increase the breeder's fitness. This has been inferred in other species through correlations of reproductive success with the number of alloparents (Macdonald, 1979; Macdonald & Moehlman, 1982; Jennions & Macdonald, 1994). These correlations can be confounded by other factors such as territory quality. Woodroffe and Macdonald (2000) demonstrated a positive relationship between the number of non-breeding females in a badger group and the number of yearlings produced, although this study did not control for the number of breeding females in the group. After controlling for territory quality, however, both the number of cubs surviving to weaning per breeding female and the number of yearlings produced within a group were negatively correlated with the number of non-breeding females (Woodroffe & Macdonald, 2000). A lack of a significant relationship between the number of females and group reproductive success, however, does not necessarily mean that alloparents do not benefit the group (Macdonald & Moehlman, 1982; Sillero-Zubiri *et al.*, 2004), especially if there are long-term advantages (Hodge, 2005; Russell *et al.*, 2007).

Sett maintenance behaviours such as bedding collection and digging are another form of cooperative behaviour in badgers (Stewart *et al.*, 1999). Only a minority of individuals carry out sett maintenance behaviour, with males digging significantly more than females, while bedding collection is equally distributed between the sexes (Stewart *et al.*, 1999). Furthermore, the males that dig are larger, older and more frequently copulating individuals (Stewart *et al.*, 1999).

We examine the frequency or duration with which group members perform the potentially cooperative behaviours of digging, bedding collection, babysitting, cub carrying, grooming of cubs, playing, non-offspring suckling and allomarking of cubs. These will be compared to parentage assignments (Chapter 2) and relatedness (Chapter 3), calculated from 22 microsatellite loci, to determine whether there is any relationship with breeding status or relatedness to the cubs in order to assess whether cooperative breeding occurs in badgers.

6.3 Materials and methods

6.3.1 Study site and population demography

We filmed from 1st February to 31st May, in 1995, 2004 and 2005, at two neighbouring social groups each year in Wytham Woods, Oxfordshire (01° 19'W, 51° 46'N). The woods are enclosed by a deer fence that contains most of the badger setts, and encompasses an area of 3.9 km² (Ellwood, 2006). The area consists primarily of deciduous woodland, surrounded by permanent pasture and mixed arable land (Kruuk, 1978). The highest density, between 1987 and 1996, was 44.3 badgers km⁻² (Macdonald & Newman, 2002).

6.3.2 Sample collection and genetic analyses

Trapping events generally took place at least four times a year, for one week in January, and two weeks in each of June, August and November (the methods are described in Tuytens *et al.*, 1999). Badgers were trapped, sedated and identified using

methods detailed elsewhere (Dugdale *et al.*, 2003). Blood or guard hair samples were collected for genetic analysis. Badgers were genotyped for 16–22 microsatellite loci: *Mel101–117* (Carpenter *et al.*, 2003), *Mel1* (Bijlsma *et al.*, 2000) and *Mel10*, *Mel12*, *Mel14* and *Mel15* (Domingo-Roura *et al.*, 2003). Samples were amplified, microsatellites analysed, parentage assigned with 80% and 95% confidence, and relatedness calculated using the methods described previously in Chapters 2 & 3. Briefly, parentage was assigned through a likelihood based approach using CERVUS 3.0.1.8 (Kalinowski *et al.*, 2007) and COLONY 1.2 (Wang, 2004), and relatedness (*R*) was estimate using RELATEDNESS 5.0.8 (Queller & Goodnight, 1989). All 24 cubs that were trapped at the filmed social groups, in the years that we filmed, were assigned a mother and a father (Table 6.1). Previous analyses revealed that the power to distinguish between the equivalent of full-siblings and unrelated individuals was high, whereas that between the equivalent of full-siblings and half-siblings, and half-siblings and unrelated individuals was low (Chapter 3). We therefore assessed whether breeding females could confidently be assigned as full-siblings or mother–offspring rather than unrelated using KINSHIP 1.3.1 (Goodnight & Queller, 1999).

6.3.3 Age of cubs

We calculated the mean implantation date of females residing in the two social groups that were filmed in the same year, using the formula calculated by Dunmartin *et al.* (1989) and the ultrasound methodology outlined elsewhere (Woodroffe, 1995; Dugdale *et al.*, 2003). Given that gestation in the badger has been reported to vary between 40 and 50 days (Canivenc, 1966; Neal & Cheeseman, 1996), we used a gestation period of 45 days to estimate the birth dates each year (Table 6.1). The first

day of the cubs' lives was recorded as day one, and the first week of the cubs' lives was recorded as week one.

Table 6.1 Composition of each social-group-year, showing the maximum number of badgers in each category that were seen on screen at the same time as cubs were seen on screen. P = Pasticks; SH = Sunday's Hill; PO = Pasticks Outlier.

Year	1995		2004		2005	
Mean birth date	04-Feb		17-Feb		03-Feb	
Social group	P	SH	P	PO	P	PO
Total observation time (hours)	1383	1242	2444	798	3872	1491
Total cub observation time (hours)	14	12	11	4	25	9
First emergence	13-Apr	26-Feb	14-Mar	23-Apr	09-Mar	25-Mar
Cubs	6	3	3	1	6	5
Adult breeding	4	2	2	1	4	2
females: non-breeding	4†	1^^	2**	3	1	1
Adult breeding ⁺	2†	1	0	1	0	0
males: non-breeding ⁺	4^†	6	2*(+1) [§]	1*	1*	2
Yearling: females	0	1	0	0	2	1
males	0	3	0	1	1	0
Unmarked (total seen on screen)	5	4	2	1	2	3
Badgers known to be unmarked	5 ^a	4 ^b	1 ^c	1 ^d	0	3 ^e
Group size (excl. cubs & incl. known unmarked badgers)	19	18	8	8	9	9

† One of these individuals was not clip-marked until May, but was included in the analyses as they babysat cubs in May.

* One additional badger was not present for all of the cub-rearing period, either because they were found dead ($n = 2$) or presumed dead as they were only seen for a maximum of 3 days and then were not seen again ($n = 3$). These badgers were excluded from the analyses and the group size estimate.

^ One more badger that was marked was present in this category, but it was not seen on screen, so they were excluded from analyses and the group size estimate.

§ One marked badger was only seen on one night when the cubs were also seen and was not seen at the same time as cubs, so was not included in the babysitting analyses but was included in the sett maintenance analysis and group size estimate.

⁺ Breeding male refers to the number that sired cubs within their own social group only.

The numbers of unmarked badgers were estimated by the maximum number seen on screen at any one time. Resident badgers known to be unmarked from trapping records are: ^a 5 non-breeding adult females; ^b 3 non-breeding adult males and 1 yearling female; ^c 1 non-breeding adult female; ^d 1 non-breeding adult male; and ^e 1 within social group father, 1 non-breeding adult male, and 1 breeding female. Marked badgers from neighbouring social groups were excluded from the analyses.

6.3.4 Behavioural analyses

Behavioural observations were made using infrared-sensitive remote video surveillance (Stewart *et al.*, 1997). Infrared illumination made observations possible in total darkness, at wavelengths of light undetectable by the mammalian eye (Lythgoe, 1979), minimising disturbance of the subjects. Data were collected continuously in 1995 (Stewart *et al.*, 1999) and in 2004 and 2005. Lost footage due to equipment failure was minimal and is unlikely to have had an effect on data recorded. Filming focused around active sett entrances, with up to three cameras per sett, and so our analyses are based on behaviours in these locations only. These fixed fields of view provide an additional problem in that some badgers may be rarely seen, although they are close by. We controlled for this in our analyses by including the number of times that each individual was seen on screen (in sett maintenance analyses only) or seen on screen with cubs (in the other analyses), in each field of view.

We analysed 960 video tapes corresponding to 319 calendar nights (11,230 hours in total). Adult and yearling badgers were identified through clip marks (Stewart & Macdonald, 1997). Cubs were not individually identifiable, as they could not be trapped until they were independent at around 15 weeks. The composition of each social-group-year is shown in Table 6.1. Unmarked badgers were recorded in a separate category, recording the maximum number seen on screen at any one time. Occasionally badgers with a clip mark could not be identified confidently, and these were recorded in a separate category as unidentifiable.

Behaviours were recorded either per incidence or per bout of activity. Bouts of badger activity commenced when the first badger appeared on screen and ended when there was at least sixty seconds without a badger on screen. We analysed footage from up to three weeks prior to cub birth and then up to and including the cub's seventeenth week of life; cubs are thought to be independent by 15 weeks (Neal & Cheeseman, 1996). The following potentially cooperative breeding behaviours were recorded:

6.3.4.1 Babysitting

Non-breeding females have been reported to babysit (Woodroffe, 1993). To analyse babysitting events we recorded the maximum number of cubs in the field of view (during a cub or cub and group-member record), along with the duration of time that cubs spent on their own (cub record) and with other non-cub group members (cub and group-member record). Records were continued if cubs went off screen and returned within ten seconds. If all group members left the field of view and the cubs followed within five seconds, a separate cub record was not made. The identities of all of the group members were recorded along with their closest proximity to the cub ($>$ one cub body length, $\leq$ one cub body length and physical contact), whether they interacted with the cub (yes / no), and whether the cub engaged with or retreated from them. We classified babysitting bouts as cub and group-member records in which group members interacted with the cub, group members moved to within at least one cub body length of the cub and the cub did not retreat from them. Additionally, we excluded records when more than two group members fulfilled these criteria, as these may correspond to social integration rather than babysitting. The total time that each individual babysat was summed over each week of the cubs' lives, with week one

being the first week of life. If an individual was seen on screen with cubs in a given week, but did not babysit, then we entered their babysitting time as zero; however, if they were not seen on screen with cubs no babysitting record was entered.

6.3.4.2 Suckling

Suckling, in badgers, is rarely seen above ground (Woodroffe, 1993; Neal & Cheeseman, 1996). We identified suckling if the cub's head was positioned at a nipple and, if a microphone was used, suckling had to be heard. Suckling females were generally laid on their back or side, or occasionally they were on their feet, in which case the cub's head was twisted upwards. A bout of suckling ended when the female moved away or when there was no suckling for at least 20 seconds. The identity of the female, the maximum number of cubs that suckled, and the total duration of the suckling bout were recorded.

6.3.4.3 Cub carrying

Group members may carry a cub (Woodroffe, 1993), usually by grasping the scruff of the cub in their mouth. The identity of the actor was recorded and a separate record made for each incidence. We considered each incidence of cub carrying to end when the actor left the field of view with the cub, the actor and cub went into the sett or the cub was left in the field of view and was not carried again by the actor in the same bout of badger activity.

6.3.4.4 *Unreciprocated allogrooming*

Unreciprocated allogrooming events (described by, Macdonald *et al.*, 2000; Stewart & Macdonald, 2003; Johnson *et al.*, 2004), in which a group member allogroomed a cub, were recorded once per cub and group-member record. The identity of the actor was recorded.

6.3.4.5 *Allomarking*

Sequential allomarking occurs when the actor lifts its tail and presses its anal region onto the body of the receiver (Buesching *et al.*, 2003). We recorded each sequential allomark between cubs and group members, along with the identity of the actor and the receiver.

6.3.4.6 *Playing*

Each group member that the cubs played with was recorded once per cub and group-member record. Playing involved the cub interacting with the group member in at least one of the following ways: nuzzling, biting their tail or ear, touching them with a paw, jumping backwards and forwards in front of the group member, climbing onto their back, or the cub rolling onto its back while interacting with the group member.

6.3.4.7 *Sett maintenance*

Sett maintenance behaviours (Stewart *et al.*, 1999) were classified as digging and bedding collection. The number of digging records were recorded such that each

record finished when the actor stopped digging and moved away, or moved back into the sett entrance to start another digging record. One bedding collection record was made each time an actor took bedding into a sett.

6.3.5 *Inter-observer reliability*

Behavioural footage were analysed by three research assistants who I trained, and seven volunteers who were trained by the four experienced researchers. Seventy percent of the tapes were analysed by the four experienced researchers. Individuals were first trained to score a catalogue of digitised behavioural clips. Following this, individuals were trained to analyse behavioural footage, on a one-to-one basis with an experienced researcher, for a minimum of two days. Individuals then analysed short clips of footage on their own, and these were then re-analysed in full by an experienced researcher. This continued for two weeks or until consistent scoring was seen. A random selection of tapes were reanalysed on a weekly basis, by different combinations of volunteers and experienced researchers to ensure consistency of scoring. In total 37% of the tapes were reanalysed by a different analyst to the original one, and a further 5% were reanalysed by the original analyst to correct errors picked up by the tape checking process.

6.3.6 *Statistical analyses*

Statistical analyses were conducted using SAS 9.1 (Littell *et al.*, 2006). We ran General and Generalized Linear Mixed Models (GLMMs) using the MIXED procedure for normally distributed responses and the GLIMMIX procedure with Poisson, negative binomial (NBD) or binomial error distributions (BED). GLMMs

enable the inclusion of fixed effects, random effects and repeated measures. Random effects control for variation between experimental units, whereas repeated measures model the covariance between pairs of observations taken on the same experimental unit at different times, which is important given that measures closer together in time are likely to be more highly correlated (Littell *et al.*, 2006). The most appropriate covariance structure was selected through graphical examination. Denominator degrees of freedom were calculated using the Kenward-Roger method (Littell *et al.*, 2006).

We analysed both the absolute number or duration of events and the relative number or duration of events. The absolute numbers of events were fitted to a NBD with a log link. The absolute durations of events were analysed in the MIXED procedure and were transformed, first adding a constant to move the minimum value to one when necessary (Osborne, 2002). We used log transformations, where appropriate, to improve conformation to the normality assumptions of the model; normality was examined using the Anderson-Darling test and by examining the histogram of residuals. MIXED models were selected according to their AIC. Relative contributions were analysed in the GLIMMIX procedure with BED and logit link. The number of times or length of time that an individual performed an event was fitted as the numerator and the total number of events or duration observed in that social-group-year as the denominator, including events by unmarked or unknown badgers. In BED models, each response data point (numerator) was weighted by its sample size (denominator), therefore controlling for unequal sample sizes (e.g. amount of babysitting observed in a social-group-year) and greater error variance with smaller samples. In the GLIMMIX procedure, the scale parameter is fixed at one with Poisson

and BED, whereas it is estimated with NBD. If the data are over-dispersed, however, setting the scale parameter to one can result in inflated test statistics, whereas under-dispersion leads to conservative estimates. Overdispersion was checked in the GLIMMIX procedure by comparing the Pearson statistic for the conditional distribution, calculated by Pearson-type residuals using the best linear unbiased predictors, to the estimate of residual dispersion. If the Pearson statistic was smaller than the estimate of residual dispersion a multiplicative overdispersion factor on the variance function was included (Littell *et al.*, 2006).

Behaviours were analysed per week, with the exception of cub carrying and suckling which were rare events. Cub carrying and suckling, as well as sett maintenance behaviours which showed no time-trend pattern over the study, were summed over the study period. Social group and badger identity were included in the models as random effects, as was badger identity within a social-group-year when analysing weekly occurrences. Random effects were removed when their variance estimate was negative. Badgers were categorised according to their sex, age and breeding status (male or female: adult breeder, adult non-breeder or yearling non-breeder); breeder refers to parentage of that year's within-group cubs. Unmarked or unidentifiable badgers were not included in the categories. Badger category was included as an independent variable, and in some analyses this was simplified to two categories of breeding female or other group-member. Average relatedness (R , Queller & Goodnight, 1989) to the cubs within the social group was included as a fixed effect in analyses that excluded breeding females. The repeated measure was badger identity within a social-group-year when analysing weekly occurrences, whereas for sett maintenance behaviours and cub carrying it was badger identity. Group size, number

of cubs and a measure that controlled for the likelihood of observing an event in the fixed field of view were included as covariates in all analyses. The number of cub and group-member records, in which an individual was observed, was used to control for the likelihood of seeing an event with cubs, and the number of bouts of activity in which the individual was observed was included in analyses of sett maintenance behaviour.

6.3.6.1 Babysitting

We ran a MIXED procedure with the absolute time each badger babysat per week as the response. We also ran a GLIMMIX procedure to analyse the relative babysitting time, with the numerator response as the time each individual babysat per week and the denominator as the total amount of babysitting observed in the social-group-year each week. The age of the cubs, in weeks, and the six categories of badger were included as fixed effects, as well as an interaction between them. Repeated measures of badger identity within a social-group-year were included with autoregressive covariance structure. An over-dispersion parameter was included in the relative babysitting analyses.

We investigated whether breeding females babysat more cubs than they were assigned maternity to, by counting the number and duration of babysitting bouts, and the maximum number of cubs in these, when the cubs were aged six to eleven weeks old, inclusive. We considered only events when one babysitter was present and we ran a Wilcoxon signed rank test on the paired differences. One social-group-year was

excluded from this analysis, as there was only one mother. We entered the mean times for females present in more than one social-group-year ($n = 2$).

6.3.6.2 *Cub carrying*

We compared the number of times that each individual was observed carrying a cub. Badgers that were seen on screen at the same time as cubs but that were never seen carrying cubs were recorded as having not carried cubs. For the absolute analysis we summed the number of carrying events observed by each individual as a response in a GLMM with NBD, while for the relative analysis the denominator was the total number of carrying events observed in that social-group-year. We entered badger category (breeding female or other group-member) as a class predictor. Social group was removed as a random factor in the absolute model, as its variance estimate was negative. Badger identity was entered as a repeated factor with compound symmetry covariance structure. An over-dispersion factor was included in the relative analysis.

6.3.6.3 *Unreciprocated allogrooming, allomarking and playing*

We fitted the number of cub and group-member records in which an individual allogroomed (without reciprocation), sequentially allomarked or played with cubs as the responses with NBD. Individuals were either breeding females or other group-members. Social group (in the allogrooming and playing analyses) and badger identity (allomarking and playing analyses) had negative estimates of variance and were removed from the absolute models. Likewise, social group was removed from the relative model in the allomarking analysis. Badger identity within a social-group-year was included as a repeated effect with compound symmetry covariance structure in all

of these analyses, except for the allomarking absolute model where a heterogeneous compound symmetry covariance structure was used.

6.3.6.4 Sett maintenance

We analysed the total number of digging and bedding collections that each of the six categories of badger were observed to do over the entire study period, and also the number of bedding collections that males versus females were observed to undertake. We analysed the absolute contribution with NBD. Social group was included as a random factor and badger identity as a repeated factor with compound symmetry covariance structure. An over-dispersion factor was included in the relative analysis.

