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Carpenter, P.J., Pope, L.C., Greig, C. et al. (2005) Mating system of the Eurasian badger, Meles meles, in a high density population. Molecular Ecology, 14 (1). pp. 273-284. ISSN: 0962-1083

https://doi.org/10.1111/j.1365-294X.2004.02401.x

Molecular Ecology (2005) **14**, 273–284 doi: 10.1111/j.1365-294X.2004.02401.x

Mating system of the Eurasian badger, *Meles meles*, in a high density population

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Abstract

Badgers are facultatively social, forming large groups at high density. Group-living appears to have high reproductive costs for females, and may lead to increased levels of inbreeding. The extent of female competition for reproduction has been estimated from field data, but knowledge of male reproductive success and the extent of extra-group paternity remains limited. Combining field data with genetic data (16 microsatellite loci), we studied the mating system of 10 badger social groups across 14 years in a high-density population. From 923 badgers, including 425 cubs, we were able to assign maternity to 307 cubs, with both parents assigned to 199 cubs (47%) with 80% confidence, and 14% with 95% confidence. Age had a significant effect on the probability of reproduction, seemingly as a result of a deficit of individuals aged two years and greater than eight years attaining parentage. We estimate that approximately 30% of the female population successfully reproduced in any given year, with a similar proportion of the male population gaining paternity across the same area. While it was known there was a cost to female reproduction in high density populations, it appears that males suffer similar, but not greater, costs. Roughly half of assigned paternity was attributed to extra-group males, the majority of which were from neighbouring social groups. Few successful matings occurred between individuals born in the same social group (22%). The high rate of extra-group mating, previously unquantified, may help reduce inbreeding, potentially making philopatry a less costly strategy.

Keywords: badger, DNA profile, mating systems, *Meles*, microsatellite, Mustelidae

Received 28 April 2004; revision received 03 September 2004; accepted 06 October 2004

Introduction

Badgers are solitary throughout most of their range, with males defending a territory that overlaps one or more females (Kruuk & Parish 1982; Woodroffe & Macdonald 1995). However, in southern England, badgers occur at high densities, and in these areas form large social groups of up to 27 individuals (Kruuk 1978; Rogers *et al*. 1997). Such high density populations are primarily associated with pastoral landscapes, and it appears likely that group-living in badgers is a recent phenomenon (Cresswell *et al*. 1989; Kruuk 1989). Group-living is rare in mustelids (Johnson *et al*. 2000), and so obtaining a better understanding of the mating system of social badgers may provide insights into factors that promote the evolution of social behaviour.

The majority of social groups form through the delayed dispersal of offspring (Emlen 1984), and this appears to be the case for badgers (Kruuk & Parish 1982; Cheeseman *et al*. 1987; da Silva *et al*. 1994). Therefore, to begin to understand social grouping we need to assess the costs and benefits of delayed dispersal by sexually mature individuals (Emlen 1984; Hatchwell & Komdeur 2000). Reasons for delaying dispersal are generally thought to be a combination of life history traits and ecological constraints (Emlen 1984; Arnold & Owens 1998; Hatchwell & Komdeur 2000). Badgers form social groups only at high density, making it likely that habitat saturation influences decisions to delay dispersal.

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Benefits from group interactions are also thought to be an important factor in selection for group formation (e.g. group hunting, Blundell *et al*. 2002; group vigilance, Clutton-Brock *et al*. 1999). The benefits of group living for badgers remain unclear, particularly as group size appears to have no influence on reproductive success once territory quality is taken into account (Woodroffe & Macdonald 2000). However, it is thought that the ecological costs to group living in badgers may also be limited (Kruuk & Parish 1982; Johnson *et al*. 2001). In southern England, badgers feed primarily on earthworms, which are distributed in patches. A territory needs to contain several patches of earthworms, and in doing so appears to provide sufficient food for more than the breeding pair (Kruuk 1989). This has led to the formulation of the resource dispersion hypothesis to explain group living in badgers and other animals (Kruuk & Parish 1982; Macdonald 1983; Carr & Macdonald 1986; Johnson *et al*. 2002; but see Revilla 2003). That environmental mechanisms are the proximate cause of social structure variation has also been proposed for canids (Geffen *et al*. 1996) and other mammals (Wrangham *et al*. 1993).

Determining reproductive success in the high-density badger populations of southern England is challenging for several reasons. If mothers cannot be assigned a priori (e.g. through the use of lactation data), there are large numbers of female candidates. Also, because of philopatry, it is likely that the candidate mothers include relatives to the cub, further reducing the power and confidence in assignment (Jones & Ardren 2003). There are also many candidate fathers, as not only are females in contact with males of the same group, but both sexes are also known to visit neighbouring setts, providing opportunities for extra-group mating. (Evans *et al*. 1989; da Silva *et al*. 1994; Woodroffe *et al*. 1995; Rogers *et al*. 1998).

The general picture of badger social groups at high density is one of limited dispersal between social groups (Neal & Cheeseman 1996; Rogers *et al*. 1998), male-biased dispersal (Rogers *et al*. 1998), delayed dispersal with preferential recruitment of offspring to their social group (Kruuk & Parish 1982; Cheeseman *et al*. 1987; da Silva *et al*. 1994) and a reproductive dominance hierarchy within social groups that may vary between years (Kruuk 1989; Cresswell *et al*. 1992; Woodroffe & Macdonald 1995). The influence of social groups on male reproductive success remains poorly known, and while extra-group mating has been inferred in earlier genetic studies (Evans *et al*. 1989; da Silva *et al*. 1994; Domingo-Roura *et al*. 2003), the extent to which this occurs is unknown. Here, we investigate the mating strategies of badgers at high density by obtaining microsatellite DNA profiles for badgers from a well-studied population. In this initial paper we ask: does multiple paternity occur in litters? What proportion of the population breeds? Does age influence reproductive success? How frequent is extra-group mating?

Materials and methods

Study site and sample collection

Woodchester Park is located on the Cotswold limestone escarpment in Gloucestershire, southwest England. The study area of approximately 11 km^2, consists of a central steep-sided wooded valley surrounded by farmland. The badger population at Woodchester Park has one of the highest recorded densities (over 25 adults per km^2), and is the site of an ongoing mark-recapture study initiated in 1976 (Cheeseman *et al*. 1987; Rogers *et al*. 1997). Twenty-one to 25 social groups within the site have been intensively studied over the last 14 years (Rogers *et al*. 1997; Delahay *et al*. 2000). In 1993 the population consisted of 27% cubs, of equal sex ratio, but becoming increasingly female-biased with age (Rogers *et al*. 1997). For many individuals, birth and death dates were known, and the reproductive status of females was recorded at capture. Previous demographic analyses of this population found that, as in other high density badger populations, social group size was large (mean 8.8 in 1993; Rogers *et al*. 1997) and the reproductive success of females was low (only 10%–42% of females bred, Rogers *et al*. 1997).

