Quantifying Contact Rates and Space Use in the Eurasian Badger (*Meles meles*): Implications for the Transmission of Bovine Tuberculosis

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I certify that all material in this thesis which is not my own work has been identified, and that no material has previously been submitted and approved for the award of a degree by this, or any other university.

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“The Mole had long wanted to make the acquaintance of the Badger. He seemed, by all accounts, to be such an important personage and, though rarely visible, to make his unseen influence felt by everybody about the place”

- Kenneth Grahame, *The Wind in the Willows*

ABSTRACT

This thesis examines the space use, movement and contact rate patterns of a high- density, group-living, Eurasian badger (*Meles meles*) population in the UK naturally infected with bovine tuberculosis (bTB). Recently developed proximity logging devices were deployed on a representative sample of 51 badgers from eight different social groups to track their movements using radio-telemetry and to quantify their within- and between-group contact rates. Whilst interactions within social groups accounted for more than 90% of contacts, the entire study population was ultimately connected through interactions among individuals from neighbouring groups. Both within and between-group contacts, and also the use of denning sites, were heavily influenced by seasonal and demographic factors, which appear to be motivated to a large extent by reproductive behaviours. Nevertheless, by using social network analysis I found that badgers that tested positive for bTB were found to interact with fewer of their group members and for a shorter amount of time. Specifically these test-positive individuals were found to associate with test-negative group members significantly less than would be expected by chance. Those animals testing positive for bTB were also found to use outlying setts significantly more frequently than those that tested negative. The within and between-group contact rates of individuals were found to correlate with their sett use patterns. Those animals that spent less time interacting with group members and those that spent more time interacting with members of foreign social groups, were found to spend a greater proportion of their time at outlier setts. The findings in this thesis suggest a link between wider roaming behaviour and the disease status of an individual. This adds support to the argument that the social disruption of badger populations, for example through culling, may promote rather than alleviate the spread of bTB as a result of increased movement and contacts between groups. State-of-the-art technology has enabled me to demonstrate the strong influence that badger social organisation may have on the transmission of an economically significant infectious disease. My findings suggest that disease control measures might be enhanced by taking into account seasonal and individual-level variation in ranging behaviour and use of outlier setts, for example, by identifying and targeting functional groups of individuals, specific areas, or times of the year that contribute disproportionately to disease spread.

KEYWORDS: Eurasian badger, bovine tuberculosis, proximity loggers, contact rates, sett use patterns, social network analysis, individual-level heterogeneity.

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I would not have completed this project without the input of numerous people, and I am very grateful for all the help that has been given to me. I need to start by saying thanks to the Woodchester Park field team, Paul Spyvee, Sarah Boxall and Jess Mayo, for being so helpful with the fieldwork and badger capturing, even putting in extra trap-ups to get all of the collars out and retrieved, and for making me feel like part of the team. I want to thank everybody at Woodchester Park for being friendly and helpful, including Gavin Wilson, Alex Tomlinson and Kate Palphramand who have all contributed data to my thesis. I based some of my ideas on work done by Emily Goodman in her PhD thesis, sadly she passed away last year. I also need to thank Darren Croft and Joah Madden for willingly helping me with last minute social network panics, and to Tore Opsahl for answering my emails so quickly. I am also grateful to Julian Drewe and Nick Royle for helpful discussions. A big thank you to Xav Harrison for 'R Wizardery'; 'Contactweld' and 'Matrix Builder' (Appendices A&B) shaved weeks off my data filtering time. Finally, thank you very much to Tim Roper and Andy Young for taking the time to examine this thesis and for very helpful comments for the future manuscripts.

Love and thanks to my family, to Sam's family, and to my friends for all of the badger-related Christmas and birthday presents – but most importantly for their support and encouragement. Thanks also to Helen Bonsor for the lovely badger illustrations in this thesis. And to Sam for his interest and ideas on my project, for help with fieldwork and the write-up, for making me happy, and for putting life on hold for the past few months - adventure time starts now.

I dedicate this thesis to my family and husband.

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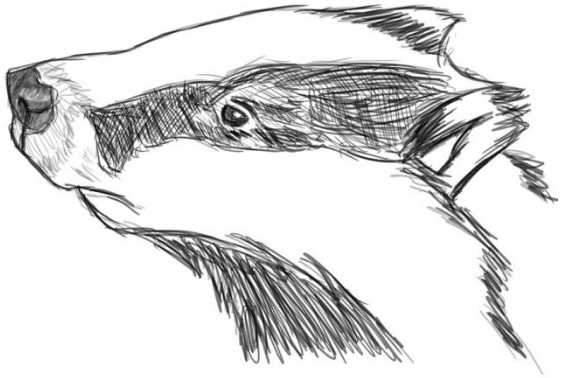
AUTHOR'S DECLARATION

The trapping records for all badgers and the data for the baitmarking maps were collected and complied by Food and Environment Research Agency (FERA) staff. Bait deployment, badger trapping, sampling and release were carried out by FERA staff, assisted by myself over the course of my study. Chapter 2 was carried out in collaboration with J. Drewe from the Royal Veterinary College who provided and analysed the entire cattle collar data presented and co-wrote the manuscript. My supervisors and others credited in the acknowledgements commented on earlier drafts of this work and/or provided unpublished data that was used in various chapters (indicated in the text). With these exceptions, I declare that the work contained in this thesis is my own and has not been submitted for any other degree or award. Trapping, anaesthesia and biological sampling of badgers were carried out under licence from the UK Home Office (licence number PPL60/3609) according to the Animals (Scientific Procedures) Act 1986. All procedures were approved by the FERA Ethical Review Panel.

Nicola Reed

Chapter 1. General Introduction



1.1 The role of host ecology and behaviour in the spread of infectious disease

Infectious diseases in wildlife have become an important challenge for ecologists, managers, policy-makers and the public alike as their prevalence and associated costs continue to rise (reviewed in Delahay, Smith & Hutchings 2009). Pathogen transmission can occur between and within species for diseases with multiple hosts, with wildlife providing a potential reservoir of infection that may spill over into domestic animals, wildlife of conservation value, or humans (Caley & Hone 2004). In such circumstances, a great deal of investment is put into developing effective disease management strategies, but these may be complicated by disease-specific parameters, such as its prevalence and pathology, and/or by host ecology and behaviours that can have a profound influence on disease dynamics (Hudson *et al*. 2002; Delahay, Smith & Hutchings 2009). The prevalence of a disease is determined by the number of infected hosts in the environment and their spatial and temporal distribution, whilst the pathology of the disease relates to the extent to which the host can produce infectious agents, and in turn the rate of disease-induced mortality. The risk of infecting/becoming infected is an interplay of several factors, including the routes and levels of infection and excretion, the infectious dose, the chance of encountering infection, and the susceptibility of the individual (Corner 2006). The studies in this thesis investigate to what extent host ecology and behaviour influence disease transmission within a mammalian host-pathogen system: bovine tuberculosis (bTB) in the Eurasian badger (*Meles meles*). I particularly focus on how host social organisation and associated behaviours in terms of space use and contact network patterns might affect the opportunities for transfer of infection between individuals in the population.

1.2 Bovine tuberculosis and badgers

Bovine tuberculosis is a chronic infectious disease caused by the bacterium *Mycobacterium bovis*. It can infect a wide range of mammalian hosts including humans, cattle, deer and wild carnivores such as badgers (Delahay, Cheeseman & Clifton-Hadley 2001). The Eurasian badger is recognised as an important wildlife reservoir for bTB in the UK and Republic of Ireland, and is implicated in its transmission to domestic livestock (reviewed in Krebs 1997; Bourne *et al*. 2007). The extent to which badgers contribute to infection in cattle is currently unclear, although the results of the recent Randomised Badger Culling Trial (RBCT) suggest they may account for around 50% of

cases in some areas (Jenkins *et al.* 2008). The badger is likely to be an efficient reservoir host for bTB for multiple reasons. In terms of physiology, it is susceptible to *M. bovis*, can survive for many years after infection, diseased females can reproduce (which presents opportunities for pseudo-vertical transmission), and badgers may be able to contain the infection for long periods of time until it is "reactivated" by physical stress (Clifton-Hadley 1996). Consequently, bTB does not appear to significantly influence the demographic profile of badger populations. In fact, incidence/prevalence of the disease, and presumably transmission rates, appear to be relatively low in badger populations, with bTB apparently having little detectable impact on badger population size or turnover (Smith *et al*. 1995; Wilkinson *et al*. 2000). In terms of ecology, badgers are group-living, sharing underground setts where conditions are probably good for the survival of *M. bovis* outside of the host, and territorial disputes resulting in bite wounding can also facilitate the transmission of bTB in the population (Courtenay *et al*. 2006; Delahay *et al*. 2006a). Indeed, findings from the RBCT highlighted the critical importance of badger ecology and behaviour in bTB epidemiology of both badgers and cattle (Bourne *et al*. 2007).

The total economic costs associated with bTB in the UK are approaching £100m per annum, with considerable investment put into management programs in an attempt to lower the prevalence of the disease in cattle stocks (McDonald *et al*. 2008; DEFRA 2010). Compulsory testing and slaughter of positive (reactor) cattle is the principal method of control in the UK and Ireland. Up until very recently there was no "intervention strategy" for badgers in the UK, although they are culled in Ireland and have been previously culled in England (pre-1997) for the purposes of disease control (reviewed in Wilson, Carter & Delahay 2011). The culling of badgers is a controversial topic, with evidence from the RBCT suggesting that it can both reduce and increase the incidence of bTB in cattle (Donnelly *et al*. 2003, 2006; see also Macdonald *et al*. 2006; Carter *et al*. 2007; **Chapter 7**). A potentially sustainable alternative is to use a vaccine. However, whilst there is considerable public support and recent evidence showing that vaccination can reduce the severity and progression of TB in badgers (Chambers *et al*. 2011), more research is needed and logistical constraints must be overcome before a vaccine could be administered on a large scale. There is general agreement that to achieve long-term disease eradication, a holistic approach is required that targets both wildlife and cattle (Wilson, Carter & Delahay 2011). The importance of cattle-based management should not be underestimated, and, in the future, vaccination of cattle may

also be a viable option. Like the majority of emerging infectious diseases, the control of bTB presents a serious challenge to the scientific community, management agencies and policy makers alike (Blanchong *et al*. 2007). Throughout this thesis, findings are considered in terms of the implications for possible management strategies and their effectiveness, with a detailed discussion presented in **Chapter 7**.

1.2.1 Routes of transmission for *M. bovis*

When considering management options for infectious diseases maintained by a wildlife reservoir, one of the first considerations is likely to be the identification of mechanisms for pathogen transmission between and within species, and their relative importance. The precise routes of transmission amongst badgers and between badgers and cattle have yet to be formally identified and described. Studies have considered transmission pathways for *M. bovis* between badgers and cattle (Benham 1993; Brown 1993; Courtenay *et al*. 2006), and among cattle (*for review* see Menzies & Neill 2000), but comparatively less attention has been given to how the pathogen is transmitted within the badger population (White *et al.* 2008). This is largely due to the logistical constraints associated with observing these secretive, nocturnal, semi-fossorial animals in the wild. A better understanding of *M. bovis* transmission within badger populations may facilitate more efficient disease control, resulting in a greater reduction in disease prevalence in both badgers and livestock. In this section, I review current evidence for the different routes of transmission between badgers, identifying the routes for which information is poor or lacking, and consider their potential significance to the spread and maintenance of bTB in badger populations. I also discuss which of these transmission routes can be quantitatively described, particularly in the light of recent advances in biotelemetry, such as proximity detection devices that facilitate the quantitative study of contact rates between individuals and which form a central part of this study.

Routes of pathogen excretion and subsequent infection vary according to the ecology and behaviour of individual species, and with social mammals in particular, host population structure is likely to play an important role in disease transmission (Altzier *et al*. 2003; Drewe *et al*. 2009). Contrary to the assumptions of earlier modelling approaches (e.g. Bentil & Murray 1993), many populations of social animals do not consist of homogeneously interacting individuals where the probability of disease

transmission is determined primarily by population density. Rather, there are many potential asymmetries in space use and contact network patterns at the individual level (e.g. Barlow 2000; Böhm *et al*. 2007; **Chapters 3-5**). Such heterogeneity, in turn, will influence the pathways through which infectious diseases can be transmitted. This was aptly demonstrated by Vicente *et al*. (2007), who found that individual and group-level bTB infection probabilities in badgers were related to changes in social group size and movement patterns of individuals between groups. Such studies highlight the importance of social structure in disease transmission dynamics. Badgers have been found to excrete *M. bovis* by several routes including respiratory, digestive, urinary and cutaneous pathways (Gavier-Widen *et al*. 2001). Thus, badger-to-badger transmission may occur via indirect methods, for example, contact with a contaminated environment such as at latrine sites, in addition to direct contacts between animals (see Fig 1.1).

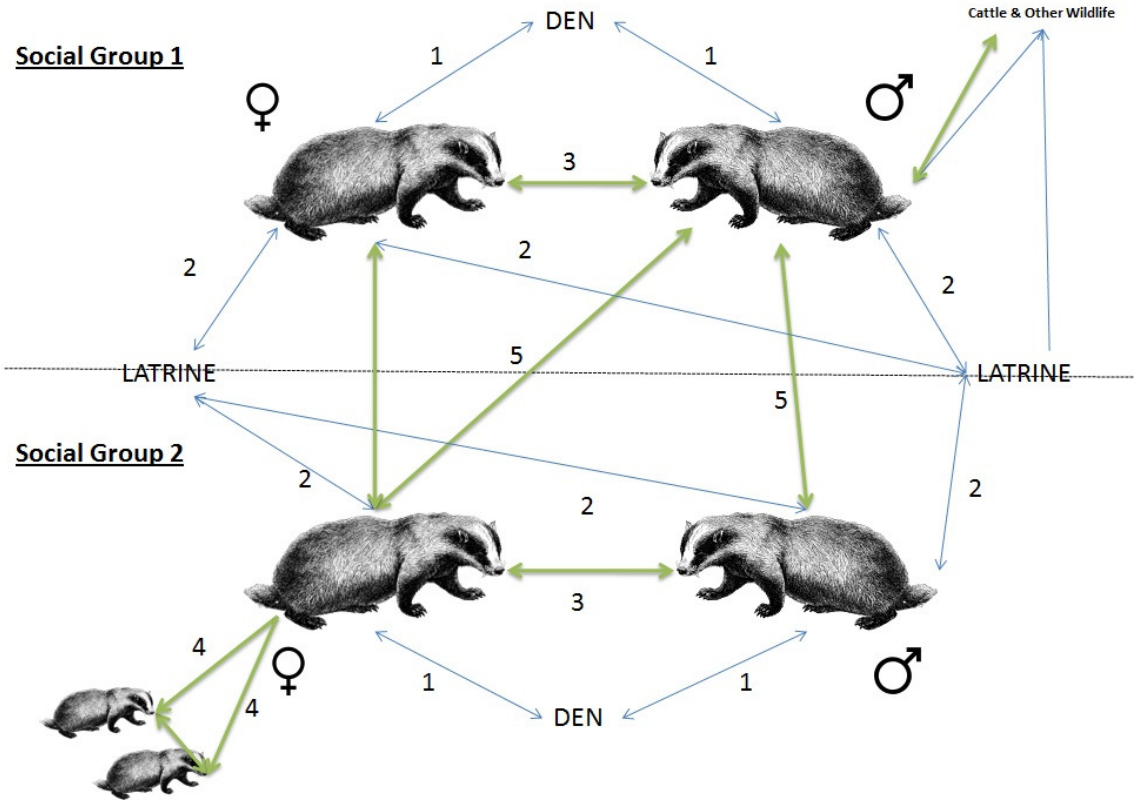


Figure 1.1 A conceptual framework for the transmission of bTB between badgers in the same social group, and between badgers in neighbouring groups. Green arrows represent direct contacts, and blue arrows represent indirect contacts. Disease transmission may occur via: 1. infected material in the den; 2. infected material at latrine sites; 3. within-group contacts (den sharing, allo-grooming etc.); 4. transmission from mother to cubs; 5. between-group contacts (mating, social, aggressive).

There is general agreement that aerosol transmission via the respiratory tract is likely to be a primary route of infection for badgers, based on the distribution of lesions at *post mortem* examination, and the observation that *M. bovis* is most frequently isolated from sputum during live sampling (Clifton-Hadley *et al*. 1993; Jenkins *et al.* 2008). Only a few bacteria are required to establish infection within the bovine lung (Menzies & Neill 2000), so aerosols are likely to represent a very effective mode of transmission. Badgers live in shared underground setts where group members are often likely to be in close proximity within a confined space, which may facilitate the efficient aerosol transmission of *M. bovis* within the social group. Individuals forage above ground during the night, and may encounter badgers from different social groups. Such encounters may be brief, and of an exploratory nature, or they could be more prolonged and related to mating and/or territorial behaviours. Longer between-group contacts that result in aggression may facilitate disease transmission transcutaneously via bite wounding (Delahay *et al*. 2006a). Thus, in order to assess the relative importance of different routes it is helpful to consider modes of transmission both in terms of indirect and direct contacts, and also in terms of within- and between-group interactions.

Indirect transmission

It is difficult to investigate, let alone quantify, rates of indirect bTB transmission within badger populations, meaning that there is little conclusive evidence for indirect routes of infection. However, it seems likely that several such routes could play an important role in the transmission and maintenance of bTB infection in badger populations. Contact with excreted pathogens in setts and at latrine sites probably represent the major routes of indirect transmission (routes 1-2 in Fig 1.1), and are discussed further below, but other indirect routes such as contaminated food stores (e.g. in farm buildings) cannot be ruled out (Garnett, Delahay & Roper 2002).

Badger Setts

Studies have shown that once excreted, *M. bovis* can survive and may remain infectious in the environment for a few weeks under most natural conditions (Young *et al*. 2005; Sweeney *et al*. 2007). Persistence is lengthened (potentially up to six months) by a number of factors such as constant temperatures, darkness and high humidity. Thus, conditions in a badger sett, from which *M. bovis* samples have been extracted, are likely to be conducive to the prolonged survival of the bacteria (Jackson, de Lisle & Morris

1995; Moore & Roper 2003; Courtenay *et al*. 2006). When infected animals shed *M. bovis* into the den environment, the setts themselves may constitute a source of infection for animals that are occupying them (route 1 in Fig. 1.1). Within this environment, when *M. bovis* is shed into bedding material from saliva, urine, bite wound discharges, or faecal material, it may persist for some amount of time. Multiple animals share a den on a regular basis (reviewed in Roper 2010; **Chapters 3 and 4**), with the greatest amount of activity at the main sett, which presumably increases the risk of inhaling or ingesting infectious pathogens. However, badgers have been observed replacing bedding on a regular basis, which may act to reduce the probability of transmission (Gallagher & Clifton-Hadley 2000).

Badger latrines

M. bovis has been detected in badger urine and faeces deposited at latrine sites (route 2 in Fig. 1.1), with the probability of detection correlated with the prevalence of excreting badgers in the population (Courtenay *et al*. 2006). The bacterium is susceptible to degradation on exposure to UV light, but badgers tend to make frequent visits to latrines that may be located in less exposed and low-disturbance habitats possibly to ensure persistence of the scent markings (Delahay *et al*. 2007). A large proportion of latrines are situated at the boundaries of territories, and play an important role in information transfer and territory delineation and defence (e.g. Roper *et al*. 1993; Delahay *et al*. 2007). Thus, badgers frequently investigate scents in the latrines belonging to other individuals; indeed, Stewart *et al.* (2002) showed that on average 44% of a badger's time at a latrine is spent sniffing. Such behaviour has associated risks of *M. bovis* being transmitted from the environment to the badger via inhalation of aerosoled aspirates and/or ingestion of contaminated material. Palphramand & White (2007) found that badgers spent a longer time investigating, and in some cases over-marking, alien-group scents compared to those that they were more familiar with, which may facilitate between-group transmission of *M. bovis*. Males have been observed visiting latrines at territorial boundaries more often than females (Roper *et al*. 1993; Stewart *et al*. 2002), with a coinciding peak in latrine use and mating behaviour (Roper *et al*. 1993). This may be to signal their commitment to defending females in their social group. In the springtime, females have been shown to increase their use of hinterland latrines, which may advertise reproductive status to the males or convey ownership of breeding areas to other females in the group (Roper *et al*. 1993).

Direct transmission

Increasing numbers of wildlife studies are highlighting the significance of heterogeneities in individual-level contact patterns to the dynamics of directly transmitted parasites and pathogenic agents (e.g. Krause *et al*. 2007; Krause, James & Croft 2010). This may be because advances in biotelemetry technology and social network analysis now allow the social networks of interacting animals to be quantitatively described, with implications for the understanding of disease transmission. Physical interactions between badgers may provide a number of transmission routes, including inhalation of *M. bovis* aspirates, and infection resulting from bite wounding. The finding that a significant number of badgers had bite wounds that were infected with *M. bovis* was originally reported by Gallagher *et al.* (1976). Badgers with severe pulmonary disease may have highly contaminated mouths and subcutaneous or intramuscular injection of *M. bovis* arising from bites from these animals can result in more rapid and extensive disease spread in the bitten animal (Gallagher & Clifton-Hadley 2000; Jenkins *et al*. 2008).

Throughout this thesis I consider two levels of social interaction: within-group contacts that primarily occur underground in and around the setts where badgers rest during the day and for extended periods over winter (route 3 in Fig. 1.1); and between-group contacts that tend to occur at night when the animals are active (route 5 in Fig. 1.1). Within-group contact rates are likely to be different in nature to those between social groups, occurring more frequently and lasting for longer (Goodman 2007; Böhm *et al*. 2008). This may increase the likelihood of disease transmission from infected group members to susceptible individuals. However, whilst within-group transmission may be more important to maintaining disease in the population, between-group transmission is likely to be more important to disease spread through the population. Patterns of badger behaviour such as space use, contact rates and their durations are likely to vary on a seasonal basis (reviewed in Roper 2010), which may have a bearing on temporal patterns of disease dynamics. In moderate to high-density badger populations in the UK, the peak breeding season occurs in late winter/early spring, as females give birth to cubs around February and are almost immediately receptive for mating (Neal & Cheeseman 1996). This is followed by animals ranging more widely through the territory when food is more limited in the summer; and then spending extended periods foraging in autumn as they build-up their fat reserves for the winter. During the harsher periods of winter,

badger activity is generally reduced (Neal & Cheeseman 1996). Such seasonal variation in behaviour is likely to profoundly affect contact patterns in badger populations. For between-group contacts, we might expect numbers and duration of contacts to be significantly higher during peak breeding periods, and relatively brief and infrequent at other times. During the winter, within-group contacts may be more frequent and of longer duration compared to other times of the year as activity and home range size have been shown to be reduced in response to harsher climatic conditions and scarcity of food (Palphramand *et al*. 2007). This may result in animals spending a greater amount of time together inside the setts, which can also confer thermoregulatory benefits (Roper *et al*. 2001). Here, I briefly discuss the nature of within- and between-group social contacts in badgers and consider their role in disease transmission and dynamics.

a) Within-group contacts

Although the social benefits of group living in badgers remain unclear (e.g. Carr & Macdonald 1986; da Silva *et al*. 1994; Johnson *et al*. 2001; **Chapter 4**), intuitively, group-living animals are more likely to contact members of their own group than those from other social groups. Hence, aerosol transmission from close contact during sett sharing may be the most prevalent route for *M. bovis* transmission. However, long-term studies have shown that the presence of a bTB-infected individual in a social group does not necessarily result in all other badgers in that group becoming infected (Cheesman *et al*. 1988; Rogers *et al*. 1998; Olea-Popelka *et al*. 2003). The reasons for this are poorly understood, but it is likely to be due to a combination of behavioural and intrinsic characteristics of individual badgers, and the chronic nature of the disease. All animals within a social group may not have an equal probability of contracting the disease due to underlying social sub-structures that could influence contact rates of individuals, and thus the dynamics of the disease within social groups (reviewed in Roper 2010; **Chapters 3-5**). Evidence suggests that within badger social groups there may be some individuals that share nest chambers within setts more frequently (and also those that share less frequently) than would be expected by chance (Roper *et al*. 2001; **Chapter 3**). Also, interactive behaviours may vary throughout the year according to factors such as age, sex and disease status (Böhm e*t al.* 2008; **Chapters 4 & 5**). Patterns of infection may also be influenced by heterogeneity in individual-level susceptibility to disease due to inherent or condition-related differences affecting the immune response.

Additionally, the probability of contracting disease may be influenced by an interplay between behaviour and condition-dependent susceptibility, for example, those animals that engage in more exploratory/aggressive behaviours may have higher levels of immunosuppressive stress hormones in their systems (Young *et al*. 2006).

Several studies have highlighted the importance of the main sett to badger sociality (reviewed in Roper 2010). For example, Böhm *et al*. (2008) found that 87% of close within-group interactions were associated with a main sett, and only around 4% with outlier setts. Individuals that have strong ties, perhaps due to relatedness or affiliations, may remain within the core social group and rest in the main sett. Although badgers do not show many of the typical cooperative behaviours of group-living mammals, they may reinforce social ties at the main sett through behaviours such as allo-grooming (Stewart 1997) and allo-marking (Buesching & Macdonald 2001). Roper *et al*. (2001) observed that some individuals ('non-movers') may use only the main sett, while others ('movers') make more or less frequent moves between the main sett and other outlier setts within the territory. Reasons proposed for the greater use of outliers by some individuals include avoidance of aggression from more dominant animals, reduction of ectoparasite burdens, movement of diseased individuals ostracised from the main sett, or simply the use of convenient resting points during extended foraging excursions (Roper 1992a; Rogers *et al*. 2003; Garnett *et al*. 2005; **Chapter 3**). Interestingly, there is increasing evidence to suggest that badgers testing positive for *M. bovis* may exhibit different behaviours to those that test negative, for example ranging more widely and using outlying setts more frequently (Cheeseman & Mallinson 1981; Woodroffe, Frost & Clifton-Hadley 1999; Garnett, Delahay & Roper 2005; **Chapters 3 & 5**). Such behaviour may reduce contact rates between infected and susceptible individuals in the main sett, thus limiting disease transmission within the group. However, at a population level, the increased ranging behaviour of infected individuals may act to increase disease spread both within the badger population and also to other susceptible wildlife. Garnett, Delahay & Roper (2005) found that infected badgers (those excreting *M. bovis* in a culture test) had home ranges that were, on average, around 50% larger than those that tested negative (presumed uninfected) for bTB, and incorporated both their own and neighbouring territories. Thus, these individuals could encounter a greater number of animals from different social groups, and be disproportionately more important in the transmission of *M. bovis* through the population. This theme is considered throughout the thesis.

Although mating in badgers can occur all year round, with females exhibiting delayed implantation, there are distinct peaks in such activity at the end of winter/early spring (immediately post-partum) and late summer/early autumn. During this time males may compete for access to females including those in other social groups, whilst the females compete for breeding status within their group (Woodroffe & Macdonald 1995). Competition for breeding status appears to be strong in females, which may be due to the high energetic costs of raising young, and it has been proposed that its intensity may reflect the availability of resources in a group's territory at the time (Macdonald *et al*. 2002). An estimated 48-70% of females of reproductive age fail to breed each season (Carpenter *et al*. 2005; Dugdale *et al*. 2007), which may be due to competition resulting in reproductive suppression, loss of litters in those individuals that are already pregnant, or infanticide after birth (Woodroffe & Macdonald 1995). In comparison, competition for breeding opportunities between male badgers from the same social group may be relatively low, with multiple males having been observed mating with the same female in the group in succession (Neal & Cheeseman 1996). The act of mating itself is likely to incorporate a risk of contracting disease if one individual is infected, due to the close contact and occasional biting that this entails (Neal & Cheeseman 1996). Infected females are known to have reproduced successfully (Clifton-Hadley 1996) and some have also been found with mammary lesions, suggesting the possibility of vertical transmission from mother to offspring through infected milk (Cheeseman, Wilesmith, Stuart 1989). However, pseudo-vertical transmission is considered to be the most important mode of transmission from sow to cub due to the high frequency and duration of their close contacts (route 4 in Fig. 1.1). The presence of infectious adult female badgers in a social group has previously been associated with new infections (Cheeseman *et al*. 1988) and has been found to positively correlate with the proportion of infected cubs in the group (Delahay *et al*. 2000a; Vicente *et al*. 2007).

b) Between-group contacts

The long-term study of an undisturbed badger population at Woodchester Park, Gloucestershire, UK has shown that bTB infection is often aggregated in certain social groups where it may persist for many years (although not all group members may be infected), with less evidence of disease being transferred between groups (e.g. Delahay *et al*. 2000a). This is believed to be due to limited between-group movement, with social (and spatial) structure reducing disease spread. However, when the infection does

spread between groups it is likely to have a significant influence on bTB prevalence in the population as a whole. This highlights the potential risks associated with management practices, such as culling, that may disrupt the normally stable badger social structure and potentially increase the frequency of between-group contacts, and also contacts with other susceptible wildlife (Tuyttens *et al*. 2000; Woodroffe *et al*. 2006a; Carter *et al*. 2007).

Territorial behaviour of badgers in undisturbed high-density populations in the UK has been shown to be ritualised with groups occupying discrete territories (e.g. Delahay *et al.*200b). On the whole, levels of aggression and direct contact between individuals of neighbouring groups appear to be kept at a comparatively low level through neighbour recognition of group-specific scent marking at latrines (Buesching, Stopka & Macdonald 2003; White *et al.* 2008). However, evidence suggests that during periods of reproductive activity, there may be a coinciding peak of aggressive encounters between members of different groups, leading to bite wounds that can be severe and are a potential route of disease transmission (Creswell *et al*. 1992; Delahay *et al*. 2006a). As aggressive and defensive behaviours are energetically costly, this may result in an increase in stress and testosterone levels, with the release of immunosuppressive hormones such as cortisol. This in turn can result in an increased susceptibility to disease (Zuk & McKean 1996; Young *et al.* 2006). Such interactions are believed to be predominantly between males that may drive the excursions into neighbouring territories for mating opportunities with females and contact a resident male. Extra-territorial mating has been well documented for badgers, with around 50% of cubs being sired by extra-group males (Carpenter *et* al. 2005; Dugdale *et* al. 2007). Thus this route of infection may be particularly important in the spread of *M. bovis* between social groups.

Many badgers in high-density populations remain in their natal social group for their lifetime which may be due to habitat saturation, with some populations approaching carrying capacity (Woodroffe *et al*. 2009). Thus, costs of dispersal in high-density populations may prove prohibitive, especially given that extra-territorial matings apparently provide the reproductive advantages of dispersal without having to actually disperse (Carpenter *et al*. 2005; Macdonald *et al*. 2008). When dispersal events are observed the trigger is generally unknown, although it has been suggested that it is driven by individuals of both sexes that receive high levels of aggression and/or

reproductive suppression from dominant animals (Rogers *et al*. 1998; Woodroffe & MacDonald 1995). Before permanent movement some individuals have been observed making a number of shorter exploratory trips to the new territory (Roper *et al*. 2003). Permanent dispersal of individuals between social groups at Woodchester Park is relatively rare, with fewer than 10% of animals per annum making permanent movements between social groups over more than 30 years of study (Rogers *et al*. 1998; **Chapter 6**). Whilst such movements may significantly enhance the opportunities for transfer of infection between groups, overall they are likely to have little role to play in the transmission of *M. bovis* within the population as they appear to be so infrequent.

In high-density badger populations in the UK, social groups are generally regarded as being discrete, relatively stable over time and contiguous (Delahay *et al*. 2000b). However, baitmarking and trapping records from long-term studies suggest that badger groups may undergo fission (da Silva *et al*. 1994; Carpenter *et al*. 2005). The number of social groups in the core 7 km^2 study area at Woodchester Park has increased from 21 in 1989 to 24/25 in 2011, and in all cases, two new territories occupied the same approximate area as the previous single territory. There is little mention in the literature of the fusion of social groups, but there is evidence from baitmarking and capture-mark-recapture studies to suggest that it occurs at a low-level, with two or three groups coming together to form one large 'supergroup' (**Chapter 6**). More data are required to discern what actually occurs in these groups, the rates of contacts (including social and aggressive interactions) between individuals and thus the potential for disease transmission (**Chapter 4**).