6.3.6.5 Does alloparenting by breeding females provide fitness benefits?

We investigated whether the number of mothers within a social group influenced their average litter size, using maternity assignments from 1988–2005 (Chapter 2). We analysed social groups in which all cubs were assigned a mother, with both 80% and 95% confidence, and in all social groups including those where not all of the cubs were assigned a mother, at both confidence levels. We entered the average litter size of the group as the response, fitted to a Poisson distribution with log link, and the year and number of mothers in the group as fixed effects. Social group was entered as a repeated factor with auto-regressive covariance structure. We also analysed whether the lifetime breeding success of a cub was related to the number of mothers present in the cub's natal social group when the cub was born. Lifetime breeding success was calculated for badgers that were first trapped as a cub and that were considered dead by the end of 2005, using parentage assignments with 80% confidence. We ran a

GLMM with Poisson error distribution, log link, birth year as a fixed effect, natal social group as a random effect, and natal social-group-year as a repeated factor with compound symmetry covariance structure. Natal social group had a negative variance estimate in the analysis of all social groups and was removed from the model. We also investigated whether the number of mothers in a group influenced the probability of a cub breeding. The analyses were exactly as in the previous models, except that the models had BED with a response numerator of did or did not breed (one or zero) and a response denominator of one.

6.4 Results

Cub behaviour was analysed over a total of 128 hours, of which 41% had only cubs on screen and 59% had cubs and other group-members on screen. Of the 75 hours that cubs were present with other group-members, 70 hours were with group members that interacted with the cubs and that the cubs did not retreat from, and furthermore, the group members were within at least one cub body length of the cubs. Considering the 70 hours that cubs were on screen with potential babysitters, 58% of this time cubs were with one group member, 20% with two, 10% with three, 7% with four and the remainder were with between five and nine group members. Babysitting events were classified as times when only one or two group members were with the cubs, which totalled 41 hours with one babysitter and 55 hours with one or two babysitters.

6.4.1 Babysitting

Observations of babysitting events up to week seven were sparse (five by breeding females, one by a non-breeding adult female, and one by an unidentifiable badger; Figure 6.1a & 6.1b). Cubs do not generally emerge until week eight (Neal & Cheeseman, 1996), therefore, we first analysed babysitting from week seven, when babysitting was first seen regularly. Female breeders spent more time babysitting than any other individual from the other age / sex / reproductive-status categories up until the cubs were in their fourteenth week, when they were the only babysitters on screen (Figure 6.1a) or when there were up to two babysitters (Figure 6.1b). Lactation lasts approximately 12 weeks (Neal & Harrison, 1958), and cubs are independent at around 15 weeks (Neal & Cheeseman, 1996). Given the greater babysitting seen by breeding females up to 14 weeks, we therefore analysed cub-rearing behaviours up to and including week 13.

Badger category had a significant effect on the amount of time spent babysitting each week ($F_{5,96.9} = 4.4$, $p = 0.0013$), but there was no overall significant interaction between category and week ($F_{5,140} = 1.7$, $p = 0.13$; Figure 6.1a). The same results were observed examining babysitting with up to two babysitters present on screen ($F_{5,52} = 3.5$, $p = 0.0080$; $F_{5,149} = 1.1$, $p = 0.34$, respectively; Figure 6.1b). There was a significant effect of relative contribution to babysitting with badger category ($F_{5,70.1} = 4.1$, $p = 0.0024$; $F_{5,66.3} = 6.1$, $p < 0.0001$) and a significant interaction between badger category and week ($F_{5,129.5} = 5.4$, $p = 0.0001$; $F_{5,219.4} = 6.5$, $p < 0.0001$) for babysitting events with one babysitter or up to two babysitters, respectively.

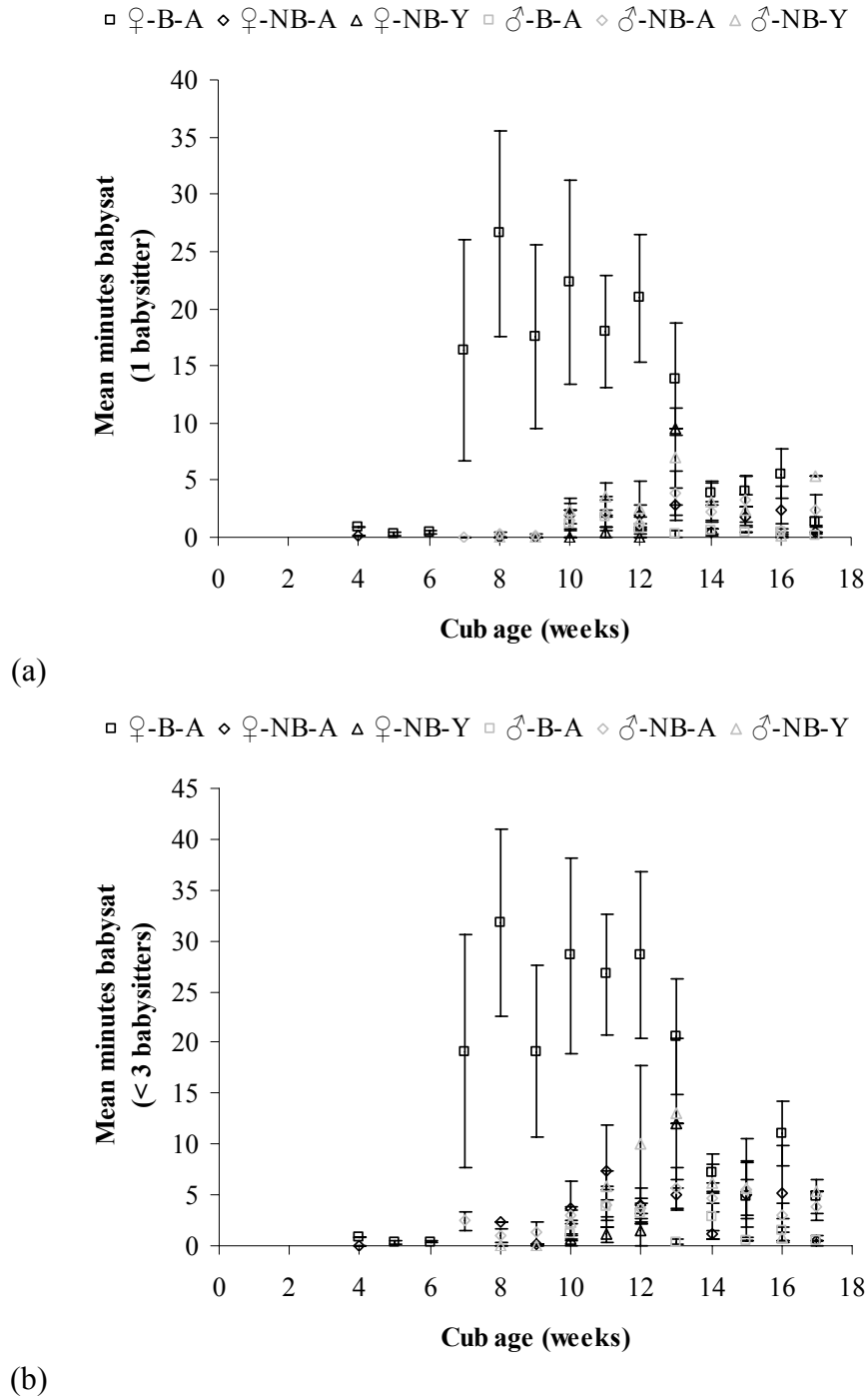


Figure 6.1 The mean number of minutes for which an individual in each category was observed (a) as the only babysitter on screen, or (b) as the only babysitter or with one other babysitter on screen, against the age of the cubs in weeks. B = breeder; NB = non-breeder; A = adult; Y = yearling. Error bars display $\pm$ one standard error. There were only two weeks in which female non-breeding yearlings babysat cubs.

We then investigated absolute and relative babysitting levels once breeding females were excluded when the cubs were up to 14 weeks old. Neither absolute nor relative babysitting levels were related to badger category (number of individuals in each category was included as a covariate: $F_{4,21.6} = 0.71, p = 0.60$; $F_{4,5.0} = 0.22, p = 0.92$) or average relatedness to cubs within the group ($F_{1,5.0} = 0.0, p = 0.91$; $F_{1,3.7} = 0.13, p = 0.73$), respectively when there was only one babysitter. The same results were seen when there were up to two babysitters present, in relation to the category of badger (absolute, $F_{4,22.5} = 0.05, p = 0.99$; relative, $F_{4,22.3} = 0.39, p = 0.81$) and average relatedness to cubs (absolute, $F_{1,19} = 0.74, p = 0.40$; relative, $F_{1,18.6} = 0.44, p = 0.52$).

Considering babysitting bouts with only one babysitter, breeding females babysat significantly more often when the maximum number of cubs on screen, during a bout, was less than or equal to their litter size ($72 \pm 6\%$ compared to $28 \pm 6\%$ of bouts where there were more cubs than they were assigned maternity to; $S_{10} = 26, p = 0.0059$). The total amount of time when a breeding female was the only babysitter, and when the maximum number of cubs equalled their litter size or less (mean = 74 ± 32 minutes), was not significantly different to the time they babysat for when the maximum number of cubs on screen was greater than their litter size (mean = 40 ± 20 minutes; $S_{10} = 21, p = 0.0674$). The low p -value, however, dictates that this result was marginal.

6.4.2 Suckling

We observed 23 occurrences of suckling, all of which were by breeding females in April 2004 (weeks 8–10) and April 2005 (weeks 9–13). Suckling lasted a mean of 1.6 ± 0.5 minutes, or 1.1 ± 0.6 minutes (taking the mean duration of suckling per mother, $n = 5$). Two females suckled more cubs than they were assigned maternity to, we refer to this as potential allonursing. One female suckled two to four cubs, on four occasions, but had a litter size of one; she had negative LOD scores for four of the other cubs within the social-group-year and a low positive score for the other cub, but five other females in the group had a higher LOD score for this cub. LOD scores were obtained from the parentage analyses. A negative LOD score suggests that the individual is less likely to be the parent of the offspring than a randomly selected individual; the most likely parent has the highest positive score (Marshall et al., 1998). A second female was observed suckling three cubs, once, when her litter size was two; she had negative LOD scores for all of the other four cubs within that social-group-year.

6.4.3 Cub carrying

We observed 186 cub-carrying events between week four and 16 (Figure 6.2), by: breeding females (72%), unmarked badgers (14%), male non-breeding adults (6%), male non-breeding yearlings (4%), female non-breeding adults (2%) and unidentifiable badgers (2%). Males were first observed carrying cubs at week 10, and this tended to be during play, possibly as an assertion of dominance. This was distinctive to cub carrying by females which was seen from week four whereby cubs were generally carried in and out of sett entrances. Breeding females carried cubs

significantly more than other group-members, over the entire study period, in absolute terms ($F_{1,39.6} = 5.7, p = 0.0215$), although the relative difference was not significant ($F_{1,43.7} = 2.6, p = 0.11$).

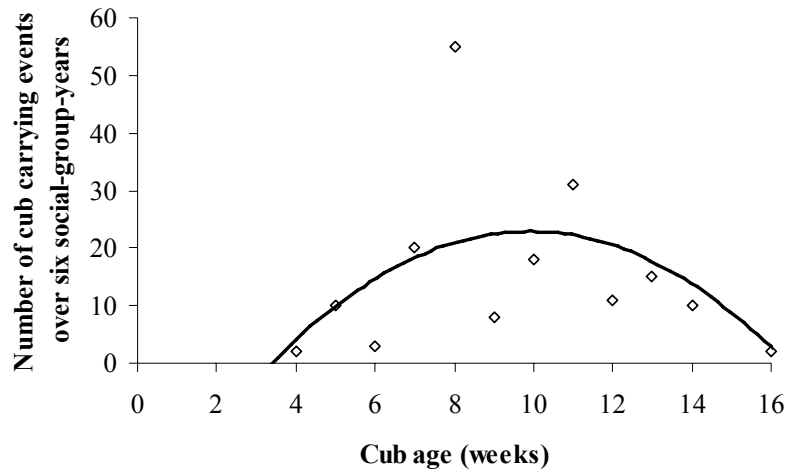


Figure 6.2 The total number of cub carrying events observed over six social-group-years, against the age of the cubs in weeks. The trend line equation is $y = -0.5415x^2 + 10.719x - 30.136$, $r^2 = 0.34$.

6.4.4 Unreciprocated allogrooming, allomarking and playing

Breeding females allogroomed cubs without reciprocation, sequentially allomarked cubs and played with cubs more than other group-members between weeks seven and thirteen, inclusive ($F_{1,7.0} = 14.7, p = 0.0064$; $F_{1,25.7} = 5.5, p = 0.0266$; $F_{1,35.9} = 3.7, p = 0.0624$; Figure 6.3). The interaction between badger category and week was not significant ($F_{1,117.3} = 1.3, p = 0.25$; $F_{1,29.9} = 3.6, p = 0.0672$; $F_{1,176.6} = 2.4, p = 0.13$, respectively). The relative contribution of breeding females to unreciprocated allogrooming, allomarking and playing with week was greater than that of other group-members ($F_{1,46.6} = 31.2, p < 0.0001$; $F_{1,47.4} = 12.3, p = 0.0010$; $F_{1,44.9} = 11.2, p = 0.0016$) and the relative proportion decreased for breeding females over the weeks

($F_{1,173.8} = 8.3, p = 0.0045$; $F_{1,173.8} = 10.7, p = 0.0013$; $F_{1,163.1} = 10.1, p = 0.0018$).

6.4.5 *Sett maintenance*

Female breeding badgers performed the most bedding collection (Figure 6.4a); however, the absolute ($F_{5,18.0} = 0.8, p = 0.58$) and relative ($F_{5,42.4} = 1.3, p = 0.27$) contributions to bedding collection did not differ significantly between the six categories of badgers. The absolute contributions did not differ between the sexes ($F_{1,23.2} = 0.2, p = 0.69$), and although the relative contribution of females was not significantly greater than males, the p -value was low ($F_{1,37.4} = 3.4, p = 0.0718$). There was a significant effect of category on the absolute ($F_{5,13.4} = 6.5, p = 0.0028$) and relative ($F_{5,44.1} = 2.8, p = 0.0297$) contribution to digging, with breeding individuals of both sexes digging the least (Figure 6.4b).

6.4.5.1 *Does alloparenting by breeding females provide fitness benefits?*

The mean litter size within a social group was not related to the number of females that were assigned maternity within a group, when groups in which all cubs were assigned a mother were analysed ($F_{1,149.9} = 0.8, p = 0.36$, with 80%; $F_{1,42.6} = 1.5, p = 0.23$, with 95% confidence). Similar results were obtained when we analysed all social groups in which at least one mother was assigned ($F_{1,218.9} = 0.5, p = 0.48$, with 80%; $F_{1,163.9} = 0.3, p = 0.57$, with 95% confidence). The number of mothers in a cub's social group was not related to the lifetime breeding success of a cub, analysing groups in which all cubs were assigned a mother ($F_{1,19.9} = 0.05, p = 0.83$) or in all social groups ($F_{1,300.6} = 0.04, p = 0.84$); neither was it related to the probability of a cub breeding ($F_{1,38.2} = 1.26, p = 0.27$, or $F_{1,297.1} = 0.00, p = 0.95$, respectively).

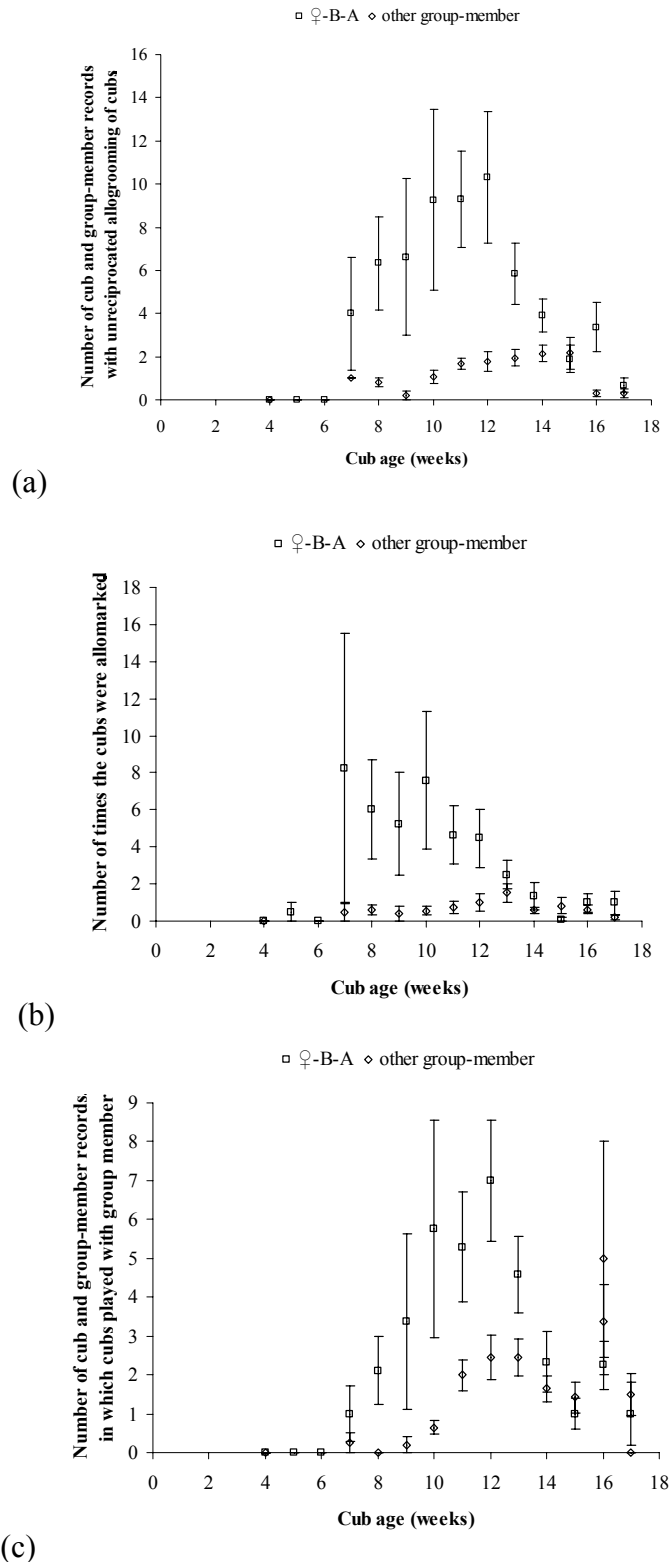


Figure 6.3 The mean number of: (a) cub and group-member records in which group members allogroomed a cub, without reciprocation; (b) times group members allomarked a cub; and (c) cub and group-member records in which cubs played with group members. Group members were classified as breeding females or other group-members. Error bars display $\pm$ one standard error of the mean. B = breeder; A = adult.