In this paper we focused on the cubs from 10 social groups and adults from 26 social groups, observed during 1989–2002 (Fig. 1). Each of these social groups is trapped four times a year, for two consecutive nights on each occasion. Traps were located on or near badger 'runs' at the active setts in each territory. Trapped badgers were anaesthetized using either ketamine hydrochloride (Vetelar: 20 mg/kg) (MacKintosh *et al*. 1976) or a combination of ketamine, butorphanol and medetomidine (de Leeuw *et al*. 2004). The sex, age class (adult, 1-year-old, cub), location of capture,

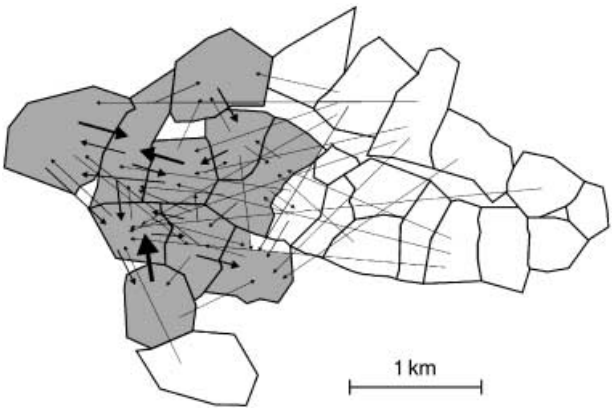

Fig. 1 Badger social group boundaries at Woodchester Park, as mapped in 1999. Paternity and maternity were determined for cubs from the 10 social groups shaded grey. Arrows represent paternity from outside the cubs' social group assigned with 80% confidence. The thickness of the arrow is proportional to the number of paternities over the 14-year period studied.

body weight and length and reproductive status were recorded for each individual at each capture. On first capture, each badger was given a unique identifying tattoo and either a blood sample or guard hairs were taken. Blood samples were stored in heparin buffer at –20 °C. Hair samples (collected since 1997) were either stored in 80% ethanol or kept at –20 °C.

The configuration of badger social group territories was established each year using bait-marking during the peak of territorial activity in spring (Kruuk 1978; Delahay *et al*. 2000). As population density has increased in the study area (Rogers *et al*. 1999), there have been some changes to badger social groups. Increases in badger density resulted in a decrease in the overlap of territories, but the locations remained fairly stable. The number of core social groups has increased over time from 21 until 1989, 22 in 1990, 23 in 1995, 24 in 1996 and 25 in 1997–2002. Territory sizes did not increase; rather in all four cases, two new territories occupied the same approximate area as the previous single territory. These fissions of territories did not take place in the 10 social groups from which we have analysed cubs. All calculations were based on the 1999 territory configuration and include an additional social group from outside the core area, leading to a total of 26 social groups from which candidate male parents were sampled.

Sample decontamination and DNA extraction

Genomic DNA was extracted from blood using a slight variation of the phenol:chloroform:isoamyl alcohol method (Sambrook *et al*. 1989). Bovine tuberculosis (*Mycobacterium bovis*) is endemic in British badgers and can cause serious disease in humans. Therefore, to reduce the potential health risks, the transfer of blood samples to extraction buffer was performed in a level 3 containment laboratory (Advisory Committee on Dangerous Pathogens *et al*. 1995). To disinfect the bacteria, blood samples were incubated at 95 °C for 30 min (Lauzardo & Rubin 2000), after the proteinase K digestion in our extraction protocol. Hair samples were either stored in 80% ethanol or transferred to 80% ethanol in a level 3 containment laboratory. This concentration of ethanol will disinfect *M. bovis* after several hours (Lauzardo & Rubin 2000). DNA was extracted from hair samples using Chelex 100 resin (Walsh *et al*. 1991). For each individual we extracted a minimum of 10 hair follicles with visible roots.

Genotyping

From the 34 polymorphic microsatellite loci isolated in the Eurasian badger (Carpenter *et al*. 2003), we used 17 that had been optimized for use on an Applied Biosystems model 377 sequencer. Approximately 50 ng of DNA and 0.25 units of *Taq* DNA polymerase (Thermoprime Plus, Advanced Biotechnologies) were added to each PCR (polymerase chain reaction) reaction. Amplification by PCR was carried out in 15 μL reactions with a final concentration of: 1 × manufacturer's PCR buffer (20 mM $(NH_4)_2SO_4$, 75 mM Tris-HCl, pH 9.0, 0.01% (w/v) Tween); 0.25 μM of each primer, 0.1 mM of each dNTP, and 1.5 mM $MgCl_2$ (Mel101, 102, 103, 105, 106, 107, 109, 111, 113, 114, 116, 117) or 2.5 mM $MgCl_2$ (Mel104, 108, 110, 112, 115). PCR amplification was carried out in a Hybaid TouchDown™ thermal cycler (Thermo Hybaid) using a touchdown program for all except two loci. The PCR conditions were 5 min at 95 °C followed by 34 cycles of 15 s at 94 °C, 20 s at 64 °C–50 °C (temperature dropping by 2 °C every two cycles with 20 cycles at 50 °C) and 30 s at 72 °C followed by a final extension stage of 10 min at 72 °C. Loci *Mel*114 and *Mel*117 were amplified with a single annealing temperature of 57 °C during all 34 cycles. Fluorescent PCR products were mixed to create two sets of multiplex loading groups. The samples were run on a 10% denaturing polyacrylamide gel in the ABI PRISM 377 DNA Sequencer. GENESCAN 3.1 and GENOTYPER 2.5 software (Applied Biosystems), were used to size alleles based on a size standard with bands at least every 50 bp (D. Paetkau, unpublished).

Mendelian inheritance of the loci used in this paper was confirmed using a set of captive individuals with a known pedigree (n = 11, E. Rafart unpublished data). Deviations from Hardy–Weinberg equilibrium (HWE) and linkage equilibrium between loci were tested by a Markov chain method (1000 dememorizations, 50 batches, 1000 iterations) with the program GENEPOP 3.3 (Raymond & Rousset 1995). This test was performed on individuals known to be alive in 1999, and for adults and cubs separately to reduce multigenerational effects. To determine how much power we had to distinguish between individuals, we used the program GIMLET (Valiere 2002) to determine PI_{sib} (Evett & Weir 1998; Woods *et al*. 1999; Waits *et al*. 2001). PI_{sib} is the probability that two siblings drawn at random from the population will have identical multilocus genotypes. This provides a conservative upper bound for the probability of observing two identical individuals sampled from a population, as the observed probability of identity is often higher than theoretically expected in natural populations (Waits *et al*. 2001).

Parentage analyses

We used a likelihood-based approach to determine parentage using the program CERVUS 2.0 (Marshall *et al*. 1998). As no parent was known, the power to assign parents using the entire population as a candidate pool was low. We therefore used mark-recapture information to define our candidate parents based on the following rules. Initially, we assigned maternity. Females were considered to be candidate mothers if they were defined as reproductive (see below) and were assigned to the same social group as

the cub in the year of birth. All males were considered to be candidate fathers if they were defined as potentially reproductive in the year before the cub was born because females conceive nearly one year before they give birth resulting from delayed embryo implantation (Cresswell *et al*. 1992). The last year of reproduction was defined as either the year a badger was found dead, or 3 years after the last recorded live observation by capture. Three years was chosen because 95% of intervals within trap records for individuals were less than 3 years in length. A previous study has shown that, in a given year, a significant number of badgers at Woodchester Park were trap 'shy' (Tuyttens *et al*. 1999). However, the majority of animals trapped only once before being subsequently found dead were also found within three years (63/69 = 91%), and so the 3-year rule was applied irrespective of the number of captures. The first year of potential reproduction was defined as the birth year plus one, or the first year of capture if caught as an adult. In this paper we assumed that one-year-old animals did not breed (i.e. 1-year-old males are able to impregnate 1-year-old females, but both will then be 2 years old when the cub is born). Other studies have found that most females do not ovulate in their first year (Ahnlund 1980; Cresswell *et al*. 1992; Rogers *et al*. 1997). Individuals were assigned to a social group if they were caught within the territory of that group. In years where individuals were not caught, but were thought to be reproductive based on the above rules, badgers were assigned to a social group based on where they were caught in adjacent years. Badgers were assigned to multiple groups if the group where they were caught differed between these years (e.g. if in 1989, an animal was caught in social group A, and in 1992 it was caught in social groups C and E, in the intervening period it was assigned to groups A, C and E).