1.3 Influence of heterogeneity in behavioural patterns on disease transmission

Heterogeneities in the behavioural patterns of individuals in animal populations have potentially important ecological, evolutionary and conservation implications (Bolnick *et al*. 2003). Behavioural patterns at the individual level are likely to have a strong influence on (and also be influenced by) both the social organisation as a whole and also rates and patterns of information and disease transmission. This theme forms a basis for analyses and data interpretation throughout this thesis, focusing primarily on individual-level heterogeneity in space use, movement and contact/interaction patterns. An understanding of how such behavioural differences influence disease dynamics may go

some way to explaining the observed spatial aggregation and density independence of bTB in badger populations (Delahay *et al*. 2000a; Woodroffe *et al*. 2009).

Identifying differences in behavioural patterns may be relatively straightforward when compared to elucidating the proximate mechanisms underlying them. The social systems of mammalian populations exhibit structure at several levels, where individuals that often share some degree of relatedness may vary in terms of reproductive and/or dominance status, and also in terms of exhibited behaviours relating to social interactions and patterns of space-use (Cross *et al.* 2010). At the demographic level it is commonplace to observe heterogeneity in behavioural patterns due to sex and age, or indeed an interplay between the two (**Chapters 3 & 4**). However, within demographic groups certain individuals may also exhibit intrinsic behavioural characteristics that can be consistent over time and/or space (Wilson *et al*. 1994). A notable example is the shyness/boldness trait, with a number of studies reporting that bolder individuals were found to be more likely to be infected by parasites and diseases than shyer individuals (e.g. Natoli *et al*. 2005; Easterbrook *et al*. 2007; Boyer *et al*. 2010). A major goal of this thesis is to explore whether the behaviour of badgers infected with bTB differs from that of non-infected conspecifics (**Chapters 3 & 5**). This might occur if animals in an advanced state of disease are in some way impaired and cannot compete and/or forage efficiently, leading to them being ostracised from a social group or forced to forage in marginal areas (Cheeseman & Mallinson 1981). Alternatively, in some cases differences in the behaviour of infected animals may be due to the pathogen altering host behaviour in a way that increases the probability of its transmission (Klein 2003; Thomas, Adamo & Moore 2005).

For many diseases, from the classic example of "Typhoid Mary" (Soper 1939) to more recent examples in HIV/AIDS (Johnson *et al*. 1994) and SARS in humans (Lloyd-Smith *et al*. 2005), West Nile virus in avian species (Kilpatrick *et al*. 2006), and Sin Nombre virus in deer mice (Clay *et al*. 2009), particular individuals ('superspreaders') in the population have been identified as contributing disproportionately to the spread of infectious agents (Shen *et al*. 2004). This is likely to be linked to consistently expressed and potentially heritable individual-level variation in behavioural/ecological and/or immunological/physiological traits. Indeed, it has been proposed that focusing half of all control effort on the most infectious 20% of cases may be up to three times more effective than random control (Lloyd-Smith *et al*. 2005). Clearly, however, the

application of such disease control strategies is limited to those animal populations where such pivotal individuals or groups can be practically identified. At present there is little knowledge of how key individuals may influence spread of bTB in badger populations.

1.4 Quantifying contact rates

As highlighted throughout this discussion, an improved knowledge of within- and between-group contact patterns and population connectivity in badgers has the potential to further our understanding of social organisation and disease transmission in this species. However, contact rates between animals in the wild have traditionally been difficult to study owing to the logistical constraints of observing these interactions directly (e.g. Totton *et al*. 2002; Hamede *et al*. 2008). Consequently, progress in this field has generally been limited to highly observable, individually marked animals that can be habituated to a human presence, which is impractical for cryptic species such as the badger. Radio-telemetry studies have offered useful insights into the general movement patterns of individuals, and can allow inferences to be made about how often animals come into close proximity (e.g. Caley *et al*. 1998; White *et al*. 2003; Böhm *et al*. 2008). However, in general, they lack the temporal and spatial resolution necessary for accurately recording contact rates (Prange *et al*. 2006; **Chapters 2 & 6**). More recently, the development of animal-borne proximity logging devices (Sirtrack Tracking Solutions, Havelock North, New Zealand) has allowed detailed, quantitative studies of interaction patterns between animals to be carried out. Data recorded by these devices can be used to address important ecological and evolutionary questions, for example about social structure and disease epidemiology, which were previously difficult to investigate. I provide a critique of these devices in **Chapter 2**, which can also be used to measure the presence or absence of animals in nest sites, dens and other places of interest, or simply to measure how often individual animals pass fixed points. Deployment of such technology provides us with the opportunity to quantitatively describe both within- and between-group contact rates in the badger population in considerable detail for the first time on such a large-scale and sample size (see also Goodman 2007; Böhm, Hutchings & White 2009).

Following the collection of interaction data, social network analysis (SNA) provides a powerful tool for exploring individual-level factors (e.g. age, sex, dominance and

disease status) that influence the social structure of animal populations (reviewed in Croft, James & Krause 2008; Whitehead 2008). The use of SNA is well established in the field of human infectious disease, particularly for the study of socially transmitted sexual infections such as the human immunodeficiency virus (HIV) (e.g. Neaigus *et al*. 2001; Liljeros *et al*. 2003). However, until recently it has received less application in the field of animal behaviour, especially with regard to diseases involving wildlife hosts (reviewed in Krause *et al*. 2007). SNA describes the relationships between interacting individuals and how observed heterogeneities relate to differences between individual attributes (Wasserman & Faust 1998). Although two studies have used proximity loggers to quantitatively describe contact patterns in badgers (Goodman 2007; Böhm, Hutchings & White 2009), they were carried out on a smaller scale and data were not analysed using SNA to relate findings to individual attributes and bTB transmission. In this thesis I expand on these previous studies by deploying a large number of proximity loggers on a representative sample of badgers in a naturally bTB-infected high-density population and use SNA to provide new insights into how contact patterns vary according to demography, environmental factors, and disease status (**Chapters 4 & 5**).

For diseases that are transmitted by direct contact between conspecifics, the probability of infection should in theory increase as a function of the frequency of interactions between hosts. For homogenously interacting animals this would be expected to result in density-dependent infection rates (Anderson & May 1992). However, in reality few species (particularly larger mammals) interact in such a way, but instead display heterogeneous or fragmented contact networks. In such cases disease dynamics are likely to be significantly more complex and perhaps less predictable (e.g. Barlow 2000; Bansal, Grenfell & Meyers 2007). For example, the rate at which a disease spreads within a heterogeneously interacting population may vary considerably according to which individuals contract the disease. If these animals are highly connected in the population then they may carry a higher risk of both contracting and rapidly transmitting disease ('superspreaders': Lloyd-Smith *et al*. 2005). In contrast, if infected animals are poorly connected then disease spread may be contained and limited to localised areas (Krause *et al*. 2007). Thus, the potential for transmission of bTB in badger populations is likely to depend crucially on the extent of between-group connectivity. The use of proximity loggers and SNA should allow me to quantitatively describe the degree of connectivity between social groups in a badger population for the first time. Such an approach has the potential to impact practical disease management:

for example, tracing an individual's contacts and potential transmission routes may allow the targeted removal, or vaccination, of certain individuals or functional groups that may pose a disproportionate risk of transmission due to their level of connectedness in the network (Cross *et al*. 2010). However, the practicalities of such an approach are discussed throughout the thesis and a more realistic approach may be to focus on certain areas or times of the year when exhibited behaviours may increase the risk of disease transmission.

1.5 Aims and structure of the thesis

Using recently developed proximity logging devices and SNA, this thesis explores the social organisation and contact patterns of a naturally bTB-infected population. In particular, I examine how heterogeneities in the behavioural patterns of individuals correlate with individual and social group-level attributes, including bTB infection status, and discuss the findings in the context of disease dynamics and management. Fieldwork was conducted over a 12-month period (June 2009 – May 2010) on a well-studied, high-density badger population at Woodchester Park, Gloucestershire, UK (51°71′N, 2°30′E) that is naturally infected with bTB (see Delahay *et al.* 2006b).

The thesis begins with a laboratory and field-based validation study of the proximity detection devices supplied by Sirtrack Tracking Solutions (Havelock, NZ) that were employed in all aspects of this study (**Chapter 2**). They are a rapidly evolving technology, with growing popularity across a range of disciplines, but the accuracy and reliability of the data that they collect remains largely un-assessed. In a collaborative study with a fellow researcher, I propose a series of recommendations related to pre-deployment settings and data analysis to guide future studies using this technology, that are universally applicable regardless of the study species and objectives.

Using proximity loggers and radio-telemetry **Chapters 3-5** then explore how space use and the social interaction behaviours of badgers correlate with demographic and individual-level factors, including bTB disease test status. Specifically, in **Chapter 3** I investigate whether space use patterns (in terms of the proportion of time spent at main or outlier setts) of individual animals vary consistently over time and whether such heterogeneities are related to demography and disease status.

In **Chapters 4 and 5** I go on to explore the contact rates and social networks of individuals and the degree of population connectivity. Using SNA I investigate how interaction patterns vary among individuals in relation to demographic factors, which may further our understanding of the social organisation of the badger (**Chapter 4**) and whether the position of an animal within the network correlates with its disease status (**Chapter 5**). I construct social networks separately to investigate within- and between-group contact patterns, and compare them across seasons.

In **Chapter 6** I quantitatively compare four methods (baitmarking, capture-mark-recapture, radio-telemetry and proximity logging devices) that are commonly employed in the study of movement and contact patterns in animals, using comparable data collected as part of the long-term study on the Woodchester Park badger population. I investigate the extent to which the data and associated conclusions drawn from these methods correspond, compliment or contradict each other, with guidelines on how to choose the most appropriate method depending on study aims and target species.

Finally, **Chapter 7** presents a synthesis of the results and assesses the importance of individual-level behaviours in the transmission of bTB in the badger population, other areas of interest for future studies and the potential implications for management and intervention strategies.

Chapter 2. Performance of proximity loggers in recording intra- and inter-species interactions: a laboratory and field-based validation study

2.1 ABSTRACT

1. Individual animals interact through networks of contact that arise from their social organisation and which may profoundly affect transmission of information and disease. Knowledge of these contact patterns may help us to address questions surrounding the ecological and evolutionary consequences of social organisation and to understand and manage the spread of infectious diseases.
2. Automated proximity loggers are increasingly being used to record interactions between animals and to quantify their contact behaviour. However, the accuracy and reliability of data collected by such devices remain largely un-assessed.
3. The aim of this investigation was to perform a validation study using both laboratory and observational field data to assess the performance of proximity loggers attached via collars to Eurasian badgers (*Meles meles*, $n = 77$), cattle (*Bos taurus,* $n = 32$), and on static base stations ($n = 19$).
4. The distances at which all loggers detected each other were found to decrease over time, potentially related to diminishing battery power that may be a function of temperature. Loggers were highly accurate in recording the identification of contacted conspecifics but less reliable at determining the duration of contacts. There was a tendency for extended interactions to be recorded as a series of shorter contacts.
5. We provide evidence from both laboratory and field trials to show that data can be manipulated to correct this discrepancy by combining records between any two loggers that occur within a 1 to 2 minute amalgamation window. This manipulation held true for both species. Furthermore, removal of remaining 1 second records post-amalgamation appears to improve the reliability of the dataset in reflecting the observed interaction patterns of the animals under study.
6. We make universally applicable recommendations for the effective use of proximity loggers, including setting pre-deployment in the field for and the preparation and analysis of the data logged after retrieval of the collar from the animal. In proposing such measures we hope to improve the validity of data arising from the use of proximity loggers in future studies of animal contact networks.

2.2 INTRODUCTION

Interactions between animals influence a broad array of social processes (reviewed in Whitehead 2008) and researchers may be interested in quantifying patterns of interactions to address important ecological and evolutionary questions. Examples include studies of the spread of information and infectious diseases (Lloyd-Smith *et al*. 2005). However, empirical data on interactions between individuals are sparse, particularly in free-ranging wild animals (Cross *et al.* 2009). Methods employed in previous studies have relied on either direct observation of contact between individuals (e.g. Cross *et al*. 2004; Drewe 2009) or the ability to infer contact using proxy measures of shared space from data collected by methods such as radio-telemetry and Global Positioning System (GPS) locations (e.g. Schauber, Storm & Nielsen 2007; Böhm *et al*. 2008). These means of directly tracking individual animals to record their interactions are expensive, time consuming and limited to animals that are readily and easily observable from a distance, or to species that habituate quickly to the presence of observers. Automated methods for gathering detailed information on animal interactions potentially confer considerable advantages in terms of the sample sizes achievable and the resolution of the data collected.

One increasingly popular method is the use of proximity detectors (e.g. proximity data logger systems, Sirtrack Tracking Solutions, New Zealand). These remote-sensing devices can be attached to animals via collars, harnesses and ear tags, or in some cases they could be glued directly on to the animal e.g. seals and hedgehogs. They carry a unique signal and can automatically record the frequency and duration of contacts when tagged animals come within a pre-set distance of one another. Proximity-logging devices have been employed in several studies of wild and domestic animals including contact networks in captive brushtail possums *Trichosurus vulpecula* (Ji *et al.* 2005); proximity detection in wild raccoons *Procyon lotor* (Prange *et al.* 2006); cow-cow, cow-calf and ewe-lamb interactions in domestic livestock (Patison *et al.* 2010; Swain & Bishop-Hurley 2007; Broster *et al.* 2010); contact rates between Eurasian badgers *Meles meles* (Goodman 2007; **Chapters 4 & 5**) and between badgers and cattle (Böhm *et al.* 2009); population network structure of wild Tasmanian devils *Sarcophilus harrisii* (Hamede et al. 2009); and revealing spatial and temporal heterogeneity in the behaviour of European rabbits *Oryctolagus cuniculus* (Marsh *et al.* 2010). Proximity loggers provide data that can be used to develop quantitative contact networks, which may offer

insights into social processes and potentially lead to improvements in disease management (White *et al.* 2008; Krause *et al*. 2010).

Despite enthusiastic adoption of this novel technology the accuracy and reliability of data collected by proximity loggers remain largely unmeasured. Proximity loggers have several user-defined parameters, such as the distance at which contacts are detected, making them amenable to investigations with different study species and objectives. Prange *et al.* (2006) investigated the performance of a prototype version in the laboratory and on raccoons in the field and reported a 43% failure rate. Whilst later generation loggers may be expected to perform better, data collected by such devices have often been used without explicit validation. Ultimately, complete precision is not possible as radio waves can be reflected, refracted and/or absorbed by naturally occurring compounds, including natural features such as vegetation, water bodies and terrain (Mullen *et al.* 2004). However, there is a need for exploring methods to minimise error and take account of it in subsequent analyses. Various suggestions for data manipulation post-collection have been made (see Prange *et al.* 2006; Hamede *et al*. 2009; Marsh *et al*. 2010, 2011), but these may not be applicable across different study systems, and are not universally employed. In particular, in previous studies proximity loggers interacting at the edge of their detection range have been shown to frequently record very short contacts (typically of 1 second duration), thought to be due to weak signal strength (Prange *et al.* 2006). Removing these records from the dataset has been reported to increase the reliability of dyadic contact records (Prange *et al.* 2006) but may have profound effects on the structure of contact networks calculated from frequency data (Hamede *et al.* 2009). If data removal is conducted after any broken records have been combined (see methods) then this could further improve the accuracy of the recorded data in terms of how they reflect 'true' patterns of interaction.

The performance of proximity loggers in recording inter-species contacts has yet to be validated. It is important that the data collected by proximity loggers are closely examined and calibrated against simultaneous observations before conclusions are drawn. In addition, as the technology advances it is likely that proximity loggers will become smaller and less expensive and will therefore become more widely adopted in studies of the social biology of wild animals, for example, from passerines to large mammals. Thus, at this stage it is important that unified methods for data collection, filtering and analyses are tested, refined and adopted. The aim of this research was to

perform a validation study using observational data to interrogate the information gathered by proximity loggers attached via collars to cattle and badgers and on static base stations in the field. Investigating contact patterns in this system is of particular contemporary interest because of the role of the badger in the perpetuation of bovine tuberculosis (bTB) in cattle herds in the UK and Ireland (see Bourne *et al.* 2007). We use our findings to make universally applicable recommendations for the effective use of proximity loggers in future studies of animal interactions.

2.3 MATERIALS AND METHODS

2.3.1 Study location and species

This study was undertaken over 18 months from April 2009 to September 2010 at Woodchester Park, Gloucestershire, UK (51°71´N, 2°30´E). This is a 7 km^2 region of Cotswold limestone escarpment consisting of a wooded valley with areas of pasture grazed by a herd of approximately 35 Welsh Black cattle. The site also contains an intensively studied population of 200-300 wild badgers (Vicente *et al.* 2007). At the time of this investigation 20 badger social groups were present in the study site, with a mean group size of 10. This badger population has been the subject of long-term ecological and epidemiological research and so their territorial organisation, individual group membership, and the methods employed for their capture are well established and described (see Delahay *et al.* 2000a,b).

2.3.2 Equipment deployed

Three configurations of the same proximity logger were used: badger collars (n = 77), cattle collars (n = 32), and static base stations (n = 19). All were manufactured by Sirtrack Tracking Solutions (Havelock North, New Zealand) and differed in packaging but operated in the same manner using the same hardware (although the badger collars also included a Very High Frequency (VHF) transmitter, see below). Proximity data-logging collars consist of an Ultra High Frequency (UHF) transceiver that broadcasts a unique ID code, whilst simultaneously 'listening' for those of others. When two or more units come within a pre-determined, user-defined distance (see individual sections below for details), a contact is recorded continuously until one or both of the receiving loggers fails to detect the signal within a user-defined separation time that is set prior to deployment. Collars were set to have a separation time of 10 seconds, meaning that a single continuous encounter would be recorded until the receiving logger(s) failed to

detect the transmitted signal for a period longer than 10 seconds. After this time, each receiving unit logged the date, starting time, and duration of interaction with the other unit(s). Interaction data stored in the loggers were periodically downloaded onto a laptop computer when the animals were recaptured, using the supplied interface and software.

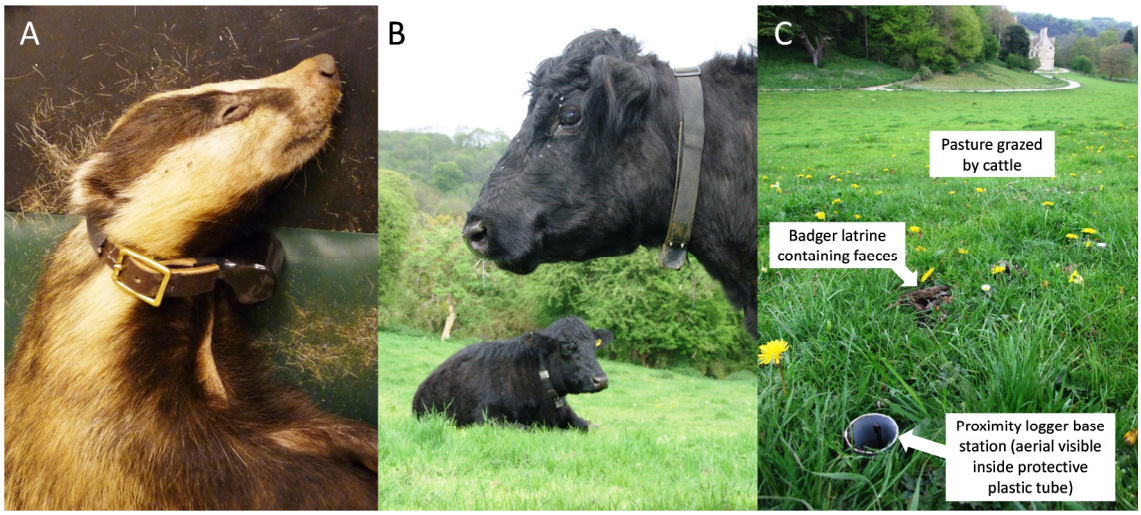


Figure 2.1 The three types of proximity logger used in this study. (A) Proximity logger on a collar fitted to an anaesthetised badger. (B) Cattle wearing proximity logger collars. (C) Proximity logger base station in situ near a badger latrine in a field grazed by the collared cattle.

Badger Proximity Loggers

Seventy-seven badgers from nine social groups were fitted with proximity loggers on adjustable leather collars whilst under anaesthesia (Fig. 2.1). These collars remained in place for up to 17 months from May 2009 to September 2010. Proximity loggers on animals that stay close to the ground, such as badgers, have a shorter expected transmission distance than those on animals of a greater height, such as cattle. We trialled two methods for setting the detection range of the badger proximity loggers: fixed UHF and variable UHF. For 16 loggers, the detection range was fixed at UHF 37, which in the trial of randomly paired collars conducted over a range of distances was found to equate to a contact initiation distance of 0.77 ± 0.27m (mean ± SD) and a contact termination distance of 0.93 ± 0.36m (Table 2.1). The remaining 61 collars were individually set using variable UHF power settings (range: UHF 34 to UHF 48) that

resulted in a contact initiation distance of 0.64 ± 0.04m and a termination distance of 0.87 ± 0.11m (Table 2.1). These short-range detection distances were chosen to record direct contacts between collared badgers such as bite-wounding and grooming, as well as to be within the likely aerosol transmission distance for *Mycobacterium bovis* (the causative agent of bTB) (Paterson & Morris 1995, Sauter & Morris 1995). Each badger logger also emitted a VHF radio signal that allowed for the animals (or collars that had been shed by animals) to be located in the field using standard radio tracking methods.

Cattle Proximity Loggers

Thirty-two cattle were fitted with proximity loggers on adjustable collars made from synthetic belting with a plastic clip for easy removal (Fig. 2.1). Collars were fitted in September 2009 and remained on for 12 months, although not all collars recorded data over this whole time period due to the logger memories reaching maximum capacity (16,384 or 32,768 records, depending on setting) before they could be downloaded. Collars were set to a detection range of UHF 45, which in the laboratory trial was found to equate to a contact initiation distance of 1.70 ± 0.12m and a contact termination distance of 1.92 ± 0.14m (n = 32 collars: Table 2.1). This distance is likely to be biologically meaningful in the epidemiology of bTB because it approximates the 1.5 – 2.0 m aerosol transmission distance postulated to occur between cattle and possums (Paterson & Morris 1995, Sauter & Morris 1995). Aerosol transmission is considered to be one of the more important transmission routes for *M. bovis* between wildlife and cattle (Corner 2006).

Static Base Stations

Nineteen static base stations were submerged in plastic tubes next to badger latrines located on the pasture grazed by the cattle (Fig. 2.1). Badger latrines may represent potentially important sources of environmental exposure to *M. bovis* in badger faeces and urine (e.g. Hutchings and Harris 1999). In addition, badgers use some communal latrines around the edge of their territories to demarcate boundaries, and so they are likely to represent nodes of interaction amongst individuals from neighbouring social groups. Base stations were set to UHF 20, which in the laboratory trial was found to equate to a contact initiation distance of 0.55 ± 0.13m (Table 2.1). Static base stations were deployed for up to 4.5 months between April and September 2010.

2.3.3 Validation in the laboratory

Proximity logger detection distances and variation over time

To ascertain the distance over which proximity loggers recorded interactions at different UHF settings, loggers were subjected to a laboratory-based trial. The badger collars, cattle collars and static base stations were randomly allocated into same-type pairs and placed 3 m apart on the ground next to an extended tape measure. Badger collars were attached to 2 litre plastic bottles filled with saline to mimic UHF wave absorption that would occur when worn by the animal (K. Lay, *pers comms*). They were positioned in the same orientation and the same height from the ground (10 cm) as would be the case when worn around a badger's neck. It was not feasible to exactly mimic the collar being worn by a cow, but it was held about 1 m above the ground, and base stations were tested at ground-level. Within each pair, one logger was moved towards the other in 1 cm increments every 20 seconds until one device detected the presence of the other. Detection was indicated by illumination of the LED on that logger for the purposes of these trials, but the LED was turned off when deployed on the free-ranging animals so as not to disrupt normal behaviours. This separation distance was recorded, being the contact initiation distance for the first logger. The distance between the pair of loggers was further reduced until the second logger detected the first. The loggers were then gradually moved apart until a long LED pulse indicated one logger had lost contact with the other (this was recorded as the contact termination distance for first logger). The distance was further increased until the second logger lost contact with the first (the contact termination distance for the second logger).

In a test to mimic inter-species contacts, 10 cattle collars and 10 badger collars were randomly allocated into pairs to investigate initiation and termination distances. In each trial the cattle collar was held 1 m above the ground and the badger collar 10 cm above the ground, and collars were moved towards each other using the same protocol detailed above. The initiation and termination distances were calculated as the hypotenuse of a right-angled triangle formed from the horizontal and vertical distances between the interacting cattle and badger collars.

To establish whether detection ranges remained constant over time, we used the same laboratory-based method to compare contact initiation and termination distances at various stages during the study (8, 12 and 17 months post-deployment) with those

recorded prior to deployment for all types of logger. In addition, at the end of the 17 month period, two cattle collars that had not been deployed (but were the same age as those that had been on cattle in the field) were tested to determine their contact initiation and termination distances so that findings could be related to battery charge. Changes in initiation and termination distances were tested against the frequency and duration of contacts recorded by the collars.

Broken Contacts

A previously identified limitation of the proximity logger technology is the tendency for a continuous contact to be recorded as a series of multiple shorter contacts (Prange *et al*. 2006). If these data are analysed without correction for this phenomenon then results and conclusions concerning the frequency and duration of interactions are likely to be misleading. A laboratory trial was undertaken whereby 25 pairs of badger proximity loggers were attached to 2-litre bottles of saline and placed facing each other at 0.30 m apart for 2 hours. As they were set to a contact initiation distance of 0.64 m (see above), they were well within detection range of one another and theoretically should have recorded the encounter as one continual contact of 2 hours (7,200 seconds) duration. If a break in the contact recording occurred, the time difference between the end of the broken contact and the initiation of the next contact was calculated and then averaged, a) for each collar individually to assess within-unit variation, and b) for all collars together in order to give an overall value that could be used as a threshold for combining the broken records into a continuous contact.

In previous studies, proximity loggers interacting at the edge of their detection range have been shown to often record very short contacts (typically of 1 second duration: e.g. Hamede *et al*. 2009), thought to be due to weak signal strength (Prange *et al.* 2006). Removing these records from the dataset has been reported to increase the reliability of dyadic contact records (Prange *et al.* 2006). If they were removed after any broken records have been combined (based on the threshold calculated above) then this could further improve the accuracy of the recorded data in terms of how they reflect the 'true' interactions. We investigated the effect of omitting 1 second records from the proximity logger dataset post-amalgamation on the dataset's similarity to the observational data.

Reciprocal contacts

To determine the accuracy of proximity loggers in correctly recording identification codes of other loggers, the databases of all recorded interactions for all three types of device were examined. To determine if the reliability of data varied between badger proximity loggers set at fixed or variable UHF settings, and therefore determine the necessity of setting each collar individually, we compared the frequency and duration of reciprocal records between five pairs of loggers in each of the three possible collar combinations (fixed-fixed, fixed-variable, variable-variable UHF) collected in the field during one calendar month (June 2010). For each pairing, a linear regression was performed on the log-transformed values for collar 1 against collar 2, for both frequency (count) and duration of contacts. The residual values were then compared using a one-way ANOVA to determine whether they varied significantly between the three different pairings in frequency and duration of shared contacts. This analysis was conducted three times using the statistical freeware R (R Development Core Team 2009): first, using the data exactly as recorded on the proximity loggers; second, after manipulating the dataset to amalgamate dyadic records occurring within 1 minute of each other (this being approximately the median gap duration for broken contacts: see Results); and third, after amalgamation followed by removal of any remaining contact records lasting 1 second (see above).

2.3.4 Validation in the field

Cattle observation study

To validate the data collected by the cattle proximity loggers focal observations of interactions between collared cattle were conducted in the field by an observer over two days in June 2010. Twelve randomly-selected cattle were each observed for 30 minutes from a distance of approximately 20 m. Cattle were considered to be interacting with each other if they were within one head's width of the other animal (this corresponded with a maximum between-collar distance of less than 1.7 m, the mean contact initiation distance to which the collars were set). All interactions were recorded during each 30 minute focal period, noting the identification of the partner (read from ear tag number using binoculars), the start and end time of the contact and the type of interaction (e.g. grooming, head butting, walking by). Observational data were compared to those recorded by the collars to determine the accuracy of the loggers in recording number of

contacts, duration of contacts and contacted logger identification. Paired t-tests in SPSS (v.18) were used to test for differences between observed and recorded data.

2.4 RESULTS

2.4.1 Validation in the laboratory

Proximity logger detection distances and variation over time

All three types of logger showed a reduction in their detection range over time (Table 2.1). The largest reduction was seen in the badger collars where logger initiation distances reduced by 50% within 8 months of deployment, but then stayed constant at this decreased value for the next 9 months (Table 2.1). In addition, badger loggers showed a pronounced reduction in mean termination distance and a shortening of the range of detection distances over this time (indicated by the decrease in standard deviations for initiation and termination distances: Table 2.1). Cattle collars showed a moderate reduction in mean initiation and termination distances and a widening of the range of detection distances over the study period (indicated by the increase in standard deviations for initiation and termination distances: Table 2.1). However, the two cattle collars that were tested after this time that had not been deployed in the field and were stored with their batteries turned off did not show any decrease. The base stations, although tested over a shorter time period than the collars, still showed an overall reduction in mean contact detection distance from 0.55 ± 0.13 m to 0.47 ± 0.15 m during the study period. For the badger and cattle collars, the decreases in detection distances were not influenced by the number ($F_{1, 60} = 1.17$, $P = 0.30$) or by the duration ($F_{1, 60} = 2.37$, $P = 0.13$) of contacts that they had recorded during deployment in the field.

Cattle and badger collars were tested against each other at different heights to mimic interspecific contacts. The detection distances were found to have a wider range than for the same collars detecting intraspecific contacts (Table 2.1). However, despite the two types of collar having different UHF settings, there was very little difference in the detection ranges for each type of collar when detecting the other (Table 2.1).

Table 2.1 Changes in the detection distances of proximity loggers over time in the field. Loggers were deployed on collars fitted to cattle and badgers, and in static base stations, for up to 17 consecutive months from May 09 – Sept. 10. Initiation distance refers to the distance between loggers when a contact starts, and termination, the distance between loggers when a contact ends. Changes in detection distances over time are given in italics; negative values indicate a reduction in detection distance over time.