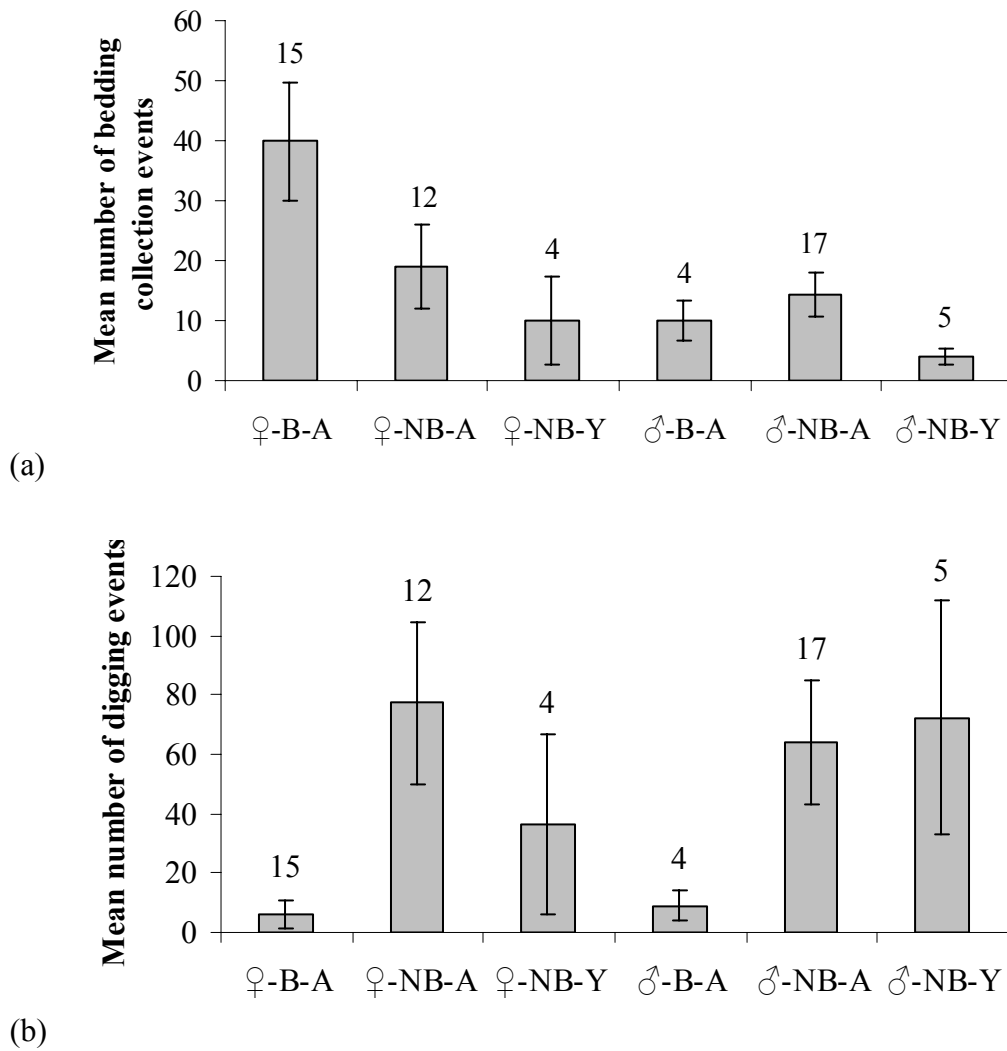


Figure 6.4 The mean number of times each category of badger was observed (a) collecting bedding, and (b) digging, over the study period. Error bars display $\pm$ one standard error of the mean. The numbers above the bars represent the number of individuals on which the means were obtained; some individuals were present in two years and therefore they are repeated in these datasets. B = breeder; NB = non-breeder; A = adult; Y = yearling.

6.4.6 Kinship of within-group breeding females

Five social-group-years contained more than one breeding female, and three of these contained breeding females that were all significantly more likely to represent dyads of full-siblings, or equivalent, than unrelated individuals (Table 6.2). The potential allonursing events were observed in one of these social groups (P 2005). Of the 16

breeding females, 10 were first trapped as adults and therefore parents could not be assigned to them. All of the other six females had both parents assigned; however, only one group (P 2005) had more than one of these breeding females and none of these was assigned the same parent.

Table 6.2 Number of dyads of breeding females within each of the six filmed social-group-years that were significantly more likely to represent a dyad of full-siblings or parent–offspring than unrelated individuals. NS = not significant.

Year	Social group	Number of dyads	Number significant $p < 0.05$	$p < 0.01$	$p < 0.001$	Total significant	Total NS	% significant
1995	SH	1	0	1	0	1	0	100%
1995	P	6	0	1	1	2	4	33%
2004	PO	0	–	–	–	–	–	–
2004	P	1	0	1	0	1	0	100%
2005	PO	3*	0	0	1	1	2	33%
2005	P	6	3	2	1	6	0	100%

* One breeding female in PO 2005 was unmarked.

6.5 Discussion

6.5.1 Who cares for cubs?

Breeding females babysat significantly more often throughout the cub-rearing period than did other group-members, controlling for time that individuals were observed on screen with cubs. Additionally, breeding females allogroomed cubs without reciprocation and allomarked cubs significantly more often than other group-members during the cub-rearing period. Furthermore, when there was only one babysitter, breeding females spent significantly more babysitting bouts with a number of cubs equivalent to, or less than, their own litter size, but they also babysat more cubs than the number to which they were assigned maternity. The total time that they babysat groups up to the size of their own litter did not differ significantly to the total time that

they babysat more cubs than the number to which they were assigned (although the p -value was low). Furthermore, although observations of suckling were rare, allonursing was potentially observed by two breeding females; however, suckling durations were short and observation of suckling does not necessarily mean that milk has been transferred (Cameron, 1998), nor that it is adaptive (Hayes, 2000). Overall, this suggests that breeding females perform the majority of the cub-rearing behaviours, but that this may benefit non-offspring as well as genetic offspring. Although group members other than non-breeding females babysat, allogroomed cubs (without reciprocation), and allomarked cubs, the duration and frequency of these behaviours represented a small proportion of the group total and may purely represent social integration of the cubs into the social group. The babysitting contribution of other group-members did not differ according to either their age / sex / reproductive-status category or their average relatedness to the cubs within their social group, controlling for the amount of time they were observed on screen with the cubs.

Breeding females spent an average of 28% of their babysitting time, when they were the only babysitter, with more cubs than the number to which they were assigned maternity, and two females potentially allonursed. For ethical reasons cubs were not trapped and marked until they were considered independent. Cubs were therefore not individually identifiable and parentage could only be assigned for those that were trapped after independence. It is possible that some cubs may have died before independence but the rate of pre-emergence mortality is unknown. The maximum number of cubs observed on screen was never greater than the maximum number of trapped cubs except on one occasion. Here, one extra cub was seen once at SH on the last day of week 13 in 1995 meaning that one cub may not have been trapped or as it

was at the end of the cub-rearing period it may have been a neighbouring cub. The general pattern, however, suggests that it is unlikely that cubs surviving to emergence died before they were trapped.

The fact that cubs were initially unidentifiable limits the conclusions of this study, but our data do suggest that breeding females may breed cooperatively, and care for non-offspring. Badger biology provides a possible explanation for this lack of differentiation: badger cubs do not produce sub-caudal odour secretion until they are between four and six months old (Buesching, 2000) and so breeding females may not be able to differentiate between own and non-offspring using this scent. Cubs from more than one breeding female may be raised in the same sett, but whether the same nest chamber is used by more than one breeding female is unknown. If they are kept in the same nest chamber then this may hinder detection of own-offspring. This has been observed in breeding female degu *Octodon degus* that breed cooperatively, and raise offspring in a communal nest (Ebensperger *et al.*, 2007). Future behavioural observations of marked offspring and of behaviour underground will improve our understanding of breeding behaviour in badgers. In particular, as allonursing is potentially costly (Lewis & Pusey, 1997), further investigation into the frequency with which this occurs in badgers, and factors that affect the likelihood of a female allonursing, such as litter size, relatedness to non-offspring and presence of own offspring (Pusey & Packer, 1994), are required.

A previous study suggested that cubs spent significantly different amounts of times with different age / sex classes of badgers in our study population (Fell *et al.*, 2006). Although, Fell *et al.* (2006) come to similar conclusions as us, in that breeding

females contribute the most to cub-rearing, their data sets were not independent, they did not have parentage data, did not observe suckling behaviour, nor have the advantage of the larger dataset at our disposal. We therefore consider ours the first study to reveal that breeding female badgers may rear young cooperatively.

6.5.1.1 Does alloparenting by breeding females provide fitness benefits?

To classify breeding females caring for non-offspring as cooperative breeding, there must be some measurable benefit to this alloparental behaviour. Woodroffe (1993) previously reported that non-breeding females babysat. Woodroffe and Macdonald (2000), however, suggested that this did not constitute alloparenting as the increase in group productivity of yearlings with the number of non-breeding female alloparents, was an artefact of territory quality (Woodroffe & Macdonald, 2000). Our study indicates that breeding females contribute significantly more to raising cubs than other group-members, and that this may include non-offspring. We therefore investigated whether the average litter size of females increased when there were more breeding females within a group; however, there was no significant relationship. Not all studies have shown relationships between the number of alloparents and short-term measures of reproductive success (Legge, 2000; Hatchwell *et al.*, 2004; Macdonald *et al.*, 2004a; Ebensperger *et al.*, 2007). Longer-term measures have been investigated in banded mongooses, where alloparents were shown to reduce the age at which offspring first breed (Hodge, 2005), and in meerkats, where alloparents increase the likelihood of offspring obtaining breeding status (Russell *et al.*, 2007).

To investigate long-term benefits we also tested whether the number of mothers in a cub's natal social group, at birth, was related to the cub's lifetime breeding success, but the relationship was not significant. Although we have not been able to demonstrate that the potential alloparenting of non-offspring by breeding females is beneficial, there may be other benefits. For example, alloparenting may also increase the survival of breeding females (Allaine & Theuriau, 2004). Lactating females are lighter than non-lactating females at the end of the cub-rearing season (Woodroffe & Macdonald, 1995a). Even though this difference disappears later in the year, two-year olds that lactate have a higher mortality rate than two-year olds that do not lactate (Woodroffe & Macdonald, 1995a). Alloparenting may also affect maternal weight which influences maternal fecundity (Russell *et al.*, 2003). In badgers, body condition affects whether female badgers breed in years of poor resource availability (Woodroffe & Macdonald, 1995b) and also affects implantation date (Woodroffe, 1995; Dugdale *et al.*, 2003). By sharing babysitting duties, breeding females may increase their potential foraging time and this may increase the resources that they can provide to offspring, perhaps increasing cub weight at independence, or increase their own survival or future reproductive success. Overall, alloparental behaviour is associated with costly reproduction (Creel & Macdonald, 1995), and it may be that the alloparental care in badgers is at a primitive stage, in evolutionary terms. Alloparental care may vary on a continuum from that seen in badgers, farm cats *Felis catus* (Macdonald *et al.*, 1987) and lions *Panthera leo* (Packer *et al.*, 2001), where alloparenting behaviour is marginal, through to canids (Macdonald *et al.*, 2004a) and meerkats (Clutton-Brock *et al.*, 1998) where alloparental care is regularly important and life-saving.

6.5.2 Why allow non-breeders to remain in the group?

If non-breeding individuals do not care for offspring, then why are non-breeding individuals allowed to remain in the group? Dominance hierarchies were not found in badgers, when behaviour at artificial feeding sites, located on social-group boundaries, was analysed (Macdonald *et al.*, 2002). One study, however, found linear dominance hierarchies in three out of six social groups, when aggressive dyadic interactions were analysed around sett entrances, with breeding females ranking highest (Appendix 2). Whether breeding females are able to control group membership is unknown. The Resource Dispersion Hypothesis (RDH) proposes that when resources are spaced patchily in the environment, the minimum defendable territory that provides sufficient resources for a minimum social unit may also accommodate more individuals (Macdonald, 1983; Carr & Macdonald, 1986). The RDH is difficult to test, however, while one study failed to reject it as a mechanism for group formation in badgers (Johnson *et al.*, 2001c) another found support (Johnson *et al.*, 2001a). As such, it may not be costly to breeding individuals, if non-breeding individuals reside in the group without alloparenting. Additionally, badgers that breed once are only assigned parentage in a third of the years in which they are potential parents, indicating a high cost to reproduction in both sexes (Chapter 4). It may be that non-breeding individuals are simply biding their time until they can afford to reproduce and so alloparenting, by non-breeding individuals, may not be selected (i.e. if the costs of tolerance are minimal, the rewards need not be great). The high-relatedness of female group-members (Chapter 3) and the potential low cost to babysitting additional cubs on top of their own may lead to alloparental behaviour between breeding females.

Overall, non-breeding individuals may contribute towards the social group through cooperative behaviours such as sett maintenance. Our analyses of sett maintenance behaviours suggest that non-breeding individuals contribute significantly more to digging during the cub-rearing season. Additionally, all group members contributed to bedding collection. These results are similar to those reported by Stewart *et al.* (1999). The finding that breeding males dig less than non-breeding males is slightly at odds with Stewart *et al.* (1999); however, breeding males were those that successfully mated the previous year, whereas Stewart *et al.* (1999) found that digging was more common in large, frequently copulating males. We did not observe a sex bias in digging behaviour; however, we only observed behaviour over a short-time period compared to the 20 months that Stewart *et al.* (1999) analysed. The cub-rearing period that we analysed also equates to the post-partum mating period, which may affect digging behaviour.

In conclusion, we have shown that breeding females potentially care for non-offspring, through babysitting and allonursing. It is also likely that breeding females allogroom and allomark non-offspring, but as cubs were unmarked we could not confirm this; however, breeding females performed the majority of these cub-rearing behaviours. These alloparental behaviours may have low-costs to breeding females that are already looking after their own young, compared to non-breeding females that may better spend their time acquiring resources to improve their likelihood of breeding themselves the next year. High relatedness between female group members may then provide indirect benefits to cooperative cub-rearing behaviours by breeding females. Further studies are required to establish the extent of such behaviours.

Discussion

7.1 Abstract

The evolution of sociality can be divided into factors that lead to the formation of groups and those that lead members of those groups to behave cooperatively (and socially). This thesis has focused upon the latter, in particular investigating factors that influence whether badgers stay in their natal group or disperse, and the reproductive trade-off between breeding now, or later. Although plural breeding occurred within badger groups there were potential costs to group living, as a low proportion of candidate parents were assigned parentage each year resulting in reproductive skew within social groups, and high-density populations were associated with reduced litter sizes. Social groups corresponded to groups of relatives, suggesting that dispersal may be required to prevent inbreeding, which was associated with a reduction in lifetime breeding success. Motivation to disperse, however, may be reduced through the occurrence of extra-group paternities which are a potential strategy to avoid or reduce inbreeding. Concerning the trade-off between breeding now or later, individuals were selected for an early age of first breeding (α) and a late age of last breeding (ω); however, there was a positive phenotypic correlation between these traits. Reproductive skew within badger groups probably resulted from limited resource availability. As groups consisted of relatives, with greater relatedness within than between groups, I investigated whether kin selection, in the form of cooperative breeding, occurred. Non-breeding individuals did not alloparent, but females that were assigned maternity probably did breed cooperatively. I did not detect any fitness benefits to cooperative breeding; however, alloparental care may represent a low-cost behaviour that is facilitated by the high relatedness among within-group females.

7.2 Reproductive trade-offs and the evolution of social behaviour

Individuals may pass on their genes either through direct reproduction, or indirectly by helping relatives to breed (Hamilton, 1964). The breeding strategy that an individual adopts should be the one that maximises their lifetime breeding success (LBS: Hamilton, 1964; Williams, 1966; Pianka, 1976). Individuals living in social groups are therefore faced with the options of whether to breed in their natal group or to disperse; whether to breed now or breed later; and whether or not to help their relatives to breed. The decisions that they make may then influence the evolution of social behaviour. In this thesis, I investigated how the mating system and social structure may affect the evolution of social behaviour in a high-density population of group-living badgers.

7.2.1 *Should I stay or should I go?*

The decision of whether to remain natally philopatric or to disperse may be influenced by ecological constraints (Emlen, 1982), benefits of philopatry (Stacey & Ligon, 1991), life-history traits (Arnold & Owens, 1998), or a combination of these factors (Hatchwell & Komdeur, 2000).

Badger groups contain 1° kin, such that overall there is high relatedness among within-group members, suggesting that dispersal is restricted (Chapter 3). These results correspond to those from analyses of trapping data that suggest badgers are natally philopatric and that dispersal is restricted (Cheeseman *et al.*, 1988; da Silva *et al.*, 1994; Macdonald *et al.*, submitted). There may be reproductive costs to group

living for both sexes as only approximately 30% of the candidate parents are assigned parentage each year (Chapter 2). Furthermore, although plural breeding is observed within groups, both behaviourally (Chapter 5 & 6) and genetically (Chapter 2), reproductive skew does occur (Chapter 3). Additionally, badgers in high-density populations have smaller litter sizes than those in lower-density populations (Chapter 2); however, further research is needed in low-density populations to substantiate this.

These results suggest that badgers should disperse to avoid factors such as reproductive conflict (Keller & Reeve, 1994; Reeve *et al.*, 1998) or competition between relatives (West *et al.*, 2001; West *et al.*, 2002), which may lower reproductive success, as well as avoiding possible fitness costs of breeding with a relative (Charlesworth & Charlesworth, 1987). There may, however, be ecological constraints on dispersal, such as: (1) prohibitive costs of individuals rearing offspring on their own; (2) a reduced chance of finding a mate; (3) a high mortality risk; or (4) habitat saturation (Emlen, 1982). Firstly, badgers do not require alloparental care in order to raise offspring, as they live singly in parts of their range (Pigozzi, 1990; Woodroffe & Macdonald, 1993; Tanaka *et al.*, 2002), so the costs of raising offspring are unlikely to constrain dispersal. Secondly, in the high-density populations around south-west England, the chance of finding a mate is unlikely to preclude dispersal. Thirdly, although the mortality risks of dispersal have not been widely investigated in badgers, there are costs associated with dispersal as females endured higher rates of bite-wounding during recolonisation (Delahay *et al.*, 2006b) and female badgers that dispersed failed to produce cubs (da Silva *et al.*, 1993). The availability of sett sites is not thought to be a limiting factor for badgers, and although the quality of setts does vary there does not appear to be a correlation between sett age and quality

(Macdonald *et al.*, 2004c). While sett sites may not be limiting, the costs incurred by digging a sett may limit dispersal.