Parentage assignment was then performed in three stages. First, mothers were assigned, and then fathers, and finally where a father was assigned without a mother, the assignment analysis for mothers was repeated with the male as a known parent. In order to assign parents, we used simulations to determine the critical value of delta [the LOD (logarithmic odds) difference between the best, and the next best parent] that we would accept. The mean number of candidate females based on the above rules was 11 (ranging from one to 25). The average percentage of reproductive females genotyped was 80%. We increased this by excluding cubs where less than 60% of potential mothers had been genotyped. For males, the mean number of candidates per year was 162, with an average of 72% genotyped. These values were used in simulations in CERVUS to determine the critical delta, the difference in LOD scores between the most likely and second most likely candidate needed to give us our required level of confidence. There was a high likelihood that there were relatives present among the candidate parents. In our simulations, we therefore also included the presence of a single relative, related to the offspring by 0.25. It is possible that the average number of relatives per social group was higher than this, and that first order relatives were present. A more accurate estimate of this number in the future would provide more accurate parentage analysis. We determined allele frequencies based on a pooled data set across the 14 years studied. Candidates were genotyped for at least eight loci. Genotyping was 87% complete. We used this value for our simulations and an error rate of 0.01. The error rate was estimated by regenotyping a subset of the samples. We analysed parentage with 80% confidence in the presence of a single relative, and performed a second, more stringent, analysis using data with 95% confidence, but without the presence of a relative. Simulations predicted that only 13% of offspring could be assigned parentage with 95% confidence in the presence of a relative, providing us with insufficient data to test our hypotheses. Unless indicated, the results presented are those from the analysis using 80% confidence in the presence of a relative.

Once mothers were assigned, we defined 'litters' as cubs assigned to the same female in the same year. From these litters we determined the rate of multiple paternity based on both 80% and 95% confident assignments. The number of females breeding per social group was determined both for social groups where all cubs were assigned a mother, and social groups with only partial assignment of maternity to cubs. The number of males breeding per social group was determined for social groups with partial assignment of cubs.

We estimate the proportion of male and female breeders in the population based on the known number of cubs, the number of parents assigned to genotyped cubs and the number of candidates. We assumed the potential number of female badgers breeding in a given year to be the same as the number of female candidates (except that females were included only once in a given year). This may be an overestimate as a result of the inclusion of individuals for three years after 'final' capture, and so may overestimate the number of nonbreeders. Male candidates were taken from 26 social groups, whereas cubs were genotyped from only 10 social groups. We therefore separately considered the number of candidate males both as (i) the number of candidates from 26 social groups, and (ii) the number from the 10 focal social groups.

We tested for an influence of age on reproduction using data based on parentage assignments made with at least 80% confidence. Analyses were performed separately for males and females. To make the results for males and females more comparable, we included only males from the 10 core social groups, rather than the entire study site as was used in paternity assignment. This subsample accounted for 89% of the males that were assigned paternity. Genotyping of this subset of males was also more

complete (80% vs. 72%), similar to that of females (80%). However, the mean number of cubs for males and females was still not directly comparable as maternity was assigned to 72% of cubs but paternity was assigned to only 47%. The number of cubs attributed to a parent was first modelled as a Poisson-distributed response (using a log link) by age alone, where age was considered to be a categorical variable (with eight levels, from 2 years to = 9 years) within a generalized linear model (GLM). Individuals of 9 years and older were pooled as a result of the small sample size. In order to account for any possible bias introduced by between-subject variation, the analysis was repeated as above within a GLMM (generalized linear mixed model) but including in addition 'individual adult badger' as a random effect. These analyses were performed in GENSTAT for Windows, 6th Edition (VSN International Ltd, Hemel Hempstead, UK).

The rate of extra-group paternity was determined by comparing the mother's social group with that of the assigned father in the year of conception. We considered both the entire data set as well as a subset of the data where both parents were actually captured in the year of conception, rather than having their social group inferred. The average 'mating dispersal' was based on the distance between main setts in the respective social groups, both including and excluding same group matings. Where the information was available, the natal social groups of assigned parents were compared to determine the rate of matings between individuals born in the same social group.

Results

Seventeen loci were used to genotype 923 badgers trapped over 14 years at Woodchester Park. Exact tests for HWE and linkage were performed on adults and cubs separately, for individuals known to be alive in 1999. Three loci showed significant departure from HWE in the adult population, the rest were in HWE after correcting for multiple tests (Rice 1989) (adults; $k = 17$, alpha = 0.01, $P = 0.003–0.841$). Only one of the three loci also showed departure from HWE in juveniles. We therefore excluded this locus, *Mel*116, from our analyses. *Mel*110 and *Mel*113 departed significantly from linkage equilibrium in adults and *Mel*110 and *Mel*111 departed significantly in juveniles however, no pairs of loci were found to be consistently in disequilibrium. All other locus pairs were in linkage equilibrium after Bonferroni correction (adults; $k = 136$, alpha = 0.05; $P = 0.0015–0.98$). The probability of identity of siblings (PI_{sib}) combined across 16 loci was 0.000008. The highest probability of drawing two matching siblings at random from the population was 0.0148.

Based on the simulation of 11 individuals with 80% sampled, we chose a critical delta of 0.71, representing 80% confidence in the presence of one relative (Table 1). For males, based on the simulation of 162 candidates in the presence of one relative, we used a critical delta value of 1.00 where a mother had been assigned and 1.68 where neither parent was known. Where a father was assigned without a known mother (delta > 1.68), a mother was then assigned if delta was greater than 0.00 (80% confidence, one parent known, 11 individuals with 80% sampled, one relative; Table 1). These values give us at least 80% confidence if one half-sibling is present among the candidate males, less if more relatives are present, and greater if no relatives are present.

In total, 425 offspring from 10 social groups were used in the parentage analysis. Maternity was assigned to 307 cubs (72%) with 80% confidence (Table 1). For the majority of cubs (413; 97%) the best candidate mother had a positive LOD score, suggesting that the rate of assignment was limited by common alleles or the presence of relatives. Data on lactation status of females showed that 91% of maternal assignments were to females known to have lactated. Paternity was assigned to 202 cubs (47%), 22 without a mother, with a mother then assigned to an additional 19

Table 1 The rate of success of parentage assignment compared to that expected from simulations

	80%				95%			
	Δ LOD	N_{obs}		E_0, E_1	Δ LOD	N_{obs}		E_0, E_1
Assigned maternity	0.71, 0.0	326	77%	79%, 92%	1.84, 1.3	185	44%	61%, 80%
Assigned paternity	1.68, 1.0	202	48%	25%, 70%	2.94, 2.74	64	15%	9%, 39%
Both parents assigned		199	47%	~60%		58	14%	~27%

The number of female candidates was simulated as 11, the number of male candidates 162 (the mean number of candidates tested). Assignments with greater than 80% confidence were determined in the presence of one relative, related to the offspring by 0.25. Assignments with greater than 95% confidence did not include the presence of a relative, as the number of parentage assignments became too low to test any hypotheses. The delta LOD values were those required when neither parent was known, followed by the value required when one parent was known, to give the required level of confidence. The expected percentage of assignment from simulations (E), is given where neither parent was known, E_0, followed by where one parent is known, E_1. The rate of assignment for both parents is approximated from these values.

cubs (total maternity 326; 77%). Both maternity and paternity were assigned to 199 cubs (47%). For our more stringent analysis, a total of 58 cubs were assigned both parents with 95% confidence (Table 1). Our rates of assignment were consistently lower than those expected from the simulations. This may indicate the presence of a greater number of relatives, higher error rate or lower proportion of candidates sampled than simulated.