Proximity logger type		UHF setting	Time (months after start of deployment)	n	Initiation distance (m)			Termination distance (m)		
					Mean (sd)	Min	Max	Mean (sd)	Min	Max
Intra-species	Cattle collars	45	0	32	1.70 (0.12)	1.47	1.94	1.92 (0.14)	1.62	2.24
			15	29	1.29 (0.30)	0.85	1.80	1.51 (0.41)	0.95	2.40
			% change in detection distances over 15 months		*-24 (145)*	*-42*	*-7*	*-21 (180)*	*-41*	*7*
	Badger collars	37 (fixed)	0	15	0.77 (0.27)	0.40	1.40	0.93 (0.36)	0.65	1.80
			8	10	0.38 (0.16)	0.10	0.60	0.49 (0.21)	0.10	0.70
			% change in detection distances over 8 months		*-51 (39)*	*-75*	*-57*	*-48 (41)*	*-85*	*-61*
		34 to 48 (variable)	0	61	0.64 (0.04)	0.57	0.71	0.87 (0.11)	0.70	1.11
			12	20	0.32 (0.03)	0.25	0.37	0.58 (0.06)	0.49	0.70
			17	20	0.31 (0.05)	0.24	0.39	0.60 (0.05)	0.46	0.71
			% change in detection distances over 12 months		*-50 (25)*	*-56*	*-48*	*-33 (45)*	*-30*	*-37*
			% change in detection distances over 17 months		*-52 (25)*	*-58*	*-45*	*-31 (55)*	*-34*	*-36*
Interspecies	Cattle collars	45	20	10	1.33 (0.57)	0.20	2.28	1.57 (0.90)	0.25	3.32
	Badger collars	37	20	10	1.22 (0.63)	0.20	2.28	1.49 (0.76)	0.25	2.75
Static	Base stations	20	0	14	0.55 (0.13)	30	75	NR	NR	NR
			4	12	0.47 (0.15)	20	60	NR	NR	NR
			% change in detection distances over 4 months		*-15 (14)*	*-33*	*-20*	NR	NR	NR

NR = not recorded

Broken contacts

In none of the laboratory trials of 25 pairs of badger collars was the contact recorded as a continuous 2 hour interaction, but rather always as a series of multiple broken contacts. Within-collar variation was found to be minimal, and across all 50 collars, the median gap duration between the end of one recorded contact and the initiation of the next was 54 s (range: 28 to 628 s; mode: 47 s), and the 95th percentile gap duration was 129 s (2 min 9 s). See below for field validation of broken contacts.

Reciprocal contacts

There was a high level of agreement in the durations of the contacts recorded by one collar and the reciprocal interacting collar under all three treatment scenarios: no amalgamation of contacts ($F_{1,14}$ = 155.0, $P < 0.001$, r^2 = 0.92); amalgamation of those less than 1 minute apart ($F_{1,14}$ = 49.4, $P < 0.001$, r^2 = 0.80); and amalgamation and removal of any remaining 1 second contacts ($F_{1,14}$ = 50.5, $P < 0.001$, r^2 = 0.80) (Fig. 2a). There was no significant difference between the three different pairing combinations based on how the UHF coefficients were set for the interacting collars (variable-variable, variable-fixed, fixed-fixed) under the three treatments: no amalgamation ($F_{1,14}$ = 0.28, P = 0.76); amalgamation ($F_{1,14}$ = 1.92, P = 0.19); amalgamation and 1 s removal ($F_{1,14}$ = 1.89, P = 0.19).

There was a weaker, albeit still significant, agreement between the number of different contacts recorded by each collar and its reciprocal under all three treatment scenarios: no amalgamation ($F_{1,14}$ = 9.00, P = 0.01, r^2 = 0.41); amalgamation of those less than 1 minute apart ($F_{1,14}$ = 11.63, P = 0.005, r^2 = 0.47); and amalgamation and removal of any remaining 1 second contacts ($F_{1,14}$ = 9.92, P = 0.008, r^2 = 0.43). A one-way ANOVA showed that there was a significant difference between the three pairing combinations (variable-variable, variable-fixed, fixed-fixed) under two of the three treatments: no amalgamation ($F_{1,14}$ = 4.82, P = 0.03); amalgamation ($F_{1,14}$ = 3.70, P = 0.06); amalgamation and 1 s removal ($F_{1,14}$ = 3.90, P = 0.05). A Tukey's post-hoc test showed that this difference was driven by variable collars recording a greater number of contacts than the fixed setting collars when paired together (All $P < 0.05$, other pairing combinations, $P > 0.20$; Fig. 2b).

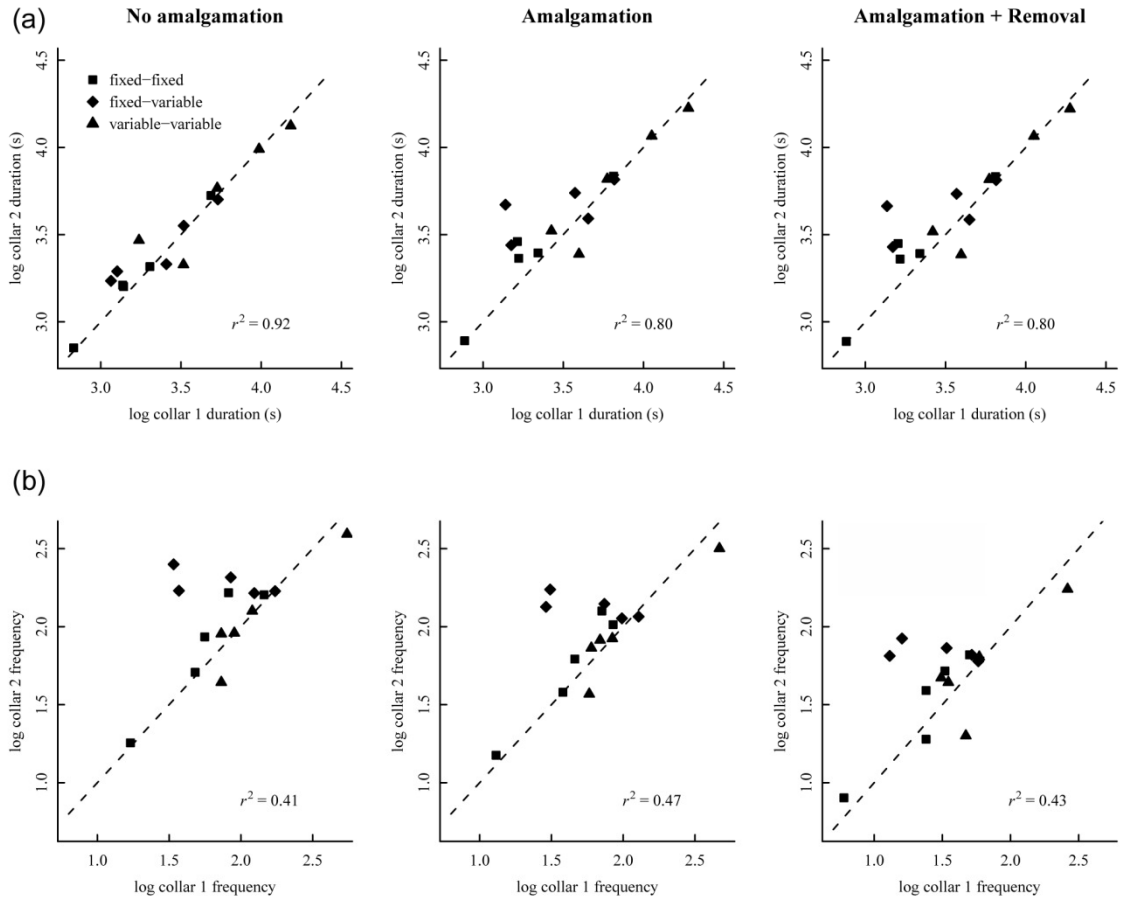


Figure 2.2 Correlations between a) the duration and b) the frequency (number) of contacts recorded by interacting pairs of badger proximity collars. Values are given for the three possible collar pairings based on their UHF settings (variable-variable (▲), variable-fixed (♦), fixed-fixed (■)) and for the three data manipulation treatments to reflect the 'real-life' contacts (no amalgamation of broken contacts, amalgamation, amalgamation and removal of remaining 1 second contacts). The dashed line is the line of equivalence (y = x), along which all points would lie if collar 1 recorded exactly the same data as collar 2.

2.4.2 Validation in the field

Accuracy of proximity logger identification

The cattle collars recorded a total of 1,290,632 interactions over a 12-month period, of which there were 471 records for spurious proximity logger identification codes. This represents an identification error rate for cattle collars of around 0.04%. These could be genuine interactions with deployed collars where for some reason the identification

code was recorded incorrectly (perhaps due to interrupted signals or 'data packet collisions' when multiple packets of data arrive at the receiver due to simultaneous interaction between several animals) or erroneous records unrelated to any interaction. Badger collars recorded 308,318 contacts of which only three (0.001%) were deemed to be erroneous, with the ID of the individual contacted being a number that had not been deployed. The base stations recorded 5,275 records, none of which had an obviously erroneous identification code. Taken together, these data suggest the identification error rate for all types of logger combined to be approximately 0.03%.

Cattle observation study

Of the 179 interactions observed during the six hours of focal observations, 129 (72%, range: 50% to 94%) were recorded by the proximity loggers (Table 2.2). The median duration of the 50 interactions that were observed but not recorded by the loggers was 2 s (range: 1 to 80 s; mode: 1 s).

Cattle observation study: broken contacts

The cattle proximity loggers split 27 of the 179 records (15%) of observed interactions into multiple shorter records. The observed duration of the shortest interaction that was recorded by the loggers as multiple shorter records was 15 s (recorded as two 1-second interactions separated by a gap of 13 s), and all interactions lasting 14 s or less were recorded as single whole records. The longest interaction recorded as one complete record was 87 s. The longest interaction recorded as a split record was 313 s (recorded as four shorter interactions separated by three gaps). The median gap duration for split records was 20 s (range: 12 to 153 s; mode: 18 s). Of the interactions recorded as split records, the 95th percentile for gap duration was 51 s. Thus on 95% of occasions, the maximum interval between logger records for interactions which were recorded as multiple shorter records was less than 51 s.

Table 2.2 Comparison of the observed number and duration of interactions of 12 randomly selected cattle within a herd of 24 (focal observation sessions each lasted 30 minutes), with data recorded by proximity logging collars worn by the animals.

Proximity logger collar identification number	**Individual cattle details**			**Number of observed interactions in 30 min**	**Number (%) of observed interactions recorded by the proximity logger**	**Total duration of observed interactions (min:sec)**	**Total duration of observed interactions recorded by the proximity logger (min:sec)**	**Number (%) of observed interactions recorded on the proximity logger as split contacts**
	Class	**Age**	**Sex**					
201	adult	11 y	F	13	7 (54)	3:46	1:42	0 (0)
230	adult	9 y 10	F	15	12 (80)	2:42	3:05	1 (7)
222	adult	6 y 5 m	F	21	12 (57)	4:39	3:30	2 (10)
205	adult	5 y 2 m	F	10	5 (50)	0:49	0:50	1 (10)
206	adult	5 y 0 m	F	14	10 (71)	3:39	4:37	3 (21)
221	adult	4 y 11	F	13	10 (77)	1:21	2:00	2 (15)
225	adult	4 y 3 m	F	18	13 (72)	9:35	7:57	5 (28)
223	adult	4 y 2 m	F	17	16 (94)	8:15	8:44	2 (12)
217	calf	0 y 1 m	F	10	5 (50)	9:34	5:31	1 (10)
214	calf	0 y 1 m	F	14	12 (86)	9:17	7:07	6 (43)
219	calf	0 y 1 m	F	19	15 (79)	1:30	1:57	0 (0)
218	calf	0 y 1 m	M	15	12 (80)	15:51	9:41	4 (27)
Total number (mean %)				***179***	***129 (72)***	***73:58***	***56:41***	***27 (15)***

y = years; m = months; F = female; M= male; min = minutes; sec = seconds

Of the 1,290,632 interactions recorded by the cattle collars and the 308,318 interactions recorded by badger collars over 58% (755,946 records) and 51% (151,076 records) respectively were of 1 second duration. We investigated what effect omitting these records from the proximity logger dataset had on that dataset's similarity with the records from the cattle observational study. We did this both for dyadic interactions 'as recorded' and for combined dyadic records if they occurred within 51 s (the 95th percentile for gap duration between pairs of cattle collars) of each other and involved the same two animals. First, observed records from all 12 loggers in the validation study were compared with proximity logger records without combining records less than 51 s apart and without filtering out 1 second contacts. The recorded dataset was significantly different to the observed contacts in the same time period (paired t-test, t = 4.64, df = 128, observed mean ± SD = 31.0 ± 50.7, edited mean ± SD = 10.8 ± 16.2, $P < 0.001$). Second, observed records were compared with proximity logger records without combining records less than 51 s apart but this time filtering out all 1 second contacts. The recorded dataset was still significantly different to the observed contacts in the same time period (paired t-test, $t_{1, 89} = 3.75$, observed = 36.2 ± 55.1, edited = 15.0 ± 17.7, $P < 0.001$). Third, observed records were compared with proximity logger records where dyadic records had been combined if they occurred less than 51 s apart, without filtering out 1 second contacts. The edited dataset was again significantly different to the observed contacts in the same time period (paired t-test, $t_{1, 127} = 2.32$, observed = 31.0 ± 50.7, edited = 26.4 ± 46.8, $P = 0.022$). Finally, observed records were compared with proximity logger records in which dyadic records had been combined if they occurred less than 51 s apart, and then 1 second contacts were removed from the dataset. This time there was no significant difference between the edited dataset and the observed contacts in the same time period (paired t-test, $t_{1, 89} = 1.71$, observed = 36.2 ± 55.1, edited = 31.7 ± 51.1, $P = 0.09$).

Recovery rates

Of the 77 badger collars fitted: 28 (36%) were retrieved by re-trapping the badgers; 25 (33%) were retrieved by locating the dropped collar in the field using radio-telemetry (in the majority of cases this was due to a snapped collar); seven (9%) were lost (no VHF signal detectable); six (8%) had fallen off underground and could not be retrieved; and 11 (14%) were still fitted on badgers at the time of writing. Of the 32 cattle collars that were deployed at the start of the study, 29 loggers (91%) were recovered undamaged (although the collar strapping of one was broken) and three loggers were

lost (collars fell off but were not found). Of the 19 base stations used in the study, 11 (58%) were recovered and eight went missing (presumed to have been dug up and removed by badgers).

2.5 DISCUSSION

Increasing recognition of the utility of proximity loggers for the collection of interaction data for free-ranging animals suggests that they are likely to become more widely used in studies in the biological sciences. These devices have several user-defined parameters, the most important being detection and separation distance, which allow them to be used in studies of different focal species and with very different aims. In the present study we have highlighted sources of inaccuracy on the basis of which we can propose unified methods for i) pre-deployment setting of proximity devices and ii) preparing derived data for analysis. In doing so we aim to improve the validity of data arising from the use of proximity loggers in future studies of animal contact networks, whilst at the same time recognising that there will always be limitations to the technology, for example, due to the physics of UHF waves with which they operate.

The recording of erroneous identification data does not appear to be a significant problem with the latest generation of proximity logger and can be considered to have a negligible impact on the data recorded. Based on the very small number of erroneous identification codes recorded, the proximity loggers appear to be extremely accurate in recording the identification of contacted collars. However, we were unable to determine what proportion of interactions recorded by the loggers as genuine identification numbers may in fact have been false. If a logger identification number existed then it was taken to be a true record. It was not possible to determine the 'false record' rate in the observational study as this would have required more accurate determination of separation distances than was achieved here.

The detection distance of all types of proximity logger decreased with time for those collars that had been deployed in the field, but not for the couple that had been kept in the laboratory with the battery turned off. Thus, rather than this being a feature intrinsic to the technology it is more likely to be related to diminishing battery power. This in turn may be a function of temperature: at temperatures above and below 25°C, the voltage of lithium thionyl chloride batteries – as used in these proximity loggers – sags

under load (K. Lay, *pers comm*). The proximity loggers fitted to the badgers are likely to have been exposed to warmer temperatures than the cattle loggers due to the sett environment and the closer fitting of collars to the badgers' necks. The reduction in detection distance was very pronounced for the badger collars where a decline of almost 50% in detection range was observed over eight months, although there was no further decrease after 12 months of deployment when a critical battery threshold may have been exceeded. Also, it was not found to be influenced by the frequency or the duration of contacts that the collars had recorded. It therefore appears that longer range interactions are less likely to be recorded by the loggers over time, but that this decrease in detection distance levels off after eight months. A possible practical solution would be to periodically re-measure the detection ranges of the loggers and recalibrate as necessary. However, this could be difficult if a large number of loggers have been deployed and is likely to be highly impractical for loggers fitted to elusive wild animals that are not amenable to frequent recapture. An alternative solution would be to apply a correction factor to the data pre-analysis to account for the decrease in detection of longer-range interactions over time and avoid biases in the interpretation of the data. Indeed, one general limitation of the technology at present is the requirement for animals to be recaptured in order to download the data stored in the internal memory. However, there is not a time limit for this and data can still be downloaded after the battery has run out (K. Lay, *pers comm*), although that situation was not encountered in this study.

Overall, the proximity loggers recorded a reasonable majority of the observed interactions although there was marked variation between individual loggers. The impact of missed interactions is likely to be very low because the modal duration of non-recorded interactions was 1 second and all contacts of this duration were later filtered out of the dataset to improve reliability after combining "broken contacts". Some interactions that were not recorded by the loggers were observed to be very close contacts – it was not just the longer-range interactions that were missed. Non-recording of contacts might be due to the varied orientation of the loggers or physical obstructions such as an animal's head, vegetation, or nearby objects deflecting the loggers' signals (Prange *et al.* 2006, Swain and Bishop-Hurley 2007). For these reasons, some spatial imprecision is likely to remain a limitation of proximity logger use in the field.

The tendency of proximity loggers to record extended duration interactions as a series of shorter contacts has been reported previously (Prange *et al.* 2006). Not all subsequent

studies appear to have accounted for this, and where they have, no consensus seems to exist on how to manipulate the data. Methods that have been applied include: joining contacts divided by periods less than the programmed 'separation time' plus 15 s (Prange *et al.* 2006); scoring the length of contact as the union time between two collars (Hamede *et al.* 2009); aggregating records from single devices over a sliding window of 20 s (Cattuto *et al.* 2010); and combining records detected within 60 s of each other (Guttridge *et al.* 2010). The findings of the present study support the proposition of a 1 – 2 minute amalgamation window for records between pairs of loggers since for 95% of the time where longer interactions were recorded as multiple shorter records the gap between records was 51 s or less for the field trial and 129 s (just over 2 minutes) or less for the laboratory trial.

Proximity loggers that interact at the edge of their detection range may record very short contacts (typically of 1 second duration) possibly due to weak signal strength (Prange *et al.* 2006). Removing these contacts from the dataset has been shown to have significant effects on contact network structure (Hamede *et al.* 2009) and increases the reliability of pairwise contact records (Prange *et al.* 2006). Despite this, removal of 1 second records has not been routinely conducted in many studies. The results of the present study indicate that proximity logger datasets should be filtered of 1 second records after combining dyadic records over a 1 – 2 minute amalgamation window, which produces a dataset that is closer to the observed values. Analysing unfiltered data may lead to erroneous conclusions; in most cases overestimating the frequency and underestimating the duration of contacts, which is also likely to impact the analysis of social networks and the metrics derived from the data.

The similar performance of the two methods for setting badger collar initiation distances (either a separate UHF setting for each collar [variable], or using the same coefficient for all collars [fixed]) suggests that it is not necessary to individually measure and set each collar to a particular UHF coefficient. An interesting result from this analysis is that interacting collars have a high level of agreement in the duration of the contacts that they record, but less of an agreement (albeit still significant) in the number of contacts that they record, suggesting that the length of the contact recorded may be a more accurate parameter to use in further analyses than the frequency of contacts recorded.

Taken together, the findings of this validation study can be summarised in a series of five recommendations which may act as guidance for researchers using proximity loggers to study animal contact behaviour in the future:

1. Barring a technological improvement to loss of battery power, measure the detection distances of proximity loggers periodically, consider recalibrating every six months if practical and consider incorporating a correction factor into data analyses if comparing across time periods (e.g. seasonal variation in behaviour) due to the decrease in the initiation and termination distances of the collars.
2. When manipulating the data collected by automated proximity loggers, contacts recorded within 1 – 2 minutes of each other should be amalgamated if they involve the same pair of loggers. For greater precision, each study could determine this amalgamation window for their devices using the methods employed here. This will give a more accurate reflection of longer duration interactions and can be easily automated, for example, with a script in the statistical programme R (provided in **Appendices A & B**).
3. Remove all records of interactions lasting 1 s from the dataset post-amalgamation as these may represent weak signals or collars interacting at the edge of their detection range and their removal increases the accuracy of the dataset.
4. Include VHF transmitters in all proximity logger devices to increase recovery rate if collars fall off or if base stations go missing.
5. As some proximity loggers are unlikely to be recovered from the field, based on the losses encountered in the present study, we suggest budgeting for 110% of the required number of large animal (in this case, cattle) loggers, 150% for medium-sized highly mobile animal (in this case, badgers) collars, and 175% for static base stations (if they are at risk of being dug up by the study animal). These budgets should be taken as a guide rather than being prescriptive because rates of collar loss are likely to differ amongst species.

In conclusion, this validation study indicates that proximity loggers are highly accurate at recording the identification of contacted loggers but less reliable at consistently determining the true frequency and duration of contacts. However, our investigations of these limitations in proximity logger performance have allowed us to quantify these sources of potential error and to suggest approaches for their mitigation. We hope that the five recommendations made here will be of use to the expanding number of

researchers using proximity loggers to determine contact patterns of animals and provide an evidence base on which data collected from these devices may be corrected to more accurately reflect the 'true-life' pattern of animal interactions.

Chapter 3. Sett use patterns of the Eurasian badger (*Meles meles*) correlate with bovine tuberculosis disease status

3.1 ABSTRACT

1. Heterogeneities in the behaviour of individuals may underpin important processes in evolutionary biology and ecology including the spread of infectious diseases. Differences in space use and contact patterns may pertain to demographic factors and also influence and/or be influenced by an individual's disease status.
2. The Eurasian badger is a wildlife reservoir for bovine tuberculosis (bTB) in Britain and the large-scale Randomised Badger Culling Trial demonstrated that badger behaviour is an important factor in the spread of bTB among badgers and from badgers to cattle.
3. Using radio-telemetry devices deployed on 51 badgers from 8 social groups, I investigated patterns of sett use in an undisturbed high-density badger population. Animals were located at their setts (classified as either 'main' or 'outlier') on 28 consecutive days in each season to investigate how patterns differed between individuals, in particular, with regard to their bTB test status. The use of main vs. outlier setts influences contact patterns among badgers and may affect the transmission of disease.
4. The badger population exhibited significant seasonal variation in sett use. However, there was considerable individual-level heterogeneity in observed behaviour, influenced by sex and age effects, which was highly repeatable across seasons.
5. When controlling for demographic effects the outcome of a serological test for bTB infection was highly correlated with sett use. Badgers that tested positive spent a significantly greater proportion of their time away from their main sett than those that tested negative. Whilst it was not possible to ascribe cause and effect, I speculate that the wider-ranging behaviour of these test-positive animals may have resulted in them contacting sources of infection more frequently, or that their behaviour may have been influenced directly or indirectly by their disease status.
6. For badgers and bTB, disease control measures might be enhanced by taking into account seasonal and individual-level variation in ranging behaviour and the use of outlier setts. For example, by identifying functional groups of individuals, specific areas, or times of the year that contribute disproportionately to disease spread and which could be targeted for disease control interventions.

3.2 INTRODUCTION

Heterogeneities in the behaviour patterns of individuals are common in social-living animal populations and may have potentially important ecological, evolutionary and conservation implications (Bolnick *et al*. 2003). Such differences may exist at the demographic level and/or be influenced by social organisation and factors such as dominance status, body condition or relatedness (Altzier *et al*. 2003). The transmission of infectious disease can be profoundly influenced by heterogeneities in exhibited behaviours, with implications for disease control (Woolhouse *et al*. 1997; Cross *et al*. 2009). Behaviours of epidemiological importance may include local movements, dispersal, intra-specific aggression, social and reproductive interactions and denning behaviour. Behavioural differences between individuals may arise pre-infection and influence an individual's risk of contracting disease. Alternatively they may arise (or continue) post-infection and influence an individual's risk of transmitting pathogenic agents. For many diseases, particular individuals in the population have been identified as contributing disproportionately to the spread of infectious agents (Shen *et al*. 2004; Lloyd-Smith *et al*. 2005). These individuals may belong to certain functional groups, for example, some studies have found that sexually mature males may be more important in the spread of parasites than females (Perkins *et al*. 2003; Skorping & Jensen 2004). Alternatively, the heterogeneities could be more individual-based, with studies showing that individuals that display more exploratory behaviours may have a greater probability of contracting and/or transmitting disease (e.g. Natoli *et al*. 2005). Overall, individual-level heterogeneities that may influence an individual's risk of becoming infected/infecting others are likely to be consistently expressed and potentially heritable, behavioural/ecological and/or immunological/physiological traits.

Whilst differences in behaviour patterns, particularly relating to social interaction and space use, can be predictable and have been shown to influence disease dynamics, identifying the underlying proximate mechanisms is extremely challenging. Social behaviour determines the social structure and framework of contacts among individuals and hence may influence the spread of disease through a population. Within populations, certain individuals may exhibit intrinsic behavioural characteristics that bring them into contact with more sources of infection and/or provide more opportunities to transmit these parasites to others (e.g. Natoli *et al*. 2005; Easterbrook *et al*. 2007; Boyer *et al*. 2010). This could be related to individual attributes such as sex,

age and/or social status, or alternatively, the behaviour of infected individuals can be particularly important if it is in some way different from that of others post-infection with a pathogen. Often this might arise because animals in an advanced state of disease are in some way impaired and cannot compete and/or forage effectively. This could result in them being ostracised from a social group or forced to forage in marginal areas and thus display different patterns of space use and movement compared to uninfected individuals (e.g. Cheeseman & Mallinson 1981). Alternatively, in some cases differences in the behaviour of infected animals may be due to the pathogens altering host behaviour in such a way that increases the probability of their transmission (Klein 2003; Thomas, Adamo & Moore 2005).

Here I focus on the behaviour of the Eurasian badger (*Meles meles*), which is a significant wildlife reservoir for the transmission of *Mycobacterium bovis* (the causative agent of bovine tuberculosis; bTB) infection to cattle in Britain and Ireland (Muirhead, Gallagher & Burn 1974; Bourne *et al*. 2007; Griffin *et al*. 2005). In recent times the incidence of bTB in British cattle has increased substantially, with significant economic consequences (Sheppard & Turner 2005). Thus, the drive to devise and implement successful and sustainable management strategies focusing on the testing and slaughtering of infected cattle, improved farm biosecurity, vaccination of cattle and badgers and/or the culling of badgers is greater than ever. In moderate-to-high density badger populations in Britain, animals live in mixed-sex territorial groups (Johnson, Macdonald & Dickman 2000). The principal route of bTB infection among badgers appears to be via the respiratory system followed by infection from bite wounding (Cheeseman, Wilesmith & Stuart 1989). Thus, close and prolonged contact between individuals would be expected to facilitate the transmission of *M. bovis*. Bovine TB infection in badger populations in Britain has been observed to be highly spatially aggregated (Delahay *et al*. 2000a; Woodroffe *et al.* 2005a) and transmission rates are likely to be non-linear with respect to host density (Smith *et al*. 1995; White & Harris 1995; Barlow 2001). The aggregated distribution of infection in badgers is likely to reflect the highly structured social organisation which typifies the moderate-to-high density populations found in the bTB-affected areas of Britain. Here badgers live in social groups, each of which occupies a group territory, so that transmission operates on two different levels: within and between groups. Field evidence strongly suggests that disease transmission risks are closely related to the extent of movement among social groups (Rogers *et al*. 1998; Vicente *et al*. 2007). Furthermore, the perturbation of social

structure is a likely reason that badger culling has resulted in complex patterns of subsequent bTB prevalence in badger and cattle populations (Donnelly *et al*. 2003; Donnelly *et al*. 2005; Woodroffe *et al*. 2006a,b). However, to date there have been only limited investigations into patterns of space use by individual badgers and how these relate to disease status and transmission risk.

In the UK badgers use communal underground burrows, or setts. These can be loosely categorised as 'main' and 'outlier' setts (Neal 1977). Main setts can be extremely large and are the primary, year-round residence of a social group (Neal & Roper 1991). Speculation remains as to the function of outlier setts, which are usually considerably smaller, tend to be only intermittently occupied and are generally found in the hinterland of a group's territory (Kruuk 1978; Harris, Cresswell & Jefferies 1989). Previous work identified a significant correlation between the bTB status of social groups and the number of setts in the territory (Rogers *et al*. 2003). This raises the possibility that outlier setts (of which there can be several in a territory) may be important to disease dynamics. Individual badgers have been shown to vary considerably in the extent to which they use main vs. outlier setts (reviewed in Roper 2010) and there is some evidence that bTB test-positive badgers range more widely and/or use outlier setts more frequently than apparently uninfected individuals (Cheeseman & Mallinson 1981; Woodroffe, Frost & Clifton-Hadley 1999; Garnett, Delahay & Roper 2005).

Previous studies have been constrained by small sample sizes, limited study periods and a lack of data on the life histories of individual badgers. Here I present the results from a larger-scale study in which badgers were tracked for continuous periods and captured regularly to collect data on factors including sex, age, body condition and bite wound score. I also consider the degree of consistency exhibited in behaviours among individuals over time and how the behaviours correlate with disease status as inferred from diagnostic test outcomes. Together, these data allow me to address the question of how sett use patterns relate to individual attributes. Findings are discussed in the context of disease transmission and management strategies.

3.3 MATERIALS AND METHODS

3.3.1 Data Collection

Study site and population

Fieldwork was conducted on a well-studied, high-density badger population at Woodchester Park, Gloucestershire, UK (51°71´N, 2°30´E). The study area comprised approximately 7 km^2 of fragmented deciduous and coniferous woodland, agricultural grassland and smaller areas of arable and scrub land (see Delahay *et al*. 2006b). In this population, individuals were trapped on average twice per year as part of a long-term study in which detailed epidemiological and morphometric data were recorded to construct relatively complete life histories (for more details: Delahay *et al.* 2006b; Vicente *et al*. 2007).

Equipment deployed

VHF transmitters were deployed on 51 badgers belonging to eight social groups as part of a collar that also included a proximity logging device (Sirtrack Ltd., Havelock, NZ). All collared animals were located while resting at their setts once every day between 08:00 and 15:00 GMT during which time the animals may move around underground but are not expected to change setts for resting on that day (Roper 2010). This was done using a R1000 receiver (Sirtrack Ltd., Havelock, NZ) and a Yagi antenna (Biotrack, Dorset, UK) on 28 consecutive days in each season (Summer [26/06/2009 – 23/07/2009]; Autumn [23/09/2009 – 20/10/2009]; Winter [10/01/2010 – 6/02/2010]; Spring [16/04/2010 – 13/05/2010]). Main setts were categorised as those with more than five entrances, large spoil heaps with obvious runs and entrance holes with signs of occupation throughout the year (Kruuk 1978; Harris, Cresswell & Jefferies 1989). Outliers were identified by the presence of fewer entrance holes, and their position in the territory hinterland. Setts in which badgers were recorded to be resting were allocated to social group territories using the results of an annual baitmarking exercise, which was based on the premise that badgers use communal latrines, which demarcate territorial boundaries and are likely to be hotspots of visits by most individuals (Delahay *et al*. 2000b). To determine the territorial configuration of the population each social group was fed a bait (peanuts and syrup) laced with a unique colour and/or shape of

plastic pellet so that the origin of faeces at latrine sites could be assigned (see Delahay *et al*. 2000b). The underground location of the animal within the sett was recorded using two different methods as part of a validation study. It was primarily determined using VHF radio-telemetry to pinpoint a location on the surface below which the badger was resting (as per: Butler & Roper 1996; Roper *et al*. 2001). This primary assessment was validated using the data recorded by the proximity loggers when two or more individuals were resting in close proximity. Both methods were found to provide the same results.