The Benefits of Philopatry Hypothesis (Stacey & Ligon, 1991) turns the Ecological Constraints Hypothesis (Emlen, 1982) on its head and proposes that the benefits of remaining in the natal group (rather than the costs of dispersal) may reduce the likelihood of dispersal. In this way, badgers remaining in their natal territory are predicted to gain benefits from familiarity of the territory, and possibly benefits from access to a presumably high-quality territory, given that it is possible to raise offspring within it. As the quality of badger territories is known to vary (Macdonald *et al.*, 2004c), benefits of philopatry, such as a high-quality natal group, are a possibility in badgers.

The Life History Hypothesis (Arnold & Owens, 1998) predicts that certain life-history traits, such as low rates of annual mortality, predispose individuals to remain in groups. This may certainly apply to badgers given their longevity (Macdonald & Newman, 2002). Ecological factors may then further reduce population turnover and opportunities for independent breeding.

The Broad Constraints Hypothesis (Hatchwell & Komdeur, 2000) highlights that the three hypotheses (Ecological Constraints, Benefits of Philopatry and Life History) are not mutually exclusive and proposes that all of these factors may play a role in the decision of whether or not to disperse. This seems plausible in badgers, as their longevity, benefits from remaining in a natal territory and constraints on establishing a new territory may all affect the decision of whether or not to disperse.

Many of the above examples rely on correlational relationships. Ideally, one would relax the ecological constraints or benefits of philopatry, to investigate whether individuals then disperse to breed elsewhere. In wild populations, especially those that are protected, this can be difficult; however, the badger culling trial provided just such an opportunity to relieve ecological constraints on dispersal. In perturbed populations, social group ranges became enlarged and overlapped considerably (Tuytens *et al.*, 2000a; Woodroffe *et al.*, 2006), as has also been observed in populations where poaching occurred (Roper & Lüps, 1993; Revilla & Palomares, 1999). After culling, initial recolonisation was almost exclusively by females (Tuytens *et al.*, 2000a; Tuytens *et al.*, 2000b), and no cubs were born in social groups following a cull, even though they had been recolonised (Tuytens *et al.*, 2000b). These culling results are consistent with the suggestion that badgers may decide not to disperse in natural populations, due to: (1) low mortality rates resulting in a surplus of individuals in the population; (2) ecological constraints, in terms of mortality risk or habitat saturation; or (3) benefits of natal territory familiarity or of remaining in a relatively good-quality natal territory.

7.2.2 To breed or not to breed?

Whether or not an individual disperses, the individual has to trade off the cost and benefits of breeding now with those of breeding later. Badgers are selected for an early age of first breeding (α) and a late age of last breeding (ω), but reproduction at an early or late age is associated with a lower annual breeding success than at other ages (Chapter 4). There is also a positive phenotypic correlation between α and ω ,

suggesting that an early α may be costly as it results in an early ω , thereby potentially lowering LBS, although the effect of autocorrelation between these two traits was not ruled out (Chapter 4). Correspondingly, trapping data suggest that breeding at an early age may be associated with a higher risk of mortality, as lactating two-year-old females displayed a higher mortality rate than two-year-old females that had not lactated (Woodroffe & Macdonald, 1995a).

The presence of relatives in a natal group may restrict breeding, as it is believed that inbreeding may lead to reduced fitness (Charlesworth & Charlesworth, 1987). As badger groups correspond to groups of relatives, inbreeding is a possibility. Inbred badgers had a reduced LBS compared to individuals that were not inbred, suggesting that the fitness costs associated with inbreeding are an important factor for badgers (Chapter 5); however, LBS did not differ when controlling for the presence of all four grandparents. Individuals may avoid inbreeding by copulating with extra-group mates or, additionally, females may reduce the chances of inbreeding through multiple-paternity litters. I observed promiscuous mounting behaviour by female badgers and observed one extra-group mounting (Chapter 5). The observed behavioural mating system corresponded to the genetic mating system, wherein there was strong evidence of multiple-paternity litters and approximately half of the paternities were assigned to extra-group males (Chapter 2). Examining the relatedness of mothers to within-group and extra-group fathers in the same litter, and to within-group and extra-group fathers in general, revealed that females that were assigned maternity were less related to extra-group mates than within-group mates (Chapter 5). Permutation tests also showed that breeding females might reduce their chances of inbreeding by mating with males from outside of their social group. Furthermore, breeding females were

also less related to candidate fathers in neighbouring groups than within their own group (Chapter 5). The high proportion of extra-group paternities obtained by neighbouring males may therefore reduce inbreeding (Chapter 2), although breeding with an extra-group male from a non-neighbouring group would reduce this chance further (Chapter 5). As potentially incestuous matings were assigned within the Wytham badger population (Chapter 5), individuals may delay breeding in order to prevent breeding with relatives. The high levels of gene flow brought about by extra-group paternities, however, may facilitate inbreeding avoidance and reduce the likelihood of delaying breeding due to the presence of related mates.

Breeding may also be restricted by reproductive suppression or reproductive restraint. I summarise, in Chapter 3, the published badger research which suggests that one group-member is unlikely to control the reproduction of other same-sex within-group members; thus, the transactional models of reproductive skew were not applicable to badger society. The assumptions of the incomplete-control models of reproductive skew did appear to fit badger society; however, the results presented in Chapter 3 were not consistent with their predictions. Badger reproduction is, however, potentially limited by resource availability in some years, whereas in other years competition between same-sex individuals may play a role (Woodroffe & Macdonald, 1995b). As a result, resource competition, rather than reproductive suppression or reproductive restraint, may play a large role in determining the degree of reproductive skew within badger social-groups.

7.2.3 *We are family*

Individuals may pass on their genes to the next generation by helping relatives to breed (Hamilton, 1964). Cooperative breeding may therefore evolve whereby group members that are not the genetic parents provide care for offspring (Brown, 1987; Solomon & French, 1997). Whether an individual disperses or remains philopatric, or breeds now or breeds later, they may also face the decision of whether or not to help their within-group relatives to breed. The presence of relatives within a group and greater relatedness within than between social groups may facilitate kin selection. Although badgers in neighbouring groups have higher relatedness than badgers in non-neighbouring groups, relatedness within groups is greater than that between groups (Chapter 3). Females that were assigned maternity were observed performing alloparental behaviour significantly more often than other group-members were, suggesting that the behaviours of other group-members are purely social integration and that they do not alloparent (Chapter 6). Non-breeding individuals may therefore decide not to provide alloparental care and instead they may increase their somatic investment to increase their chance of breeding in the following year. Females that were assigned maternity probably bred cooperatively as they were observed babysitting and allonursing more cubs than the number to which they were assigned maternity. As breeding females are already caring for their own offspring, it may not be prohibitively costly to extend this care to non-offspring within the group, especially given that the majority of within-group mother dyads were more likely to represent 1° kin than unrelated individuals. It was not possible to determine whether allonursing was adaptive, as it was rarely observed. Furthermore, I did not detect an increase in litter size, probability of offspring breeding, or LBS of offspring with an increased number of mothers in a group. Cooperative breeding among mothers may or

may not be helpful, as other benefits, such as improved body condition of mothers, in groups with more mothers, cannot be ruled out. Overall, cooperative breeding among within-group mothers represents a low-cost behaviour that is probably facilitated through high kinship between within-group breeding females.

7.3 Conclusions

Long-term studies of wild populations are essential to improve understanding of how natural selection and kin selection mould the evolution of social behaviour. Genetic studies are also important in order to authenticate the behavioural mating system and to assess the validity of social structure inferred from behavioural data. In this thesis I have demonstrated how understanding the mating system and social structure enable the factors that most probably facilitate the evolution of social behaviours, such as cooperative breeding and promiscuous mounting, to be elucidated. Badgers live in groups that contain relatives and their dispersal is restricted. Extra-group paternity increases gene flow between groups, which decreases the chances of inbreeding. Promiscuous mounting by females may have evolved to reduce male–male aggression and the risk of males committing infanticide, but it may also function to promote sperm competition and reduce inbreeding. Reproductive skew within groups appears to be constrained by resource availability and although non-breeding individuals do not alloparent, cooperative breeding probably does occur among breeding females. Overall, the social behaviour of badgers is influenced by their mating system and social structure and these behaviours probably represent an early stage in the pathway to social evolution.

7.4 Future research

The application of molecular genetics to studying the behavioural ecology of badgers has provided a window of opportunity for future research. There are still many unanswered questions and here I briefly mention the specific questions that lead on from this thesis.

7.4.1 Population genetic structure

Examination of the population genetic structure would provide a firmer understanding of the dispersal patterns within badger populations. Isolation by distance measures (Hardy & Vekemans, 2002) and the comparison of results from Bayesian cluster analysis of genotypes with the geographical location of individuals (Pritchard *et al.*, 2000), have been applied to badger populations (Pope *et al.*, 2006), and could be used to describe the genetic structure of the Wytham Woods badger population. The effects of physical or paternity dispersal could then be investigated in relation to social structure to examine how they affect the genetic structuring of the population.

7.4.2 Group fission and relatedness patterns

There are both costs and benefits to group living. In species such as the badger, in which dispersal is restricted, costs associated with group living may be reduced through group fission. Badger social groups do undergo fission, wherein an outlier or annex sett develops into the main sett of a new social group, splitting the territory of the old social group into two new smaller territories (da Silva *et al.*, 1993). The residency choice that individuals make during this process may shed light on the

processes underlying the evolution of social behaviour in badgers. In particular, investigations could focus upon whether individuals choose to reside in the social group that contains their closest relatives. This has been observed in rhesus macaques *Macaca mulatta* (Widdig *et al.*, 2006) and savannah baboons *Papio cynocephalus* (van Horn *et al.*, 2007), although social bonds may be influential in savannah baboons when kin are not abundant.

7.4.3 Breeding success

Although breeding success was influenced by age, reproductive skew within cohorts of similarly aged individuals suggested that traits other than age may influence breeding success (Chapter 4). Quantitative genetic analyses of traits that are potentially associated with breeding success, such as body condition (Chapter 5), may be possible using the animal model (Kruuk, 2004) and the long-term Wytham badger data. Genotyping the extra DNA samples that have accumulated since I finished genotyping in 2005 would increase the pedigree depth. Analysis of traits, such as body condition, in badgers of known age may also facilitate understanding of why male breeding success initially increases with age. Power and sensitivity analyses should be run to investigate the effect of potential pedigree errors, pedigree paucity and genetic architecture on estimates of quantitative genetic parameters (Morrissey *et al.*, 2007). Further investigation is also required into the reasons underlying why many individuals were not assigned parentage over their lifetime. Breeding at an early age may be costly, and individuals may increase somatic investment instead of reproductive investment to increase their chance of survival to the next year. Random factors such as road fatalities may then remove these individuals from the population

before they have a chance to breed. Analysis of morphological traits such as testes size and descent, or vulva status, may confirm whether these individuals attempted to breed or whether they did indeed refrain from breeding.

7.4.4 Offspring sex ratio variation

Male and female offspring are expected to be produced in a 50:50 sex ratio, all other things being equal (Fisher, 1930). This ratio may deviate from 50:50 if one sex is more costly to produce, if differences in the condition of individual offspring at the end of parental investment persist into adulthood, and if the most costly sex displays greater variation in reproductive success because of its variance in quality. Mothers may then produce offspring according to their investment ability (Trivers & Willard, 1973). Alternatively, if there is sex-biased dispersal mothers may produce the dispersing sex when there is competition for local resources (Clark, 1978; Silk, 1983). Badgers are slightly sexually dimorphic (Johnson & Macdonald, 2001; Abramov & Puzachenko, 2005). When female badgers are in better body condition they implant earlier (Woodroffe, 1995; Dugdale *et al.*, 2003). When the mean annual implantation date within Wytham Woods was early then the population post-emergence cub sex ratio was male biased, and when the population cub sex ratio was male biased the cub cohort was larger (Dugdale *et al.*, 2003). As these correlations were at the population level and they were affected by outliers, research is required into whether individuals vary their offspring sex ratio in relation to their investment ability (Trivers & Willard, 1973), or whether individuals vary their offspring sex ratio according to local resource competition (Clark, 1978; Silk, 1983). The latter is less likely given that there is no evidence of sex-biased dispersal in the Wytham badger population.

7.4.5 Genetic compatibility versus genetic diversity

Promiscuous mounting by female badgers may increase the genetic diversity or the genetic compatibility of their litters (Chapter 5). The Genetic Diversity Hypothesis proposes that promiscuous mating should increase genetic diversity at the level of the litter (Williams, 1975), whereas the Genetic Incompatibility Hypothesis proposes that the increase will be at the level of the individual (Zeh & Zeh, 1994; Jennions, 1997; Jennions & Petrie, 2000). Comparison of the relatedness of offspring to their mothers and of litters to their mothers would enable these two hypotheses to be tested (Cohas *et al.*, 2007), and may provide further clarification of the reasons underlying promiscuous mounting behaviour in badgers.

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**Female receptivity, embryonic diapause and superfoetation
in the European badger (*Meles meles*): implications for the
reproductive tactics of males and females⁶**

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FEMALE RECEPTIVITY, EMBRYONIC DIAPAUSE, AND
SUPERFETATION IN THE EUROPEAN BADGER (*MELES MELES*):
IMPLICATIONS FOR THE REPRODUCTIVE TACTICS
OF MALES AND FEMALES

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ABSTRACT

The European badger *Meles meles* is thought to mate throughout the year, with two mating peaks occurring in late winter/spring and summer/autumn. After mating, fertilized ova enter embryonic diapause (delayed implantation) at the blastocyst stage, which lasts up to eleven months. Even if mating is successful, however, the estrous cycle may continue during embryonic diapause, which suggests that female badgers are capable of superfetation (conception during pregnancy). This may increase female fitness by facilitating polyandry, and reduce the risk of infanticide by resident males through paternity confusion. Detailed understanding of female receptivity, specifically the association of superfetation with embryonic diapause, may explain field observations of seemingly inconsistent reproductive

tactics of male badgers with regard to, for instance, whether or not they guard mates or defend territories. The combination of embryonic diapause and superfetation may occur in other mustelids; if so, the sociobiology of mustelids will need re-evaluating, and the Mustelidae may prove to be a good model taxon for studies of sexual conflict in the reproduction of eutherian mammals.

NATURAL SELECTION and sexual selection act on both sexes. However, emphasis on sexual selection as a directional evolutionary force acting on males has diverted attention from the selective processes acting on females, whose discrete mating tactics may have masked the extent of the potential for reproductive conflict between the sexes (Zeh and Zeh 2003). Recent evidence suggests that the reproductive interests of males and females frequently differ, thereby generating sexual conflict rather than cooperation (Chapman et al. 2003; Montrose et al. 2004). This is highlighted in polyandrous mating systems, which may be the norm across various taxa (Chapman et al. 2003; Zeh and Zeh 2003). Such sexual conflict is manifested as a “tug-of-war” at both precopulatory and postcopulatory stages, with males attempting to monopolize access to the females’ ova and manipulate their physiology, while females attempt to control their own reproductive options (Chapman et al. 2003; Zeh and Zeh 2003; Hosken and Stockley 2004; Martin et al. 2004). Therefore, it is important to understand the mechanisms through which males and females achieve reproductive success (Zeh and Zeh 2003).

Understanding sociobiology requires knowledge of the tactics that maximize individual survival and reproductive success, which are determined by the availability of food and shelter for both sexes and the receptivity of females for males (Macdonald 1983; Sandell 1989). In spite of the theoretically accepted importance of the pattern of female receptivity, empirical information, particularly on reproductive physiology and endocrinology, is rudimentary for many species. Furthermore, it has recently been suggested that the unusual reproductive phenomenon of superfetation (conception during pregnancy; Shackelford 1952) that occurs in female American mink *Mustela vison* may, in combination with embryonic diapause (delayed implantation of embryos), play a crucial role in sexual con-

flict in American mink reproduction, and this phenomenon may occur in other members of the Mustelidae (Thom et al. 2004b; Yamaguchi et al. 2004). The Mustelidae is unusual amongst eutherian families (placental mammals), as not only is it comprised of approximately one-third of the species known to exhibit embryonic diapause (Mead 1981; Sandell 1990; Ben-David 1998; Renfree and Shaw 2000; Thom et al. 2004a), but also all additional transitions of the evolution of embryonic diapause amongst the Carnivora occur within the Mustelidae (Lindenfors et al. 2003). The possible connections between embryonic diapause and superfetation, and their importance with regards to sexual conflict in the Mustelidae, merit consideration. The aim of this paper is to shed light on the importance of female reproductive physiology for the evolution of reproductive tactics of both sexes by focusing on another mustelid, the European badger *Meles meles*, for which relatively robust information is available in terms of its ecology, behavior, and reproductive physiology.

DISTRIBUTION AND SOCIAL ORGANIZATION

The European badger (*Meles meles*) is a large, stocky mustelid that weighs around 10 kg. It is widely distributed across Eurasia, from the U.K. to Japan and from Palestine to the Russian Arctic Circle. It exhibits large variation in social organization, being solitarily, pair, or small group living in many parts of Eurasia and group living in parts of the U.K. This is unique among badgers as all others are solitary (e.g., the American badger *Taxidea taxus*; Macdonald 2001). Social groups of badgers can be composed of up to 30 individuals that share a large communal “sett” or den (a network of underground tunnels and chambers; Neal and Cheeseman 1996; Johnson et al. 2002). However, cooperative behaviors amongst group members are less developed than those seen in highly social mammalian species such as wolves *Canis lupus*

(da Silva et al. 1994; Woodroffe and Macdonald 2000; Macdonald et al. 2002b; Revilla and Palomares 2002; Rogers et al. 2003). Currently a considerable bias exists in the literature on *Meles meles* because a disproportionate number of studies have been conducted in the U.K. where these badgers live in social groups. The reported reproductive biology thus may not be applicable throughout its range (Neal and Cheeseman 1996; Johnson et al. 2002). The identity of the population from which data were derived is therefore specified throughout this paper.