Multiple paternity

From the 326 maternity assignments, 75 'litters' of more than one cub could be defined. The average assigned litter size was 1.46 (range one to six; one to four with 95% confidence). More than one cub was assigned a father in 38 of these litters. Of these, nine litters were assigned only one father and 29 more than one father. However, this estimate of multiple paternity will be inflated because of our use of only 80% confidence. Only 39 litters were assigned with 95% confidence, and in the seven litters of greater than two cubs, none were assigned paternity. In the 31 litters of two cubs, one litter had two different fathers assigned, four litters had both cubs assigned the same father and 12 litters had only one cub assigned a father. By considering the LOD score of the male assigned paternity to one cub, we determined that in four cases this male had a negative LOD score for the second cub (–3.3 to –0.51), indicating it was unlikely to be the father. Based on this result, five of 31 litters suggested multiple paternity (16%).

Number of breeding females and males per social group

More than two cubs were assigned a mother for 59 social group years. In 20 of these cases, all cubs were assigned a mother. For 41 social-group-years (70%) the number of cubs was greater than the number of mothers, indicating that there was uneven reproductive success among breeding females per year. The maximum number of females thought to be mothering cubs was six with 80% confidence and five with 95% confidence (Fig. 2). Based on social groups where all cubs were assigned a mother, the modal number of mothers per social group was one, and the mean 2.0 (n = 39). If we include social groups where some cubs were not assigned a mother, which may underestimate the number of breeding females, the modal number of mothers was one and the mean 2.4 (n = 93) (95% confidence, mode = 1, mean = 1.78, n = 78) (Fig. 2).

The mean number of cubs assigned to a male within a year (ignoring males not assigned paternity) was 1.4 (SD = 0.9) and ranged from one to seven (1.3 with 95% confidence, SD = 0.7, range 1–4). Data was available on paternity for 83 social group years with 80% confidence, 44 social group years with 95% confidence. The modal number of fathers per social group was one and the mean was 2.0 (95% confidence, mode = 1, mean = 1.2). The maximum number of males thought to be fathering cubs in a social group was seven with 80% confidence and four with 95% confidence. If we consider the strategies of males that gained more than one cub in a given year, we find that, with 80% confidence, 11 males sired all offspring in other groups, 10 sired all offspring within their own social group and 12 had a mixed strategy. While the sample size was much lower, these proportions were similar when we considered results with 95% confidence (three internal, four external and four mixed).

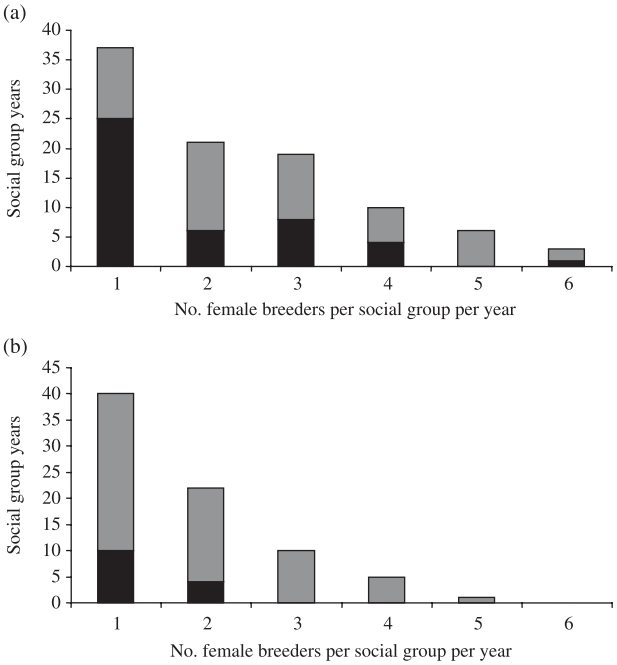

Fig. 2 The estimated number of female badgers reproducing per social group per year. Black bars represent social group years in which all cubs were assigned a mother, grey bars indicate social group years where only some cubs were assigned mothers. (a) assignments with greater than 80% confidence in the presence of a relative; (b) assignments with greater than 95% confidence.

Number of breeders in the population

If we assume that the number of breeding females determined through maternity assignments can be used to estimate the total number of breeding females in the population, on average 29% (range 15%–50%) of the candidate female population reproduced in a given year (Table 2). If we consider only candidate males from the 10 social groups studied then the mean number of breeders was similar to that for females (31%; range 12%–49%; Table 2). The proportion of male breeders is reduced to about half this if we consider the candidate pool to derive from the surrounding 26 social groups (Table 2). It is likely that the true proportion of male breeders lies somewhere between these two values.

Table 2 Estimation of the percentages of female and male badgers that bred at Woodchester Park

		Maternity					Paternity						
Year	All cubs	Cubs	Mothers	$E_{Mothers}$	Females (10sg)	% breeding	Cubs	Fathers	$E_{Fathers}$	Males (10sg)	Males (26sg)	% 10sg breeding	% 26sg breeding
1989	40	21	14	26.7	66	40%	13	6	18.5	119	56	28%	16%
1990	33	14	9	21.2	72	29%	8	7	28.9	137	63	40%	21%
1991	61	36	22	37.3	75	50%	22	15	41.6	148	80	55%	28%
1992	36	23	13	20.3	81	25%	19	9	17.1	153	79	21%	11%
1993	44	25	18	31.7	104	30%	13	12	40.6	155	80	39%	26%
1994	35	19	14	25.8	114	23%	10	9	31.5	144	68	28%	22%
1995	40	20	15	30.0	110	27%	12	8	26.7	164	84	24%	16%
1996	19	5	4	15.2	103	15%	1	1	19.0	177	85	18%	11%
1997	36	23	16	25.0	114	22%	18	13	26.0	183	87	23%	14%
1998	54	45	27	32.4	97	33%	25	22	47.5	157	75	49%	30%
1999	46	31	19	28.2	85	33%	17	12	32.5	165	72	38%	20%
2000	39	25	21	32.8	88	37%	16	11	26.8	186	87	30%	14%
2001	34	26	22	28.8	96	30%	18	16	30.2	194	91	31%	16%
2002	21	13	10	16.2	102	16%	10	6	12.6	186	86	12%	7%

The expected numbers of parents ($E_{Mothers}$; $E_{Fathers}$) is an estimate for all known cubs (All cubs), extrapolated from the number of parents assigned to cubs in the Maternity and Paternity columns. The number of female candidates (Females 10sg) is based on the biological rules used to determine potentially reproductive individuals in the population. The percentage breeding, for both males and females, is then the expected number of breeding females in relation to the number of candidates. For males, candidates from 10 social groups (10sg) as well as 26 social groups (26sg) are shown, as cubs were only genotyped from 10 groups, but paternity was allowed from 26 social groups.