Badger sampling

For each badger we recorded sex (male/female) and age class, which was categorised as sub-adult (sexually immature yearling/young-adult >1< 2.5 years), or a reproductive/ breeding adult (≥ 2.5 years). Juvenile badgers (cubs) were still growing and so for welfare reasons could not be collared. When females were captured in the 2010 spring and summer trapping sessions it was noted whether they were lactating and if they were, they were assumed to have given birth at the start of the year. Body condition index (BCI) was calculated using the relationship between body length and weight (Le Cren 1951); BCI = observed W / aL^n, where W is weight, L is body length, and a and n are constants. Linear regression of ln W against ln L from badgers captured during the long term study at Woodchester Park from 1997-2009 allowed the constants a and n to be estimated separately for male and female badgers (A. Tomlinson, *unpublished data*). Animals were given a score based on the number of fresh bite wounds that they had on their bodies when captured during the 2009/10 trapping season (0 = 0 wounds, 1 = 1-2 wounds, 2 = 3-4 wounds, 3 = 5+ wounds) (Delahay *et al*. 2006a).

I had the results from three diagnostic tests available to use in the study: 1) BrockTB Stat-Pak lateral flow serum antibody test (Chambers *et al.* 2008; Chambers *et al.* 2009); 2) an enzyme immunoassay for interferon-gamma (IFNγ) (Dalley *et al.* 2008; Chambers *et al*. 2009); and 3) culture testing. I did not include the results from the culture test as only one animal was found to be actively excreting *Mycobacterium bovis* (the causative agent of bTB) at the time of the study. Culture is a much less sensitive predictor of infection than either the StatPak or IFNγ, both of which have been used to indicate infection in free-living badgers (see Chambers *et al.*, 2011). Moreover, the Stat-Pak detects the presence of *M. bovis* antigens, the production of which has been shown to

positively correlate with the extent and severity of TB infection in both naturally and experimentally infected badgers (reviewed in Chambers 2009). I chose to use the results from the Stat-Pak immunoassay alone as this test is the best available indicator of established infection in badgers and the sensitivity of the test increases considerably with disease severity (Chambers *et al.* 2008).

The disease status of each badger was inferred from the positive or negative outcome of a badger-specific lateral flow immunoassay carried out at any time prior to and at the start of the study period (BrockTB Stat-Pak; Chembio Diagnostic Systems, New York, USA) (Kampfer *et al*. 2003; Chambers *et al.* 2009). A positive Stat-Pak result was interpreted as evidence of current infection with *M. bovis* as antibody production is positively correlated with the extent and severity of bTB infection in badgers (Chambers *et al.* 2009) and thus is an effective test for identifying animals with established infection. The Stat-Pak assay used to assess bTB status has a sensitivity of approximately 58%, increasing to more than 80% with disease progression, and 93% specificity (Dalley *et al*. 2008; Chambers *et al*. 2009). There is potential for the frequency of testing to influence the determination of disease status, with animals that had been captured more frequently having a greater chance of being considered positive. Consequently, the number of capture events for test-positive and test-negative animals were compared to ensure that both were sampled with comparable intensity. The possibility exists for false negative results due to the sensitivity of the test. However, there is an equal probability of getting these results for individuals using outlier setts as there is for those using the main sett and thus it is unlikely that they will bias any trends that may be detected.

The number of different individuals captured in each territory during the 2009/10 trapping year was used as a proxy measure of group size (Delahay *et al*. 2006b) and the demographic structure of each social group was summarised using the ratios of adults/cubs and males/females. The territory size of each social group was estimated in terms of the true surface area (correcting for topography and expressed in m^2) using data from baitmarking studies conducted in spring 2009 (for details: Delahay *et al*. 2000b; K. Palphramand, *unpublished data*). Finally, territories were surveyed to determine the number of main and outlier setts available to each social group.

3.3.2 Statistical Analysis

Relationships between the measure of proportional sett use (number of days out of 28 spent resting at main vs. outlier sett(s)) and characteristics of individual badgers and social groups detailed above were investigated by fitting generalized linear mixed models (GLMMs) with a binomial error structure and logit link function. To take into account repeated sampling within individuals and social groups, badger ID nested within social group were included as random factors. A minimum adequate model including biologically meaningful interactions was constructed by retaining the significant and deleting the non-significant terms from a maximal model on the basis of likelihood ratio tests (compared against a χ^2 distribution) where $\alpha = 0.05$ (Crawley 2007). Models were checked for overdispersion.

Repeatability of among-individual differences in the use of the main vs. outlier setts was assessed using pair-wise comparisons across the seasons in which each individual was tracked. This was done by means of the intra-class correlation coefficient (ICC) using the function 'rpt.binomGLMM.add' in the R package rptR. This produces GLMM-based repeatability estimates from additive models fitted by Markov Chain Monte Carlo (MCMC) sampling with standard errors and Bayesian credibility intervals (for details: Nakagawa & Schielzeth 2010). All analyses were carried out using R v. 2.11.1 (R Development Core Team 2010).

3.4 RESULTS

Of the 51 badgers collared, 40 were tracked across two or more seasons before the collars either fell off or were removed 12 months post-deployment (two seasons $n = 9$, three seasons $n = 13$, four seasons $n = 18$) and were used in analyses of sett use patterns (summer $n = 33$, autumn $n = 30$, winter $n = 35$, spring $n = 27$; see Figures 3.1 and 3.2 for sample sizes of different age classes, sexes and disease status). The social group to which a badger belonged accounted for a significant, albeit relatively small, amount of the variance in the proportion of time spent at the main sett, although none of the measures of social group size, demography or territorial characteristics included were significant determinants of variation in use of sett types (Table 3.1). In contrast, a considerable amount of variation in sett use patterns could be attributed to differences among individual badgers within a group. Of the individual characteristics measured, an

interaction between age class, sex and season, and the bTB test outcome were all significantly associated with sett use patterns, whilst body condition index (BCI) and bite wound score were not (Table 3.1). Even after accounting for the individual attributes above, considerable differences between individuals still remained (likelihood ratio test, χ_1^2 = 184.5, p < 0.001; Table 3.1). The proportion of time spent at the main sett vs. outlier setts was highly repeatable for individuals across the seasons in which they were surveyed (R = 0.71 – 0.89 in pair-wise contrasts between seasons using measures of ICC; Table 3.2).

Table 3.1 Factors determining the proportion of time spent by badgers at the main sett vs. outlier setts. Main effects and significant interactions from binomial GLMM models are presented. *P* values were obtained by step-wise deletion from maximal model, starting with the least significant term. Minimum adequate model was: Proportion of time at main sett ~ bTB test outcome × season + sex × age × season + (1 | social group / badger ID) (model 1).

Variables	χ^2	**d.f.**	***P***
Fixed Effects			
bTB test outcome × Season	24.8	3	**< 0.001** ***
Sex × Age × Season	12.9	3	**0.01** **
Number of outlier setts	3.11	1	0.08
Group size	2.77	1	0.10
Proportion of males	1.65	1	0.20
BCI	1.18	1	0.28
Bite wound score	0.64	1	0.42
Territory size	0.38	1	0.54
Number of main setts	0.36	1	0.55
Proportion of adults	0.23	1	0.64
Random Effects			
Individual	185	1	**< 0.001** ***
Social Group	3.85	1	**0.05** *

Table 3.2 Repeatability (as given by the intra-class correlation coefficients (ICC)) in individual badgers of the proportion of time they each spent at the main sett vs. outlier setts between the different seasons. *R* is the estimate of repeatability, SE the standard error, CI the Bayesian credibility intervals, and *n* the number of pairwise comparisons.

	Summer	**Autumn**	**Winter**	**Spring**
Summer	-	-	-	-
Autumn	***R* = 0.77** SE = 0.11 CI = [0.55, 0.95] *n* = 28	-	-	-
Winter	***R* = 0.84** SE = 0.11 CI = [0.61, 0.97] *n* = 28	***R* = 0.87** SE = 0.09 CI = [0.62, 0.95] *n* = 25	-	-
Spring	***R* = 0.71** SE = 0.09 CI = [0.52, 0.89] *n* = 21	***R* = 0.89** SE = 0.10 CI = [0.62, 0.99] *n* = 20	***R* = 0.85** SE = 0.08 CI = [0.66, 0.96] *n* = 28	-

When controlling for the effects of age and sex, bTB test outcome in interaction with season emerged as the most significant correlate of time spent at the main sett (Table 3.1 and Fig 3.1). In all seasons badgers that had tested positive for bTB prior to or at the start of the study were found to use the main sett less than badgers that tested negative, with the difference being most pronounced in spring and summer (Fig 3.1). Capture frequency did not differ significantly between badgers that were test-positive or negative for bTB (for test-positive badgers, mean captures ± SE = 7.90 ± 3.31, test-negative: 7.10 ± 3.10, t-test for unequal sample sizes, t = 0.672, d.f. = 15, $P = 0.512$). In addition, of the 10 badgers that tested positive for bTB, four were adults and six were sub-adults, suggesting that the results were not biased by the fact that older animals may have an increased probability of having acquired disease during their lifetimes.

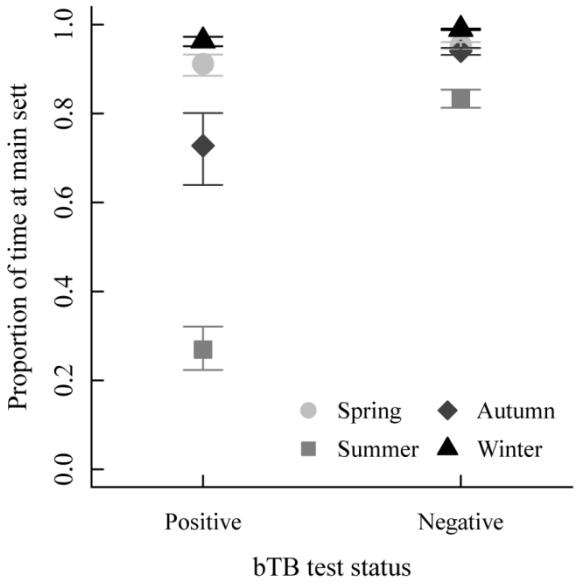


Figure 3.1 The proportion of time spent at the main sett across the four seasons in relation to bTB test outcome (estimates ± 1 SE from model 1 where sex, age and social group identity are controlled for). For the 10 badgers that tested positive; Sp *n* = 7, S *n* = 9, A *n* = 5, W *n* = 8. For those 30 that tested negative; Sp *n* = 20, S *n* = 24, A *n* = 25, W *n* = 27.

The proportion of time that badgers spent using the main sett varied significantly according to a three-way interaction between age class, sex and season (Table 3.1 and Fig 3.2). In the winter and spring months both sub-adult and adult females spent a greater proportion of their time at the main sett when compared to both age classes of males (Fig 3.2). Of the 13 adult females collared, 12 were found to be lactating in summer 2010 and were thus assumed to have given birth to cubs that year. Within the male age classes, sett use patterns of sub-adults and adults were similar during the winter but in spring and autumn adult males spent a greater proportion of time away from their main sett when compared to sub-adult males (Fig 3.2). During the summer adults and sub-adults of both sexes spent a greater proportion of their time away from the main sett than in other seasons (Fig 3.2).

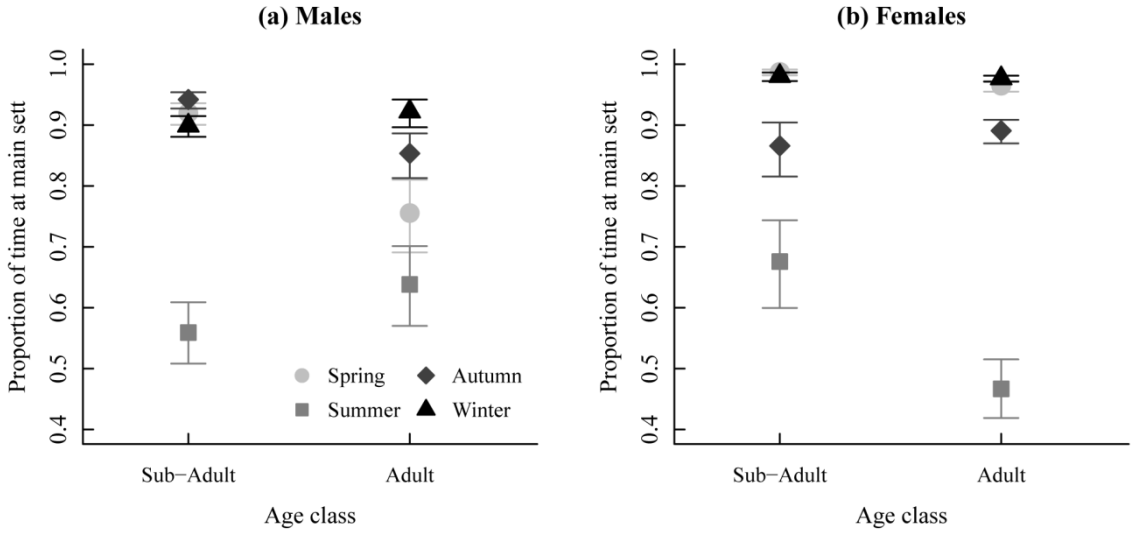


Figure 3.2 The proportion of time spent at the main sett in relation to sex, age and season (estimates ± 1SE from model 1). For sub-adult males; Sp n = 8, S n = 10, A n = 8, W n = 12. For adult males; Sp n = 5, S n = 6, A n = 6, W n = 6. For sub-adult females; Sp n = 6, S n = 6, A n = 5, W n = 6. For adult females; Sp n = 8, S n = 11, A n = 11, W n = 11.

3.5 DISCUSSION

This study has shown an association between infection status and individual behaviour in a wildlife host of a disease of zoonotic, agricultural and conservation significance. Whilst bTB status was inferred from an imperfect diagnostic test it is the most sensitive test currently available for identifying established infection in a live animal and, crucially, the rate of false positives would not have expected to vary according to the degree to which individuals used main or outlier setts. The study also suggests a link between ranging behaviour, age class, sex and season, which provides valuable insights into how these animals behave and utilise the space available to them when living in a relatively undisturbed population. The heterogeneity observed at the level of the individual animal, relating to both demographic factors and also disease status, was found to be consistent across the seasons in which the animal was tested. This highlights the potential importance of variations in behaviour for the dynamics of disease transmission in wildlife populations. Such findings could inform management options, perhaps by identifying functional groups of individuals, specific areas or times of the year that contribute disproportionately to disease spread and/or could be effectively targeted for disease control interventions.

In terms of demographic factors, sett use was found to be influenced by an interaction between sex, age and season, which can be explained by reproductive behaviours. From February to May cubs are born and females are suckling dependent young (Woodroffe *et al*. 2005b). During this time females utilised the main sett to a greater extent than both sub-adult and adult males. None of the sub-adult females were found to be lactating when captured in spring/summer but spent the same amount of time at the main sett as the breeding adult females. There is, however, little evidence that badgers exhibit allo-parental behaviours (but see Woodroffe 1993; **Chapter 4**) or where it has been observed that it provides any detectable benefits (Woodroffe & Macdonald 2000). In spring, males spent a greater proportion of their time away from the main sett than females, with adult males spending more time away than sub-adult males. Both females and males may seek extra-group mating opportunities as demonstrated in genetic studies (Carpenter *et al*. 2005; Dugdale *et al*. 2007) and the use of outlying setts by both sexes may be a symptom of spending more time at the edges and/or outside their resident territory. These results are consistent with mature males making forays into the territories of other social groups for mating opportunities in spring and females doing the same in autumn when they are not rearing young.

A considerable amount of variation in sett use patterns was attributed to differences among individual badgers that were found to be consistent across the seasons in which the badgers were sampled. After controlling for demographic factors discussed above, bTB test outcome emerged as the most significant factor that was correlated with sett use patterns. Badgers that tested positive for bTB were found to utilise outlier setts to a greater extent in all seasons than animals that tested negative. This provides a putative mechanism that might help to explain the correlation between bTB status of badger social groups and the number of setts in the territory observed in a previous study (Rogers *et al*. 2003). It may also relate to previous observations of wider ranging behaviour in infected badgers (Garnett *et al*. 2005). I consider these findings in relation to two (non-mutually exclusive) causal mechanisms relating to a) the behaviour of an individual influencing its risk of contracting disease and b) the disease status of an individual influencing its displayed behaviours either directly or indirectly via the actions of others.

The findings suggest that outlier setts may play an important role in bTB transmission dynamics in badger populations. Main setts tend to occupy a relatively central position

within a badger territory whereas outliers are located throughout the hinterland and sometimes near to boundaries with neighbouring groups (reviewed in Roper 2010). It seems probable, therefore, that encounters between animals from different social groups are more likely to take place at or near to outlier setts than at main setts. Hence, more frequent use of outliers may be associated with an enhanced risk of exposure to *M. bovis* either from contact with an infected neighbour or a contaminated sett environment. Thus, there may be a link between wider ranging behaviour and more frequent use of outlier setts, which may also result in an increased risk of exposure to infectious agents. For example, a number of studies have reported that bolder individuals that elicited a greater number of encounters were more likely to be infected by parasites and diseases than shyer individuals (e.g. Natoli *et al*. 2005; Easterbrook *et al*. 2007; Boyer *et al*. 2010). This question is addressed in **Chapter 5** using contact data collected by the proximity logger function of the collars and social network analysis to investigate whether individuals that use outliers more frequently and those that test positive for bTB, have higher (or lower) levels of connectivity in terms of both within- and between-group contacts.

Alternatively, the observation could either be attributed to, and/or, exacerbated in combination with other mechanisms if infection with bTB resulted in individuals spending more time away from the main sett. This could be driven by reduced ability to hold social status or compete for resources. Cheeseman & Mallinson (1981) radio-tracked three badgers in an advanced stage of bTB infection (they were found to be excreting *M. bovis* and were in poor body condition). These animals ranged more widely and were more likely to rest in outliers than uninfected individuals. However, in my study, animals that used outlier setts to a greater extent did not exhibit a greater number of bite wounds, which is another indicator of inter-specific aggression (Delahay *et al*. 2006a), nor were they found to be in poorer body condition, as would be expected if these were individuals in the very late stages of disease. Too few of the individuals sampled (only two of the 10) were found to be actively excreting *M. bovis* at the time of a culture test and thus, this measure of disease status could not meaningfully be included in our analyses. An alternate, albeit more speculative, mechanism could be direct host manipulation from the pathogenic agent, such as that exhibited by animals infected with the rabies virus (Rupprecht, Hanlon & Hemachudha 2002). Although not previously documented in *M. bovis*, it is conceivable that the pathogen could induce

wider-ranging behaviour in the badger, thereby increasing opportunities for onward transmission in the population.

This study highlights the potential for an understanding of heterogeneity in behaviour between infected and uninfected individuals to inform disease management strategies. In the case of badgers the correlation between use of outlier setts and bTB test outcome suggests that a proportion of animals using these secondary setts may have disproportionate epidemiological significance and that specific consideration of outlier setts and their occupants should be included in the evaluation of any management strategy (e.g. vaccination and/or culling) that involves targeting badgers at their setts. Focusing effort on outlier setts might expedite the objectives of any management strategy and prove a useful addition to targeting main setts. In the case of an oral vaccine delivered in bait, deployment at main setts in the autumn may increase uptake as a greater proportion of animals were found to be resident at the main sett during this time, although this should be weighed against the benefits of targeting susceptible cubs when they first emerge from the setts in spring (Palphramand *et al*. 2011). The abundance of natural food sources in autumn may also result in lower uptake of baits at this time despite more of the resident badgers being at the main sett. Regardless of which strategy is adopted (culling, vaccination or a mixed-approach) findings suggest that the identification of particular functional groups of individuals, locations of effort and the time of the year that they are carried out are important considerations that should be addressed for maximum success and cost effectiveness.

Chapter 4. Social life of the Eurasian badger (*Meles meles*): Proximity loggers provide new insights into contact patterns within and among social groups

4.1 ABSTRACT

1. Social organisation plays an important role in the lives of many animals, shaping their ecology and evolution, but is often inherently difficult to study in any detail.
2. The Eurasian badger (*Meles meles*) exhibits flexibility in social structure across its geographic range, living in mixed age and sex groups in many high-density UK populations. However, owing to the nocturnal and cryptic behaviour of this species, much of what is known about interactions within and between social groups has either been inferred indirectly or described in only qualitative terms.
3. Recently-developed proximity logging devices were deployed on 51 badgers from eight social groups in order to directly record contacts made over a one-year period, and use social network analysis to quantify how social behaviour varies across the annual cycle and among different demographic groups (sex and age classes).
4. Interactions within social groups accounted for the majority of contacts, but the entire study population was ultimately connected through interactions among individuals from neighbouring groups. These interactions were largely nocturnal and appeared to be significantly motivated by reproductive behaviour: contacts peaked in line with known breeding seasons and had a higher probability of involving males and females, with males apparently initiating more of these encounters. However, evidence was also found of possible dispersal events and social group fusion, suggesting that badger social structure is more dynamic than is often assumed.
5. Within-group contacts were heavily influenced by seasonal and demographic factors: younger individuals had higher intra-group contact rates than adults, which may facilitate integration into the group, while adult females appeared to largely segregate themselves after giving birth, perhaps to reduce the risk of infanticide. However, there was also evidence of individually consistent differences in contact behaviour (both within and between social groups) that were independent of age and sex and that were highly repeatable across seasons.
6. This study provides direct support for many previously held assumptions regarding the social structure of badger populations. It also highlights how novel technologies can be used to provide new insights into the social lives of otherwise cryptic species. Individual-level heterogeneities in social behaviour are particularly difficult to detect using more indirect methods such as capture-mark-recapture and radio-telemetry, and as illustrated here may play a large role in how animal populations are structured.

4.2 INTRODUCTION

Social structure is a fundamental characteristic of most animal populations. Individuals in a population interact as part of a network of associations that can vary in strength, type and dynamics at different times of the year (Croft, James & Krause 2008). Such ties between individuals may be influenced by a variety of extrinsic (e.g. resource availability, habitat quality and climate) and intrinsic (e.g. age, sex and dominance) factors (Whitehead 2008). The structure of social networks is defined by, but also has significant implications for, the ecology and evolution of individuals, populations and species (Croft, James & Krause 2008), and is often an important factor in management and conservation practices (Sutherland 1998). An array of behaviours relating to the sociality of a population (e.g. individual affiliations, foraging and reproduction) may influence and be influenced by the social structure of a population (Croft, James & Krause 2008). Such behaviours may in turn affect ecological and evolutionary processes including how information and infectious disease are spread through a population. However, despite its importance in animal ecology, evolution and conservation, social behaviour is often difficult to study in natural populations owing to the elusive and wide-ranging behaviour of many species and the risks of observer interference to naturally displayed behaviours.

The complex and dynamic nature of social behaviour and interactions is perhaps best exemplified by group-living animals (Kutsukake 2008). The Eurasian badger (*Meles meles*) is particularly interesting among such species due to the flexibility in its social organisation, which varies from pair- to group-living in different parts of its geographic range (Johnson, Macdonald & Dickman 2000). In the UK the majority of social groups contain from two to eight adults of mixed age and sex, although as many as 19 have been recorded (da Silva, Woodroffe & Macdonald 1993). There is still considerable debate surrounding the evolutionary origins and functional significance of social behaviour in these high-density badger populations (reviewed in Roper 2010). Indeed, it is only within the last 40 years that we have begun to fully understand the social organisation of such groups (Kruuk 1978). Since the pioneering work of Kruuk (1978), our understanding of the social organisation of badger populations has advanced considerably. We know, for example, that badgers live in relatively stable family groups which share and defend a discrete territory (da Silva, Woodroffe & Macdonald 1993; Delahay *et al*. 2000b) and exhibit low levels of permanent dispersal (Woodroffe 1993; Rogers et al. 1998). Reproduction is polygynandrous, with males

and females mating with multiple individuals from both their own and foreign social groups (Carpenter *et al*. 2005; Dugdale *et al*. 2007). We also know that although badgers live in clearly defined social groups and share nest chambers for diurnal resting they generally do not exhibit the level of cooperative behaviour found in many other group-living social carnivores (Jennions & Macdonald 1994; Clutton-Brock 2002). Females are thought to raise their young without assistance from other group members (reviewed in Roper 2010),despite some anecdotal evidence to the contrary (Woodroffe 1993) and foraging appears to be a largely solitary activity (reviewed in Roper 2010).

However, despite these advances, due to their secretive and nocturnal behaviours our understanding of the social organisation of badger populations is still limited by a lack of direct, quantitative information on interactions between individuals. Instead, most of what is known has been inferred indirectly from other methods such as radio-telemetry (e.g. Böhm *et al*. 2008) and capture-mark-recapture studies with trapping at setts (e.g. Rogers *et al*. 1998; Macdonald *et al*. 2008) or is based on direct observation which is generally qualitative and is subject to observer bias. Data from such studies lack resolution and are generally limited by small sample sizes and short observation periods (reviewed in **Chapter 6**). Consequently, relatively little is known about how social behaviour varies across the annual cycle, whether individual-level attributes such as age and sex influence social interactions or the level of connectivity among different social groups within populations. Radio-tracking has shown that individuals encroach into each other's territories (e.g. Böhm *et al*. 2008), trapping of animals at foreign groups' setts indicate that contacts among groups do occur (Rogers *et al*. 1998; Vicente *et al*. 2007) and genetic studies have suggested that around 50% of a female's litter may be sired by extra-group males (Dugdale *et al*. 2007). However, the frequency and extent of such interactions are poorly understood.

In recent years the development of lightweight proximity detection devices has provided new opportunities for continuously and remotely recording social interactions among free-living animals that were previously difficult to study owing to their cryptic and/or elusive nature (**Chapter 2**). This new technology is being rapidly adopted by ecologists but has been used predominantly to address applied questions relating to wildlife disease epidemiology (e.g. Hamede *et al*. 2009; Böhm, Hutchings & White 2009; **Chapter 5**). In contrast relatively few studies have used proximity loggers to address fundamental ecological questions related to how social behaviour varies over the annual cycles of animals, and among demographic

groups and individuals (but see Swain & Bishop-Hurley; Marsh *et al*. 2011). Here, proximity loggers were used to explore the social organisation of a high-density badger population in more detail than has previously been possible. Specifically, by combining contact data from proximity loggers with formal social network analyses (SNA), I examine how the frequency and duration of interactions within and among social groups vary according to individual-level attributes (e.g. age, sex, dominance status), environmental factors (e.g. season/climate) and social group composition. Although two previous studies have attached these devices to badgers, they were limited by small sample sizes and did not analyse the data within a SNA framework (Goodman 2007; Böhm, Hutchings & White 2009). In this study proximity loggers were deployed on a large, representative sample of badgers (n = 51) from eight social groups in order to record social interactions made over a one-year period. The length of the study period combined with the high temporal resolution of data from the proximity loggers permitted an assessment of how social behaviour varies throughout the annual cycle and whether such behaviour is repeatable for individuals or demographic groups over time.

4.3 METHODS AND MATERIALS

4.3.1 Data collection and field procedures

Study site and population

Fieldwork was conducted over a 12 month period (June 2009 – May 2010) on a well-studied, high-density badger population at Woodchester Park, Gloucestershire, UK (51°71´N, 2°30´E). The study area comprised approximately 7 km^2 of fragmented deciduous and coniferous woodland, agricultural grassland and smaller areas of arable and scrub land. The study population consisted of 20 social groups in 2009/10 and has been the subject of long-term ecological and epidemiological research; consequently their territorial organisation, individual group membership and the methods employed for their capture are well established and described (see Delahay *et al.* 2006b).

Measuring contact rates

Badgers were trapped during May and June 2009 and anaesthetised as part of on-going epidemiological research in this population. Whilst under anaesthetic, proximity logging

devices (Sirtrack Tracking Solutions, Havelock North, New Zealand) mounted on leather collars were deployed on 51 badgers from eight different social groups, ensuring that a representative sample of sexes and age classes from each social group was included wherever possible (see Table 4.1 for sample sizes). The proximity detection devices operate by transmitting a unique Ultra High Frequency (UHF) code whilst simultaneously 'listening' for the codes of other loggers (for details see Prange *et al*. 2006; **Chapter 2**). Loggers were individually set to begin recording a contact when two or more animals came within 0.64 ± 0.04m of each other (UHF settings range 34 - 48). This short-range detection distance was chosen to record direct contacts between collared badgers such as aggression, mating and allo-grooming. After the collars had been out of this range for 30 seconds or more, the ID of the individual(s) that had been encountered, the start time of the interaction (in GMT) and the contact duration in seconds were logged to memory. Proximity loggers thus yielded two types of information: the number of discrete contacts between pairs of individuals ('contact frequency') and the total duration of interactions between them ('contact duration').

Table 4.1 The numbers of collars deployed (black) and the total number of individuals present (*italics*) of different demographic classes across social groups in the Woodchester Park high-density badger population during the study period 2009/10.

Social	**Adults**			**Sub-Adults**			**Total**
Group	Male	Female	Total	Male	Female	Total	
Beech	1	2	3	3	3	6	**9**
	3	*3*	*6*	*3*	*3*	*6*	***12***
Cedar	0	3	3	0	0	0	**3**
	0	*3*	*3*	*0*	*0*	*0*	***3***
Kennel	1	1	2	0	4	4	**6**
	3	*1*	*4*	*1*	*4*	*5*	***9***
Larch	1	2	3	2	0	2	**5**
	1	*6*	*7*	*2*	*1*	*3*	***10***
Septic Tank	1	0	1	2	1	3	**4**
	1	*0*	*1*	*2*	*1*	*3*	***4***
Top/Yew	4	4	8	2	2	4	**12**
	5	*5*	*10*	*2*	*2*	*4*	***14***
Wych Elm	1	2	3	3	1	4	**7**
	1	*2*	*3*	*3*	*1*	*4*	***7***
West	1	2	3	2	0	2	**5**
	1	*2*	*3*	*2*	*0*	*2*	***5***

Whenever collared badgers were recaptured, data were downloaded to a laptop computer using software supplied by the manufacturers. Prior to analysis all contacts recorded during trapping periods were removed. In addition, the first 12 hours of data recorded after the end of each trapping event (the release of the animal) were removed as the period was treated as a re-acclimatisation time after which the animals were likely to be exhibiting natural behaviours again. Prior to analysis these data were adjusted by amalgamating contacts that were recorded within 1.5 minutes of each other if they involved the same pair of individuals, and then removing any remaining interactions that lasted for 1 second to construct a more realistic representation of what had most likely occurred in the field (Prange *et al*. 2006; **Chapter 2**).

Individual and group-level attributes

For each collared badger we recorded sex and age class, categorised as either sub-adult (i.e sexually immature yearling or young-adult, >1 and < 2.5 years) or reproductively active, breeding adults (≥ 2.5 years). Of the 15 adult females that were collared, 14 were found to be lactating when re-trapped in the following spring/summer and were thus assumed to have successfully given birth. Juvenile badgers (cubs) that are still growing rapidly could not be collared for welfare reasons. In addition to demographic attributes, for each study animal we also recorded its body condition and the number of fresh bite wounds, which can be used as a measure of aggressive encounters among conspecifics (e.g. Delahay *et al*. 2006a). Body condition index (BCI) was calculated using the relationship between body length and weight (Le Cren 1951); BCI = W / aL^n , where W is weight, L is body length, and a and n are constants. Linear regression of ln(W) against ln(L) for badgers captured during the long-term study at Woodchester Park from 1997-2009 allowed the constants a and n to be estimated separately for male and female badgers (A. Tomlinson, *unpublished data*). Bite wound scores were assigned based on the number of fresh wounds that animals had on their bodies when captured during the 2009/10 trapping season as follows: 0 = 0 wounds, 1 = 1-2 wounds, 2 = 3-4 wounds, 3 = 5+ wounds (see Delahay *et al*. 2006a for details).

The number of different individuals captured in each social group's territory during the 2009/10 trapping year was used as a proxy measure of group size (Delahay *et al*. 2006b) and the demographic structure of each social group summarised using the ratios of adults/cubs and males/females. The territory size of each social group was estimated in terms of the true

surface area (correcting for topography and expressed in m^2) using data from bait marking studies conducted in spring 2009 (Delahay *et al*. 2000b; K. Palphramand, *unpublished data*). Finally, territories were surveyed to determine the number of main and outlier setts available to each social group.