MATING AND BIRTH

Since the classic work of Neal and Harrison (1958), based on about 85 animals from southern England, post mortem studies of the reproductive biology of free-ranging badgers include: Canivenc and Bonnin-Lafargue (1963: about 600 from France), Canivenc (1966: about 700 from France), Ahn-lund (1980: 1095 from central Sweden), Wandeler and Graf (1982: 230 from Switzerland), Cresswell et al. (1992: 650 from southern England, U.K.), Whelan and Hayden (1993: 548 from central Republic of Ireland), and Page et al. (1994: 1875 from southern England).

Badgers can mate during any month in Britain (Neal and Cheeseman 1996). Seasonal changes in the wet weight of testes combined with epididymides, and the presence or absence of spermatozoa in the caput epididymis, of males suggest that testicular activity in southern England is, on average, highest during late winter to summer and lowest during late autumn to early winter (Figure 1) (Neal and Harrison 1958; Page et al. 1994; Neal and Cheeseman 1996). There are always some males that are physiologically capable of reproduction at any time of the year, however, and this capability has been confirmed in vivo in Wytham Woods, Oxford, southern England (Woodroffe and Macdonald 1995b). Although many matings last less than two minutes, some last more than 15 minutes (up to 90 minutes; Johnson 2001), and these are speculated to represent successful matings (Neal and Harrison 1958; Neal and Cheeseman 1996). Although the existence of such

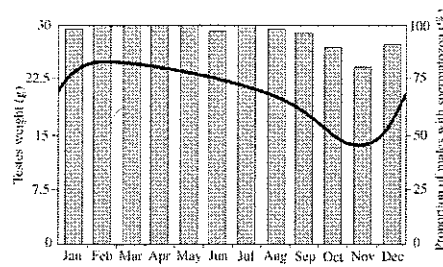


FIGURE 1. SEASONAL VARIATION IN TESTES WEIGHT AND THE PROPORTION OF MALES WITH SPERMATOZOA

The generally high levels of testicular activity as shown by testes weight (bar chart) and the proportion of animals with spermatozoa (solid line) among adult males in their third year or older. Testes weight is the average wet weight of testes combined with epididymides, both with and without spermatozoa.

long-duration matings may suggest that stimulation associated with intromission is important in badger reproduction, whether or not the badger is an induced ovulator remains unconfirmed. It has been suggested that ovulation could occur without copulatory stimulation, and that spontaneous ovulation might occur in the American mink, a related species and an induced ovulator whose reproductive biology has been well studied in captivity (Sundqvist et al. 1988). Therefore, these two forms of ovulation may not be mutually exclusive and further studies are necessary to establish whether or not badgers are induced ovulators.

Observations of long-duration matings, both in the field and in captivity, are most frequent between February and May in Britain (Neal and Cheeseman 1996; Johnson 2001), which suggests this is an important period in the reproduction of British badgers. Cresswell et al. (1992) demonstrated that in southern England, U.K., the proportion of females carrying large (>1.0 mm diameter) pre-ovulatory follicles peaks twice a year: one peak occurs in late winter to spring and another in summer to autumn (Figure 2), suggesting that badgers in that region have two peaks of reproductive activity. However, as one may speculate from Figure 2, it is possible that throughout most of the year some females are always physiologically receptive to

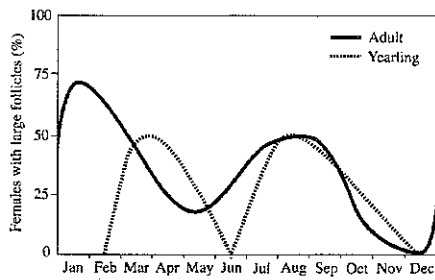


FIGURE 2. SEASONAL OCCURRENCE OF LARGE (>1.0 mm) FOLLICLES IN FEMALE BADGERS

The general occurrence of large follicles in female badgers in both their third year or older (adult) and in their second year (yearling) throughout the year, showing two peaks of reproductive activity.

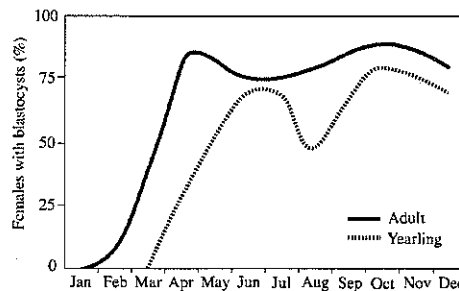


FIGURE 3. SEASONAL OCCURRENCE OF BLASTOCYSTS IN FEMALE BADGERS

The general trend in the occurrence of blastocysts in female badgers throughout the year. The majority of females both in their third year or older (adult) and in their second year (yearling) are pregnant by May.

mating. Nevertheless, the majority of females are pregnant by early summer (April to June) in southern England, as well as in central Sweden and in Switzerland (Figure 3) (Ahnlund 1980; Wandeler and Graf 1982; Cresswell et al. 1992; Whelan and Hayden 1993; Page et al. 1994). Unfortunately, conducting year-round behavioral observations of badgers is very difficult even at the setts because they are a nocturnal species that spend much of their time underground. Badgers are not individually identifiable on the basis of their natural markings, and consequently, there is no published behavioral information on the frequency of matings throughout the year by identified individuals. The application of appropriate observation methods, such as infrared video surveillance to record badger activities at the setts with each individual identified by fur clip marks (Stewart and Macdonald 1997; Stewart et al. 1997), should be encouraged widely to collect such data.

Due to well-synchronized implantation dates facilitated by embryonic diapause, regardless of the timing of successful matings, the great majority of births occur within a short period during late winter–spring (Neal and Cheeseman 1996). This holds true throughout the badger's range, although the timing of the peak may vary from one geographical region to another in terms of the calendar month (Neal and Cheeseman 1996). In contrast to the potential for year-round mating activity, in southern England,

the most accurately estimated birth dates (76% of the 97 litters examined) fall between mid-January and mid-March, with a peak during the first fortnight in February (Neal and Cheeseman 1996). The modal number of cubs at birth is estimated to be three, and the estimated mean is 2.8 with a range of one to five (Neal and Cheeseman 1996). By the time cubs are seen above ground at eight to ten weeks after birth, the average number in a litter is reduced to 2.4. In Wytham Woods, Oxford, mean fetal litter size is 1.8 (Woodroffe and Macdonald 1995b) and postemergence litter size is 1.6 (Macdonald and Newman 2002). There is speculation that this decrease may be due to infanticide by other breeding females in the sett (Cresswell et al. 1992), although in Wytham some result from coccidial infection (Newman et al. 2001).

EMBRYONIC DIAPAUSE

Reproductive delay can also be achieved through delayed fertilization and delayed development, but the most widespread means in mammals is embryonic diapause. During embryonic diapause, a newly fertilized egg (or eggs) temporarily ceases development and remains free in the uterus lumen instead of being directly implanted into the uterus (Sandell 1990; Mead 1993; Bernard and Cumming 1997; Renfree and Shaw 2000; Lindfors et al. 2003). Embryonic diapause has been found in 69 eutherian (including 3 spe-

cies of Chiroptera, 4 Insectivora, 2 Edentata, 18 Rodentia, 41 Carnivora, and 1 Artiodactyla) and 28 marsupial species among about 4,600 mammalian species (Mead 1993; Renfree and Shaw 2000). Two different forms of embryonic diapause are distinguished. Obligate (or seasonal) diapause usually occurs in species that have a single litter per year, and it is controlled by extrinsic factors such as day length. Facultative (or lactational) diapause is usually associated with multiple litters per year, and it is controlled by intrinsic factors such as the duration of lactation of the previous litter (Sandell 1990; Mead 1993; Renfree and Shaw 2000).

In the European badger, ovulation and fertilization can occur a few days after parturition, in late winter–early spring (January to March); implantation does not occur until mid-winter (December to January), however (Canivenc and Bonnini 1981; Woodroffe 1995; Dugdale et al. 2003). Therefore, due to this embryonic diapause, which is known to occur in 22 (and is considered not to occur in 11) of the 55 species of the family Mustelidae (Mead 1981; Sandell 1990; Ben-David 1998; Amstislavsky and Ternovskaya 2000; Lindenfors et al. 2003; Thom et al. 2004a), the badger's gestation period, which includes the reported 40 to 49 days postimplantation (Canivenc 1966; Neal and Cheeseman 1996), is hugely inflated to almost 11 months (Canivenc and Bonnini 1981; Mead 1981; Sandell 1990; Ben-David 1998). The existence of unimplanted blastocysts in the badger was first reported by Fries (1880). Later Fischer (1931) showed that embryonic diapause occurs in the badger. Endocrinologically, it is thought that embryonic diapause in mustelids results from insufficient hormonal secretion from the pituitary gland, including prolactin and LH (luteinizing hormone), which causes incomplete differentiation of the corpora lutea and reduced luteal hormonal secretion (Canivenc and Bonnini 1981; Sundqvist et al. 1988; Mead 1993; Renfree and Shaw 2000). In the badger, corpora lutea are formed after ovulation, but they are considerably smaller than those seen during the postimplantation pregnancy, and appear less active during embryonic diapause (Canivenc and Bonnini 1981; Wandeler and Graf 1982).

Renewed luteal development is associated with a change in photoperiod and increased pituitary secretion, triggering resumption of luteal cell differentiation and increased secretion of luteal hormones, including progesterone. This in turn is probably responsible, through uterine development and secretion, for the resumption of embryonic development and implantation (Canivenc 1966; Sundqvist et al. 1988; Mead 1993; Renfree and Shaw 2000). As summarized in Figure 4, progesterone levels, although still low, are significantly elevated when embryogenesis is renewed, which suggests progesterone-dependent implantation; however, attempts have failed to stimulate implantation artificially by administering progesterone (Canivenc 1966; Canivenc and Bonnini 1981).

During embryonic diapause, embryogenesis is retarded at the blastocyst stage (where the hollow ball of embryo cells has developed into two layers of cells), and further development does not occur until the embryos are implanted in the uterus. However, it is incorrect to consider that blastocysts are in complete suspended animation during diapause (Mead, 1993; Renfree and Shaw, 2000). In carnivores, blastocysts are not totally metabolically inactive during embryonic diapause as oxygen consumption is continuous, and the synthesis of RNA, DNA, and protein continues, although at reduced rates compared to activated blastocysts (Mead 1993; Renfree and Shaw 2000). Additionally, unimplanted blastocysts undergo a gradual increase in diameter due to fluid accumulation within the blastocoele and increased cell numbers in the trophoblast (the outermost cell layer in the blastocyst) (Neal and Harrison 1958; Mead 1993; Renfree and Shaw 2000). This gradual increase in the size of unimplanted blastocysts is thought to be useful for investigating the time of year at which ovulation and fertilization occurred. Cresswell et al.'s (1992) report of seasonal changes in the size of unimplanted blastocysts (diameter range: about 0.1–0.2 mm in February, about 1.2–2.8 mm for the "first cohort" and about 0.2–0.7 mm for the "second cohort" in September, and about 2.5–4.0 mm for the "first cohort" and about 1.4–1.8 mm for the "second cohort" in December) in female badgers in southern

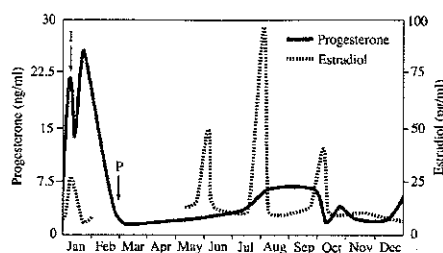


FIGURE 4. SEASONAL VARIATION IN PLASMA PROGESTERONE AND ESTRADIOL PROFILES

Elevated progesterone levels, although low, at embryogenesis renewal, which suggests progesterone-dependent implantation. Captive female badgers were housed separately and were not introduced to males during the monitoring period. Parturition is indicated with an arrow labeled "P" and estimated time of implantation is indicated by an arrow labeled "I."

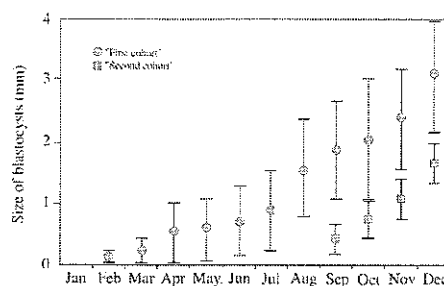


FIGURE 5. SEASONAL VARIATION IN THE DIAMETER OF UNIMPLANTED BLASTOCYSTS

The seasonal changes in the median and the range of the diameter of unimplanted blastocysts. There appears to always be a distinct gap between the size ranges of the larger "first cohort" and the smaller "second cohort" blastocysts, the later of which occur from September onwards.

England suggests the existence of two distinguishable peaks of ovulation and fertilization corresponding with the occurrence of large follicles (Figures 2 and 5). When the oviducts of badgers were severed in March, however, blastocysts recovered at autopsy were of unequal size, although they were thought to belong to the same ovulation (Canivenc 1966). Although Canivenc (1966) did not give further details (e.g., how large the size discrepancy was and proof that all of the blastocysts belonged to the same ovulation), these are grounds for caution when timing ovulation(s) solely from the size of blastocysts. This is further reinforced by the observed variation (ranging 0.6–1.1 mm in diameter) in the size of unimplanted blastocysts apparently from the same ovulation observed in western spotted skunk *Spilogale putorius latiformis* (Rodney Mead personal communication).

LOSS OF BLASTOCYSTS AND SUPERFETATION

Throughout embryonic diapause, the average number of blastocysts per female is fairly constant (Figure 6; Creswell et al. 1992; Page et al. 1994). However, the average number of corpora lutea increases during the same period (Figure 6) (Neal and Harrison 1958; Ahnlund 1980; Page et al. 1994). Furthermore, direct comparison between these two figures, based on the same females, shows

that in general there are more corpora lutea than blastocysts, as highlighted in extreme cases where more than ten corpora lutea, but no blastocysts, were observed (Neal and Harrison 1958; Ahnlund 1980; Page et al. 1994). However, small blastocysts, formed just before examination, may have been missed during dissection, which may account for the large difference observed between the number of corpora lutea and blastocysts in March (Figure 6). The difference in the number of corpora lutea and blastocysts throughout the mating season tentatively suggests that more ova are ovulated than are retained as blastocysts (Ahnlund 1980; Wandeler and Graf 1982; Creswell et al. 1992; Page et al. 1994). Additionally, there are females that possess blastocysts that visibly differ in size, which has been interpreted as an indication of superfetation (Neal and Harrison 1958; Creswell et al. 1992; Neal and Cheeseman 1996), but the possibility that these blastocysts of different sizes belong to the same ovulation (Canivenc 1966) cannot be dismissed. Furthermore, in addition to normal follicular development leading to corpora lutea vera (corpora lutea that are formed following ovulations primarily by the proliferation and hypertrophy of the membrane granulosa), atresia can also occur and lead to the production of corpora lutea atretica (corpora lutea that are primarily formed without ovulations from

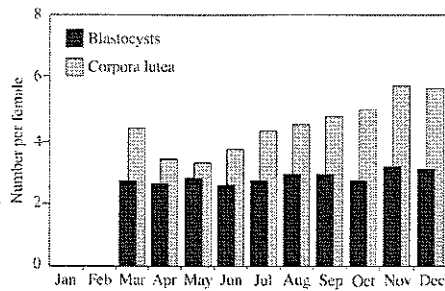


FIGURE 6. SEASONAL VARIATION IN THE AVERAGE NUMBER OF BLASTOCYSTS AND CORPORA LUTEA

The seasonal changes in the numbers of blastocysts and corpora lutea among adult females in their third year or older. The number of blastocysts tends to remain relatively constant while the numbers of corpora lutea tend to increase towards the end of the breeding season.

undifferentiated stromal and thecal cells) (Mead 1968). These are very difficult to distinguish between, and can also be mistaken with corpora lutea accessorium (formed by the lutenization of the granulosa cells of unovulated follicles) (Mead 1968). This should be considered when interpreting the difference in the observed number of corpora lutea and blastocysts (Wandeler and Graf 1982; Rodney Mead personal communication). The foregoing arguments suggest that, although superfetation is thought to occur in the European badger (Neal and Harrison 1958; Cresswell et al. 1992; Neal and Cheeseman 1996), definitive evidence is still required.

Superfetation has been well studied in the American mink. In ranched mink, ova from a single ovulation can be fertilized by more than one male when other males mate within one to three days of the initial mating, a phenomenon known as superfecundation (Shackelford 1952; Venge 1973). Furthermore, female American mink continue to ovulate after the initial fertilization if more than six days elapse between matings; thus, kits can be sired by more than two males that fertilize the ova of different ovulations: superfetation (Shackelford 1952; Mead 1994). Superfetation is a phenomenon where ovulation and fertilization (and usually implantation) of the

second (and subsequent) set(s) of ova occurs during pregnancy whether or not the subsequent parturition is successful. Superfetation has been reported in humans (Steck and Busen 1997) and in some domestic animals (ewe: Schuyt 1981; pig: Hall 1987; cow: Rotenstein 1989), however, these are rare cases that have usually occurred under medical/veterinary pregnancy treatments. Confirmed reports of natural superfetation during normal reproduction of a species resulting in successful parturition are extremely rare in eutherian mammals. Apart from the American mink, and possibly the European badger, superfetation has only been reported in the casiragua *Proechimys semispinosus* (Weir 1974), the North African gundi *Ctenodactyles gundi* (Gouat 1985), the brown hare *Lepus europaeus* (Caillol et al. 1991), and possibly the common tenrec *Tenrec ecaudatus* (Poduschka 1996). However, superfetation in these four species, which occurs without embryonic diapause, is characterized by a second estrus just before parturition of the fetuses that developed from the first set of ova. Therefore, although the second ovulation occurs before parturition, each set of ova develop separately in essentially two different pregnancies leading to two different parturitions, in a fundamentally different process from that occurring in association with embryonic diapause. This means that among eutherian mammals, there are only two species where biologists currently know or presume that both embryonic diapause and superfetation occur, the American mink and the European badger. This is due to a lack of evidence of superfetation rather than proof that superfetation does not occur in other species that possess embryonic diapause.