Age structure

There was a significant influence of age on the mean number of cubs produced by males (GLM $P = 0.001$; GLMM $P < 0.001$) and females (GLM $P = 0.004$; GLMM $P = 0.02$) (Table 3, Fig. 3). This appeared to be resulting from a deficit in the number of cubs produced by 2-year-old individuals and, to a lesser extent, by animals of 9 years and older. It is possible that the use of the three year rule may have lead to a different age structure among candidates in comparison to the actual population, particularly in the 2-year and older age classes which are likely to experience increased mortality. However, the proportion of individuals whose fate was known did not differ greatly among age classes 2–5, 6–8 and above 8, suggesting that this bias may be small. A further point is that many 2-year-old males were assigned in the second round, where paternity was assigned first, followed by maternity (16 from first round, 9% of total; six from second round, 27% of total). This may have erroneously inflated our estimate of 2-year-old male paternity.

Table 3 Number of cubs attributed to parents by age for adult reproductive badgers from 10 social groups

	Mean number of cubs	
Age	Male	Female
2	0.07	0.19
3	0.17	0.27
4	0.21	0.30
5	0.17	0.32
6	0.25	0.34
7	0.19	0.35
8	0.14	0.21
9 +	0.12	0.14
Probability		
GLM	0.001	0.004
GLMM	< 0.001	0.02

Reproductive adults were defined using biological rules (See Methods). The male mean is based on 202 cubs, the female average on 307 cubs. Probability values are for a Generalized Linear Model, with the number of cubs as a function of age (GLM) and for a Generalized Linear Mixed Model, where 'individual' was included as a random effect (GLMM).

Mating dispersal

Of the 199 that were cubs assigned both parents, 108 were assigned to a male from a different social group (54%,45% with 95% confidence; Table 4). If we consider only individuals who were trapped in the year of conception, our results are similar (53% with 80% confidence; 54% with 95% confidence; Table 4). The majority of the extra-group paternities were by males from a neighbouring social group (67% of extra-group paternities; 36% of all paternity), with a few taking place over greater distances (18% of all

Table 4 Summary of mating system results from parental assignment in a high-density badger population

	80%			95%		
	N_{obs}*	*N*†		N_{obs}	*N*	
Same natal group mating	42	188	22%	17	54	31%
Extra-group paternity‡	108	199	54%	26	58	45%
Extra-group paternity (trapped only)	55	103	53%	19	35	54%
Mean extra-group boundaries crossed§	1.62	108		1.27	26	
Mean extra-group mating distance (sd)	775 (545) m	108		551 (254) m	31	

*N_{obs} is the number of times the observation was recorded; †*N* being the total sample size; ‡Extra-group paternity of trapped only individuals represents a subset of the data in which individuals were caught in the year of conception, rather than having their location inferred; §Boundaries refer to territorial boundaries as determined through bait marking.

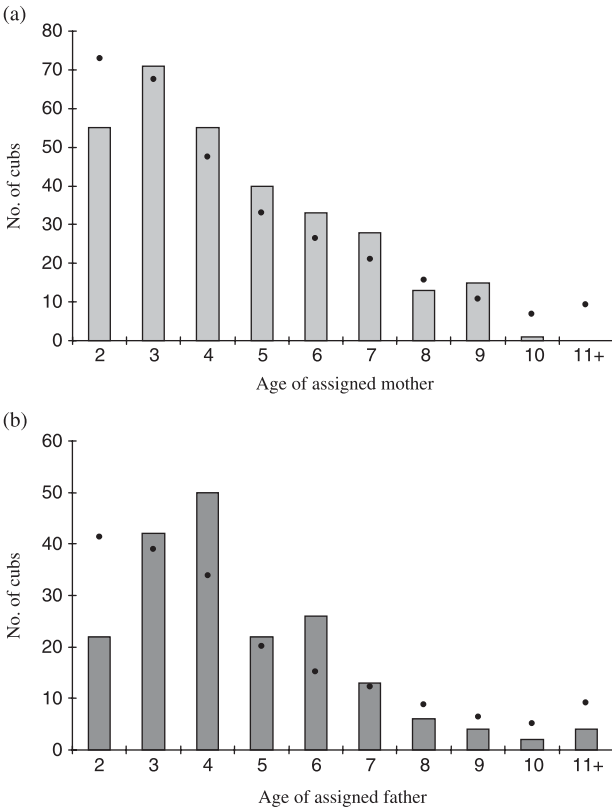

Fig. 3 The age distribution of (a) female and (b) male badgers who obtained parentage. The *x*-axis represents the age of the breeding individual in years, based on the year in which the cubs' birth occurred. The dots indicate the number of candidates in each age category, adjusted to match the number of parentage assignments. Individual breeders are included more than once. Data shown represent assignments with greater than 80% confidence in the presence of a relative.

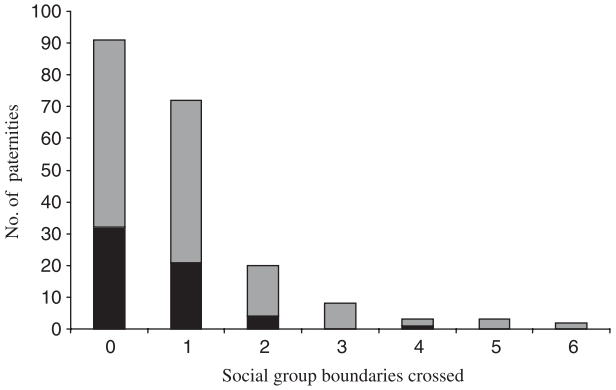

Fig. 4 The number of social group boundaries that a male or female badger must have travelled across to obtain paternity based on the distance of the known father's social group from that of the mother ($n = 199$). Black bars represent assignments with greater than 95% confidence, grey bars assignments with greater than 80% confidence in the presence of a relative. The 10 social group main setts were on average 570 m apart. The first column represents fathers from the same social group.

paternity) (Figs 1 and 4). The mean distance between an assigned father and mother, based on the distance between the main setts of the fathers' and the mothers' social groups, was 417 m (SD 556 m). If we consider extra-group paternity only, the mean dispersal distance was 769 m (SD 546 m). These distances might have been lower had residence in outlying setts been able to be taken into consideration, but the number of social boundaries crossed would remain the same. The mean distance between neighbouring social group main setts was 570 m. The greatest inferred mating dispersal distance was 3.2 km. This was to a 1-year-old male and may represent assignment to a sibling rather than an offspring, however, the two badgers did not share the same natal group. The next greatest distance was 2.4 km. In 188 cases the natal social group of both parents of a cub was known. Of these, only a small proportion (42 = 22%) of matings occurred between individuals born in the same social group.

Discussion

Determining the mating system of badgers in a high-density population was made challenging by a number of

factors. Neither parent was known a priori, and there were a large number of candidate parents (Rogers *et al*. 1997). There was also the likelihood that the candidates included a relative of the true parent or offspring, and a full-sibling would on average have a higher likelihood of parentage than the true mother if neither parent were known (Thompson 1976a; Thompson 1976b). To improve our success, we assumed that yearling females do not have cubs (Ahnlund 1980; Cresswell *et al*. 1992) and that mothers were resident in the social group in which the cub was first trapped. Using these rules, we achieved an overall rate of parentage assignment of 47%, although this will include errors as a result of our chosen level of confidence and possible inaccuracies in our simulation parameters. This rate of parentage assignment is similar to other studies of parentage where neither parent was known (e.g. 32% in a population of kangaroo rats; Winters & Waser 2003).