4.3.2 Statistical analyses

Two complementary methods were used to analyse contact data recorded by proximity loggers: standardised contact rates (SCRs) and social network analysis (SNA). SNA is increasingly used for analysing the structure of animal populations (Croft, James & Krause 2008; Whitehead 2008) and produces a range of network metrics which quantitatively describe the connectedness of individuals within the population (see below). However, the outputs cannot be analysed using standard linear modelling approaches due to non-independence of data points, meaning that interactions among explanatory variables cannot currently be tested (Croft *et al*. 2011). SCRs, on the other hand, directly quantify the number and duration of contacts among individuals adjusted for the number of recording days and contactable conspecifics and can be analysed using standard modelling techniques. However, SCRs can greatly oversimplify the structure of social networks and ignore individual heterogeneities and asymmetries in social behaviour. Although SCRs have been used to quantify contact rates in badger populations previously (Goodman 2007; Böhm, Hutchings & White 2009), SNA has never been applied in this species.

Standardised contact rates

SCRs for within-group contacts were calculated by dividing the total number of contacts or the total contact duration by the number of days for which the proximity logger was attached to yield the mean daily contact duration (C_{DUR}) and frequency (C_{FREQ}) for each individual badger (see below for details of analyses of between group contacts). The number of individuals collared in a group was also included as a covariate in all subsequent analyses (see below), so C_{FREQ} and C_{DUR} represent the average daily contact frequency and duration for a specific badger with any other collared individual within its social group on any one day. C_{FREQ} and C_{DUR} were calculated separately for each season (summer: June – August 2009; autumn: September – November 2009; winter: December 2009 – February 2010; spring: March – May 2010) and were found to be significantly positively correlated in all

cases (Pearson's product-moment correlation; all $r > 0.93$, all $P < 0.001$). Consequently, only statistical analyses for C_{DUR} are presented as the results are qualitatively similar for C_{FREQ}.

General linear mixed models (GLMMs) were used to investigate the relationship between C_{DUR} and characteristics of individual badgers (age, sex, body condition, bite wounds) and social groups (group size, territory size, demographic structure) across seasons. Badger identity and social group were included as random effects (badger nested within group) and the number of collared animals per group and the proportion of collared sub-adults/adults and males/females were included as covariates. The level of correlation in the residuals was inspected to assess whether there was any degree of time-dependence among successive study periods. No significant correlation was detected at any point, so the inclusion of autocorrelation structures was not deemed to be necessary (Crawley 2007). It was necessary, however, to normalise the data using Box-Cox transformations prior to analysis, but no models were found to display overdispersion. A minimum adequate model, including biologically meaningful interactions pertaining to season and demographic factors (beginning with a three-way interaction between season × age × sex) was constructed by retaining significant terms or deleting those that were not, starting with the least significant terms from a maximal model in stepwise manner on the basis of likelihood ratio tests (compared against a χ^2 distribution) where $\alpha = 0.05$ (Crawley 2007). Where a significant effect was found, differences between factor levels were assessed using post-hoc Tukey's tests implemented using the 'multcomp' package for mixed effects models (Hothorn, Bretz & Westfall 2008).

Unfortunately, SCRs could not be analysed for between-group contacts as they occurred comparatively infrequently and the distribution of the values (including a large number of zeros) could not be accommodated by conventional error structures. Instead, between-group contacts were analysed using the total number of foreign individuals encountered over the course of the study as a response variable in a GLMM with a Poisson error structure. For those animals that did have such encounters, a separate analysis was performed for the individual C_{DUR} measures. These responses were analysed in relation to demographic and social group factors as described above for within-group contacts. Finally, GLMMs were employed to test whether an individual's between-group measure of C_{DUR} could be predicted by its measure of within-group C_{DUR}. All analyses were carried out using R v. 2.11.1 (R Development Core Team 2010).

Social network analysis

Due to the 'completeness' and high temporal resolution of data collected by proximity loggers, association matrices for SNA were constructed directly from the raw values for contact duration and frequency (Hinde 1976). A custom-written R script (**Chapter 2**; **Appendices A & B**) was used to construct symmetrical matrices, with the largest value recorded by any one of an interacting pair of collars taken as the dyadic value. Association matrices constructed for contact duration and frequency were highly significant correlated in all seasons (Pearson's product-moment correlation; all $r > 0.93$, all $P < 0.001$), therefore results are only presented for SNA of contact duration data. Within- and between-group population-level networks were analysed separately and for each of the four seasons (see above for definitions).

Three network metrics were calculated for each individual: degree centrality, closeness centrality and flow-betweenness (for further details see: Hanneman & Riddle 2005). Degree centrality measures the number and strengths (durations/frequencies) of immediate ties that an individual has, whilst closeness centrality provides an overall measure of the geostadic distance of an individual to all others in the network. These two metrics were calculated using the R package 'tnet' (Opsahl 2009) which allows the relative importance given to tie number (i.e. number of individuals to which an individual is connected) and tie weight (i.e. the amount of time spent interacting with other individuals) to be varied using a tuning parameter (ranging from $\alpha = 0 - 1$; for a detailed discussion see Opsahl *et al.* 2010). For comparison, analyses were conducted using values of $\alpha = 0$ (solely tie number; as per Freeman's (1979) measure to a binary network), $\alpha = 1$ (solely tie weight), and $\alpha = 0.5$ (equal weighting to both). The third network metric, flow-betweenness, is a measure of the extent to which an individual lies on the shortest (geodesic) pathway between other pairs of individuals and was calculated using binary ties in UCINET (Borgatti, Everrett & Freeman 2002). Individuals at the edges of the study area may have had higher between-group network metrics as they were likely to be connected to un-surveyed social groups around the periphery. However, as this bias applied equally to all demographic groups it was assumed that it would not influence trends discussed in the Results.

As network parameters derived for individual members of the network are not independent, standard linear modelling approaches are generally not appropriate for the analysis of such

data (James, Croft & Krasue 2009; Croft *et al*. 2011). Instead, data were analysed using a series of randomisation tests that are robust to autocorrelation between data points in the network. The tests involve permuting the network (10,000 times) to generate a series of random data sets, against which the 'real' network can be compared using a range of standard statistical tests. This allows one to determine the probability that the observed contact patterns occurred due to chance alone or whether there was real and consistent variation in social behaviour linked to individual-level attributes. I began by testing whether animals within a group interacted assortatively (or disassortatively) with respect to age and sex in the different seasons. The probabilities of non-random associations between individuals based on these attributes were calculated using the UCINET function 'Join-Count' for the categorical factors. Next, I investigated whether the three network measures that were calculated (degree, closeness and flow-betweenness) varied according to an individual's attributes. The probabilities of differences in network measures between individuals differing in age and sex were calculated using a node-level t-test with 10,000 permutations. Relationships between network measures and continuous variables (BCI and bite wound score) and also relationships between an individual's within and between-group values of degree were tested using node-level OLS regression tests, again with 10,000 permutations. All analyses were carried out using UCINET (Borgatti, Everrett & Freeman 2002; for details on statistical tests used see Hanneman & Riddle 2005) and visual representations of the weighted association networks were produced using NetDraw (Borgatti 2002).

4.4 RESULTS

Of the 51 badgers fitted with proximity-logging devices, 45 retained fully-functioning collars that collected data across two or more seasons before the collars either fell off or were removed 12 months post-deployment (two seasons $n = 8$, three seasons $n = 7$, four seasons $n = 30$). Data from these individuals were used in analyses of contact rate patterns (summer $n = 44$, autumn $n = 45$, winter $n = 38$, spring $n = 33$). This sample contained similar numbers of both males ($n = 24$) and females ($n = 27$), and of adults ($n = 27$), and sub-adults ($n = 24$).

Standardised contact measures

Average daily within-group contact durations (C_{DUR}) varied significantly across seasons and among different sexes and age classes, with significant two-way interactions found between

age × sex, and age × season (Fig 4.1 and Table 4.2). For all sex and age classes, C_{DUR} was highest in summer and winter (Fig 4.1). However, sub-adult animals, particularly sub-adult males, had markedly higher C_{DUR} during these seasons when compared to adults (Fig 4.1). The differences between sub-adult and adult males were greater than that for sub-adult vs. adult females (i.e. age × sex interaction; Fig 4.1). C_{DUR} of adults was also considerably lower than sub-adults in spring, with very little within-group contact recorded for adult males or females during these months (i.e. age × season interaction; Fig 4.1).

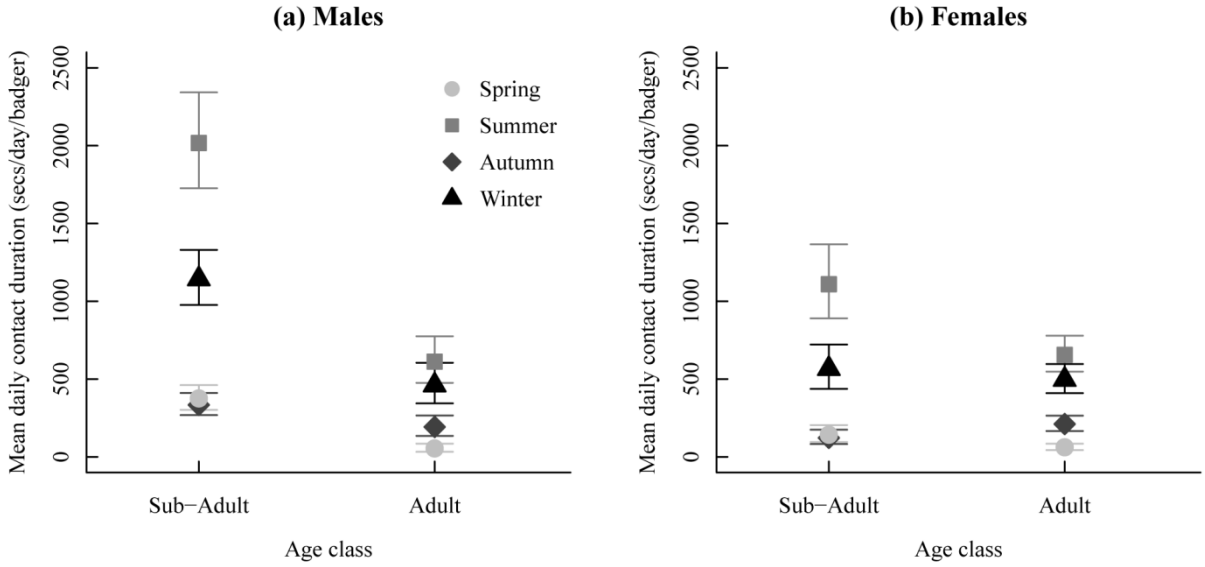


Figure 4.1 Average daily contact duration for (a) male and (b) female badgers with any other collared individual within the same social group in relation to season and age class (estimates ± 1SE from GLMM).

Within-group contacts accounted for > 90 % of an individual's total interactions in all seasons, both in terms of the frequency and duration of contacts (summer: 90.4 %; autumn 92.5 %; winter: 91.4 %; spring: 96.1 %; values comparable for frequency and duration). However, 91% of collared individuals recorded interactions with one or more animals from a different social group at some point during the study period, 97 % of which were made with animals from an adjoining social group. The number of individuals from another group that was encountered ranged from 0 – 13 (median = 1) and varied considerably among seasons, but was not affected by demographic parameters (Table 4.2) mirroring the effect shown in Fig 4.2 for contact duration. Individuals from groups containing a higher proportion of adults vs. cubs also tended to have more between-group contacts (Table 4.2). For those animals that

had between-group contacts, the duration of these contacts varied significantly among seasons, but again was not affected by demographic parameters (Table 4.2; Fig. 4.2). Between-group contact durations were longest in summer and winter, coinciding with peaks in reproductive hormones (Neal & Cheeseman 1996; Buesching, Heistermann & Macdonald 2009) and were shortest in autumn and spring (Fig. 4.2).

Table 4.2 Factors determining within- and between-group contact duration (C_{DUR}) and between-group contact numbers of Eurasian badgers. Main effects and significant interactions from GLMM models are presented. *P* values were obtained by step-wise deletion from the maximal model, starting with the least significant term.

	Within-group C_{DUR}			**Number of Between-Group Contacts**			**Between-group C_{DUR}**		
	χ^2	**d.f.**	***P***	χ^2	**d.f.**	***P***	χ^2	**d.f.**	***P***
Fixed Effects									
BCI	2.08	1	0.15	1.76	1	0.19	1.82	1	0.18
Bite wound score	0.63	1	0.43	9.52	1	0.09	8.92	5	0.06
Group size	0.05	1	0.94	0.04	1	0.85	0.14	1	0.71
Prop. adults	0.74	-	0.39	9.92	1	**0.002****	3.48	1	0.06
Prop. males	1.24	-	0.27	2.42	1	0.12	0.56	1	0.65
Season × Sex	3.07	3	0.38	7.79	3	0.09	6.81	3	0.08
Season × Age	7.97	3	**0.05***	5.35	3	0.10	1.72	3	0.63
Age × Sex	4.75	1	**0.03***	0.09	1	0.76	1.67	1	0.20
Age	N/A	N/A	N/A	0.05	1	0.82	1.09	1	0.30
Sex	N/A	N/A	N/A	0.07	1	0.79	0.12	1	0.85
Season	N/A	N/A	N/A	14.1	3	**< 0.003****	20.5	3	**< 0.001****
Random Effects									
Individual	4.23	1	**0.04***	7.00	1	**0.008****	23.6	1	**< 0.001****
Social Group	13.8	1	**< 0.001****	1.88	1	0.17	24.2	1	**< 0.001****

N/A – these values were significant in an interaction and hence not tested individually

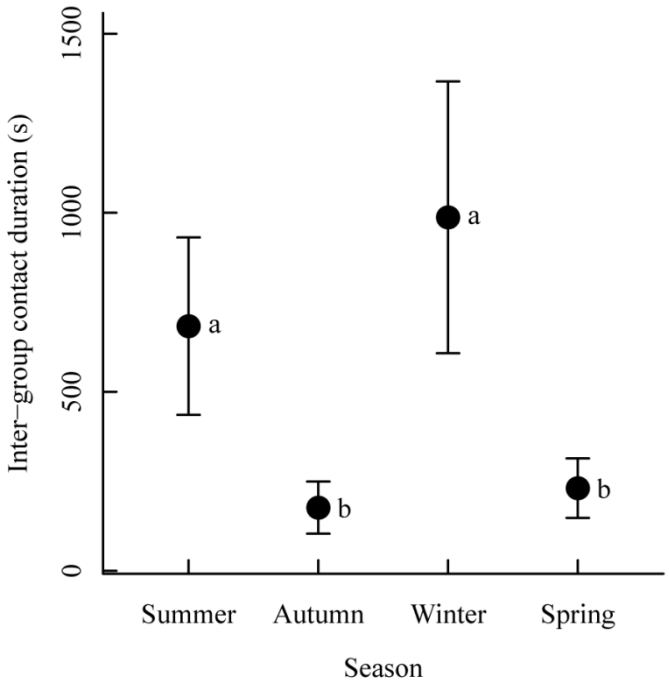


Figure 4.2 Average between-group contact durations (estimates ± 1SE from GLMM) of study badgers during different seasons: summer (minor mating peak), autumn (reproductive quiescence), winter (mating season) and spring (end of mating season). Unshared letters (a, b) denote significant differences between seasons in post-hoc Tukey's test ($P < 0.05$).

Even after controlling for demographic factors, differences among individuals and social groups continued to explain a significant proportion of the variance in C_{DUR} (i.e. significant random effects in GLMMs; Table 4.2), indicating consistent differences in social behaviour across seasons. Individual identity was marginally significant as a predictor of within-group C_{DUR}, but explained a highly significant proportion of variation in the number and duration of contacts with individuals from neighbouring social groups (Table 4.2). In contrast, differences among social groups accounted for a highly significant proportion of the variance in C_{DUR} for both within- and between-group contacts, but did not affect the number of individuals encountered from other groups (Table 4.2). No relationship was found between an individual's measure of within-group C_{DUR} and its measure of between-group C_{DUR} (χ^2_1 = 0.68, P = 0.41). Individual- and group-level differences in between-group contacts may be partly due to dispersal and social group fusion events which are described in more detail later.

Social Network Analysis

A diagrammatic representation of the population-level social network corroborates inferences made from SCRs: animals contact members of their own social group most often and where between-group contacts do occur they overwhelmingly involve interactions between individuals from neighbouring social groups (Fig 4.3a). Dividing the network into diurnal (06:00 – 19:59) and nocturnal contacts (20:00 - 5:59) shows that the diurnal network is generally limited to within-group contacts (but with some notable exceptions detailed in the Discussion; Fig. 4.3c), whereas the nocturnal network is consists of both within- and between-group contacts (Fig. 4.3b).

Within social groups badgers did not interact assortatively (or disassortatively) with respect to age or sex in any season (Table 4.3). However, there were significant differences in the within-group network parameters of different demographic groups. For example, sub-adults had significantly higher degree centrality scores than adults in all seasons except summer, indicating stronger direct connections to other group members (Table 4.4). This difference was due to sub-adults spending a greater amount of time interacting with others, rather than an increase in the number of within-group ties (evident from different α values in Table 4.4). Indeed, closeness scores (i.e. a measure of the extent to which the animals lie on the shortest path between two other group members) did not differ between age classes, with the exception of spring when the values for sub-adults were significantly higher. Few differences were found in the within-group contact patterns of male and female badgers. Whilst males generally had higher measures of degree (Table 4.4) and closeness centrality (Table 4.5) there were large amounts of variation in these, so that the values were only significantly different in spring in relation to the strength of the ties (i.e. the amount of time spent interacting). Variation in within-group flow-betweenness scores did not differ according to age or sex (Table 4.8).

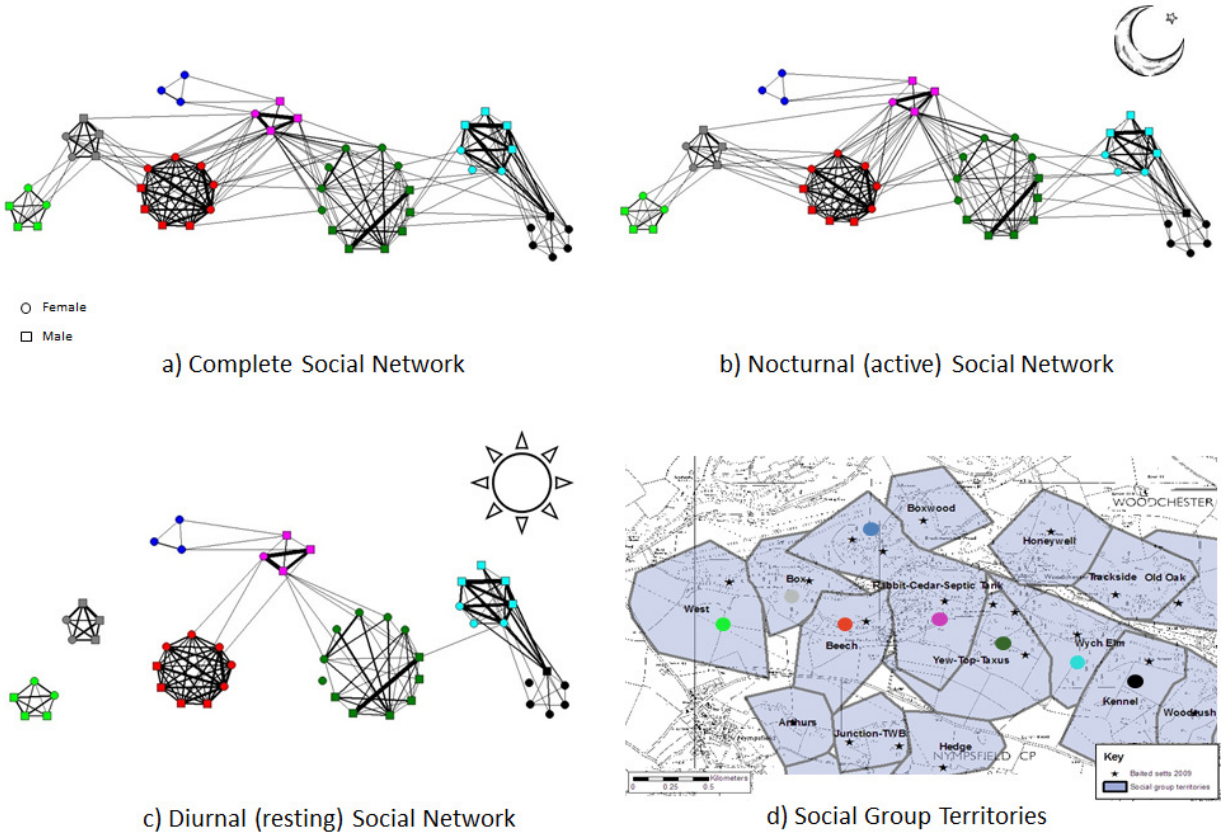


Figure 4.3 Contact patterns and social group territories of a wild badger population. (a) Social network diagram for the whole study period (June 2009 – June 2010), and then divided into (b) nocturnal (20:00 – 05:59) and (c) diurnal contacts (06:00 – 19:59). Nodes represent individuals and are coloured according to the social group to which they belong (distances between nodes have no meaning other than visual representation). The thickness of the edges is proportional to the association strengths as determined by the cumulative amount of time spent in close proximity. Nodes are arranged to correspond with the geographical layout of different social group territories, as shown in panel (d).

Table 4.3 Tests for assortative interactions within and between social groups in relation to demographic factors and across different seasons. For each possible association the probability is given that interactions were more frequent ($P >$) or less frequent ($P <$) than would be expected by chance. Significant values are indicated with asterisks ($P \leq 0.05$).

	Sex						**Age**					
	Male / Male		Male / Female		Female / Female		Sub-Adult / Sub-Adult		Adult / Sub-Adult		Adult / Adult	
Season	*P* >	*P* <	*P* >	*P* <	*P* >	*P* <	*P* >	*P* <	*P* >	*P* <	*P* >	*P* <
Within-group contacts												
Summer	0.25	0.80	0.26	0.83	0.96	0.07	0.56	0.55	0.54	0.55	0.51	0.57
Autumn	0.14	0.90	0.58	0.50	0.92	0.13	0.87	0.20	0.42	0.68	0.33	0.73
Winter	0.13	0.91	0.69	0.40	0.86	0.25	0.83	0.30	0.36	0.75	0.53	0.58
Spring	0.41	0.68	0.67	0.43	0.53	0.59	0.54	0.58	0.21	0.90	0.85	0.26
Between-group contacts												
Summer	0.08	0.94	0.75	0.37	0.91	0.15	0.34	0.74	0.70	0.43	0.61	0.45
Autumn	0.78	0.38	0.09	0.96	0.84	0.31	0.79	0.37	0.35	0.81	0.53	0.63
Winter	0.52	0.62	**0.04***	0.98	0.97	**0.04***	**0.03***	0.99	0.67	0.47	0.96	0.07
Spring	0.86	0.25	0.72	0.44	0.14	0.93	**0.02***	0.99	0.84	0.28	0.97	0.07

Table 4.4 Within-group degree centrality measures of male/ female and sub-adult/adult badgers calculated at three different levels: α = 0 (solely based on the number of ties), α = 0.5 (equal weighting given to tie number and tie strength) and α = 1 (solely on the basis of tie strengths). Values are means ± 1 SD. *P*-values for differences between categories of individuals were obtained using randomisation tests (see Materials and Methods for details).

		Sex			Age		
Season		Male	Female	*P*	Sub-Adult	Adult	*P*
Summer	α = 0	5.38 ± 2.21	4.48 ± 2.50	0.25	4.90 ± 2.45	4.92 ± 2.38	0.99
	α = 0.5	1186 ± 625	997 ± 767	0.38	1228 ± 783	980 ± 626	0.25
	α = 1	286443 ± 186989	246079 ± 248377	0.56	341298 ± 206460	215219 ± 176172	**0.05***
Autumn	α = 0	5.55 ± 2.17	4.44 ± 2.64	0.16	4.64 ± 2.57	5.30 ± 2.35	0.38
	α = 0.5	654 ± 337	593 ± 432	0.60	634 ± 415	611 ± 363	0.85
	α = 1	86537 ± 62081	85230 ± 71802	0.95	92285 ± 71564	79732 ± 62186	0.55
Winter	α = 0	4.28 ± 1.45	3.55 ± 1.77	0.21	3.77 ± 1.67	4.00 ± 1.66	0.70
	α = 0.5	904 ± 547	649 ± 472	0.14	879 ± 580	682 ± 456	0.26
	α = 1	229627 ± 216363	135775 ± 124293	0.12	240300 ± 223583	131603 ± 114276	**0.05***
Spring	α = 0	3.89 ± 1.84	3.75 ± 2.89	0.88	4.00 ± 2.09	3.65 ± 2.25	0.70
	α = 0.5	620 ± 395	364 ± 344	0.07	668 ± 428	334 ± 270	**0.01***
	α = 1	130690 ± 124691	52165 ± 75091	**0.04***	146603 ± 130615	41807 ± 49632	**0.004****

Table 4.5 Within-group closeness centrality measures of male/ female and sub-adult/adult badgers calculated at three different levels: α = 0 (solely based on the number of ties), α = 0.5 (equal weighting given to tie number and tie strength) and α = 1 (solely on the basis of tie strengths). Values are means ± 1 SD. *P*-values for differences between categories of individuals were obtained using randomisation tests (see Materials and Methods for details).

		Sex			Age		
Season		Male	Female	*P*	Sub-Adult	Adult	*P*
Summer	α = 0	5.60 ± 2.22	4.98 ± 2.53	0.41	5.21 ± 2.38	5.32 ± 2.43	0.90
	α = 0.5	5.74 ± 2.88	5.13 ± 3.71	0.55	6.09 ± 3.73	4.91 ± 2.92	0.26
	α = 1	6.07 ± 3.49	5.46 ± 4.74	0.64	6.94 ± 4.75	4.85 ± 3.49	0.10
Autumn	α = 0	5.55 ± 2.17	4.44 ± 2.64	0.16	5.24 ± 2.62	5.76 ± 2.53	0.51
	α = 0.5	5.54 ± 2.60	5.23 ± 3.52	0.75	5.57 ± 3.17	5.20 ± 3.03	0.70
	α = 1	5.48 ± 3.61	5.50 ± 4.29	0.98	5.92 ± 4.11	5.08 ± 3.79	0.49
Winter	α = 0	4.50 ± 1.40	3.95 ± 1.73	0.32	4.00 ± 1.62	4.38 ± 1.58	0.48
	α = 0.5	4.54 ± 2.57	3.57 ± 2.41	0.25	4.56 ± 2.80	3.60 ± 2.21	0.25
	α = 1	5.22 ± 4.78	3.15 ± 2.80	0.12	5.42 ± 4.93	3.08 ± 2.63	0.07
Spring	α = 0	4.00 ± 1.75	3.88 ± 2.42	0.88	4.06 ± 2.05	3.82 ± 2.15	0.80
	α = 0.5	4.89 ± 2.89	3.04 ± 2.81	0.08	5.22 ± 3.21	2.84 ± 2.23	**0.02***
	α = 1	5.77 ± 5.33	2.53 ± 3.35	**0.05***	6.40 ± 5.56	2.13 ± 2.49	**0.008****

In contrast to within-group contacts, for those animals that had encounters with individuals from a different social group(s), interactions were found to be assortative in some seasons. For example, interactions between males and females were more frequent (and female - female interactions less frequent) than would be expected by chance during the winter breeding season (Table 4.3). Between-group interactions were also assortative with respect to age in some seasons, with sub-adults interacting with other sub-adults significantly more than would be expected by chance in winter and spring, and a marginal but non-significant effect for adult – adult encounters during the same seasons (Table 4.3). There were no significant differences in the number or length of between group contacts of male and female badgers, as evidenced by the lack of significant differences in degree and closeness centrality scores. However, males did have significantly higher between-group flow-betweenness scores than females during summer and winter (Table 4.8). This suggests that whilst males and females appear to encounter similar numbers of individuals during these times, males may be encountering animals from further afield than the females, and so are responsible for linking together a greater proportion of the population. Age also affected some between-group network measures. For example, adult badgers interacted with a greater number of animals from other social groups than sub-adults in winter, and vice versa in spring (with greater contact durations also), with no significant differences in the other two seasons (see Table 4.6). Consequently, sub-adults had higher closeness centrality measures in spring than those of adults (Table 4.7).

Body condition had no discernible effects on contact patterns in any season (within-group interactions: all $P > 0.38$; between-group interactions: all $P > 0.31$) and there was no relationship between BCI and any of the network metrics calculated for within-group interactions ($R^2 = 0.10 – 0.29$, $F = 0.99 – 6.97$) or between-group interactions ($R^2 = 0.09 – 0.20$, $F = 0.50 – 2.68$) in any season. Similarly, network measures were not correlated with bite wound score in terms of either within-group ($R^2 = 0.05 – 0.31$, $F = 0.82 – 7.09$), or between-group interactions ($R^2 = 0.14 – 0.39$, $F = 1.05 – 9.09$). As with the measures of SCR, no significant correlations were found between an individual's value for within-group degree and its value for between-group degree either in terms of the number of different animals contacted ($R^2 = 0.05 – 0.12$, $F = 0.21 – 2.36$) or in terms of the length of interactions ($R^2 = 0.09 – 0.19$, $F = 0.95 – 4.41$).

Table 4.6 Between-group degree centrality measures of male/female and sub-adult/adult badgers calculated at three different levels: α = 0 (solely based on the number of ties), α = 0.5 (equal weighting given to tie number and tie strength) and α = 1 (solely on the basis of tie strengths). Values are means ± 1 SD. *P*-values for differences between categories of individuals were obtained using randomisation tests (see Materials and Methods for details).

		Sex			Age		
Season		Male	Female	*P*	Sub-Adult	Adult	*P*
Summer	α = 0	2.29 ± 1.22	1.22 ± 0.78	0.08	1.90 ± 2.94	1.60 ± 1.33	0.79
	α = 0.5	118 ± 270	69.8 ± 102	0.55	107 ± 274	82 ± 121	0.79
	α = 1	9510 ± 23709	7603 ± 14888	0.79	10914 ± 26153	6688 ± 12255	0.55
Autumn	α = 0	0.77 ± 1.35	0.83 ± 0.92	0.91	0.77 ± 1.38	0.83 ± 0.87	0.91
	α = 0.5	21.7 ± 47.4	18.1 ± 45.6	0.83	18.4 ± 48.4	21.3 ± 44.6	0.87
	α = 1	1268 ± 3760	1211 ± 5083	0.88	1243 ± 5197	1236 ± 3679	0.98
Winter	α = 0	1.94 ± 2.04	1.35 ± 1.42	0.33	1.10 ± 1.11	2.29 ± 2.16	**0.04***
	α = 0.5	70.9 ± 102	54.4 ± 91.8	0.63	48.3 ± 68.4	79.4 ± 122	0.35
	α = 1	3617 ± 6965	3231 ± 7688	0.87	2936 ± 6189	4005 ± 8546	0.66
Spring	α = 0	1.18 ± 1.50	1.88 ± 1.62	0.25	2.19 ± 1.78	0.88 ± 1.08	**0.02***
	α = 0.5	19.7 ± 27.8	25.4 ± 30.5	0.60	35.4 ± 35.0	10.3 ± 14.3	**0.01***
	α = 1	391 ± 581	409 ± 664	0.93	644 ± 757	170 ± 323	**0.02***

Table 4.7 Between-group closeness centrality measures of male/female and sub-adult/adult badgers calculated at three different levels: α = 0 (solely based on the number of ties), α = 0.5 (equal weighting given to tie number and tie strength) and α = 1 (solely on the basis of tie strengths). Values are means ± 1 SD. *P*-values for differences between categories of individuals were obtained using randomisation tests (see Materials and Methods for details).