We present four hypotheses to explain the difference in the number of blastocysts and corpora lutea. The first hypothesis assumes that badgers are induced ovulators and that some of the blastocysts from early ovulations may be lost and replaced through superfetation during embryonic diapause. Detailed studies on the reproductive biology of female American mink also indicate that during embryonic diapause, a longer delay until implantation decreases the survival of fertilized ova (Shackelford 1952; Venge 1973). The extent

to which this occurs in badgers is unknown. Studies in a few species of the genus *Martes* suggest, on the basis of the similar numbers of corpora lutea and unimplanted embryos, that there is little evidence for preimplantation loss of embryos (Mead 1994). Based on these studies, it may be speculated that most embryo loss occurs during postimplantation in many mustelids that delay implantation, and the American mink is an exception (Rodney Mead personal communication). A presumed preimplantation loss of blastocysts is reported in the European badger (Creswell et al. 1992), strongly suggesting that further detailed studies are necessary to obtain a better understanding of the possible blastocyst turnover during the embryonic diapause and superfoetation. The only published study to investigate atrophying blastocysts used live animals and found that marked blastocysts from postpartum ovulations were all present and implanted in January (Canivenc and Bonnin-Laffargue 1963). However, ultrasound of female badgers in Wytham Woods, Oxford revealed that a female in January 2003 had one vesicle that was being reabsorbed, and a female in January 2004 had one embryo in the right uterine horn and a vesicle in the same horn that was being reabsorbed (Dugdale unpublished data). Although at an advanced stage in development, this suggests that atrophy of blastocysts may also occur. This first hypothesis explains the fairly constant number of blastocysts per female throughout embryonic diapause in spite of the increase in the number of corpora lutea.

The second hypothesis is that if spontaneous ovulation occurs in badgers, the surplus corpora lutea could come from ova that are not fertilized, and hence not retained. In American mink (an induced ovulator), it is suggested that intromission may not always be necessary for ovulation and that rough fighting associated with courtship foreplay may lead to ovulation (Dunstone 1993). This suggests a third hypothesis that the surplus corpora lutea are the result of induced ovulation(s) where ovulated ova are not subsequently fertilized. The presence of degenerate and apparently unfertilized ova along with healthy blastocysts (Harrison and Neal 1956)

accords with either (or both) of the latter two hypotheses.

The fourth hypothesis is that the surplus corpora lutea are due to the three types of corpora lutea, mentioned above, being incorrectly classified together as indications of ovulations. Neal and Cheeseman (1996) appear to consider that the first hypothesis is the most likely. It seems possible that female European badgers ovulate more than once within a single breeding season, which lasts for nearly a year, regardless of whether or not ovulation and fertilization occur in the early stage of that breeding season.

In ranched American mink, more than four waves of follicles mature at approximately eight day intervals during the mating season (Sundqvist et al. 1988), although the maximum number of times a female can ovulate after successful matings is unknown. It is not known how many times a female badger ovulates during one breeding season. Service et al. (2002) suggest, based on the estradiol concentration of urine samples from two unmated females monitored daily for a year, that up to five estrous cycles may occur in late winter/spring and autumn with an average interval of 28 days. Plasma estradiol levels in free-ranging female badgers from southwest France show three recognizable peaks during embryonic diapause in June, August, and October. These peaks correspond extremely well with the seasonal changes in plasma estradiol levels, monitored on average once a week, in five captive females from the same region (Figure 4) (Mondain-Monval et al. 1980). The observed peaks in plasma estradiol (along with another estrogen: estrone) may be linked to the keratinization and epithelial proliferation of vaginal mucosa, which occurs at the same time (Mondain-Monval et al. 1980). However, it is not clear if such estradiol peaks are associated with estrus, which is defined as the period of sexual receptivity, since the five observed females (Mondain-Monval et al. 1980) were housed separately and without any contact with males. Similarly, although spontaneous ovulation was not observed in any of the females during these estrus-like periods (Mondain-Monval et al. 1980), it does not necessarily follow that badgers do not ovulate after the postpartum mat-

ing season, especially if induced ovulation is the main form of ovulation in badgers. At present, we do not know how many times a female badger (in captivity or various natural circumstances) can physiologically ovulate during the year-long breeding season and how long the interval is between these ovulations.

IMPLICATIONS FOR THE REPRODUCTIVE TACTICS OF MALES

Territorial males are expected, theoretically, to expel intruders in order to maximize their own reproductive success (e.g., Maynard-Smith 1978). However, a lack of understanding of female receptivity seems to have thwarted a consistent explanation of seemingly contradictory reproductive tactics of male badgers.

Roper et al. (1993) monitored latrines intermittently throughout the year while radio-tracking four males and two females in southern England. Whereas the spring peak (March and April) in scent-marking was attributable to both sexes, the autumn peak (September and October) was mainly attributable to males. They concluded that both sexes are territorially most active in spring, but that males also showed a minor secondary increase in territorial activity in autumn. Cresswell et al. (1992) reported that bite wounding in males, interpreted as indicative of increased intrasexual competition among males, followed a roughly bimodal pattern, with one distinct peak in February and March and a less defined peak around September, and that bite wounding of females has two peaks in April and between November and December, in the badger populations of southern England. On the other hand, Macdonald et al. (2004) found no seasonal bite-wounding pattern in either sex in the Wytham Woods population. Macdonald et al. (2002a) reported that, in general, individual body mass, body condition, and fecundity decreased with increasing group size in their study population. Interestingly, female badgers exhibited the greatest effects of density-dependent constraints on body mass and condition in autumn, whereas for males the effect was most pronounced in spring (Mac-

donald et al. 2002a). Additionally, whereas females showed no sex-related preferences when scent-marking other badgers within their social group, males preferred females (Buesching et al. 2003). These reported sexual and seasonal differences in latrine usage, bite-wounding patterns, body condition, and scent-marking patterns suggest that male and female specific reproductive tactics do exist. These are therefore grounds to expect behaviors such as mate-guarding and territory defense in male badgers (Roper et al. 1986; Roper et al. 1993; Christian 1995). Nevertheless, a general consensus has not been reached in terms of male reproductive tactics, although male-male aggression appears to occur.

Revilla and Palomares (1999) reported the expansion of a male badger's territory after the removal of a neighboring male, which suggests that the male was attempting to gain access to females in the adjacent territories. There is also a report of a resident male fighting and chasing away an outsider male that was attempting to mate in the resident male's territory in early February (Christian 1995). However, in general there is little evidence to support the occurrence of mate-guarding by males (Cresswell et al. 1992). Indeed, behavioral observations suggest that male-male aggression is rare (Macdonald et al. 2002b), and males are even seen grooming each other before mating with the same female (Johnson 2001). Furthermore, it is also suggested that territories may not be exclusive even during the peak breeding periods when males are known to make sorties into neighboring territories (Woodroffe 1993; Neal and Cheeseman 1996).

In general, male mammals associating with certain females either socially or spatially try to guard, or conceal, those females from other males (Brotherton and Manser 1997; Jennions 1997). However, female badgers may advertise their receptivity widely through scent marking (Stewart et al. 2001, 2002; Buesching et al. 2002), and are themselves easy to locate during the day at either the main or outlier (smaller setts within the territory) setts of their social group (Roper et al. 2001), although they may range widely at night. The general lack of cooperative behav-

iors among group members (da Silva et al. 1994; Woodroffe and Macdonald 2000; Macdonald et al. 2002b; Revilla and Palomares 2002; Rogers et al. 2003) suggests that it is uncommon for resident males to act cooperatively to evict trespassing males; thus, a single male outsider may manage to mate in neighboring territories (Woodroffe 1993; da Silva et al. 1994; Woodroffe et al. 1995). Indeed, on the rare occasions that a mating male was observed to chase away another male, a third male then mated with the female (Johnson 2001). However, mating success will depend upon female receptivity.

In the American mink, the earlier fertilization occurs in the breeding season, the longer the delay until implantation, which decreases the survival of fertilized ova (Shackelford 1952; Venge 1973). Hence, the later a male mates, the greater the proportion of the litter he is likely to sire (Shackelford 1952; Venge 1973). When female American mink mate again within 7 to 28 days of the first mating, less than 10% of kits are sired by the first male (Shackelford 1952; Mead 1994). In badgers, it is not known if males mating later have better chances of siring a larger proportion of the litter. Based on the size of unimplanted blastocysts in females sampled between September and December, Cresswell et al. (1992) show that about 65% and 35% of all blastocysts are from winter/spring and summer/autumn matings respectively. As the average number of blastocysts (about 3.2) appears to be greater than the estimated average litter size at birth (about 2.8) (Cresswell et al. 1992; Page et al. 1994; Neal and Cheeseman 1996), the relative importance of winter/spring matings and summer/autumn matings needs further investigation, although these figures suggest that if there is a reproductive advantage of later matings, it is less than that in American mink. Furthermore, there is no convincing evidence to reject the possibility that female badgers ovulate more than once within each of the two recognized mating seasons. These factors raise questions concerning the reproductive tactics of male badgers, as well as offering possible explanations as to why a consensus has proved elusive regarding the territorial and reproductive tactics of male badgers. Males may attempt to

mate guard females only when they are receptive and only from unrelated males. As field observations of mating tend to be at the sett where potentially closely-related males reside, this might explain why little male aggression has been observed when females mate with multiple males. When there are no estrous females in a social group, males may increase their reproductive fitness by attempting to mate with receptive females from neighboring groups instead of guarding their own territory and the nonreceptive females within it.

The foregoing arguments, along with those in previous sections, suggest that good evidence for blastocyst turnover/mortality during embryonic diapause in the female is essential for understanding the evolution of the reproductive tactics of the male. Furthermore, we need to determine whether blastocyst turnover occurs within the mating season immediately after parturition, which is potentially the highest peak of reproductive activity in the European badger (e.g., Cresswell et al. 1992). Unfortunately, hard evidence is currently lacking, and the necessary analytical resolution may not be achieved by postmortem analysis that is limited by the availability of carcasses. In the American mink, significant turnover of blastocysts and occurrence of superfetation was documented on the basis of experiments using males of different coat color genotypes to mate single females at different times, and surgically marking the corpora lutea from the first of the two ovulations (Shackelford 1952). Confirmation of superfetation, and to a certain extent estimation of blastocyst turnover during embryonic diapause in females, could be assessed using similar controlled manipulations on captive badgers of known genotype. Genetic fingerprinting could then be employed using a large number of animals to compare several different treatments—for example, comparison between females mating only during the early part of the breeding season and those doing so throughout the breeding season. Once the general trends have been discovered, further experiments could be designed to answer more specific questions. However, confirmation of the rate of blastocyst turnover, especially during the early stages of

pregnancy, may require surgical examination that raises animal welfare issues as well as potentially impairing the condition of the females.

The majority of females that ovulate in the later stage of embryonic diapause (e.g., late summer onward) may be less than three years old (Ahnlund 1980). However, few females under the age of three years breed (da Silva et al. 1994; Woodroffe and Macdonald 1995b), and those that do appear to have lower reproductive success than older females (Creswell et al. 1992). Also, males sustaining testicular activity later into the summer acquire more bite wounds by autumn than other males, and may even become anemic (Woodroffe and Macdonald 1995a), which suggests that there may be a physiological cost associated with extended breeding activity in male badgers. These factors may therefore influence the reproductive tactics of male badgers, and different tactics may exist depending on an animal's age, physical condition, and circumstances.

Although male "status" (e.g., dominant or subordinate) may be an important element associated with mating tactics, feeding experiments with wild European badgers found no compelling evidence for dominance hierarchies within social groups, suggesting that identifying the conventional "dominance" hierarchy in badgers (if indeed there is one) is difficult (Macdonald et al. 2002b). Male badgers may be classified into "high" or "low" status (Stewart et al. 1999), but this is on the basis of body weight, age, and copulating frequency based on observations of individually identified male badgers around setts. Detailed observations of whether males differ in their mating efforts throughout the year, in relation to their physical and social status, are required to improve our understanding of male reproductive tactics in the European badger. As conventional field observations may not provide the appropriate resolution (e.g., temporal resolution and individual identification) to answer these questions, explorations of new types of observation techniques should be encouraged, such as continuous video surveillance (Stewart et al. 1997) and individual identification by noninvasive fur clipping (Stewart and Macdonald 1997).

The foregoing arguments shed light on the reproductive tactics of the male European badger at the time of analysis. The evolution of a reproductive tactic may not necessarily lead the process to a terminus, however, and the tug-of-war may continue both intra- and intersexually. For example, in the European badger, the n th stage of selection may favor those males that compete aggressively for mating opportunities during the postpartum mating period, and yet, the $(n + 1)$ th stage of selection may favor those males that compete aggressively for mating opportunities all year round. Therefore, unless we have a fairly good idea of the evolutionary stage/history of a species, we may easily be lured into suggesting another just-so story.

The phylogenetic norm of the social system in badgers (consisting of eight species) appears to be solitary, and the European badger itself is unlikely to have evolved under circumstances where group living is the norm (Macdonald 2001). Thus, it is not clear whether the European badger has fully adapted to the high-density group-living society commonly found in the U.K. For example, the general lack of cooperative behaviors among group members (da Silva et al. 1994; Woodroffe and Macdonald 2000; Macdonald et al. 2002b) may simply suggest that the European badger has not evolutionarily adapted to such a society yet. Most behavioral observations of the European badger have been carried out in the U.K. Unless relevant observations become available from areas where badgers do not live in groups, it is difficult for researchers to truly tackle the evolution of reproductive strategies in the European badger.

IMPLICATIONS FOR THE REPRODUCTIVE TACTICS OF FEMALES

Several advantages to females mating with multiple males have been suggested in terms of female reproductive fitness, including direct benefits such as fertilization assurance, mate retention, obtaining material benefits, avoiding male harassment, and devaluing a previous male's sperm, and genetic benefits such as promoting sperm competition, obtaining "good genes," increasing the genetic diversity of the litter, and avoiding genetic in-

compatibilities (Stockley et al. 1994; Reynolds 1996; Jennions 1997; Arnqvist and Nilsson 2000; Jennions and Petrie 2000; Johnson 2001; Chapman et al. 2003; Wolff and Macdonald 2004). A female may achieve polyandry more successfully by prolonging the window of opportunity for mating, both to increase access to more males and to diminish the chance of any one male securing a monopoly. Without doubt, a female's window of opportunity is greatly extended by superfetation. In terms of fertilization assurance, considering the possible gradual death of blastocysts during the embryonic diapause, a female badger capable of superfetation would clearly be at an advantage.

Stewart et al. (1999) report that individuals of both sexes with a high sett fidelity perform more digging and collect more bedding than do transients and badgers of low site fidelity. Additionally, males of "high" status (large, mature, frequently copulating individuals) are more likely to dig than males of "low" status, notwithstanding the difficulty of identifying status in badgers (Macdonald et al. 2002b). Stewart et al. (1999) hypothesize that while highly resident adult females benefit from extending the sett to avoid direct reproductive competition among females, males of "high" status and site fidelity might extend the sett to encourage receptive breeding females into their group and/or to improve survivorship of sired litters. This suggests that resident females benefit from the labor provided by resident males, which they might not receive if they did not mate with them. The possibility of infanticide by other breeding females sharing the same sett (Cresswell et al. 1992) may lead to intense competition within a group for breeding space (Woodroffe et al. 1995; Macdonald et al. 2002a; Domingo-Roura et al. 2003). This is reinforced by the observation that when an entire social group was removed, initial recolonization was almost exclusively by females (Tuytens et al. 2000). Therefore, from a resident male's point of view, it would be desirable to eliminate cubs sired by male outsiders to improve the survival of their own cubs; however, multiple mating may disguise the true paternity of cubs (Wolff and Macdonald 2004). This is advantageous for females as it potentially re-

duces the risk of infanticide from resident males. The foregoing argument suggests that multiple matings and superfetation benefit female reproductive fitness. Endocrinological studies suggest that embryonic diapause is probably a prerequisite for superfetation resulting in a single litter (such as that seen in the American mink and the European badger). As discussed above, following the initial fertilization, the corpora lutea of badgers do not initially produce sufficient progesterone to induce implantation, and this enables later estruses and ovulations (Canivenc and Bonnin 1981; Sundqvist et al. 1988; Mead 1993; Renfree and Shaw 2000). This embryonic diapause is terminated by the reactivation of corpora lutea that secrete progesterone, which is stimulated by the pituitary secretion (Canivenc and Bonnin 1981; Sundqvist et al. 1988; Mead 1993; Renfree and Shaw 2000). From this stage onwards, female badgers do not ovulate as the blastocysts are implanted and postimplantation pregnancy begins (Sundqvist et al. 1988; Mead 1993; Renfree and Shaw 2000). Therefore, without embryonic diapause, superfetation cannot occur while there is only one parturition.

Currently, mainstream adaptive hypotheses for the evolution of embryonic diapause deal principally with how it fine tunes mating and parturition times, and not with the origin of embryonic diapause (Kim King and Roger Powell personal communication). However, the foregoing arguments suggest that embryonic diapause, associated with superfetation, benefits females in the context of sexual conflict over reproduction, whether or not its function is to tune mating and parturition times. This may be the case if a female bears more than one offspring per litter, as seen in the Mustelidae. The existence of sexual conflict over reproduction may lead to the establishment of embryonic diapause in association with superfetation, and longer delays may have evolved thereafter. Obligate embryonic diapause is invariably found in species that breed annually at the most (Sandell 1990). The stoat *Mustela erminea* breeds once a year and exhibits embryonic diapause, whereas the ecologically and phylogenetically similar weasel *M. nivalis* usually breeds twice

a year and does not possess embryonic diapause (Sandell 1990; Mead 1993). The combination of embryonic diapause and superfetation may therefore benefit females, regardless of their social system, by enabling cryptic polyandry. If a female has a small litter size, however, the potential benefits of the combination of embryonic diapause and superfetation may need further evaluation.

All species of the Phocidae and the Otariidae, whose reproductive biology is known, possess embryonic diapause (Lindenfors et al. 2003). They have a typical litter size of one, however, so they may not benefit from superfetation as much as the Ursidae (typical litter size of 1 to 3) and the Mustelidae (2 to 10). Unfortunately, knowledge on the combination of embryonic diapause and superfetation is limited, so an analysis of the life-history parameters that favor this combination is not possible. The occurrence of embryonic diapause in combination with superfetation is clearly testable though, and if this combination does occur broadly across other species, their sociobiology may require re-evaluation (Yamaguchi et al. 2004).