This paper represents the largest analysis of maternity and paternity in a badger population to date, and provides a significant advance in our understanding of the mating system of the Eurasian badger. This is the first paper to provide evidence that multiple paternity occurs within litters. The number of female breeders per social group and the proportion of females breeding had previously been estimated from lactation data (e.g. Cresswell *et al*. 1992; Woodroffe & Macdonald 1995; Rogers *et al*. 1997), and for a few groups from genetic data (Domingo-Roura *et al*. 2003). This paper extends these observations across many social group years, and this is the first time that such estimates have been possible for males. Some influence of age on the reproductive success of females was known previously (Cresswell *et al*. 1992; Rogers *et al*. 1997), but this is the first time such effects have been shown in males. Extra-group paternity has been previously reported (Evans *et al*. 1989; da Silva *et al*. 1994; Domingo-Roura *et al*. 2003), but that it comprised as much as 50% of matings was unknown.

Age structure and the number of breeders

Previous studies of high-density badger populations have shown that a large proportion of females may not reproduce in a given year (70% Cresswell *et al*. 1992; 58%–92%; Rogers *et al*. 1997), and that females compete for reproductive status (Woodroffe & Macdonald 1995). Genetic studies have shown that at least three females can reproduce in a social group (da Silva *et al*. 1994; Domingo-Roura *et al*. 2003). Field studies relying on teat morphology and sonograms have concluded that up to four females may breed in a social group (Cheeseman *et al*. 1987), but that the average in southern England is between two and three (Woodroffe & Macdonald 1995; Rogers *et al*. 1997). Our results support these earlier studies, showing that at least five females may breed in a social group, the modal number being one and the mean between two and three. Our results are similar for the number of male breeders within a social group. Our estimate of the proportion of females breeding in any given year (29%; range 15%–50%) agrees with the estimate for this population based on lactation data (10%–48%; Rogers *et al*. 1997), and supports the idea that there are considerable reproductive costs to group-living for females. This study is the first to estimate the amount of reproductive skew among badger males, and shows a similar range to that of females (Table 3). The level of reproductive skew may decrease if lifetime reproductive success is considered, but even so, this yearly reproductive cost to both males and females must play an important role in the decision process of whether to disperse (Hatchwell & Komdeur 2000).

While the modal number of breeders within each social group in a given year is one, the mean is higher. Reproductive skew does not seem as extreme in badgers as in some other social mammals (Solomon & French 1997, e.g. meerkats, Griffin *et al*. 2003; alpine marmots, Hacklander *et al*. 2003). This may be because the costs are small, breeding incentives are required or because dominants have limited control over the breeding of subordinates (Clutton-Brock *et al*. 2001b). The banded mongoose forms an egalitarian society where reproductive skew is low and there appears to be little evidence for the suppression of subdominants (De Luca & Ginsberg 2001). However, in this species, dispersal remains a viable option, making the enforcement of dominance hierarchies difficult (Emlen 1995; De Luca & Ginsberg 2001). Further investigation into the conditions in which more than one female badger breeds in a social group should bring greater insights into whether low reproductive skew in some badger social groups results through low costs, optimal skew or through an inability of dominants to suppress subordinates.

Previous studies have suggested that, in badgers, female reproductive success will be related to body condition in difficult years, and social status, as measured by the size of an exclusive home range within the social group territory, rather than age, in good years (Woodroffe & Macdonald 1995). However, other studies have found that the proportion of females reproducing did differ among age-classes (Cresswell *et al*. 1992; Rogers *et al*. 1997). If the conditions for reproductive success vary with the environment, we would expect the number of breeders to vary across years, and that age may not remain a significant factor across years (assuming variable ecological conditions). In our study, both males and females showed a trend for 2-year-old badgers and those older than 8 years to be assigned parentage less often than other age categories. Despite likely variation in reproductive success dependant on environmental conditions, our results suggest that age is an important factor in reproductive success at our study site, for both males and females.

This paper has focused on annual reproductive success, yet clearly badgers remain reproductive for several years.

A more detailed study of lifetime reproductive success, the relative age of females that reproduce in a social group, whether females queue to obtain reproductive success at a later stage and how this affects dispersal, will be the subject of future research.

Mating dispersal

While it had previously been demonstrated that extra-group matings take place (Evans *et al*. 1989), the extent of extra-group mating and the distances over which this occurs was unknown. Extra-group matings occurred mostly between neighbouring social groups, although a reasonable proportion (18%) was assigned over greater distances, as much as 3.2 km. This dispersal across six social group boundaries was observed only twice (Fig. 4). One male assigned paternity at this distance was only 1 year of age when inseminating the female, and it is possible that this represents assignment to a sibling rather than the true father. However, this type of error will generally lead to an underestimate of dispersal, rather than an overestimate, because of the prevalence of delayed dispersal by badgers (Kruuk & Parish 1982; Cheeseman *et al*. 1987; Rogers *et al*. 1998). It is also possible that both assignments resulted through error, based on our use of 80% confidence, however, it is worth noting that our sample size, at 95% confidence, was too small to detect such a low rate of long distance dispersal. A further limitation, as in all studies of dispersal, is the restricted area over which the study was conducted, which is again likely to lead to an underestimate of gene flow.

Previous studies of badger movement have described visits between social groups, with both males and females visiting or moving permanently (Woodroffe & Macdonald 1995; Woodroffe *et al*. 1995; Rogers *et al*. 1998; Tuyttens *et al*. 2000). While the frequency of adult movement was found to be high throughout their lifespan (50% of adults had moved in their known lifetime, Rogers *et al*. 1998), within-year movements were less common. Our results suggest that the visiting of other setts may be even more frequent than has been revealed by mark-recapture data. As badger trapping takes place only near setts, mark-recapture data does not record movements between social groups that do not result in visits to setts (especially main setts). Indeed, males seeking extra-group matings might encounter less opposition if they were able to approach females away from the males resident in her own sett.

A similar result was observed in a population of banner-tailed kangaroo rats, where 'gamete dispersal' was greater than natal dispersal (Winters & Waser 2003) and contributed to increasing genetic neighbourhood size. In a study of chimpanzees, the observed social groups did not represent exclusive reproductive units, with roughly half of matings occurring outside the group (Gagneux *et al*. 1999). In this case, extra-group mating was thought to provide females with a greater choice of mates. The high rate of extra-group paternity observed in badgers suggests that within group relatedness might be low, as has been shown for spotted hyenas (Van Horn *et al*. 2004). However, low levels of relatedness do not necessarily restrict kin selection for group formation (Rousset 2004). The relevance of kin selection will depend on the relative costs and benefits of group formation. Unlike slender meerkats who experience both large benefits (Clutton-Brock *et al*. 2001c) and large costs from group living (Clutton-Brock *et al*. 1998), with relatedness playing little part (Clutton-Brock *et al*. 2001a). Badgers, as suggested by Woodroffe & Macdonald (2000), appear experience low-costs and low-benefits from group living. While it appears unlikely that kin selection will be an important factor in the evolution of group formation in the badger, it is still a possibility worth further investigation. Finally, the level of extra-group mating recorded in the present study will undoubtedly reduce inbreeding within social groups, making delayed dispersal a less disadvantageous strategy than might have been expected if inbreeding depression occurs (but see Duarte *et al*. 2003).