		Sex			Age		
Season		Male	Female	*P*	Sub-Adult	Adult	*P*
Summer	α = 0	8.23 ± 5.62	6.65 ± 4.68	0.33	8.01 ± 5.34	6.95 ± 5.06	0.51
	α = 0.5	3.92 ± 4.61	4.66 ± 4.86	0.62	4.41 ± 5.22	4.23 ± 4.37	0.91
	α = 1	2.40 ± 5.18	3.06 ± 5.20	0.69	2.96 ± 5.95	2.58 ± 4.54	0.83
Autumn	α = 0	1.35 ± 2.17	1.87 ± 2.18	0.44	1.25 ± 2.20	1.97 ± 2.12	0.28
	α = 0.5	1.23 ± 2.44	1.20 ± 2.27	0.97	0.99 ± 2.35	1.44 ± 2.34	0.55
	α = 1	1.21 ± 3.56	0.86 ± 3.35	0.89	0.84 ± 3.43	1.21 ± 3.48	0.86
Winter	α = 0	7.17 ± 4.18	5.77 ± 4.48	0.33	7.86 ± 3.50	5.28 ± 4.70	0.07
	α = 0.5	6.01 ± 4.42	4.84 ± 4.55	0.44	6.15 ± 4.17	4.78 ± 4.71	0.36
	α = 1	3.59 ± 4.55	2.86 ± 4.41	0.64	3.00 ± 4.37	3.46 ± 4.62	0.77
Spring	α = 0	4.71 ± 3.71	6.06 ± 4.23	0.34	6.70 ± 3.59	4.10 ± 4.01	0.07
	α = 0.5	4.56 ± 3.93	5.01 ± 3.94	0.75	6.34 ± 3.89	3.13 ± 3.39	**0.03***
	α = 1	3.57 ± 3.63	3.63 ± 3.74	0.97	5.00 ± 3.87	2.28 ± 3.00	**0.04***

Table 4.8 Within and between-group flow-betweenness score for male/ female and sub-adult / adult badgers calculated using binary ties. Values are means ± 1 SD. Significance of differences between categories of individuals were assessed using randomisation tests (see Materials and Methods for details).

	Sex			Age		
Season	Male	Female	*P*	Sub-Adult	Adult	*P*
Within-group						
Summer	5.79 ± 3.78	3.78 ± 6.72	0.48	3.73 ± 7.03	5.50 ± 10.1	0.55
Autumn	6.35 ± 7.86	5.00 ± 5.27	0.51	6.16 ± 8.18	5.18 ± 4.82	0.63
Winter	6.19 ± 8.62	3.05 ± 5.11	0.19	4.98 ± 8.26	4.17 ± 6.12	0.75
Spring	4.17 ± 6.54	3.98 ± 5.31	0.93	4.63 ± 5.78	3.56 ± 6.11	0.63
Between-group						
Summer	79.7 ± 140	6.34 ± 15.3	**0.001****	49.8 ± 139	35.0 ± 66.0	0.74
Autumn	5.35 ± 17.3	2.79 ± 5.46	0.65	5.63 ± 17.3	2.53 ± 5.31	0.55
Winter	70.1 ± 135	15.2 ± 52.7	**0.04***	76.5 ± 132	24.9 ± 60.5	0.15
Spring	20.1 ± 49.1	43.3 ± 60.1	0.25	52.0 ± 63.9	11.9 ± 38.1	**0.03***

4.5 DISCUSSION

This study integrates high resolution contact data with social network analyses to explore the social organisation of a high-density population of Eurasian badgers in more detail than has previously been possible. The findings help to reinforce some established orthodoxies relating to the social organisation of badger populations but also provide new insights into the dynamics and demographic determinants of within and between-group contact rates over the annual cycle. The population-level social network clearly illustrates the strong and discrete group organisation of badgers that is typical throughout much of the UK (Fig 4.3a), where within-group contacts accounted for more than 90% of an individual's social interactions in all seasons. However, the entire population was ultimately connected via predominantly nocturnal encounters between members of neighbouring groups (Fig 4.3b). Indeed, over 97% of between-group

interactions involved pairs of individuals from adjoining social groups, indicating that badgers seldom encounter animals that are not from their own group or direct neighbours.

The extent of between-group contacts varied dramatically across the annual cycle and among different demographic groups and appeared to be predominantly driven by reproductive behaviours. Between-group contacts peaked in winter and summer, which coincides with known increases in male and female sex hormone levels (Neal & Cheeseman 1996; Buesching, Heistermann & Macdonald 2009), were disproportionately represented by sexually mature adults and had a higher probability of involving animals of opposite sexes. Genetic studies have confirmed inter-breeding between badgers from neighbouring social groups, which is thought to be an evolved mechanism for reducing inbreeding depression in highly related social groups (Carpenter *et al*. 2005; Dugdale *et al*. 2007; MacDonald et al. 2008). There is little evidence to suggest whether these inter-group matings are solicited by one sex e.g. males that have been shown to have larger home range sizes (Tuyttens *et al*. 2000), or equally by both sexes e.g. capture-mark-recapture data has shown an equal probability of capturing both males and females in foreign social groups (Rogers *et al*. 1998). Since proximity logger data is directionless (i.e. it does not reveal who contacted whom) it is hard to firmly establish which sex is driving between-group sexual contacts. Males and females did not differ significantly in terms of the number and durations of between-group contacts during the winter and summer mating seasons. However, males were found to have significantly higher flow-betweenness scores than females during these periods indicating that they were connected to more groups, and hence may be more influential in initiating sexual forays. This is consistent with findings from previous studies that the home range sizes of male badgers were larger (Tuyttens *et al.* 2000) and that in certain seasons males use outlier setts close to territorial margins more frequently than females (**Chapter 3**).

Reproductive behaviours also appeared to have a strong bearing on within-group contact patterns. During the spring when females give birth (Neal & Cheeseman 1996; Roper 2010), their contacts with other group members dropped to very low levels suggesting that they almost totally segregate themselves at this time. Indeed, all but one of the adult females were found to be lactating when captured the following summer, indicating that they had given birth earlier in the year. This reduction in social

interactions could be a by-product of mothers nursing their highly altricial young, but could also stem from a more active decision to guard their cubs against potential threats from other group members. Female-female competition is thought to be prevalent in badger social groups (e.g. Woodroffe & Macdonald 1995), with the more dominant individuals giving birth and aggression or stress-induced abortions and infanticide suppressing the reproduction of others (Woodroffe & Macdonald 1995; Fell, Buesching & Macdonald 2006; also documented in other group-living carnivores e.g. spotted hyenas (*Crocuta crocuta*); East *et al*. 2003, and also meerkats (*Suricata suricatta*); Young & Clutton-Brock 2006). Indeed, there is indirect evidence to suggest that younger female badgers may actively segregate themselves during the breeding season to avoid intra-sexual aggression from conspecifics (Cresswell *et al*. 1992; Rogers *et al*. 2003). In some group-living social animals females that have not given birth that year may increase their fitness by caring for the young of the others, to which they may be related (reviewed in Komdeur 2010). The prevailing view is that badgers do not exhibit the same level of reproductive cooperation (reviewed in Roper 2010); however, allo-parental care has been reported in at least one badger population in the UK (Woodroffe 1993). The results of my study appear to support the consensus view, with the low within-group contact rates of adult females suggesting that allo-parental behaviours are absent or very uncommon during the spring breeding season in the Woodchester Park study population. Indeed, I am not aware of any other evidence of allo-parental care in Eurasian badgers and even in the single population where it was claimed, helping yielded no detectable fitness benefits (Woodroffe & Macdonald 2000).

Within-group contacts were found to be at a maximum for both sexes and age classes in the summer, which may reflect longer periods of social interaction at the sett before leaving to forage. For example, badgers are known to exhibit allo-grooming behaviour, with group members helping to remove ectoparasites from one another, in particular, from inaccessible areas (Stewart 1997; Macdonald *et al*. 2000). Ectoparasite levels have been shown to be highest in the summer, with grooming bouts lasting for up to 40 minutes post-emergence at this time (Macdonald *et al*. 2000). The costs of parasitic infections associated with group-living are believed to be comparatively high in badgers, and cooperative allo-grooming to minimise ectoparasite infestation is considered an important factor in their sociality (Johnson, Stopka & Macdonald 2004). At the start of summer, cubs also begin to leave the sett more frequently and at the same time as the adults (Neal & Cheeseman 1996; Fell, Buesching & Macdonald 2006), so

the higher level of within-group social interaction at this time may facilitate social integration of the cubs into the group (e.g. Buesching, Stopka & Macdonald 2003). Interestingly, whilst adults and sub-adults encountered the same number of individuals, the younger animals that were collared were found to interact for significantly longer with other group members particularly during the summer (Fig 4.1 and Table 4.4), which could be related to these animals completing the process of full integration into the social group (see also Fell, Buesching & Macdonald 2006; Böhm *et al*. 2008). More frequent social interactions of younger juvenile animals (or "playing") have been documented in a number of group living species and may help to cement social ties important in later life (Graham & Burghardt 2010; Thornton & Clutton-Brock 2011). Indeed, with badgers the members of a social group often have a favourite place for "playing", which can be easily identified by its lack of vegetation (Roper 2010). Interestingly, social groups remained highly connected both at night and during the day (Fig 4.3a, b). Badgers share communal diurnal resting setts so contacts during day might be expected (e.g. Roper *et al*. 2001). However, nocturnal foraging is thought to be largely solitary (e.g. Neal & Cheeseman 1996). The results suggest that interactions remain common throughout the night but because of the lack of a spatial component to proximity logger data it is not possible to determine whether these interactions occurred back at the sett or outside whilst foraging. This could be investigated further in future studies by using stationary proximity loggers to 'gate' sett entrances in order to examine where these contacts took place.

Unlike within-group contacts which occurred both during the day and at night, between-group interactions were almost exclusively nocturnal when badgers are active above ground (Fig 4.3b). However, there were two notable exceptions involving prolonged between-group contacts which merit further consideration. These examples, most likely driven by separate factors, suggest that while stable social groups comprise a majority of the social interactions among individuals, the social structure of badger populations is also dynamic to some extent. In the first example, there was evidence of very strong diurnal ties between multiple individuals from two adjacent social groups (Wych Elm and Kennel; cyan and black symbols in Fig 4.3), suggesting that these groups may potentially be functioning as a 'super group'. There is little mention of the fusion of badger social groups in the literature but there is evidence from baitmarking and capture-mark-recapture studies on the Woodchester Park population to suspect that it occurs at a low level (R. Delahay, *unpublished data*; **Chapter 6**). A 'super group' has

been loosely defined as an association of badger groups that may use different setts for breeding but have overlapping ranges and frequently spend the day in one another's setts (Evans *et al*. 1989). In the case of the two 'fusing' groups in this study the driving force could be related to the skewed sex ratios of the groups (Kennel comprised of 70% adult females, and Wych Elm 70% adult males) bringing benefits in terms of mating opportunities through closer association. Indeed, using capture-mark-recapture data, Vicente *et al*. (2007) also found evidence to suggest that the relationship between inter-group movements was stronger when the proportion of males in the group was higher. Moreover, the territories of the two groups encompass farmland, and individuals from these groups have been observed feeding on crops and animal supplemental feed, suggesting that constraints on food resources are unlikely to limit closer ties if they bring benefits. This example is not analogous to the fission-fusion societies documented in e.g. spotted hyenas (Holekamp *et al*. 1997) for instance, but it does bear some resemblance in that the dynamic joining and splitting of these groups may be driven by within-group competition and the availability of resources. In the second example there was evidence of prolonged between-group diurnal contacts that may be related to prospecting prior to dispersal. Over the course of this study two collared animals appeared to initiate dispersal from their natal group (Septic Tank; pink symbols Fig 4.3) to a neighbouring group (Top/Yew; dark green symbols Fig 4.3), maintaining frequent interactions (including diurnal contacts presumably related to sett sharing) with members of both social groups. Permanent dispersal may be a lengthy process during which dispersing animals use both their old and new territories and setts, potentially to keep open the option of returning to their original social group (Christian 1994; Roper, Ostler & Conradt 2003). The two dispersing animals, both sub-adults males, were moving from a smaller group where only one female was caught that year, to a larger group containing at least eight adult females, perhaps to increase reproductive success (Rogers *et al*. 1998; Macdonald *et al*. 2008). Using capture-mark-recapture from 2010 and 2011 it appears that one of the individuals did in fact disperse, whilst the other appears to have remained in its natal group.

The unusual behaviour discussed above might help to explain why individual and social group identity explained a significant proportion of the variance in between-group contact rates, even after controlling for the effects of sex, age and group composition. These strong individual- and group-level effects, that were independent of demographic factors, indicate that social behaviour was highly repeatable for individuals and social

groups across seasons. While we can only speculate about the strong individual-level differences in contact behaviour of badgers, several studies in social species have referred to the existence of sociable/un-sociable and/or bold/shy personality types (introduced in **Chapter 1**). Longer term studies are needed to examine whether the behaviours in badgers are consistent across years. Individually consistent social behaviour may play a significant role in how animal populations are structured over and above the more obvious effects of sex and age and may have important implications for disease transmission within networks (see **Chapter 5**).

This study demonstrates how recently-developed proximity logging devices can be used to provide new insights into the social lives of species that were previously difficult to study due to their nocturnal and secretive behaviour. The results show that while social groups form the major focus of social interactions in badger populations, there are also significant numbers of interactions between neighbouring social groups that connect the entire population. The degree of between-group (and overall population) connectivity may have previously been underestimated due to the logistical difficulties of recording such behaviour and may have important implications for disease transmission through the network (**Chapter 5**). Such contacts may often be transient interactions motivated by mating opportunities, perhaps instigated by males, but may also involve dispersal or fusion events that lead to permanent alterations of group structure. Why badgers form social groups is currently unclear, but the results support the view that there is little cooperation during cub rearing. In the absence of truly cooperative behaviour it seems likely that an interplay of factors relating to the availability and defence of resources, including food (da Silva *et al*. 1993), mates (Roper, Shepherdson & Davies 1986), suitable habitat and the economics of constructing and sharing denning sites (Roper 1993), may play an important role in driving social cohesion. A better understanding of contact patterns within and among social groups may allow the testing of alternative hypotheses for why group living has evolved in only certain parts of the badger's geographic range, in addition to providing an empirical basis for predictive models of disease transmission and population dynamics.

Chapter 5. Contact patterns of the Eurasian badger (*Meles meles*) vary according to bovine tuberculosis disease status

5.1 ABSTRACT

1. Whilst the transmission of infectious diseases is strongly influenced by rates of contact, host behaviours may play an important role. Rates of transmission may be influenced by the social and spatial organisation of the host, reflecting demographic factors and individual-level heterogeneities in behaviour.

2. In the UK and Ireland the group-living Eurasian badger is a wildlife host for bovine tuberculosis (bTB) and is implicated in its transmission to cattle. However, the effects of badger group structure on disease transmission are not well understood. Proximity loggers were deployed on 51 badgers from eight social groups to record within- and between-group contact rates. Social network analysis was employed to quantify the role that individuals play in the transmission of bTB within a badger population based on their rates of social interaction and network positions.

4. Within a social group, individuals that tested positive for bTB were found to associate with test-negative members less frequently than would be expected by chance. These animals had lower centrality measures than their counterparts, interacting with fewer group members for less time. These measures correlated with sett use patterns where those animals with lower centrality measures spent more time at outlying setts. These findings could be related to wider roaming behaviours of the individuals that increase their exposure to sources of infection, or they could be driven by avoidance/exclusion tactics from other group members.

5. Whilst animals had similar rates of between-group contact, bTB test-positive animals had higher flow-betweenness scores and thus, may be more influential as an intermediary for contact between groups. Between-group network measures correlated with sett use where those animals with longer between-group contacts spent more time at outlying setts. Whilst intrinsic behaviours of an individual may influence their risk of contracting disease, the infection could also affect the behaviour of individual animals.

6. Quantifying the network positions of social animals can identify 'high-risk' functional groups and/or individuals for bTB transmission and lead to more accurate predictions of disease dynamics. With the badger there appear to be animals displaying different behaviours that may be more influential in disease transmission, but at the same time the relatively stable social organisation may act to limit its spread. These findings have important implications for culling practices that have been shown to disrupt this social structure and may increase transmission rates between badgers at unpredictable levels.

5.2 INTRODUCTION

Animals that live in social groups and interact through complex patterns of associations that often bring them into close contact with one another may be especially vulnerable to parasites and infectious diseases (Altizer *et al*. 2003). The dynamic social structure of groups of animals has a strong influence on who contacts whom at different times of the year and hence on the transmission and maintenance of diseases in the population (Perkins *et al*. 2008; Drewe 2009). Indeed, whilst conventional epidemiological theory treated hosts as randomly (and often homogenously) interacting entities (Anderson & May 1991) more recent network theory recognises that infectious agents are likely to be spread via structured, but often dynamic, interaction networks in social animals (Newman *et al*. 2006). An individual's risk of infection may change according to its position in the social network, which may also vary over time. Knowledge of host social structure and individual interaction patterns is therefore important for making informed and effective management strategies. From an ecological perspective, studying interaction patterns may allow us to address fundamental questions relating to how a disease is spread and how this is influenced by demographic factors and/or behaviours intrinsic to the individual (**Chapters 3 & 4**). For example, for many diseases there may be individuals that are disproportionately more important to disease transmission ('superspreaders'; Lloyd-Smith *et al*. 2005). This may be due to their number of contacts or because they play a pivotal role in connecting other individuals, groups, populations or species (Krause, James & Croft 2010). By studying individual behaviour over time it is also possible to examine whether certain behaviours, such as increased roaming, influence the likelihood of contracting disease (e.g. Clay *et al*. 2009) and/or, once infected, whether the host's behaviour is modified to increase the probability of the pathogenic agent being transmitted to others (e.g. Klein 2003); or alternatively, whether infected animals are marginalised within a group (e.g. Cheeseman & Mallinson 1981). Such information can give us insights into how host behaviours and social structure can potentially evolve to self-regulate disease spread within populations (Altzier *et al*. 2003).

The Eurasian badger (*Meles meles*) is a social, group-living mammal throughout most of its geographic range and is a known wildlife reservoir for *Mycobacterium bovis* (the causative agent of bovine tuberculosis: bTB) in parts of the UK and Ireland (e.g. Bourne *et al*. 2007). Infection with *M. bovis* can occur via inhalation of aerosolised bacilli,

ingestion of contaminated materials or via bite wounding (e.g. Jenkins *et al*. 2008). The high incidence of pulmonary infection found during *post mortem* examinations suggests that infectious aerosols may be the primary route. If this is indeed the case then direct contacts between individuals are likely to have a strong influence on disease transmission. TB infection in badgers has gained prominence in recent years due to more robust evidence of transmission to domestic cattle, and the large and rising economic costs to both the farming industry and the taxpayer (Defra 2010). Whilst there is increasing pressure to combat the disease, identifying an effective method to control it in cattle is proving difficult. Recently, the Independent Scientific Group (Bourne *et al*. 2007) led a large-scale field experiment to investigate the effects of badger culling on bTB in cattle. A key finding arising from this trial was that culling sometimes altered badger behaviour in such a way that it became counterproductive for disease controls in both badger and cattle populations (Donnelly *et al*. 2005). Also, epidemiological studies have failed to find a relationship between badger population density and bTB prevalence (e.g. Vicente *et al*. 2007; Woodroffe *et al*. 2009). Such findings demonstrate that infection rates are not simply a function of population density and highlight the importance of studying host ecology and social behaviour in order to better understand disease dynamics and develop effective control strategies (for review see, Cross *et al*. 2009).

Although group-living animals may frequently contact other group members, the division of individuals into discrete social units may mitigate against the rapid transmission of an infectious disease through the population as a whole (Altizer *et al*. 2003; Wilson *et al*. 2003). In such cases, the connectedness of the population may be determined by a relatively small number of individuals that mediate contacts between groups e.g. through dispersal, mating or shared use of resources (Cross *et al*. 2005; Craft *et al*. 2011). Thus, whilst the majority of individuals belonging to different groups may be considered spatially separated from each other and may never actually meet, the entire population may in fact be connected indirectly through a few pivotal animals that can also exert a disproportionate influence over the transmission of parasites/disease through the network (Lloyd-Smith *et al*. 2005; Fenner, Godfrey & Bull 2011). In the case of the badger this may explain the lack of simple density-dependent disease prevalence. In high-density badger populations in the UK, individuals live in social groups of around six-to-eight animals that actively defend a territory (Johnson *et al*. 2000). Within these populations disease incidence is often spatially clustered and is not

necessarily present in all groups (Delahay *et al*. 2000a). Even within groups containing infected individuals only a proportion of members carry the disease, suggesting the presence of either within-group differences in contact behaviours or differences in individual susceptibility. However, owing to their secretive and nocturnal habits, very little is currently known about contact patterns within and among badger social groups and how this relates to disease transmission through the population. Previous studies have demonstrated a link between movement patterns and disease status, with more frequent between-group movements generally increasing the likelihood of *M. bovis* infection (Rogers et al. 1998; Vicente et al. 2007). Similarly, badgers infected with bTB have been shown to range more widely (Garnett, Delahay & Roper 2005), use outlier setts more often (**Chapter 3**) and venture more frequently into neighbouring territories (Cheeseman & Mallinson 1981). Although it was not possible to infer causation from these studies, it has been suggested that wider-ranging individuals may have a greater probability of encountering other individuals and sources of infection and thus of contracting disease.

This study attempts to bridge these important gaps by using recently-developed proximity logging devices to directly determine the within- and between-group contact patterns of a bTB-infected badger population. These devices operate remotely and continuously record interactions between tagged animals, thus overcoming longstanding problems associated with studying social interactions in this elusive species. Using social network analyses I investigate how numbers of within- and between-group connections, their relative strengths and an individual's position within the population network compare for animals testing positive or negative for bTB across seasons. These data allowed me to directly test whether individual differences in social behaviour correlate with bTB status in badgers in a population, and may shed light on the apparent lack of simple density-dependent disease prevalence. By providing the most extensive direct measurements of contact rates in an infected badger population to date, this study aims to provide an empirical basis for epidemiological models and 'science-led' management strategies aimed at the control of bTB in badgers and cattle.

5.3 METHODS

5.3.1 Data Collection

Study site and population

Fieldwork was conducted over a 12-month period (June 2009 – May 2010) on a well-studied, high-density badger population at Woodchester Park, Gloucestershire, UK (51°71´N, 2°30´E). The study area comprised approximately 7 km^2 of fragmented deciduous and coniferous woodland, agricultural grassland and smaller areas of arable and scrub land (see Delahay *et al*. 2006b). At the time of this investigation 20 social groups were present at the study site, with a mean group size of 10. This badger population had been the subject of long-term ecological and epidemiological research; consequently their territorial organisation, the group membership of individuals, and the methods employed for their capture were well established and described (see Delahay *et al.* 2006b).

Contact rates

Badgers were trapped in May and June 2009, anaesthetised and sampled (as detailed below). Proximity logging devices (Sirtrack Tracking Solutions, Havelock North, New Zealand) mounted on leather collars were deployed on 51 badgers belonging to eight social groups. Animals were collared in such a way as to provide a representative sample of demographic classes in each social group wherever possible (see Table 5.1). The proximity loggers operate by transmitting a unique Ultra-High Frequency (UHF) code whilst simultaneously 'listening' for the codes of other loggers (for details: Prange *et al*. 2006; **Chapter 2**). Loggers were individually set to begin recording a contact when two or more animals came within 0.64 ± 0.04m of each other (UHF settings range 34 to 48; see **Chapter 2**), and to log it to memory after the animals had been out of this range for 30 seconds or more. This short-range detection distance was chosen to record direct contacts between collared badgers such as bite-wounding and grooming, as well as to be within the likely aerosol transmission distance for *M. bovis* (e.g. Sauter & Morris 1995).

Table 5.1 The numbers of collars deployed (black) and the total number of individuals present (*italics*) of different demographic classes across social groups in the Woodchester Park high-density badger population during the study period 2009/10.

Social	**Adults**			**Sub-Adults**			**Total**
Group	Male	Female	Total	Male	Female	Total	
Beech	1	2	3	3	3	6	**9**
	3	*3*	*6*	*3*	*3*	*6*	***12***
Cedar	0	3	3	0	0	0	**3**
	0	*3*	*3*	*0*	*0*	*0*	***3***
Kennel	1	1	2	0	4	4	**6**
	3	*1*	*4*	*1*	*4*	*5*	***9***
Larch	1	2	3	2	0	2	**5**
	1	*6*	*7*	*2*	*1*	*3*	***10***
Septic Tank	1	0	1	2	1	3	**4**
	1	*0*	*1*	*2*	*1*	*3*	***4***
Top/Yew	4	4	8	2	2	4	**12**
	5	*5*	*10*	*2*	*2*	*4*	***14***
Wych Elm	1	2	3	3	1	4	**7**
	1	*2*	*3*	*3*	*1*	*4*	***7***
West	1	2	3	2	0	2	**5**
	1	*2*	*3*	*2*	*0*	*2*	***5***

When two or more collared animals were within range, each proximity logger recorded the ID of the individual that it encountered, the start time of the interaction (in GMT) and the contact duration in seconds. Whenever collared badgers were recaptured, data were downloaded to a laptop computer using software supplied by the manufacturers (Sirtrack Tracking Solutions). Prior to analysis all contacts recorded during trapping periods were removed. In addition, the first 12 hours of data recorded after the end of each trapping event (the release of the animal) were removed as the period was treated as a re-acclimatisation time after which the animals were likely to be exhibiting natural behaviours again. Prior to analysis, these data were adjusted by amalgamating contacts that were recorded within 1.5 minutes of each other if they involved the same pair of loggers and then removing any remaining interactions that lasted for 1 second to construct a more realistic representation of what had most likely occurred in the field (Prange *et al*. 2006; **Chapter 2**). Proximity loggers yield two types of information: the number of discrete contacts between pairs of individuals ('contact frequency') and the total duration of interactions between them ('contact duration').

Disease Status

I had the results from three diagnostic tests available to use in the study: 1) BrockTB Stat-Pak lateral flow serum antibody test (Chambers *et al.* 2008; Chambers *et al.* 2009); 2) an enzyme immunoassay for interferon-gamma (IFNγ) (Dalley *et al.* 2008; Chambers *et al*. 2009); and 3) culture testing. I did not include the results from the culture test as only one animal was found to be actively excreting *Mycobacterium bovis* (the causative agent of bTB) at the time of the study. Culture is a much less sensitive predictor of infection than either the StatPak or IFNγ, both of which have been used to indicate infection in free-living badgers (see Chambers *et al.*, 2011). Moreover, the Stat-Pak detects the presence of *M. bovis* antigens, the production of which has been shown to positively correlate with the extent and severity of TB infection in both naturally and experimentally infected badgers (reviewed in Chambers, 2009). I chose to use the results from the Stat-Pak immunoassay alone as this test is the best available indicator of established infection in badgers and the sensitivity of the test increases considerably with disease severity (Chambers *et al.* 2008).

The disease status of each badger was inferred from the positive or negative outcome of a badger-specific lateral flow immunoassay carried out at any time prior to and during the study period (BrockTB Stat-Pak; Chembio Diagnostic Systems, New York, USA) (Kampfer *et al*. 2003; Chambers *et al.* 2009). A positive Stat-Pak result was interpreted as evidence of current infection with *M. bovis* as antibody production is positively correlated with the extent and severity of bTB infection in badgers (Chambers et al. 2009). As the Stat-Pak assay used to assess bTB status has a sensitivity of approximately 58%, increasing to >80% with disease progression, with 94% specificity (Dalley *et al*. 2008; Chambers *et al*. 2009), there is potential for the frequency of testing to influence the determination of disease status, with animals that had been captured more frequently having a greater chance of being considered positive. Therefore, the number of capture events for test-positive and test-negative animals were compared to ensure that both were sampled with comparable intensity. Whilst the possibility exists for false negative results due to the sensitivity of the test, there is an equal probability of getting these results for animals with many contacts, as for those with few, thus it seems unlikely that they will bias any trends that may be detected.

5.3.2 Network measures and analytical methods

Previous studies have used standardised contact measures to study interaction patterns; calculated by dividing the total number of contacts and/or total contact duration by the number of days over which data were collected to give daily estimates, and then dividing these resultant values by the number of individuals available for contact at any one time (see Böhm, Hutchings & White 2009; Marsh *et al*. 2011). However, it has been suggested that standardised contact measures can potentially disguise patterns by assuming that all animals within a group contact each other and that interactions occur on a daily basis (Goodman 2007; **Chapters 4 & 6**). Here social network analysis was employed to explore relations among individuals. Due to the 'completeness' and high temporal resolution of the data collected by the proximity loggers in each season, association matrices could be constructed directly from the raw data values for contact duration and frequency (Hinde 1976). A custom-written R script (**Appendices A & B**) was used to construct symmetrical matrices, with the largest value recorded by any one of an interacting pair of collars taken as the dyadic value. Association matrices constructed from the duration and frequency data were highly significantly correlated in all seasons (Pearson's product-moment correlation; all $r > 0.93$, all $P < 0.001$). Thus, results are only presented for the networks constructed and analysed based on the values of contact duration as all patterns were the same for contact frequency. Within-group and between-group population-level networks were analysed separately and in each of the four seasons (summer: June – August 2009; autumn: September – November 2009; winter: December 2009 – February 2010; spring: March – May 2010).

Three network metrics were calculated for each individual: degree centrality, closeness centrality and flow-betweenness (for further details see: Hanneman & Riddle 2005). Degree centrality measures the number and strengths (durations/frequencies) of immediate ties that an individual has, whilst closeness centrality provides an overall measure of the geostadic distance of an individual to all others in the network. These two metrics were calculated using the R package 'tnet' (Opsahl 2009) which allows the relative importance given to tie number (i.e. number of individuals to which an individual is connected) and tie weight (i.e. the amount of time spent interacting with other individuals) to be varied using a tuning parameter (ranging from $\alpha = 0 – 1$; for a detailed discussion see Opsahl *et al.* 2010). For comparison, analyses were conducted using values of $\alpha = 0$ (solely tie number; as per Freeman's (1979) measure to a binary

network), $\alpha = 1$ (solely tie weight), and $\alpha = 0.5$ (equal weighting to both). The third network metric, flow-betweenness, is a measure of the extent to which an individual lies on the shortest (geodesic) pathway between other pairs of individuals and was calculated using binary ties in UCINET (Borgatti, Everrett & Freeman 2002). Animals with high flow-betweenness scores are expected to be important to disease transmission (Klovdahl *et al*. 2001), acting as an intermediary for contact between others. All network metrics were calculated at the population-level and separately for the within- and between-group contact networks. I am aware that individuals at the edges of the study area may potentially have higher between-group network metrics than recorded due to interactions with un-surveyed animals around the periphery. However, as this bias would be expected to apply equally to the different categories of individual it should not influence trends.

As network parameters derived for individual members are not independent of each other, standard linear modelling approaches cannot be used to analyse these data (James, Croft & Krasue 2009; Croft *et al*. 2011). Instead, data were analysed using randomisation tests that account for autocorrelation between data points in the network (Hanneman & Riddle 2005). The tests involve permuting the network (10,000 times) to generate a series of random data sets against which the 'real' network can be compared using a range of standard statistical tests. This allows me to determine the probability that observed contact patterns occurred due to chance alone or whether there was real and consistent variation in the behaviour of individuals linked to their disease status. However, using this approach there is not yet a proven method of considering multiple factors or interactions between factors (e.g. sex × bTB test status) equivalent to a general linear model. I began by testing whether animals interacted assortatively with respect to their bTB test statuses, both within and between groups and in different seasons. This analysis can tell us whether infected animals were connected to a greater number of other infected animals than would be expected by chance, or vice versa. The probabilities of non-random associations between individuals based on their infection status (positive or negative) were calculated using the UCINET function 'Join-Count' for categorical factors with 10,000 permutations. Next, I tested whether the network measures that were calculated for each individual (i.e. degree, closeness and flow-betweenness) varied according to an individual's disease test status. The probabilities of differences in network measures between positive and negative individuals were calculated using a node-level *T*-test with 10,000 permutations. All analyses were carried

out using UCINET (Borgatti, Everrett & Freeman 2002; for details on statistical tests used see Hanneman & Riddle 2005) and visual representations of the weighted association networks were produced using NetDraw (Borgatti 2002).