Previous studies have tried to explain the emergence of embryonic diapauses based on adaptive hypotheses (Sandell 1990; Mead 1993). However, recently, it has been suggested that there is one basal origin of embryonic diapause in the Carnivora phylogenetic tree at the point where the Canidae splits from the rest of the caniforms (doglike families; Lindenfors et al. 2003). This suggests that: (1) phylogenetic influence may be as strong as natural history parameters; and (2) the question should be why some species have lost it rather than developed it (Lindenfors et al. 2003). The phylogenetic effect clearly explains why no feliforms (catlike families) or Canidae possess embryonic diapause, whereas most of the caniform species (whose reproductive information is known) do possess it, regardless of their natural history parameters. For example, five of the six phylogenetic groups of the caniforms (except the Canidae) are invariable in terms of embryonic diapause within the family: Ursidae, Phocidae, Otariidae (including the walrus *Odobenus rosmarus*), and red panda *Ailurus fulgens*

all have embryonic diapause, whereas the Procyonidae do not (Sandell 1990; Mead 1993; Renfree and Shaw 2000; Lindenfors et al. 2003). The only exception is the Mustelidae (including the closely-related Mephitidae), where considerable intrafamily variation exists (Sandell 1990; Mead 1993; Renfree and Shaw 2000; Lindenfors et al. 2003). This probable monophyletic origin of embryonic diapause in the Carnivora and the invariability of it in the other families suggest that the Mustelidae is the only group in which adaptive hypotheses of embryonic diapause can be tested (Lindenfors et al. 2003). Detailed research into the reproductive physiology (especially the occurrence of superfetation) of Mustelidae species, along with ecological studies, would enhance our understanding of why some species have lost (or regained) embryonic diapause, making the Mustelidae a good model taxon for studying sexual conflict in the reproduction of eutherian mammals.

Interdisciplinary research should be encouraged by combining detailed behavioral observations and genetic or surgical investigations into the occurrence of superfetation and blastocyst turnover during embryonic diapause in the Mustelidae, such as manipulative mating experiments using captive animals under controlled conditions. Once hard evidence has been collected in terms of embryonic diapause, research may be extended to other forms of reproductive delay, such as delayed fertilization and delayed development. This would give biologists greater insight into the evolution of delay in mammalian reproduction from both the natural and sexual selections' perspectives. As the Chiroptera achieve reproductive delay through all three of these mechanisms (Bernard and Cumming 1997; Renfree and Shaw 2000), captive bat breeding facilities suitable for mating manipulation experiments may benefit the study of the evolutionary biology of mammalian reproductive tactics.

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**Do social groups of European badgers *Meles meles*
exhibit dominance hierarchies?⁷**

⁷ A slightly modified version of this appendix is currently under review as: Hewitt S, Macdonald DW, Dugdale HL (submitted) Do social groups of European badgers *Meles meles* exhibit dominance hierarchies? *Behavioral Ecology*

A2.1 Abstract

A social hierarchy is generally assumed to exist in those mammalian societies in which the costs and benefits of group-living are distributed unevenly among group members. Here, infra-red CCTV footage, collected over three years of filming in Wytham Woods, Oxfordshire, was analyzed to test whether social groups of European badgers display linear hierarchies. Analysis of aggressive dyadic interactions between group members revealed that three out of six social-group-years were structured according to significantly linear dominance relationships. In these three groups, there was an effect of sex on dominance rank, with females gaining significantly higher rank than males in two of them. There was no effect of age on rank, however, in any of the groups, nor did social rank appear to affect the likelihood of an individual being wounded, or an individual's reproductive success. There was some evidence of a negative correlation between levels of aggression and increasing relatedness between group members. Positive correlations between levels of aggression and unreciprocated allogrooming, and aggression and sequential allomarking, were also found, but were not consistent across all groups. The European badger is thought to represent an early stage of social evolution, where social structures may not yet be well established. We further suggest that dominance structures may be situation dependent, with hierarchies arising in only those groups where (and when) competition is highest.

A2.2 Introduction

Group living is hypothesized to evolve when individuals derive net benefits from group membership, which enhance their fitness above the level that they would achieve as solitary individuals (Alexander, 1974; Koenig et al., 1992). Benefits arising from group living may include increased vigilance and protection from predation (Rasa, 1986), or increased resource acquisition (Creel & Macdonald, 1995). Once a group has been established, social behavior may then evolve, which further enhances these original benefits (Alexander, 1974). However, within a group certain individuals may exert disproportionate influence over others and as such egalitarian societies are thought to be rare. Competitive asymmetries may generate a social hierarchy, with those individuals that monopolize contested resources classed as dominant. The definition of dominance often varies between studies, leading Drews (1993) to suggest the following structured definition:

“Dominance is an attribute of the pattern of repeated, agonistic interaction between two individuals, characterised by a consistent outcome in favour of the same dyad member and a default yielding response of its opponent rather than escalation.”

Societies may therefore display strong linear hierarchies, where the costs and benefits of group living are divided unequally between group members; with dominant individuals gaining the majority of the benefits (such as increased access to food or mates), while subordinate individuals suffer the majority of the costs (such as decreased access to those same resources). These differences in dominance status may be related to factors such as size (e.g. McElligott et al., 2001) or age (e.g. Berdoy et al., 1995) and may have large consequences for the fitness of individuals.

Dominance hierarchies are generally assumed to exist in mammalian society and have been demonstrated in, for example, brown hyenas *Hyaena brunnea* (Owens & Owens, 1996) and patas monkeys *Cercopithecus patas* (Goldman & Loy, 1997). Hierarchies may also be limited to one sex within a species, as is the case in male capybaras *Hydrochaeris hydrochaeris* (Herrera & Macdonald, 1993), female mountain goats *Oreamnos americanus* (Cote, 2000) and bighorn rams *Ovis canadensis* (Pelletier & Festa-Bianchet, 2006). Here, we investigate whether evidence can be found of dominance hierarchies within social groups of the European badger *Meles meles*.

European badgers display marked intra-specific variation in their behavioral ecology across the species' extensive geographical range (Johnson et al., 2002). In lowland Britain they live at high densities, forming social groups of up to 29 individuals of both sexes (da Silva et al., 1994). Groups appear to form by the retention of offspring in their natal territory, with low dispersal rates among adults (da Silva et al., 1994; Pope et al., 2006). Individuals in groups share communal setts, within which they sleep by day, and are sociable around sett entrances, where they greet, mark, groom and play with each other (Kruuk, 1989). However, individuals typically spend much of their time foraging alone (Kruuk, 1978a) and there is limited evidence that group members benefit from cooperation (Woodroffe & Macdonald, 2000). This, together with the fact that group living is rare in mustelids, has led to the suggestion that the European badger is at an early stage of social evolution (Kruuk, 1989; da Silva *et al.*, 1994).

The degree of sociality found in European badgers also varies across their geographic range, with individuals in continental Europe commonly living alone or in pairs, while

within the British Isles badgers are more typically group living (Woodroffe & Macdonald, 1993; Johnson *et al.*, 2000). Kruuk (1978b) first proposed that the Resource Dispersion Hypothesis (Macdonald, 1983) explained why badgers form groups in some areas (Woodroffe & Macdonald, 1992). The RDH suggests that if resources are heterogeneous in space and time, this may enable several individuals to share them without imposing large costs on each other. Ecological constraints may also play a role in group formation by the costs that they impose on dispersal and independent breeding (da Silva *et al.*, 1994). However, while the role of ecological factors in the variability in sociality in this species has been explored (Johnson *et al.*, 2002); the effect of behavioral factors is less well understood.

Although it is often implied in the literature that dominance hierarchies exist within European badger social groups (by allusion to dominant group members), no empirical evidence of such hierarchies has yet been found (Macdonald *et al.*, 2002). This aspect of the structuring of badger societies therefore remains open to question. Furthermore, understanding whether badger groups exhibit dominance hierarchies is of relevance, as, firstly, it remains uncertain why badgers form groups to begin with (Woodroffe & Macdonald, 1992), and also, more widely, because it may offer important insights into the evolution of mammalian societies.

This study examines behavioral data, collected over three years of filming of badgers in Wytham Woods, Oxfordshire, to investigate: (a) through analysis of aggressive dyadic interactions between group members, whether evidence of linear hierarchies can be found in social groups in this area; (b) whether the sex and age of an individual (demonstrated to be related to dominance status in some other species) are correlated

with rank in these social groups; (c) whether high ranking individuals gain any benefit (in the form of increased reproductive success) and low ranking group members suffer a cost (in the form of increased bite wounds) due to their position in the hierarchy; (d) whether the degree of aggression displayed by badgers is correlated with relatedness between individuals; and, (e) whether patterns of aggressive behavior in these badgers are reflected in other behaviors, in order to assess wider behavioral strategies within these social groups.

A2.3 Methods

A2.3.1 Study site

This study was conducted in Wytham Woods, an area of mostly deciduous woodland 5km north-west of the city of Oxford, U.K. A detailed description of the site is given by Kruuk (1978a). European badgers in this area have been studied since the 1970s and trapping events have occurred annually since 1987 (Macdonald & Newman, 2002). Territory borders are mapped every 2 years using bait marking techniques (Kruuk, 1978b; Delahay *et al.*, 2000).

Filming was carried out at three social groups of European badgers within Wytham Woods: Sunday's Hill (SH), Pasticks (P) and Pasticks Outlier (PO). Pasticks Outlier was originally part of Pasticks but bait marking and trapping records revealed that it had become a separate social group by 2003.

A2.3.2 Data collection

Badgers were trapped at least four times a year, generally around January, June, August and November (Macdonald & Newman, 2002). No trapping took place between February to April, to avoid disturbing the badgers during the final trimester of gestation and in the period that cubs are highly dependent on their mothers. Individuals were caught using swing-door box-traps that were baited with peanuts (Tuytens et al., 1999).

Once caught, badgers were transferred to a holding cage and transported to a processing area. They were then sedated by an intra-muscular injection of approximately 0.2 ml Ketamine hydrochloride (Vetlar; Pharmacia and Upjohns, Crawley, UK) per kg bodyweight. All badgers were marked with a unique tattoo number upon first capture. Individuals from the three social groups studied here were also given unique clip-marks to allow identification on camera (Stewart et al., 1999). Badgers were allocated to social groups based on where they were most frequently trapped. A range of measurements were also taken from each badger, providing information on sex, age and any visible fresh wounds (Macdonald et al., 2004). In addition, guard hairs or a blood sample were collected from each individual for DNA analysis.

Filming was carried out using infra-red CCTV equipment (Stewart et al., 1997), with cameras set up to record all nocturnal, above-ground activity in the locations selected. As behavior occurred sporadically, with periods of no activity, passive infra-red (PIR) detectors were used so that video-recording (in 3 hr real-time mode) was only triggered when activity was detected.

In this study, we analyzed video footage from three years of filming (1995, 2004 and 2005). Cameras were placed at two social groups each year; SH and P in 1995 and P and PO (by this point a separate group) in 2004 and 2005. Each camera was placed to capture social behavior in the vicinity of the sett entrances. As there were more sett entrances at P than the other setts, three cameras were placed at P and one camera at SH and PO per year.

A2.3.3 Behavioral analysis

We analyzed a total of 615 nights of video footage, from the period 1st February to 31st May each year. Cubs are born underground around February, and breeding females immediately enter a period of estrus post-partum (Cresswell et al., 1992). Although mating can occur all year round, this is the first of two distinct peaks in mating activity, with a second period of estrus in late summer / autumn. However, not all males remain fertile for this second period (reviewed in Appendix 1). As males may compete for access to females, while females compete for breeding status (Woodroffe & Macdonald, 1995b), dominance hierarchies may therefore be expected to be most apparent during the spring period considered in this study.

Each night's footage was analyzed according to a strict ethogram with random tape checks between observers to confirm consistency. Behavior was recorded on an ACCESS database (Microsoft Corporation, Redmond, WA), along with the identity of the badgers involved (as deduced from clip-marks). In this study we consider three behaviors; directed aggression, unreciprocated allogrooming, and sequential

allomarking. All are clear unidirectional behaviors, with one badger initiating the behavior, and the other receiving it without reciprocating.

A2.3.4 Genetic analysis

DNA was extracted from hair samples using a Chelex protocol (Walsh et al., 1991) and from blood samples using a GFX Genomic Blood DNA Purification Kit (Amersham Biosciences, Little Chalfont, UK). Individuals were genotyped for 22 microsatellite loci and amplified using methods described in Chapter 2.

The Queller and Goodnight (1989) index of relatedness (R) was estimated for each badger dyad in this study using RELATEDNESS 5.0.8 (see Chapter 3 for details). R ranges from -1 (unrelated) to +1 (identical). Due to natal philopatry, group members may be closely related (Cheeseman et al., 1988). Parentage of cubs was therefore determined by assigning both or one parent through a likelihood-based approach using the program CERVUS 3.0.1.8 (Kalinowski et al., 2007) beta test version, which takes into account the presence of relatives within the group. Cubs were assigned to social groups based on the location in which they were trapped, and candidate parents selected using biological rules (Chapter 2).

The breeding status of each group member was obtained from the parentage analysis, with badgers that had bred within their own social group classed as reproductively active in that year. Although males may father cubs outside their own group, such instances were excluded from analysis, as such reproduction is outside the confines of

any dominance hierarchy. Group members were divided into three categories; breeding, non-breeding or not capable of breeding (i.e. yearlings).

A2.3.5 Statistical analysis

We constructed actor-receiver matrices (Macdonald et al., 2000) for all instances of directed aggression, unreciprocated allogrooming and sequential allomarking that were recorded within each social group, in each year of filming. All matrices were square, with an undefined diagonal. To take into account variation between dyads in their opportunity to interact on camera, each matrix was also weighted by the number of ‘bouts’ in which each member of the dyad was seen. A bout is here defined as a period of badger activity captured on camera, ending when no further activity is detected within a minute of the last badger leaving the screen.

Only badgers that were present, and identifiable, for the entire study period each year were included in the analyses. Any unmarked individuals (those that eluded trapping or whose clip-mark was lost through molting) were excluded, as were all cubs. One badger was seen infrequently at P until early May 2004 and is thought to have subsequently died, while another individual (also from P) was found dead in April 2005. Both badgers were therefore excluded in these respective years. In addition, two individuals were excluded from the matrices for P 1995 as their presence led to ‘structural zeros’ (where two individuals were never observed together and so did not have the opportunity to perform the behavior). As such values would invalidate the matrix analyses as currently performed, the decision was made to exclude these badgers.

A2.3.5.1 *Analysis of dominance*

We tested the directed aggression matrices for the presence of linear hierarchies using MATMAN 1.1 (de Vries et al., 1993). Where there were unknown relationships between dyads the strength of linearity within each group was tested using the improved linearity index h' (de Vries, 1995); otherwise Landau's h index was used. For groups where the degree of linearity was found to be significant, the matrices were then reordered using the I & SI method (de Vries, 1998) to give the most consistent rank order for each group.

As the matrix for PO in 2005 was made up of only five individuals, it was not possible to obtain a statistically significant level of hierarchy in this group when using either the h or h' index (Appleby, 1983). However, it has been suggested by some authors (e.g. Isbell & Young, 2002) that the directional consistency (DC) index (van Hooff & Wensing, 1987) and transitivity of relationships across dyads may be used as an alternative to the h or h' indices. These values have been used to provide a measure of dominance in another study where group sizes were small (Archie et al., 2006).

The DC index ranges from 0 to 1 and is calculated across all dyads as: $(H-L) / (H+L)$, where H is the number of times the behavior was performed in the main direction within each dyad and L is the number of times the behavior occurred in the opposite direction. A high value therefore indicates that, for those individuals observed to interact multiple times, exchanges are highly unidirectional. The transitivity of relationships across dyads is measured as the number of circular triads observed within a social group. A circular triad occurs when, for any three individuals in a

social group, A dominates B, B dominates C, but C dominates A. Therefore, at least one of these three pairs will be inconsistent with a linear order. The observed number of circular triads in a group can be compared with that expected if relationships within the group were totally random, with a smaller than expected number of observed triads thus indicating non-random relationships, and the strength of linearity in the group increasing as the number of circular triads decreases.

Measuring the DC index and transitivity of relationships therefore provides a means of commenting on dominance structure in the smallest group in this study, even if it cannot be tested for the presence of significant linearity. However, it should be noted that the former value can be greatly affected by dyads in which the behavior is performed frequently, suggesting caution should be used in its interpretation.

A2.3.5.2 Correlates of rank

For those social-group-years where significant linear hierarchies were found, we analyzed the relationship between dominance rank and minimum age (considered to be one if first trapped as an adult), wounding status (classified as wounded or unwounded from the presence of fresh wounds in the January and June trapping data; see Macdonald et al., 2004) and breeding status (did or did not breed, with yearlings excluded) using Spearman's Rank correlations and unequal variance *t*-tests. Zimmerman and Zumbo (1993 – cited in Ruxton, 2006) argue that, when carried out on ranked data, the unequal variance *t*-test performs better than the Mann-Whitney *U* test when variances are unequal. This test was therefore considered preferable here.

All analyses were conducted in the program MINITAB 14 (Minitab Inc., State College, PA, USA).

A2.3.5.3 Correlates of aggression

As individuals might be expected to show lower levels of aggression towards relatives, we entered R into matrices for all social-group-years and Kendal's (Kr) row-wise correlations (de Vries, 1993) were used to compare the resulting relatedness matrices with those for directed aggression. To assess the significance of the resulting Kr statistic a permutation procedure, in which the rows and columns were permuted simultaneously 10,000 times, was employed using MATMAN 1.1.

Finally, we investigated whether individuals were more likely to direct aggression at partners that they allomarked more often, or whether they received more unreciprocated allogrooming from those partners to which they were more aggressive. To achieve this, we used Kr row-wise correlations to compare the matrices for directed aggression with their corresponding matrices for unreciprocated allogrooming and allomarking. We observed a total of 659 instances of directed aggression, compared to 838 instances of unreciprocated allogrooming and 1476 of sequential allomarking.

A2.4 Results

A2.4.1 Analysis of dominance

Of the six social-group-years investigated, three showed a significant degree of linearity (SH 1995: $h' = 0.393$, $df = 13$, $P_{\text{right}} < 0.05$; P 2004: $h = 0.929$, $df = 6$, $P_{\text{right}} < 0.05$; P 2005: $h' = 0.683$, $df = 8$, $P_{\text{right}} < 0.05$). Two groups displayed no significant hierarchy (P 1995: $h' = 0.286$, $df = 7$, $P_{\text{right}} = 0.635$, PO 2004 - $h' = 0.607$, $df = 6$, $P_{\text{right}} = 0.201$), while one further group (PO 2005) contained too few individuals for testing using this method.

DC indices for the six groups studied here were high (ranging from 0.7 to 1), with a DC index of 0.84 for PO in 2005 suggesting a high degree of unidirectionality within dyads. The number of circular triads observed were also lower than expected in all groups except P 1995, although not greatly so in PO 2005 (observed = 1.75, expected = 2.5). This suggests that members of this social group year have asymmetric relationships, although the degree of linearity within the group may be low.