That there is a much higher rate of contact among social groups than previously thought may also have implications for the spread of bovine tuberculosis. Previous models of *Mycobacterium bovis* infection in badgers and cattle have shown that if culling leads to social perturbation that increases contact rates, it is possible there would be a significant increase in the prevalence of the disease (Swinton *et al*. 1997; Smith 2001). Studies to date at Woodchester Park have shown *M. bovis* to remain localized within the badger population (Cheeseman *et al*. 1988; Delahay *et al*. 2001), suggesting that mating between social groups has not encouraged the disease to spread. Nevertheless, this potentially high contact rate among undisturbed social groups should be considered when interpreting the effects of culling on the spread of bovine tuberculosis in badgers (Donnelly *et al*. 2003).

Acknowledgements

We are indebted to the field team at Woodchester Park for kindly obtaining blood and hair samples, in particular Peter Mallinson, Paul Spyvee, Dave Handoll, Anton De Leeuw, and John Howell. We thank Elaine Cairns and Andrew Parham for assistance with marker development and optimization, and Andy Krupa for general laboratory help. Neil Walker kindly performed the GLM and GLMM analyses. This work was supported by the Natural Environment Research Council, UK, the Sheffield Molecular Genetics Facility, and the Department for Environment, Food and Rural Affairs, UK.

References

Advisory Committee on Dangerous Pathogens (1995) *Categorisation of biological agents according to hazard and categories of containment*.

Advisory Committee on Dangerous Pathogens and HSE, Sudbury, Suffolk, England.

Ahnlund H (1980) Sexual maturity and breeding season of the badger, *Meles meles*. Sweden. *Journal of Zoology, London*, **190**, 77–95.

Arnold KE, Owens IPF (1998) Cooperative breeding in birds: a comparative test of the life history traits hypothesis. *Proceedings of The Royal Society of London Series B-Biology Sciences*, **265**, 7.

Blundell GM, Ben-David M, Bowyer RT (2002) Sociality in river otters: cooperative foraging or reproductive strategies? *Behavioral Ecology*, **13**, 134–141.

Carpenter P, Dawson DA, Greig C *et al.* (2003) Isolation of 39 polymorphic microsatellite loci and the development of a fluorescent marker set for the Eurasian badger (*Meles meles*) (Carnivora: Mustelidae). *Molecular Ecology Notes*, **3**, 610–615.

Carr GM, Macdonald DW (1986) The sociality of solitary foragers: a model based on resource dispersion. *Animal Behaviour*, **34**, 1540–1549.

Cheeseman CL, Wilesmith JW, Ryan I, Mallinson PJ (1987) Badger population dynamics in a high-density area. *Symposium of the Zoological Society, London*, **58**, 279–294.

Cheeseman CL, Wilesmith JW, Stuart FA, Mallinson PJ (1988) Dynamics of tuberculosis in a naturally infected badger population. *Mammal Review*, **18**, 61–72.

Clutton-Brock TH, Brotherton PNM, O'Riain MJ *et al.* (2001a) Contributions to cooperative rearing in meerkats. *Animal Behaviour*, **61**, 705–710.

Clutton-Brock TH, Brotherton PNM, Russell AF *et al.* (2001b) Cooperation, control, and concession in meerkat groups. *Science*, **291**, 478–481.

Clutton-Brock TH, Gaynor D, Kansky R *et al.* (1998) Costs of cooperative behaviour in suricates (*Suricata suricatta*). *Proceedings of The Royal Society of London Series B-Biology Sciences*, **265**, 185–190.

Clutton-Brock TH, O'Riain MJ, Brotherton PNM *et al.* (1999) Selfish sentinels in cooperative mammals. *Science*, **284**, 1640–1644.

Clutton-Brock TH, Russell AF, Sharpe LL *et al.* (2001c) Effects of helpers on juvenile development and survival in meerkats. *Science*, **293**, 2446–2449.

Cresswell P, Harris S, Bunce R, Jefferies DJ (1989) The badger (*Meles meles*) in Britain: present status and future population changes. *Biology Journal of the Linnean Society*, **38**, 91–101.

Cresswell WJ, Harris S, Cheeseman CL, Mallinson PJ (1992) To breed or not to breed: an analysis of the social and density-dependant constraints on the fecundity of female badgers (*Meles meles*). *Philosophical Transactions of the Royal Society of London*, **338**, 393–407.

De Luca DW, Ginsberg JR (2001) Dominance, reproduction and survival in banded mongooses: towards an egalitarian social system? *Animal Behaviour*, **61**, 17–30.

Delahay RJ, Brown JA, Mallinson PJ *et al.* (2000) The use of marked bait in studies of the territorial organization of the European badger (*Meles meles*). *Mammal Review*, **30**, 73–87.

Delahay RJ, Cheeseman CL, Clifton-Hadley RS (2001) Wildlife disease reservoirs: the epidemiology of *Mycobacterium bovis* infection in the European badger (*Meles meles*) and other British mammals. *Tuberculosis*, **81**, 43–49.

Delahay RJ, Langton S, Smith GC, Clifton Hadley RS, Cheeseman CL (2000) The spatio-temporal distribution of *Mycobacterium bovis* (bovine tuberculosis) infection in a high-density badger population. *Journal of Animal Ecology*, **69**, 428–441.

Domingo-Roura X, Macdonald DW, Roy MS *et al.* (2003) Confirmation of low genetic diversity and multiple breeding females in a social group of Eurasian badgers from microsatellite and field data. *Molecular Ecology*, **12**, 533–539.

Donnelly CA, Woodroffe R, Cox DR *et al.* (2003) Impact of localized badger culling on tuberculosis incidence in British cattle. *Nature*, **426**, 834–837.

Duarte L, Bouteiller C, Fontanillas P, Petit E, Perrin N (2003) Inbreeding in the greater white-toothed shrew, *Crocidura russula*. *Evolution*, **57**, 638–645.

Emlen S (1984) Cooperative breeding in birds and mammals. In: *Behavioural Ecology* (eds Krebs J, Davies N), pp. 301–337. Blackwell Science, Oxford.

Emlen S (1995) An evolutionary theory of the family. *Proceedings of the National Academy of Science U.S.A.*, **92**, 8092–8099.

Evans PGH, Macdonald DW, Cheeseman CL (1989) Social structure of the Eurasian badger (*Meles meles*): genetic evidence. *Journal of Zoology, London*, **218**, 587–596.

Evett I, Weir B (1998) *Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists*. Sinauer, Sunderland.

Gagneux P, Boesch C, Woodruff DS (1999) Female reproduction strategies, paternity and community structure in wild West African chimpanzees. *Animal Behaviour*, **57**, 19–32.

Geffen E, Gompper ME, Gittleman JL *et al.* (1996) Size, life-history traits, and social organization in the Canidae: a re-evaluation. *American Naturalist*, **147**, 140–160.

Griffin AS, Pemberton JM, Brotherton PNM *et al.* (2003) A genetic analysis of breeding success in the cooperative meerkat (*Suricata suricatta*). *Behavioural Ecology*, **14**, 472–480.

Hacklander K, Mostl E, Arnold W (2003) Reproductive suppression in female Alpine marmots, *Marmota marmota*. *Animal Behaviour*, **65**, 1133–1140.