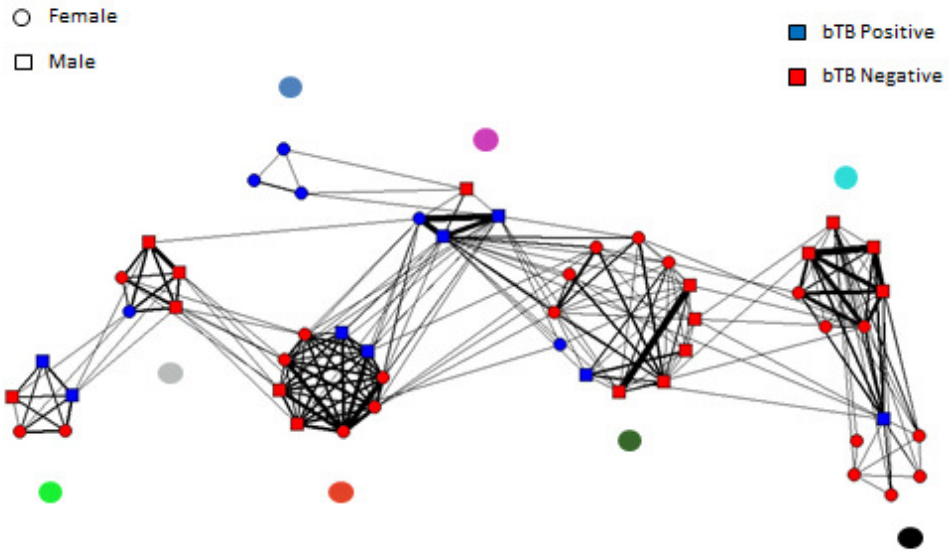
In an earlier study it was found that bTB test status correlated with sett use patterns, where those animals that spent a greater proportion of their time at outlier (as opposed to main) setts were more likely to test-positive for disease (**Chapter 3**). To link those data with this study, generalised linear mixed effects models (with individual nested within social group as random error terms) were used to test whether an individual's degree centrality network measure could be predicted by the proportion of time spent at outlier setts. These analyses were carried out using R v. 2.11.1 (R Development Core Team 2010).

5.4 RESULTS

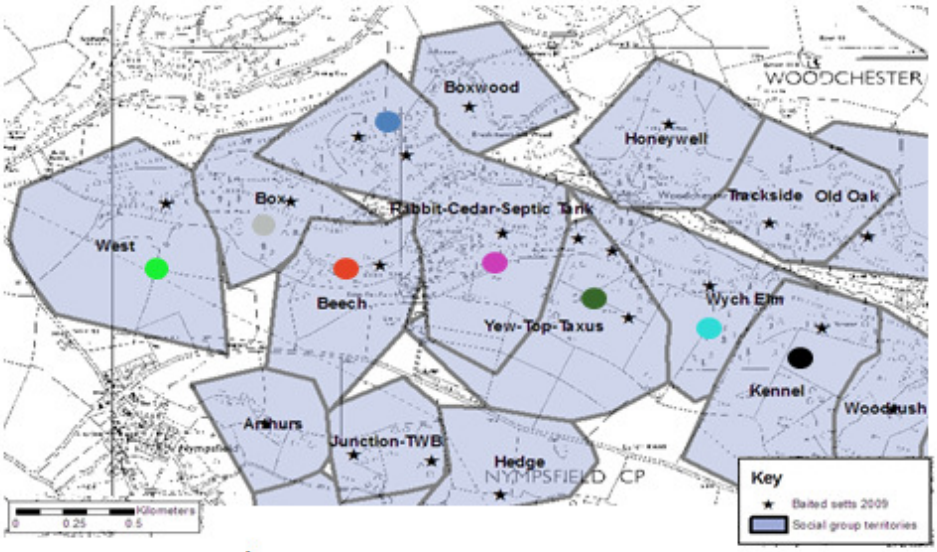
Of the 51 badgers fitted with proximity-logging devices, 45 retained fully-functioning collars that collected data across two or more seasons before the collars either fell off or were removed 12 months post-deployment (two seasons $n = 8$, three seasons $n = 7$, four seasons $n = 30$). Data from these animals were used in analyses of contact rate patterns (summer $n = 44$, autumn $n = 45$, winter $n = 38$, spring $n = 33$). This sample contained similar numbers of males ($n = 24$) to females ($n = 27$), and of adults ($n = 27$) to sub-adults ($n = 24$). Of the 45 animals, 14 tested positive for bTB (summer: $n = 12$, autumn: $n = 13$, winter: $n = 10$, spring: $n = 9$). Numbers of animals testing positive for bTB did not differ significantly among sexes (male, $n = 8$; female, $n = 6$; Pearson's χ^2 test: $\chi^2_1 = 0.33$, $P = 0.57$) or age classes (adult, $n = 6$, sub-adult, $n = 8$; Pearson's χ^2 test: $\chi^2_1 = 0.33$, $P = 0.57$). Similarly, test-positive and test-negative badgers did not differ significantly in terms of capture frequency (mean captures ± SE, positive: 7.90 ± 3.31; negative: 7.10 ± 3.10; Student's t-test, t = 0.67, d.f. = 15, $P = 0.51$), body condition (Student's t-test: t =1.03, df = 28.4, $P = 0.31$) or bite wound score (Wilcoxon rank-sum test: W =, $P = 0.49$).

A visual representation of the contact network over the entire study period is provided in Fig. 5.1. On the whole, it appears that animals contacted members of their own social group most often and where between-group contacts did occur they appeared to be primarily between neighbouring social groups (see **Chapter 4** for more information on

the demographic structure of the network). Subsequent analyses are divided into within- and between-group interactions to reflect these two different levels of contacts.



a) Social Network with Disease Status



b) Social Group Territories

Figure 5.1 Contact patterns and social group territories of a wild badger population. (a) Social network diagram for the whole study period (June 2009 – June 2010). Nodes represent individuals and are coloured according to bTB test status (proximity of nodes to each other are purely illustrative and have no spatial relevance). The thickness of the edges is proportional to the association strengths as determined by the cumulative amount of time badgers spent in close proximity to each other. Nodes are arranged to correspond with the geographical layout of different social group territories, as shown in panel (b).

Within-group contacts

Within social groups, bTB test-negative animals interacted significantly less with test-positive individuals and significantly more with other test-negative individuals than would be expected by chance in all seasons apart from summer (pair-wise association tests; Table 5.2). Less frequent within-group contacts for test-positive animals were also supported by network metrics. In all seasons, within-group degree centrality measures of test-positive animals were significantly lower than their test-negative counterparts both in terms of the number of individuals encountered and the amount of time spent in contact with other animals (Table 5.3). However, due to high levels of variation among individuals in some seasons, these differences were only found to be significant at the $P < 0.05$ level in autumn and winter. The same pattern was observed for closeness centrality measures (Table 5.4). Test-positive animals occurred on the shortest path between two individuals less frequently than those that tested negative for bTB in all seasons, with significant differences detected in autumn and winter (Table 5.4). Perhaps not surprisingly, therefore, within-group flow-betweenness scores were also generally lower for bTB test-positive badgers, although this difference was only significant in autumn (Table 5.5).

Between-group contacts

Results for between-group contact patterns were generally the opposite of those observed within groups. For example, animals that tested positive for bTB were found to interact with test-negative individuals from other social groups more frequently than would be expected by chance in both spring and summer (Table 5.2). In all seasons, values of degree centrality for between-group contacts were also higher for test-positive individuals, although the difference was only significant in winter and when calculated in terms of the number of individuals encountered (Table 5.3). This was due to high levels of variation in between-group contact patterns of both test-positive and test-negative badgers. Closeness centrality measures differed little among test-positive and test-negative individuals (Table 5.4). However, values of flow-betweenness were considerably higher for test-positive animals when compared with their negative counterparts, with the differences being highly significant in summer and winter (Table 5.5). Thus, whilst animals generally appeared to have similar numbers of between-group contacts irrespective of their disease status, test-positive animals tended to

encounter individuals from more than one different social group (high flow-betweenness scores), whilst test-negative individuals tended to encounter individuals from just one other group (the neighbouring group in most cases; see Fig 5.1).

Social networks and sett use patterns

The different contact patterns of infected and uninfected animals may be related to different space use and ranging behaviour of infected and non-infected individuals. For example, in **Chapter 3** I showed that test-positive animals spent more time at outlier setts and hypothesised that this may increase contact among groups. Consistent with this, animals that spent a greater proportion of time at outlier setts interacted for longer with badgers from other social groups (GLMM controlling for season, χ^2_1 = 7.87, P = 0.005, Estimate +/- SE = 130 +/- 46.3) and for less time with badgers from their own group (χ^2_1 = 21.1, P < 0.001, Estimate +/- SE = -2612 ± 534), although the patterns did not correlate with the number of individuals contacted (within-group: χ^2_1 = 1.71, P = 0.19; between-group: χ^2_1 = 0.30, P = 0.84).

Table 5.2 Tests for assortative interactions between bTB test-positive (TB+) and test-negative (TB-) badgers across different seasons. For each possible association the probability is given that interactions were more frequent ($P >$) or less frequent ($P <$) than would be expected by chance. Significant values are indicated with asterisks ($P \leq 0.05$).

	Within-group contacts						**Between-group contacts**					
	TB- / TB-		TB- / TB+		TB+ / TB+		TB- / TB-		TB- / TB+		TB+ / TB+	
Season	$P >$	$P <$	$P >$	$P <$	$P >$	$P <$	$P >$	$P <$	$P >$	$P <$	$P >$	$P <$
Summer	0.19	0.85	0.82	0.23	0.78	0.40	0.93	0.11	**0.05***	0.95	0.31	0.80
Autumn	**0.01***	0.99	0.99	**0.02***	0.66	0.50	0.76	0.36	0.26	0.85	0.73	0.57
Winter	**0.001****	0.99	0.99	**0.003****	0.50	0.71	0.97	0.06	0.10	0.94	0.18	0.93
Spring	**0.04***	0.97	0.98	**0.03***	0.37	0.78	0.92	0.13	**0.03***	0.99	0.90	0.23

Table 5.3 Degree centrality measures of bTB test-positive (TB+) and test-negative (TB-) badgers calculated at three different levels: α = 0 (solely based on the number of ties), α = 0.5 (equal weighting given to tie number and tie strength) and α = 1 (solely on the basis of tie strengths). Values are means ± 1 SD. *P*-values for differences between TB+ and TB- individuals were obtained using randomisation tests (see Materials and Methods for details).

		Within-group			**Between-group**		
Season		TB-	TB+	*P*	TB-	TB+	*P*
Summer	α = 0	5.13 ± 2.33	4.33 ± 2.53	0.37	1.44 ± 1.14	2.50 ± 3.62	0.17
	α = 0.5	1104 ± 712	1042 ± 699	0.80	73.0 ± 109	146 ± 338	0.31
	α = 1	262838 ± 205403	266284 ± 228080	0.96	7182 ± 13775	12063 ± 29808	0.51
Autumn	α = 0	5.44 ± 2.41	3.85 ± 2.82	**0.05***	0.78 ± 0.89	0.85 ± 1.61	0.89
	α = 0.5	699 ± 384	434 ± 336	**0.04***	19.3 ± 48.1	21.2 ± 42.2	0.90
	α = 1	97380 ± 68432	57533 ± 54671	**0.05***	964 ± 2797	1351 ± 5007	0.82
Winter	α = 0	4.36 ± 1.49	2.60 ± 1.43	**0.004****	1.22 ± 1.14	2.90 ± 2.69	**0.05***
	α = 0.5	863 ± 548	509 ± 336	**0.05***	49.9 ± 90.2	96.8 ± 107	0.21
	α = 1	200303 ± 146009	124030 ± 107911	**0.04***	3109 ± 8054	4268 ± 4803	0.76
Spring	α = 0	4.17 ± 2.19	2.89 ± 1.85	0.16	1.42 ± 1.35	1.79 ± 2.10	0.64
	α = 0.5	521 ± 430	431 ± 260	0.58	19.9 ± 26.2	29.1 ± 35.5	0.44
	α = 1	90981 ± 114849	96980 ± 99195	0.90	346 ± 587	543 ± 689	0.46

Table 5.4 Closeness centrality measures of bTB test-positive (TB+) and test-negative (TB-) badgers calculated at three different levels: α = 0 (solely based on the number of ties), α = 0.5 (equal weighting given to tie number and tie strength) and α = 1 (solely on the basis of tie strengths). Values are means ± 1 SD. *P*-values for differences between TB+ and TB- individuals were obtained using randomisation tests (see Materials and Methods for details).

		Within-group			**Between-group**		
Season		TB-	TB+	*P*	TB-	TB+	*P*
Summer	α = 0	5.63 ± 2.26	4.33 ± 2.53	0.12	7.22 ± 4.88	7.90 ± 5.96	0.70
	α = 0.5	5.58 ± 3.34	4.98 ± 3.34	0.60	4.41 ± 4.50	4.03 ± 5.39	0.82
	α = 1	5.91 ± 4.29	5.33 ± 3.91	0.69	2.67 ± 4.70	2.95 ± 6.34	0.88
Autumn	α = 0	6.10 ± 2.39	4.04 ± 2.47	**0.02***	1.86 ± 1.03	1.03 ± 2.09	0.26
	α = 0.5	6.05 ± 2.94	3.73 ± 2.88	**0.02***	1.30 ± 2.43	1.03 ± 2.16	0.74
	α = 1	6.27 ± 3.94	3.57 ± 3.34	**0.04***	1.07 ± 3.66	0.94 ± 2.90	0.89
Winter	α = 0	4.71 ± 1.33	2.80 ± 1.47	**0.001****	6.33 ± 3.86	6.72 ± 5.64	0.81
	α = 0.5	4.50 ± 2.63	2.70 ± 1.62	**0.05***	5.19 ± 4.25	5.97 ± 5.19	0.64
	α = 1	4.62 ± 4.34	2.76 ± 1.38	**0.04***	3.05 ± 4.79	3.64 ± 3.50	0.75
Spring	α = 0	4.33 ± 2.06	2.89 ± 1.85	0.09	5.60 ± 3.80	4.72 ± 4.52	0.59
	α = 0.5	4.11 ± 3.24	3.67 ± 2.20	0.73	4.81 ± 3.64	4.70 ± 4.65	0.94
	α = 1	4.16 ± 5.01	4.32 ± 4.04	0.93	3.48 ± 3.49	3.92 ± 4.23	0.77

Table 5.5 Flow-betweenness scores for bTB test-positive (TB+) and test-negative (TB-) badgers calculated using binary ties. Values are means ± 1 SD. Significance of differences between TB+ and TB- individuals were assessed using randomisation tests (see Materials and Methods for details).

	Within-group			**Between-group**		
Season	TB-	TB+	*P*	TB-	TB+	*P*
Summer	5.09 ± 9.28	3.81 ± 8.06	0.70	19.6 ± 39.6	99.5 ± 176.2	**0.02***
Autumn	7.12 ± 7.18	2.07 ± 3.20	**0.02***	3.15 ± 7.06	6.23 ± 20.9	0.58
Winter	5.42 ± 7.44	2.07 ± 5.66	0.23	17.0 ± 35.9	134 ± 162	**0.002****
Spring	4.68 ± 6.13	5.21 ± 2.47	0.36	28.4 ± 53.0	39.1 ± 62.4	0.65

5.5 DISCUSSION

The results indicate clear differences in the social behaviour of infected and non-infected badgers in certain seasons of the year. The findings have implications for understanding how bTB spreads within badger populations, and potentially on to domestic cattle and for management practices. Badgers that tested positive for bTB interacted less frequently with members of their own social group (particularly test-negative individuals) compared to those animals that tested negative for bTB, both in terms of the number of animals encountered and contact duration and frequency. However, test-positive animals interacted more frequently with negative animals from other social groups and had considerably higher between-group flow-betweenness scores than their negative counterparts in summer and winter, which correspond with times of high mating activity (Neal & Cheeseman 1996; Buesching, Heistermann & Macdonald 2009; Roper 2010). These animals fall on the shortest pathways between other pairs of individuals, linking social groups together, and may therefore be highly influential in the spread of *M. bovis* through the population.

The different contact patterns of test-positive and test-negative animals appear to be, at least in part, linked to differences in space use and movement behaviour. Several studies have reported increased roaming activity in infected badgers (Cheeseman & Mallinson 1981; Garnett, Delahay & Roper 2005), and I previously showed that test-positive individuals in this study population spend more time using marginal outlier setts and less time at their social group's main sett when compared to negative conspecifics (**Chapter 3**). Here these findings were extended by showing that animals using outlier setts more frequently also spend longer interacting with individuals from neighbouring social groups and have shorter contacts with members of their own group in all seasons (albeit significant only in autumn and winter). Thus, it seems likely that a tendency for test-positive animals to spend more time at territorial margins affects their within and among-group contact networks. Although it was not possible to directly infer causation from we can speculate on the underlying mechanism(s) that may be driving these findings. They could be operating at different levels: firstly, becoming infected with bTB could directly or indirectly alter the social behaviour and movement patterns of test-positive animals, or these individuals may have intrinsic behaviours such as wider roaming that increased their probability of contracting disease. Alternatively, the

differences could be driven by other members of the social group actively avoiding/ ostracising infected animals.

Exploitation of hosts by parasites has been documented in a number of host-parasite systems and can directly influence host feeding rates, sociability, movement patterns and success in sexual selection (Kortet, Hedrick & Vainikka 2010). If parasites and other pathogens are transmitted through social contact then natural selection should favour those parasites that manipulate the expression of social behaviours that increase their own transmission rates. This may include increased aggressiveness (as in rabies; Niezgoda *et al*. 2002) or roaming behaviour (Cheeseman & Mallinson 1981). To my knowledge there is no evidence of *M. bovis* actively manipulating host behaviour, but it remains possible that the altered social behaviours of bTB infected badgers are a direct consequence of the pathogen.

A second possibility is that intrinsic differences in the roaming behaviour of individuals affect space use, contact patterns and therefore the probability of contracting disease. 'Personality' has been described in a number of animal species whereby individuals display behaviours that are consistent across time and/or contexts (Stamps & Groothius 2010). Individuals with 'bold' personality types are often found to be consistently incautious, prone to risk-taking and may be more likely to contract disease (Sih *et al*. 2004; Clay *et al*. 2009). In studies of other species with defined social organisation there are often references to 'floater individuals' that move between a number of groups and can have a profound influence on the spread of infectious disease (e.g. Craft *et al*. 2011). For example, Drewe (2009) showed that the incidence of intergroup roving by male meerkats (*Suricata suricatta*) influenced their probability of subsequently testing positive for bTB. Thus, in this case it was the intrinsic behaviours of the host that put them at a greater risk of contracting bTB rather than the disease causing them to roam more. Animals that range more widely are likely to increase their probability of encountering infection in other conspecifics, in other host species or in the environment (Woodroffe *et al*. 2009). In the case of the badger this could include encountering a greater number of badgers from other social groups, contacting cattle on the pasture or in barns or investigating a greater number of latrines that demarcate territorial boundaries and which are potential sources of infection (e.g. Courtenay *et al*. 2006). Indeed, bTB test-positive badgers were found to have higher flow-betweenness scores than those that tested negative, suggesting that they were interacting with individuals

from more than one foreign social group. Flow-betweenness was significantly higher for test-positive animals in winter and summer coinciding with known breeding seasons (February and June; e.g. Buesching, Heistermann & Macdonald 2009), suggesting that mating is responsible for many of the between-group contacts of these individuals (**Chapter 4**). No sex differences were found in bTB infection levels (and it was not possible to test for an interactions as this is at present a limitation of social network analyses) but there is the suggestion that males may be key hosts for infectious pathogens if they are the ones that are driving the contacts (Perkins *et al*. 2003; **Chapter 4**).

Alternatively, bTB infection may indirectly affect social interactions through avoidance or exclusion of positive animals by uninfected group members or reduced competitive ability forcing infected animals into more marginal habitats. However, there was no evidence that these animals were in poorer body condition from external examination, and only two individuals were found to be actively excreting *M. bovis* by a culture test. It has been demonstrated in a number of species, including humans, that individuals are able to detect disease status in others using behavioural cues or social odours (such as urine, faeces and scent secretions; Penn & Potts 1998; Kavaliers *et al*. 2004; Curno et al. 2009) and take measures to avoid areas or individuals that may expose them to parasites (Moore 2002; Ezenwa 2004). Badgers frequently use olfactory cues in territorial defence and for conveying information such as group membership, individual identify and/or reproductive state (Buesching & Macdonald 2001; Palphramand & White 2007) and it is possible they may also derive information on disease status from such odours. This may allow active avoidance of infected group members or exclusion via aggressive encounters (although there was no evidence that test-positive animals had higher bite wound scores) resulting in reduced within-group contacts. However, current evidence suggests that the costs of bTB infection in badgers in terms of fecundity, competitive ability and mortality are comparatively low (e.g. Wilkinson *et al*. 2000), making the selective benefits of disease avoidance mechanisms unclear. Moreover, test-positive animals had higher between-group connectivity (higher flow-betweenness scores) than bTB test-negative animals which might not be expected if avoidance behaviour is commonplace. Unless, however, group members could detect the disease status of these individuals due to more time spent in close proximity than between-group animals that may have limited encounters with them.

Overall, therefore, animals that spend more time on the periphery of social group territories may increase their risk of becoming infected due to increased contacts with individuals from other groups and/or, which may be exaggerated at certain times of the year, such as during the breeding seasons. These animals may therefore have a disproportionate influence on the connectivity of social groups, with important implications for the spread of disease through the population as a whole.

5.5.1 Implications for disease transmission and management practices

Although it is not possible to firmly establish the causal mechanism for these findings, they suggest that social structure is likely to play an important role in regulating disease transmission at both the within- and between-group level. Disease dynamics are complex and there are likely to be many factors involved (e.g. individual-level susceptibility). However, at the population level, the stable structure of badger social groups appears to mitigate against infection in all groups (Delahay *et al*. 2000a). Most animals had low flow-betweenness scores suggesting that their between-group contacts were primarily limited to their neighbouring groups, with the exception of those individuals that tested positive for bTB. At the group-level, fewer contacts between infected and uninfected badgers may be a contributing factor to the observation that not all members in the group become infected with the disease. This behavioural sub-structure is likely to be one of the reasons for the non-density dependence and aggregation of bTB into certain social groups in the population that have been previously documented in a number of badger populations (Delahay *et al*. 2000a; Woodroffe *et al*. 2009).

A theoretical study by Bonds *et al*. (2005) suggested that increased prevalence of infectious diseases could induce greater sociality. Related to this is the idea that the spatial organisation of badgers may limit disease transmission under certain circumstances. Woodroffe *et al*. (2009) found that *M. bovis* prevalence was consistently higher at low badger densities and in small social groups, which appears to be at odds with the general perception that the prevalence of directly transmitted pathogens increases in larger groups and at higher population densities. Also Vicente *et al*. (2007) found that as groups decreased in numbers there was a higher probability of disease, which may be attributable to badgers in smaller groups interacting more frequently with members of neighbouring groups. This is also supported by other studies that observed

the incidence of infected badgers increasing following years of high between-group movement (Rogers *et al*. 1998). Indeed my study found that bTB test-positive animals tend to interact with animals from more social groups and spend more time at outlier setts (**Chapter 3**), thus suggesting that disease status is related to between-group contacts and ranging behaviour. Overall, it appears that higher density badger populations exhibit a greater degree of spatial organisation and territoriality with less spatial overlap between groups (Woodroffe *et al*. 2009). This is likely to result in fewer between-group contacts, which could promote a lower level of disease transmission in the population.

The potential link between stable host structure and lower incidence of disease in badger populations urges caution when implementing control measures, such as culling, that may result in disruption of this structure. Culling may cause animals to roam further, thus bringing them into contact with a greater number of conspecifics, cattle and with more sources of environmental infection (e.g. Woodroffe *et al*. 2006a,b; Carter *et al*. 2007). The results of this study suggest that increased roaming behaviour and between-group contacts is correlated with bTB infection and that social structure, both within groups and at the population level, may mitigate against the uncontrolled spread of disease through the population. Culling could alter these structures in unpredictable ways and the effects could be long lasting as it may take some time for the population to return to previous levels of comparative stability (Cheeseman *et al*. 1993; Jenkins, Woodroffe & Donnelly 2010). In terms of practical management practices, targeting 'floater' individuals that may be disproportionately more influential in the spread of infectious disease is an attractive idea. However, whilst this may work successfully in humans and domestic livestock, it is unlikely that it could be practically applied to badgers due to the inherent difficulties of identifying and capturing such individuals. However, culling could perhaps be carried out at outlier setts, where a higher proportion of animals are likely to be already infected, and then vaccinating badgers at the main sett, to minimise any associated perturbation. A more practical/agreeable option may be the use of vaccination, for example, deployed in an oral bait form at both main and outlier setts to target all individuals.

Conclusion

The advent of new technology has enabled the quantitative study of contact networks in an animal population that was otherwise difficult to observe. This study suggests that host social structure and behaviours may generally act to reduce bTB transmission both within and among badger social groups. However, disease transmission may be strongly influenced by the behaviours of a few, wider-roaming individuals that are disproportionally more likely to become infected and spread the disease through the population. Research effort should now work towards establishing the causation of the behavioural differences that have been documented. This could be achieved through long-term studies of the contact patterns of individuals before and after infection with the disease. Such data could be used to establish whether social interactions and roaming behaviour affect the risk of contracting the disease, or whether such behaviours are altered post-infection. At a practical level, this study adds indirect support to the argument that any social disruption of badger populations, for example through culling, that increase movement and contacts between groups could promote rather than alleviate the spread of disease.

Chapter 6. A quantitative comparison of methods used to study space use, movement and contact patterns in free-ranging animals using the Eurasian badger (*Meles meles*) as a test case

6.1 ABSTRACT

1. Behavioural studies of space use, movement and contact patterns among individuals can provide insights into the social organisation of animal populations, which is central to many areas of behavioural ecology, evolutionary biology and the conservation or management of wildlife.

2. Here I compare four methods that have been used to study such behaviours: baitmarking, capture-mark-recapture, radio-telemetry, and proximity detection devices. I highlight the strengths and weaknesses of each method, with a focus on the level of data resolution that they provide at the individual, group and population-level.

3. Using comparable quantitative data collected as part of a long-term field study of a high-density Eurasian badger (*Meles meles*) population, and social network analysis, a number of case studies are presented to investigate the extent to which the data and associated conclusions drawn from these methods correspond and whether they compliment or contradict each other.

4. Whilst the methods provide comparable data and conclusions, differences were found in the level of detail that was returned, which could largely be attributed to the spatial and temporal resolution of the methods. In particular, intricate movement and contact patterns that occur on a day-to-day basis were only consistently detected by the proximity loggers. This resulted in the social networks constructed using these data having a higher overall level of population connectivity than those from other methods.

5. Fine-scale data from the proximity loggers also suggested the presence of a within-group sub-structure where all individuals did not necessarily encounter each other to the same extent, thus decreasing overall within-group connectivity measures. This is contrary to assumptions made by coarser methods that infer a relationship based on shared group membership, and hence may overestimate the level of connectedness within and between groups for some species.

6. While the different approaches broadly agree with one another, it is clear that assumptions of within-group connectedness may be wrong. Thus, choosing the most appropriate cost- and time-effective method (or combination of methods) will largely depend on the aims of the investigation, the study species, and the degree of spatial and temporal resolution that is required of the data for the necessary conclusions to be drawn.

6.2 INTRODUCTION

Quantifying the social structure of populations and investigating the underpinning mechanisms is often a central part of studies relating to behavioural ecology, evolutionary biology, wildlife management and conservation (Whitehead 2008; Croft *et al.* 2011). An individual's movement and contact patterns are likely to have a strong influence on access to information and resources and also on the spread of infectious disease within and amongst populations (Croft *et al*. 2008). In some cases, pathogen transmission may occur between species, which poses problems for understanding the dynamics of the disease and for informing intervening management strategies (Caley & Hone 2004). Whilst some species may lend themselves to direct observation by a recorder or using remote video surveillance, e.g. birds and habituated mammals (Bradley *et al.* 2004; Drewe 2009), others may lead particularly secretive and/or nocturnal lives. In either case there are the additional limitations that individuals may not be readily recognisable/distinct and logistical/financial constraints are likely to prevent the collection of continuous data for extended periods and over an extensive area. This necessitates other approaches for assessing movement patterns, which may be passive or may require capture of the animals and the attachment of measuring devices. Four commonly employed and well established methods and a recently emerging method (in particular for mammalian species) are; a) baitmarking (Delahay *et al*. 2000b), b) capture-mark-recapture/re-sight (Macdonald *et al*. 2008), c) molecular/genetic studies (Watts & Paetkau 2005), d) radio-telemetry (Samuel & Fuller 1994) and e) recently developed proximity detection devices (Prange *et al*. 2006). A brief outline of these methods, their applications and their pros and cons is provided in Table 6.1.

Table 6.1. Methods for the study of movement and/or contact patterns in free-ranging animals.