A2.4.2 Correlates of rank

Females had significantly higher dominance ranks than males in two of the three social-group-years for which hierarchies were detected (unequal variance t -tests: SH 1995: $t' = 5.02$, $N_1 = 10$, $N_2 = 4$, $df = 10$, $P = 0.001$; P 2004: $t' = 4.04$, $N_1 = 3$, $N_2 = 4$, $df = 4$, $P = 0.016$; Figure A2.1). While there was no significant difference between male and female rank in P 2005 ($t' = 1.56$, $N_1 = 2$, $N_2 = 7$, $df = 6$, $P = 0.170$), sexually mature females did occupy the top ranks in this group, although the sexually

immature female yearlings were ranked bottom (Figure A2.1b). Age, however, was not related to rank in any of the three social-group-years (Spearman's Rank correlations: SH 1995: $r_s = 0.336$, $N = 14$, $P = 0.240$; P 2004: $r_s = -0.222$, $N = 7$, $P = 0.632$; P 2005: $r_s = 0.630$, $N = 9$, $P = 0.069$).

A significant relationship between rank and breeding status was seen in one social group year (unequal variance t -tests: SH 1995: $t' = -0.28$, $N_1 = 3$, $N_2 = 5$, $df = 2$, $P = 0.803$; P 2004: $t' = -4.04$, $N_1 = 2$, $N_2 = 5$, $df = 4$, $P = 0.016$; P 2005: $t' = -2.94$, $N_1 = 4$, $N_2 = 2$, $df = 1$, $P = 0.209$). However, the small sample sizes, and the low rank of the sole breeding male in SH, were likely to have been influential. There would appear to be non-orthogonality between sex and breeding status in these social groups, with only one male seen to reproduce within his own group (in SH 1995). Thus any apparent relationship between rank and breeding status may well be artificial, instead reflecting the difference in rankings between males and females (Figure A2.1).

Small sample size was also a problem for these analyses when testing the relationship between rank and wounding status, with no badgers documented as carrying wounds during the period of this study in P 2005. No significant relationship was found between the two variables in the remaining two social-group-years (unequal variance t -tests: SH 1995: $t' = -0.25$, $N_1 = 6$, $N_2 = 8$, $df = 11$, $P = 0.806$; P 2004: $t' = 1.83$, $N_1 = 2$, $N_2 = 5$, $df = 4$, $P = 0.140$).

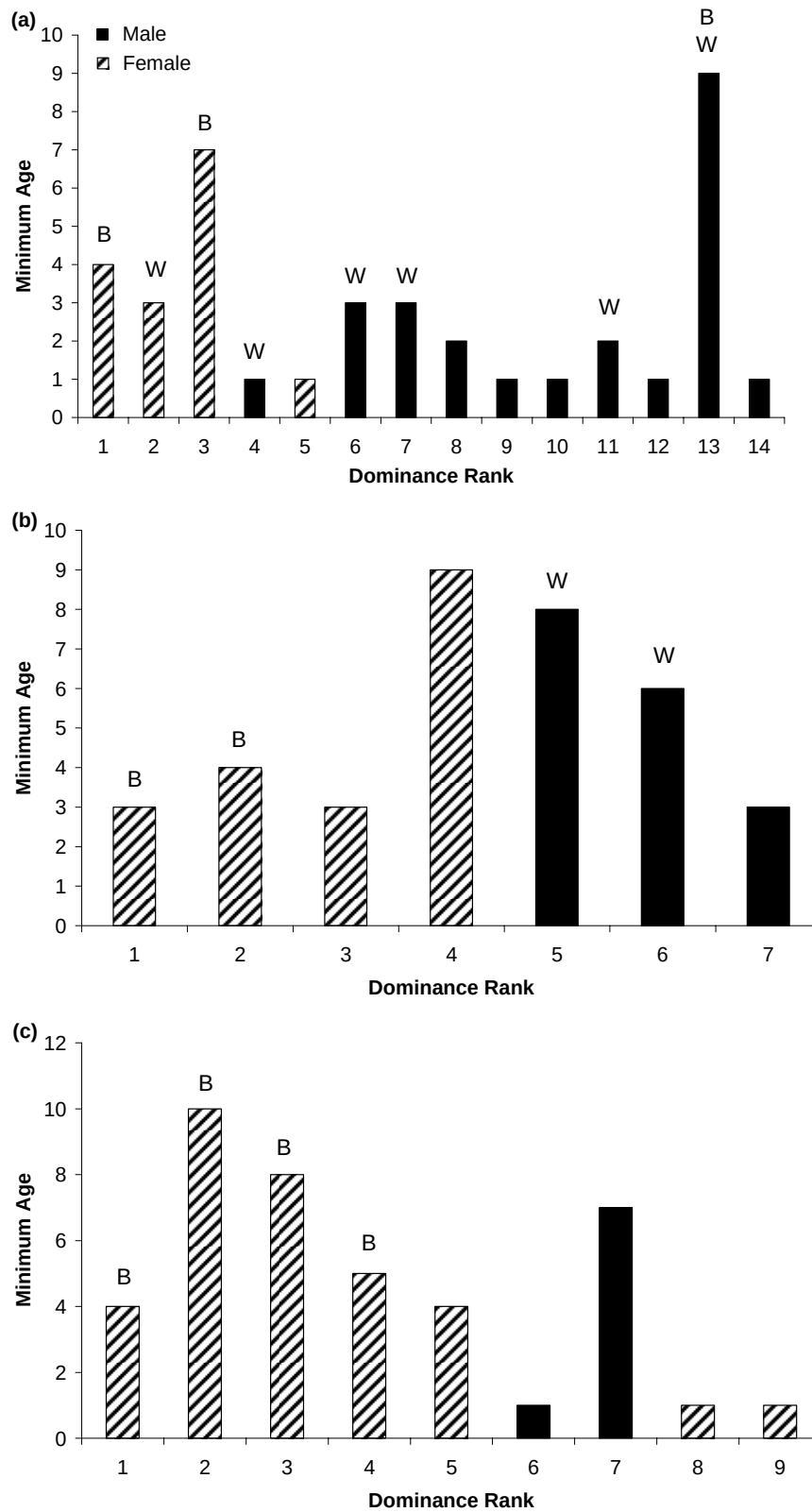


Figure A2.1 The age (years), sex, breeding status and wounding status of badgers of each dominance rank in: (a) SH 1995; (b) P 2004; and (c) P 2005. A rank of 1 is the highest rank. Individuals that successfully bred in each social group year are indicated by a B, wounded individuals by a W.

A2.4.3 Correlates of aggression

There was a negative correlation between levels of directed aggression and relatedness in the three social-group-years that were structured according to linear dominance hierarchies, although this was significant for only P 2005 (SH 1995: $K_r = -93$, $P_{\text{left}} = 0.070$; P 2004: $K_r = -19$, $P_{\text{left}} = 0.111$; P 2005: $K_r = -37$, $P_{\text{left}} < 0.05$). The remaining three social-group-years showed no evidence of this relationship (P 1995: $K_r = 1$, $P_{\text{left}} = 0.616$; PO 2004: $K_r = 17$, $P_{\text{left}} = 0.917$; PO 2005: $K_r = 3$, $P_{\text{left}} = 0.652$).

There was some evidence that badgers were more likely to receive unreciprocated allogrooming from those individuals to which they were more aggressive. Positive correlations between the two behaviors were seen in four social group years, although these were significant for only P 1995 and PO 2004 (SH 1995: $K_r = 23$, $P_{\text{right}} = 0.2988$; P 1995: $K_r = 22$, $P_{\text{right}} < 0.01$; PO 2004: $K_r = 27$, $P_{\text{right}} < 0.05$; P 2004: $K_r = -22$, $P_{\text{right}} = 0.923$; PO 2005: $K_r = 12$, $P_{\text{right}} = 0.072$; P 2005: $K_r = 0$, $P_{\text{right}} = 0.5116$). Similarly, four of the six social-group-years showed evidence of a positive correlation between levels of directed aggression and sequential allomarking, suggesting that badgers more frequently allomark individuals towards which they are more aggressive. However, this relationship was significant for only one group; P 2005 (SH 1995: $K_r = -3$, $P_{\text{right}} = 0.527$; P 1995: $K_r = 7$, $P_{\text{right}} = 0.219$; PO 2004: $K_r = 25$, $P_{\text{right}} = 0.076$; P 2004: $K_r = 20$, $P_{\text{right}} = 0.178$; PO 2005: $K_r = -2$, $P_{\text{right}} = 0.649$; P 2005: $K_r = 55$, $P_{\text{right}} < 0.01$).

A2.5 Discussion

Although dominance hierarchies are often alluded to in studies of the European badger (Kruuk, 1989; Woodroffe & Macdonald, 1995b; Revilla & Palomares, 2002), empirical evidence of such hierarchies has to date proved elusive (Macdonald et al., 2002). European badgers do not seem to gain from many of the benefits often suggested to arise from group living, such as greater hunting efficiency or cooperative defense (Kruuk, 1989), although benefits from allogrooming have been established (Johnson et al., 2004). This lack of cooperation and competition between group members may explain the apparent lack of dominance hierarchies previously found in social groups. Here we present evidence of significant linear hierarchies in social groups from southern England, as determined from analysis of aggressive interactions in the vicinity of sett entrances. The hierarchies were detected during the mating and cub rearing season, suggesting that dominance structures may be situation dependant, with hierarchies arising where (and when) competition is highest.

Of the six social-group-years studied, half exhibited significant linear hierarchies. Another (PO 2005) showed evidence of asymmetric relationships, with a high degree of unidirectionality in aggressive behavior within dyads, but, due to small group size, it was not possible to test for linearity within this group. Of the three groups found to display significant linearity, the h' value for SH 1995 was somewhat low. This may be due to the number of individuals in this group, as models predict that linearity will decrease with group size. Furthermore, obtaining high values of linearity is problematic in groups of more than around nine individuals (Mesterton-Gibbons & Dugatkin, 1995). However, there was no evidence of linear dominance hierarchies in two of the social-group-years studied here. This may suggest that different groups

display different social structures. Of the groups studied, PO contained the fewest individuals (in both years); thus, competition in this social group may be lower, with less opportunity for individuals to assert dominance. This social group was also only identified as a separate group from P in 2003 and so social dynamics may not yet have been well established. For P 1995, however, it seems likely that the relatively low frequency of interactions between group members had an effect, with a high proportion of unknown relationships, and the further exclusion of two individuals due to structural zeros, making it difficult to detect any evidence of a hierarchy. In addition, an unusually high number of unmarked individuals were observed within this social group, with a maximum of five unmarked badgers seen on screen together. As unmarked individuals could not be included in the analysis, important interactions with additional group members were potentially missed, decreasing the likelihood of detecting any hierarchy even further.

A2.5.1 Correlates of rank

For those social-group-years in which linear hierarchies were seen, a further particularly striking finding of this study was the clear difference in dominance rankings between males and females. Species in which females are dominant or equal in status to males are generally rare in mammals (Kappeler, 2000) yet the older, sexually mature female badgers were consistently ranked above males in all social groups found to have hierarchies (although the same did not hold for immature female yearlings; see Figure A2.1). Macdonald et al. (2002) suggest that the high competitive ability of female European badgers may result from the high energetic costs of raising young (resulting in a payoff asymmetry), or from their potential to withhold mating

opportunities from males. Additionally, intra-sexual competition for breeding opportunities may limit competitive asymmetries in this species. Competition for breeding status appears to be high in female badgers within social groups, with da Silva et al. (1994) finding that at least 48% of females of reproductive age failed to breed within Wytham Woods, while the number was estimated to be as high as 69% in Chapter 2. Additionally there is significant reproductive skew within badger social groups (Chapter 3).

One resource for which females may compete is breeding dens (Kruuk, 1989; Stewart et al., 1999) and it has been suggested that females may monopolize such areas during cub rearing (Cresswell et al., 1992). Females may therefore display territorial behavior around sett entrances, providing an explanation for the high dominance rankings of females of reproductive age seen here. The increased aggression observed in these females may be explained both by competition for the resource in question, and due to the risk of infanticide by other group members. Females that have bred successfully will fight to maintain their hold on breeding space and to protect their cubs, while those females who have not bred successfully in the current year will compete to obtain breeding space for future seasons.

Overall there appears to be no relationship between female dominance rank and reproductive status in this study. Although only the two highest ranking females reproduced in P 2004, suggesting that social rank may be linked to reproductive success in this group, only one reproductively active female (out of five) failed to breed in P 2005. Similarly, the top and third ranking of three sexually mature females in SH 1995 successfully raised cubs; again suggesting no relationship. Previous

studies of this population have indicated that the females that breed (or fail to breed) in one year will not necessarily do the same the next year (da Silva *et al.*, 1994; Woodroffe & Macdonald, 1995b), although they do have a greater probability of breeding in the following year (Chapter 4). It is thought that resource availability plays a key role, with da Silva *et al.* (1994) observing that in areas of high food availability several females may raise cubs within a group, and that there was a trend for the proportion of females that breed to decline with increased group size. It is likely that it is the availability of food in autumn that is important, with females that are heavier at this time more likely to successfully raise cubs the next year, due to their reliance on fat reserves during gestation and early lactation (Cheeseman *et al.*, 1987; Woodroffe & Macdonald, 1995a; Dugdale *et al.*, 2003).

Where though, do males fit in? Males may be expected to compete for access to females, with dominant males securing more mating opportunities, as seen in capybara (Herrera & Macdonald, 1993), and the brown lemming *Lemmus trimucronatus* (Huck & Banks, 1982). However, females have been observed to mate with multiple males during a single estrus (Johnson, 2001), and extra-group paternity is common (Carpenter *et al.*, 2005), with approximately 50% of cubs in Wytham sired by extra-group males (Chapter 2). Three of the identifiable males included in this study sired cubs outside of their own group (in SH 1995; rank 6 / 14, P 2004; rank 7 / 7, and in P 1995; unranked), and four males sired a cub within their own social group (two in P 1995; unranked, one in SH 1995; rank 13 / 14 and one in PO 2004; unranked). The SH male was the oldest male in this group (9 years) but the second lowest ranking (rank 13), suggesting that for this individual at least, dominance rank had little effect on reproductive success. Although we observed some instances of

fight breaking out over females on the video footage, and males were sometimes seen to harass mounted males (presumably in an attempt to break up the mating pair and gain access to the female themselves), competition between males may be relatively low in this context.

The effect of dominance ranking on reproductive status therefore remains uncertain in this study. There would also appear to be no relationship between dominance rank and the likelihood of receiving bite wounds in these social groups, although few wounds were recorded overall, again hindering any firm conclusion. However, the patterns of wounding seem to provide support for the findings of Macdonald *et al.* (2004) that bite wounds are more likely for males and that wounds are more common in larger groups (see Figure A2.1). Dominance rank would seem to be inconsistent between years for those individuals that were present at P in both 2004 and 2005, although the introduction and loss of several individuals in the second year may have affected social dynamics. Age does not appear to be related to dominance rank, either within groups as a whole (as shown by the Spearman's rank correlations for each social group) or within the sexes (not tested for statistical significance due to small sample sizes but see Figure A2.1). The influence on dominance status of size / weight (McElligott *et al.*, 2001; Holand *et al.*, 2004) could not be tested in this study because badgers undergo marked weight changes over the seasons (Kruuk & Parish, 1983); so the weights of badgers caught at one trapping event cannot be justifiably compared with the weights of those caught at another. This factor therefore remains to be considered.

A2.5.2 Correlates of aggression

There would appear to be some evidence that individuals are less aggressive towards closer relatives, with negative correlations between levels of aggression and relatedness values found within those social groups which displayed linear hierarchies. Belisle and Chapais (2001) found that female Japanese macaques *Macaca fuscata* were more tolerant of co-feeding by close relatives, with the aggression levels of dominant females towards subordinate females decreasing with increasing degree of kinship. Due to natal philopatry and restricted dispersal, however, relatedness levels between group members in these badger social groups are high and one third of group members are parent–offspring or full-siblings (Chapter 3). Any potential fitness benefit badgers derive from reducing aggression to closer kin may therefore be negligible.

Positive correlations between aggression and unreciprocated allogrooming were seen for four of the six groups, although only significantly so in two. This suggests that badgers in these groups are more likely to be groomed by individuals to which they direct greater aggression, a result that has also been observed in meerkats *Suricata suricatta* (Kutsukake & Clutton-Brock, 2006). Unreciprocated allogrooming may be considered costly to the actor (Johnson et al., 2004) and so may reflect a form of tolerance payment, with individuals attempting to appease dominants. However, if this is the case in badger social groups, it might be expected that the relationship between aggression and unreciprocated allogrooming should be strongest in those groups for which hierarchies were found, and weak or non-existent in those groups without hierarchies. Such a pattern was observed in female chacma baboons *Papio cynocephalus ursinus* (Barrett et al., 2002). In a period where aggression was lower in

the troop and the dominance gradient appeared to be shallower, dominants no longer appeared to attract grooming from subordinates, and instead reciprocal exchange of grooming increased. This was not reflected in the badger social groups studied here. Although reciprocated allogrooming between badgers has not been considered in this study, it would seem that the motivation behind unreciprocated grooming behavior in these social groups is unclear.

The relationship between aggression and sequential allomarking also seems to vary between groups. However a positive correlation was again seen in four groups (significantly so in P 2005), suggesting that these badgers more frequently allomark individuals to which they are more aggressive. Buesching et al. (2003) found that sequential allomarking in badgers was related to sex and age, with males marking more than females and yearlings and sub-adults marking the most frequently. They therefore suggest that, in addition to distributing a “group-smell”, which helps individuals to distinguish between group members and outsiders, this type of allomarking also advertises individual-specific information. It is possible that sequential allomarking may serve as a means of ‘reinforcing’ aggression, with individuals following aggressive acts with sequential allomarking and perhaps also conveying information on social status in the process. However, the results seen here again do not allow any broad conclusions about motivational strategies associated with this behavior.

The European badger is thought to represent an early stage of social evolution and a full understanding of the social system of this species remains incomplete. In this study we have presented evidence of female led dominance in the vicinity of sett

entrances within badger social groups during the mating and cub rearing season. However, it remains unclear what factors may promote dominance, or what benefits / costs individuals may receive because of their dominance status. Further research on the social structure of European badgers is needed, both because it may add to our understanding of how sociality in badgers develops and is maintained, and because it may offer insights into the evolution of mammalian societies generally.

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