Hatchwell BJ, Komdeur J (2000) Ecological constraints, life history traits and the evolution of cooperative breeding. *Animal Behaviour*, **59**, 1079–1086.

Johnson DDP, Kays R, Blackwell PG, Macdonald DW (2002) Does the resource dispersion hypothesis explain group living? *Trends in Ecology and Evolution*, **17**, 563–570.

Johnson DDP, MacDonald DW, Dickman AJ (2000) An analysis and review of models of the sociobiology of the Mustelidae. *Mammal Review*, **30**, 171–196.

Johnson DDP, Macdonald DW, Newman C, Morecroft MD (2001) Group size versus territory size in group-living badgers: a large-sample field test of the Resource Dispersion Hypothesis. *Oikos*, **95**, 265–274.

Jones AG, Ardren WR (2003) Methods of parentage analysis in natural populations. *Molecular Ecology*, **12**, 2511–2523.

Kruuk H (1978) Spatial organisation and territorial behaviour of the European badger, *Meles meles*. *Journal of Zoology (London)*, **184**, 1–19.

Kruuk H (1989) *The Social Badger: Ecology and Behaviour of a Group Living Carnivore (Meles Meles)*. Oxford University Press, Oxford.

Kruuk HH, Parish T (1982) Factors affecting population density, group size and territory size of the European badger, *Meles meles*. *Journal of Zoology, London*, **184**, 1–19.

de Leeuw ANS, Forrester GJ, Spyvee PD, Brash MGI, Delahay RJ (2004) Experimental comparison of ketamine with a combination of ketamine, butorphanol and medetomidine for general anaesthesia of the Eurasian badger (*Meles meles* L.). *Veterinary Journal*, **167**, 186–193.

Lauzardo M, Rubin J (2000) Mycobacterial Disinfection. In: *Disinfection, Sterilization and Preservation* (ed. Block S), pp. 513–528. Lippincott Williams & Wilkins, Philadelphia, PA.

Macdonald D (1983) The ecology of carnivore social behaviour. *Nature*, **301**, 379–384.

MacKintosh C, MacArthur J, Little T, Stuart P (1976) The immobilisation of the badger (*Meles meles*). *British Veterinary Record*, **132**, 609–614.

Marshall T, Slate J, Kruuk L, Pemberton J (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.

Neal E, Cheeseman CL (1996). *Badgers*, T. & AD Poyset Ltd, London.

Raymond M, Rousset F (1995) GENEPOP (v. 1.2): a population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Revilla E (2003) What does the Resource Dispersion Hypothesis explain, if anything? *Oikos*, **101**, 428–432.

Rice WR (1989) Analysing tables of statistical tests. *Evolution*, **43**, 223–225.

Rogers LM, Cheeseman CL, Mallinson PJ, Clifton Hadley RS (1997) The demography of a high-density badger (*Meles meles*) population in the west of England. *Journal of Zoology London*, **242**, 705–728.

Rogers LM, Delahay R, Cheeseman CL *et al.* (1998) Movement of badgers (*Meles meles*) in a high-density population: individual, population and disease effects. *Proceedings of the Royal Society of London, Series B*, **265**, 1269–1276.

Rogers LM, Delahay RJ, Cheeseman CL, Smith GC, Clifton Hadley RS (1999) The increase in badger (*Meles meles*) density at Woodchester Park, southwest England: a review of the implications for disease (*Mycobacterium bovis*) prevalence. *Mammalia*, **63**, 183–192.

Rousset F (2004) Inclusive fitness, cooperation, and alturism. In: *Genetic Structure and Selection in Subdivided Populations* (ed. Rousset F). Princeton University Press, Princeton.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring. Harbour Laboratory Press, Cold Spring, New York.

da Silva J, MacDonald DW, Evans PGH (1994) Net cost of group living in a solitary forager, the Eurasian badger (*Meles meles*). *Behavioural Ecology*, **5**, 151–158.

Smith GC (2001) Models of *Mycobacterium bovis* in wildlife and cattle. *Tuberculosis*, **81**, 51–64.

Solomon NG, French AJ (1997) *Cooperative Breeding in Mammals*. Cambridge University Press, Cambridge.

Swinton J, Tuyttens F, MacDonald D *et al.* (1997) A comparison of fertility control and lethal control of bovine tuberculosis in badgers: the impact of perturbation induced transmission. *Philosophical Transactions of the Royal Society of London B Biology Sciences*, **352**, 619–631.

Thompson E (1976a) Inference of genealogical structure. *Social Science Information*, **15**, 477–526.

Thompson E (1976b) A paradox of genealogical inference. *Advances in Applied Probability*, **8**, 648–650.

Tuyttens FAM, MacDonald DW, Delahay R *et al.* (1999) Differences in trappability of European badgers *Meles meles* in three populations in England. *Journal of Applied Ecology*, **36**, 1051–1062.

Tuyttens FAM, Macdonald DW, Rogers LM, Cheeseman CL, Roddam AW (2000) Comparative study on the consequences of culling badgers (*Meles meles*) on biometrics, population dynamics and movement. *Journal of Animal Ecology*, **69**, 567–580.

Valiere N (2002) GIMLET: a computer program for analysing genetic individual identification data. *Molecular Ecology Notes*, **2**, 377–379.

Van Horn RC, Engh AL, Scribner KT, Funk SM, Holekamp KE (2004) Behavioural structuring of relatedness in the spotted hyena (*Crocuta crocuta*) suggests direct fitness benefits of clan-level cooperation. *Molecular Ecology*, **13**, 449–458.

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: Cautions and guidelines. *Molecular Ecology*, **10**, 249–256.

Walsh P, Metzger D, Higuchi R (1991) Chelex100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*, **10**, 506–513.

Winters JB, Waser PM (2003) Gene dispersal and outbreeding in a philopatric mammal. *Molecular Ecology*, **12**, 2251–2259.

Woodroffe R, Macdonald DM (1995) Female–female competition in European badgers *Meles meles*: Effects on breeding success. *Journal of Animal Ecology*, **64**, 12–20.

Woodroffe R, Macdonald DW (2000) Helpers provide no detectable benefits in the European badger (*Meles meles*). *Journal of Zoology, London*, **250**, 113–119.

Woodroffe R, Macdonald D, Silva JD (1995) Dispersal and philopatry in the European badger, *Meles meles*. *Journal of Zoology London*, **237**, 227–239.

Woods JG, Paetkau D, Lewis D *et al.* (1999) Genetic tagging of free-ranging black and brown bears. *Wildlife Society Bulletin*, **27**, 616–627.

Wrangham R, Gittleman JL, Chapman C (1993) Constraints on group size in primates and carnivores: population density and day-range as assays of exploitation competition. *Behavioural Ecology and Sociobiology*, **32**, 199–209.

Petra Carpenter conducted this research as part of her PhD, supervised by Terry Burke and Lucy Rogers. Genotyping of additional samples was performed by Lisa Pope, Kristien Erven, Carolyn Greig and Deborah Dawson. Some statistical analyses were performed by Lisa Pope. Lucy Rogers, Gavin Wilson and Richard Delahay and provided extensive knowledge of the Woodchester Park badger population and helped define the biological rules for candidates. This paper was initiated by Terry Burke, who heads the molecular ecology laboratory at the University of Sheffield, and Chris Cheeseman, who leads the Wildlife Disease Ecology team at Woodchester Park.