METHOD	DESCRIPTION	APPLICATIONS	PROS	CONS	REFERENCES
Baitmarking	Bait is deployed that contains markers such as radioactive isotopes, dyes, fluorescent powders, chemical biomarkers, metallic flakes and plastic beads. The surrounding areas are then surveyed for evidence of where they have been deposited in faeces.	- Home range estimates - Population densities - Migration/dispersal patterns - Territorial configuration of social groups e.g. the Eurasian badger - Estimating rates of bait uptake for wildlife control measures e.g. for vaccination or poison delivery.	- Can be carried out passively with little or no disturbance to the animal - Relatively inexpensive.	- Resolution limited to the period over which marked bait was given – 'snap-shot' of behaviour - Relies on the observer detecting the majority of deposited markers - Biases related to habitat e.g. bait is easier to locate in short grassland areas vs. woodland.	Hobson (1999) Delahay *et al*. (2000a) Webbon, Baker & Harris (2004) Rösner & Selva (2005) Palphramand *et al*. (2011)
Capture-mark-recapture/ re-sight (CMR)	Animals are given a unique identifying mark so that they can be recognised on subsequent re-sighting and/or recapture. Marks may be external attachments e.g. rings and ear tags, internal devices e.g. sub-cutaneous microchips, or on the animal itself e.g. dyes, tattoos, fur clips, fin/fluke shape (in cetaceans).	- Population size estimates - Population dynamics - Survival rates - Studying patterns of movement and dispersal - Disease monitoring.	- Can be carried out on a vast array of organisms e.g. insects to large mammals. - Animals do not necessarily have to be recaptured, but can be re-sighted by an observer or remotely using e.g. camera-traps.	- Potentially biased by individual-level effects e.g. 'trap-happy/shy' that introduces a resampling bias - Can be labour intensive - Can be invasive with welfare implications.	Rogers *et al*. (1998) Straley, Quinn & Gabriele (2008) Grosbois *et al*. (2009) Sharma *et al*. (2009) Junker *et al*. (2010) Doligez *et al*. (2011)
Molecular/ Genetics	DNA extracted from sample tissue/materials is used to genotype individuals at varying numbers of microsatellite loci depending on the species. From this, multiple measures of e.g. genetic diversity and kinship can be assigned.	- Population size estimates - Population-specific parameters of genetic variability - Rates of out-breeding and dispersal.	- Samples can be collected non-invasively e.g. faeces, hair or feathers.	- Difficult to get individual-level effects unless each animal is captured - Comparatively expensive and potentially time-consuming.	Dugdale *et al*. (2008) Prugh *et al*. (2005) Scheppers *et al*. (2007) Hogan *et al*. (2008) Hájková *et al*. (2009) Frantz *et al*. (2009)
Radio-telemetry	Animals are given tags that emit unique Very High	- Estimation of individual home ranges	- Tags have been miniaturised and can be	- Large amount of effort to track a small number of	Harris *et al*. (1990) Garton *et al*. (2001)

	Frequency (VHF) wavelengths. This allows individuals to be tracked, or their location estimated using a directional antenna.	- Resource use and selection - Population abundance and density - Survival and fecundity - To infer social interactions and contact rates through shared space use.	attached to animals ranging in size from passerine birds to large mammals - High spatial resolution data.	animals - Observer is in close proximity to the animal, potentially influencing behaviours.	Aarts *et al*. (2008) Böhm *et al*. (2008) Dillon & Kelly (2008) Marker *et al*. (2008) Kesler (2011)
Global Positioning System (GPS) telemetry	Animals wearing these tags can have their geographical position tracked remotely via satellite technology. Paths of movement can be visualised on a computer.	- As above	- Not very labour-intensive as an animal's position is tracked remotely. - Provides spatial and temporal data.	- Difficulty in getting fixes in cluttered habitats (e.g. woodland in leaf). - High expense limits sample sizes - Weight prohibits deployment on small-to-medium sized mammals and below.	Bandeira de Melo *et al*. (2007) Cagnacci *et al*. (2010) Tomkiewicz *et al*. (2010)
Proximity detection devices	Collect contact data between tagged individuals in one or more populations and/or species. When animals come within range of each other, each tag can record the ID of the individual that it encountered, the start time of the interaction (in GMT), and the contact duration in seconds.	- Quantitative study of social interactions/ contact rates - Visualisation of even complex social network systems - Investigating information or disease transmission in and between populations.	- User defined settings e.g. detection distance allows use in studies with different aims. - Continuous, remote recording with high temporal resolution.	- Spatial resolution is at present limited – location can be inferred based on which animals are interacting, from static devices at specific locations, or by conventional radio-telemetry. New generations are seeking to also incorporate a GPS function. - Population should ideally be saturated with detectors. - Loggers need to be recovered to download data.	Prange *et al*. (2006) Ji *et al*. (2005) Böhm, Hutchings & White (2009) Drewe *et al*. (*In Prep*) Swain & Bishop-Hurley (2007) Hamede *et al*. (2009) Marsh *et al*. (2010)

Techniques such as those outlined in Table 6.1 offer a wealth of opportunities for investigating movement and interaction patterns in animals that were previously difficult to study, but also present dilemmas when selecting the most appropriate method. Previous reviews have tended to provide only qualitative descriptions of how such techniques are carried out, the type of data that can be collected and, in some cases, how those data can be analysed. However, when selecting between monitoring methods it is important to consider whether different approaches provide consistent data and whether they support similar conclusions on movement patterns and population connectivity. Methods vary in terms of cost- and time-effectiveness and the level of disturbance caused to the animal. It is likely that the most appropriate techniques will vary depending on the particular study species or research question. Nonetheless, a quantitative assessment of how movement, contact and connectivity data and associated conclusions compare across methods may be invaluable to researchers when selecting an approach.

As part of on-going studies into the ecology of the Eurasian badger (*Meles meles*) data were simultaneously collected by means of baitmarking, capture-mark-recapture, radio-telemetry and proximity detection devices. Moderate-to high-density badger populations in the UK provide a good model system for such a comparison as they live in relatively stable social groups that actively defend a shared territory, with the general perception that contact between groups is limited (Delahay *et al*. 2000b; Johnson, Jetz & Macdonald 2002). This presents the opportunity to use baitmarking to determine the configuration of the social group territories, capture-mark recapture to investigate both short-term and long-term movement/dispersal between groups, radio-telemetry to study the space use/movement patterns of individuals and proximity logging devices to investigate both within and between-group contact rates (the inclusion of molecular/genetic data was not possible within the scope of this study, however, see results/discussion). In the UK and Ireland the Eurasian badger is a wildlife reservoir for bovine tuberculosis (bTB) and is implicated in its spread to domestic cattle (Muirhead, Gallagher & Burn 1974; Griffin *et al*. 2005; Bourne *et al*. 2007). The transmission of bTB in badger populations has been correlated with individual heterogeneities in space use (Cheeseman & Mallinson 1981; **Chapter 3**), movement (Rogers *et al*. 1998; Vicente *et al*. 2007) and contact rate patterns (**Chapter 5**). Thus, the study of badger movement patterns at multiple scales is likely to be important for answering ecological and evolutionary questions surrounding badger social organisation (**Chapter 4**), for

understanding disease transmission dynamics and informing intervention strategies for management of the disease in both badgers and cattle (**Chapter 5**).

Here quantitative methods are used to determine how the data and conclusions drawn from the four techniques outlined above correspond with one another in the context of space use, movement and contact patterns at a) the social group level and b) the individual level. In particular, I address the question of whether methods with greater resolution simply provide more data on trends that are detected by all methods, or whether they provide fundamentally new insights. The results from these comparisons should aid in decision-making when selecting the most appropriate method, or combination of methods, for a particular study species or research question(s).

6.3 MATERIALS AND METHODS

6.3.1 Study Site and Population

Fieldwork was conducted on a high-density badger population at Woodchester Park, Gloucestershire, UK (51°71´N, 2°30´E). The study area comprised approximately 7 km^2 of fragmented deciduous and coniferous woodland, agricultural grassland and smaller areas of arable and scrub land (see Delahay *et al*. 2006b). In this population individuals were trapped on average twice per year as part of a long-term study in which detailed epidemiological and morphometric data are recorded to construct relatively complete life histories (for more details: Delahay *et al.* 2006b; Vicente *et al*. 2007).

6.3.2 Techniques Employed

Baitmarking

Badgers use communal latrines that demarcate territorial boundaries and are likely to be hotspots of visitation by most individuals (Delahay *et al*. 2000b). To determine the territorial configuration of the population each social group was fed a bait (peanuts and syrup) laced with a unique colour and/or shape of plastic pellet so that the origin of faeces at latrine sites could be assigned. Bait was placed at the main sett for a period of ten days in spring (23/02/2009 – 05/03/2009) when territorial activity, particularly by males, is considered to be at its peak. Following this, all known latrines were surveyed

and the colours of the plastic beads in the faeces were noted. Bait returns were then used to map the territorial boundaries of each social group. To construct territorial boundaries the outermost latrines for each social group were joined to construct a Minimum Convex Polygon (MCP) which was then corrected using field data of 'boundary runs' (Delahay *et al*. 2000b). Thus, in the majority of cases each territory was surrounded by a series of shared latrines with neighbouring groups. The size of these MCPs acts as a proxy measure for social group territory size. If there was a clear overlap between two or more groups with bait returns being distributed evenly then these groups were classified as a 'super group'. This is loosely defined as an association of badger groups that may use different setts for breeding but have overlapping ranges and frequently spend the day in one another's setts (Evans *et al*., 1989; **Chapter 4**). If there was not a clear association between the groups, or if there were insufficient bait returns to assign a territory with confidence, then MCPs were either not constructed for the particular group(s), or assigned with low confidence. ArcGIS (v.10) was used to create the baitmarking maps.

Capture – Mark – Recapture

A regular capture-mark-recapture programme has been in operation at this site since 1978 for the purposes of investigating badger ecology and the epidemiology of bTB infection in badgers. Cage traps were placed at active setts and pre-baited for five days prior to trapping, after which they were set for two consecutive nights. Each social group was trapped four times per year, with each individual being captured on average twice in this period (Delahay *et al*. *Unpublished Data*). Animals were anaesthetised and permanently marked with a unique tattoo in the inguinal region for identification on subsequent re-trapping events. The location of capture (sett and social group) of each individual was recorded. The 2009/2010 trapping season referred to in the results ran from May 2009 until January 2010 (no trapping takes place for welfare reasons during February, March and April when females may be giving birth or suckling young cubs (Woodroffe *et al*. 2005b)).

Radio-telemetry

Collars containing Very High frequency (VHF) transmitters were deployed on 51 badgers (of which 40 were tracked and are included in the analysis) belonging to eight

social groups (Sirtrack Tracking Solutions, Havelock North, New Zealand). The VHF transmitter emits a signal every few seconds on a narrow frequency band, which is different for each individual. As part of a study investigating diurnal resting patterns (**Chapter 3**), collared animals were located whilst resting at their setts once every day between 08:00 and 15:00 GMT using a R1000 receiver (Sirtrack Ltd., Havelock, NZ) and a Yagi antenna (Biotrack, Dorset, UK) on 28 consecutive days in each season (Summer: 26/06/2009 – 23/07/2009; Autumn: 23/09/2009 – 20/10/2009; Winter: 10/01/2010 – 6/02/2010; Spring: 16/04/2010 – 13/05/2010). The precise underground location of each animal within the sett or nest chamber was estimated as per Butler & Roper (1996) and Roper *et al*. (2001) using the VHF signal to pinpoint a location on the surface below which a radio-collared badger was resting. A 'fix' for this location was then taken by measuring its distance from three markers (two trees and the entrance hole) for which the GPS co-ordinates were known. If the fixes of two or more badgers located at the same sett fell within 1 m^2 of each other then they were deemed to be resting in the same nest chamber (Butler & Roper 1996).

Proximity Loggers

Collars containing VHF transmitters were also equipped with recently-developed proximity logging devices (Sirtrack Tracking Solutions, NZ) which allow detailed information on rates of contact and patterns of interaction to be collected. The proximity loggers operate by transmitting a unique Ultra High Frequency (UHF) code whilst simultaneously 'listening' for the codes of other loggers (for details: Prange *et al*. 2006; **Chapter 2**). Loggers were individually set to begin recording a contact when two or more animals came within 0.64 ± 0.04 m of each other and to log it to memory after the animals had been out of this distance for 30 seconds or more. When two or more collared animals were within range, each proximity logger recorded the ID of the individual that it encountered, the start time of the interaction (in GMT) and the contact duration in seconds. Whenever collared badgers were recaptured (see above) data were downloaded from the proximity loggers to a laptop computer using software supplied by the manufacturers. Prior to analysis these data were adjusted by amalgamating contacts that were recorded within 1 minute 30 seconds of each other if they involved the same pair of loggers, and then by removing any remaining interactions that lasted for 1 second to construct a more realistic representation of what had most likely occurred in the field (Prange *et al*. 2006; **Chapter 2**).

6.3.3 Data Analysis

Comparison of networks

Social network analyses (SNA) were carried out at a) the social group-level for data collected by all four of the field methods and b) at the individual-level for the capture-mark-recapture, radio-telemetry and proximity logger data (baitmarking does not provide information on individual-level patterns). For each method, data were presented from the same individuals so as to allow comparison across the social networks, which were constructed in the following ways:

1) Social group territories for the 2009/2010 season were assigned using baitmarking as described above. A binary network was then constructed with groups considered to either be connected (1) if one or more of the bait returns from one groups was found within the territory of the other group (as delineated by the corrected MCPs), or unconnected (0) if their bait returns were only found within their own territory. As mentioned above, the majority of territories are delineated by a series of shared latrines around the border, which do not constitute an extra-group bait return and do not directly link two groups.

2) A binary network was also constructed using capture-mark-recapture data collected during the 2009/2010 trapping season. Badgers that were caught in the same social group at one or more of the trapping events were deemed to be associated (1). Such an interaction network is based on the "gambit of the group" (i.e. individuals that were found together in the same group are connected to each other in the network graph; Croft *et al.* 2004).

3) For radio-telemetry data, precise diurnal resting locations were used to determine which animals were sharing nest chambers on a day-to-day basis. At the group level a binary connection was inferred if animals from different social groups had shared a sett and at the individual level a connection was present if particular individuals had rested together. A second weighted network was constructed based on the number of days (out of the 112 sampled; 28 in each season) that individuals were found to be sharing a nest chamber.

4) For proximity logger data, social networks were constructed based on the individuals that had encountered each other during the 2009/2010 study period. For comparison with the capture-mark-recapture network a 'full' binary network was constructed that comprised of all of the data collected in the study period. For comparison with the radio-telemetry data a binary diurnal network was constructed based on contacts made between 06:00 and 20:00 that are likely to represent sett sharing. A final weighted diurnal network was constructed based on the cumulative amount of time that any two badgers (a dyad) spent in proximity in the sett in order to compare the strength of the ties with that given by the radio-telemetry data.

To allow comparison across methods at the social group and the individual level all social networks were un-weighted (i.e. not adjusted for duration or frequency of contacts). At the individual level, the higher resolution data from radio-telemetry and proximity loggers were compared separately using weighted values to investigate if the strength of the connections between individuals varied. Data collected using different methods were compared both qualitatively using network diagrams and quantitatively by a) measuring the correlation between the association matrices given by each method in a pairwise manner using a Mantel test (with 10,000 permutations) and b) comparing the network measure of degree centrality given by each method. The average degree centrality value of a network measures how many direct connections an individual has, and when used alone can indicate an individual's importance within the network. Degree centrality measures were analysed to determine whether they differed according to method for the group-level and the individual-level networks using general linear mixed effect models (GLMMs) in the statistical freeware, R v. 2.11.1 (R Development Core Team 2010). This is not the typical way for analysing network data; interdependence of data points normally favours permutation approaches with the observed network being compared to a large number of randomly generated networks. I too have adopted this approach when analysing the data at an individual level (**Chapters 4 & 5**). However, here I am simply comparing among networks of the same individuals over the same time period. Thus, the analysis is unlikely to be confounded by non-independence of points in the same way as when looking for differences among individuals, with the behaviours of animal 'a' being dependent on those of animal 'b'. GLMMs take into account repeated measures with group and then individual nested within group included as random factors. Values were transformed to conform to normality and significance was assessed on the basis of likelihood ratio tests where $\alpha =$

0.05 (Crawley 2007). Where a significant effect was found differences between factor levels (i.e. the different methods) were assessed using post-hoc Tukey tests in the multcomp package for mixed-effects models (Hothorn *et al*. 2008).

Method Specific Analyses

Baitmarking

Annual baitmarking maps from 1977 to 2010 were used to record the total number of bait returns per group per year and to calculate the proportion of those returns observed outside of each social group's territory. In addition, the number of social groups that could not be assigned a territory was noted for each year.

Capture-mark-recapture

Using the capture-mark-recapture data, badgers that had been caught at least five times over three years were allocated to one of four categories according to the number of social groups in which they were trapped (cf. Rogers *et al*. 1998). Each animal was assigned a main residence based on the group in which it was first captured (normally as a cub) and an examination of the groups where it appeared most over the course of its trapping history. If it had been captured an equal number of times between two groups then it was counted in the group in which it was first captured (presumably the group in which it was born). 'Non-movers' were only ever caught in a single social group; 'occasional movers' visited only one or two social groups other than their main residence; 'frequent movers' moved between more than two groups other than their main residence and 'permanent movers' were those for which a movement led to permanent subsequent residence in a different group. This classification was used by Rogers *et al*. (1998) for badgers captured between 1978 and 1995. I extend this dataset to include animals captured up to 2009 to determine whether the inclusion of an extra fourteen years of data altered the findings of the earlier study. Data were initially analysed using individual social groups as per Rogers *et al*. (1998) and secondly using the 'super group' category.

6.4 RESULTS

Comparison of Networks

Group-level:

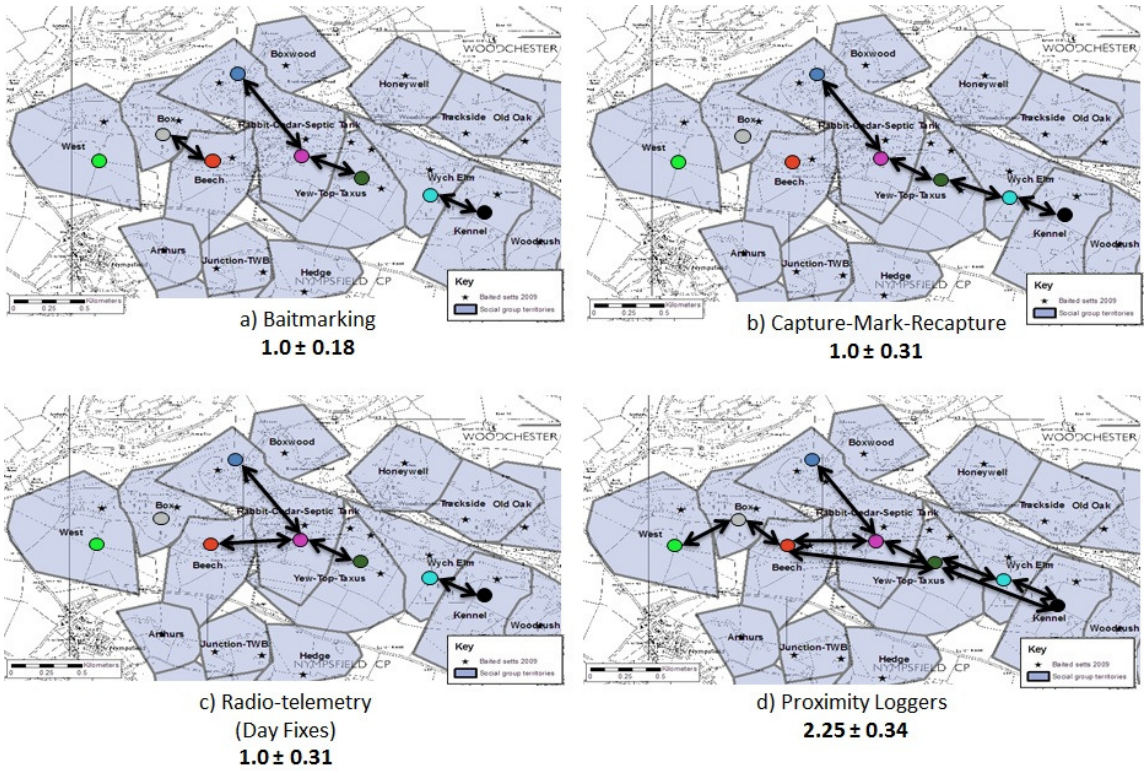


Figure 6.1. Connectivity of badger social groups at Woodchester Park, Gloucestershire calculated for 2009–2010 using four different field methods (overlaid onto a map of their territories). Groups were considered connected using: a) baitmarking, if one or more of the plastic pellets fed to one group was found in a latrine(s) within the territory of a foreign group; b) capture-mark-recapture, if a badger from one group was trapped in the territory of another group; c) radio-telemetry, if badgers were located resting in setts during the day in the territory of another social group; and d) proximity loggers, if one or more badger interacted with another from a different social group. Values for average node degree centrality (the number of social groups to which each group is connected) are given in bold (mean ± s.e.).

The level of social group connectivity from data collected by four different field methods is represented visually in Fig 6.1. Values for node degree (mean ± s.e. given in

Fig. 6.1) were also found to differ significantly between the four networks (GLMM: χ_3^2 = 19.3, $P < 0.001$). However, a Tukey's post-hoc test showed that there were no differences between the values given by baitmarking, capture-mark-recapture and radio-telemetry methods (all $P > 0.9$), but all differed to a large extent when compared with the values from proximity loggers (all $P < 0.001$). Although outside of the scope of this study molecular/genetics work has previously been carried out on this badger population. As illustrated in Fig 6.2, over the course of 14-years all of the studied social groups were either directly or indirectly connected as a result of one or more successful extra-group mating events.

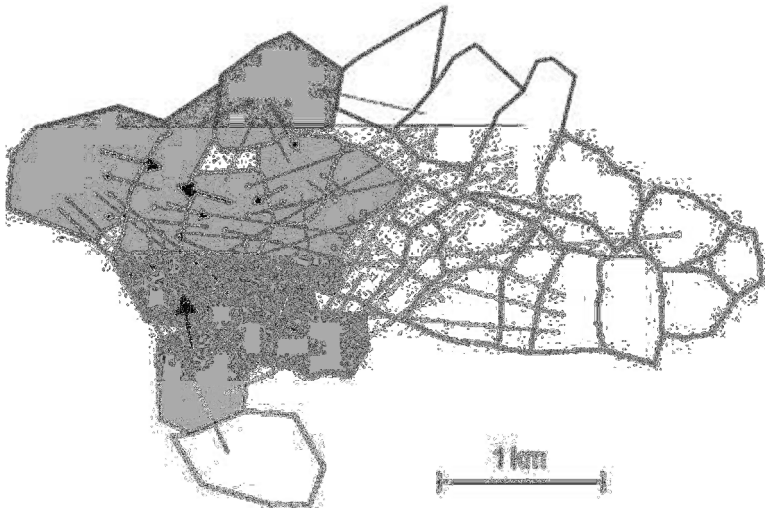


Figure 6.2 Connectivity of badger social groups at Woodchester Park in relation to successful between-group matings. Paternity and maternity were determined for cubs from the 10 social groups shaded grey. Arrows represent paternity from outside the cubs' social group assigned with 80% confidence. The thickness of the arrow are proportional to the number of paternities over the 14-year study period. Taken from Carpenter *et al*. (2005).

Individual-level:

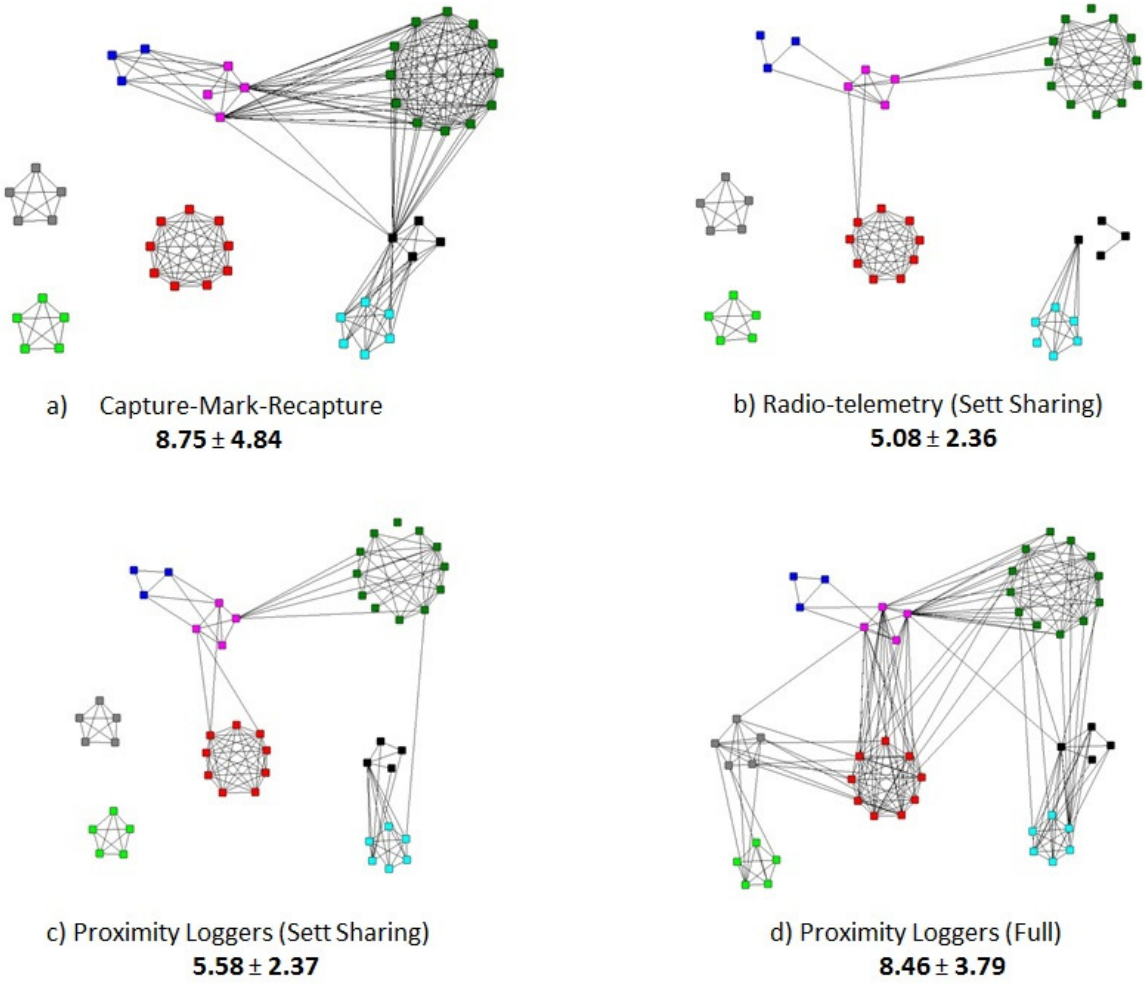


Figure 6.3 Average connectivity of individual badgers at Woodchester Park as determined by the four different methods over the 2009/2010 season (animals are in approximately the same positions in each diagram). Individuals were considered connected using: a) capture-mark-recapture data, if the animals were trapped in the same social group at one or more of the trapping events; b) radio-telemetry, if badgers were found resting together in the same nest chamber during the day; c) proximity loggers, if badgers were recorded resting in close proximity during the day-time; and d) proximity loggers, if individuals encountered each other during the day or the night. Values for average node degree centrality (i.e. the number of animals to which each individual is connected) are given in bold (mean ± s.e.).

Individual connectivity estimated from the data collected by different methods is represented visually in Fig. 6.3. Mantel tests showed a significant correlation only between the network matrices of the capture-mark-recapture and proximity logger data (P = 0.004), with all other pairwise comparisons being found to have no significant correlation (All $P > 0.25$). Values for node degree (mean ± s.e. given in Fig. 6.3) were found to differ significantly between the four networks (GLMM: $\chi^2_3 = 64.7$, $P < 0.001$). A Tukey's post-hoc test showed that all networks differed significantly from each other (all $P < 0.001$), with the exception of the full proximity logger and capture-mark-recapture networks ($P > 0.90$) and the radio-telemetry and proximity logger sett use network (P = 0.43). A visual representation of these two networks is given in Figure 6.4, including a weighted measure whereby the thickness of the connecting line represents the cumulative time that two individuals were recorded as resting together over one calendar year. Whilst it is not possible to compare these values statistically as they were measured on unrelated scales, and hence weighted network metrics are given on non-comparable scales, it does appear that both methods infer similar strength ties between interacting pairs of individuals (given by the thickness of the connecting edge).

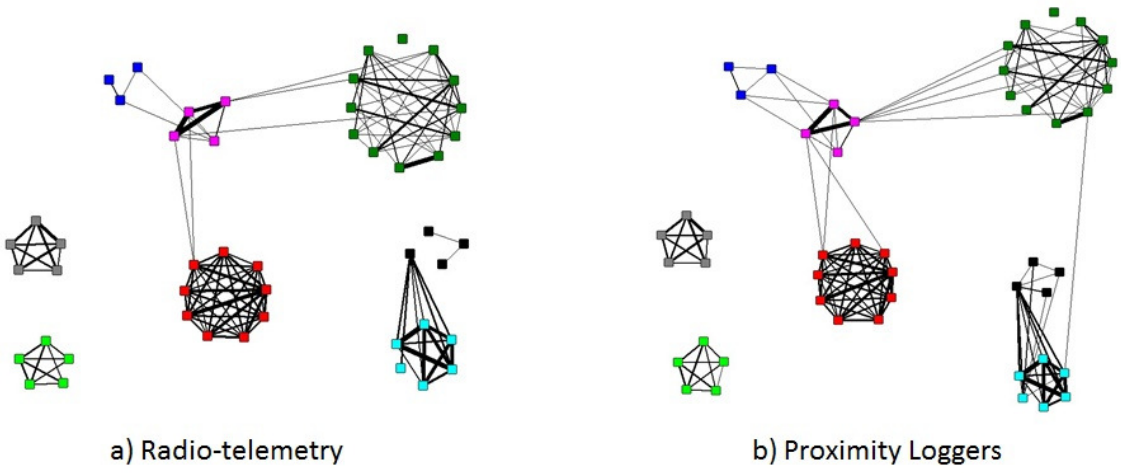


Figure 6.4 Weighted diurnal networks for individuals as determined by: a) radio-telemetry, where weightings shown in terms of the thickness of the connecting edges represent the proportion of days out of 112 spent resting in the same nest chamber; and b) proximity logging devices, where weightings represent the cumulative time individuals were recorded as being in close contact with each other (presumably sharing the same nest chamber) during the day over one calendar year.

Method Specific Analyses

Baitmarking

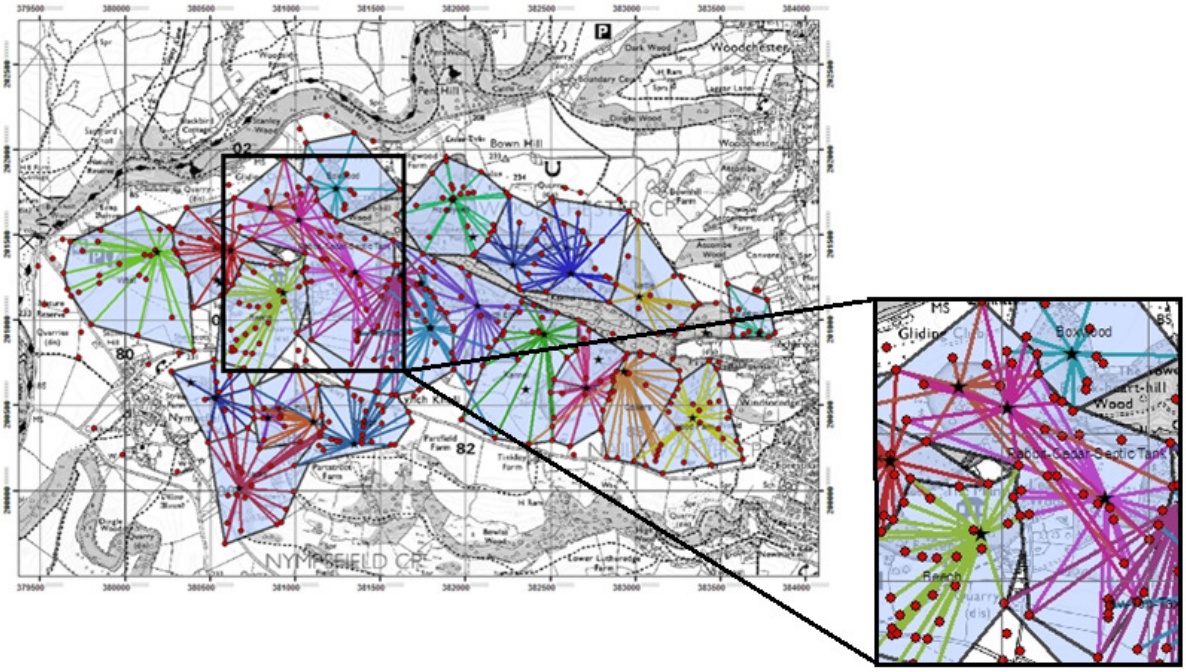


Figure 6.5 A map showing the configuration of badger territories (shaded areas) estimated from baitmarking carried out in March 2009 at Woodchester Park. Each group was fed bait laced with different coloured indigestible plastic beads. The lines (spokes) radiate from the main sett where bait was fed to the latrines (red circles) where marked droppings were found. MCPs represent the extent of each social group's territory. The enlargement shows two social groups, Septic Tank (pink lines) and Cedar (brown lines) that cannot be distinguished from each other due to the overlap of bait returns and can be considered a 'super group'.

Using historical data and baitmarking maps constructed each year from 1977–2010 for the 24 social groups shown in Fig. 6.5 it was found that on average 5.52 % ± 2.57 % (mean ± SD) of the bait returns per year were retrieved from a foreign group's territory (range: 1.68 % - 12.87 %). Also, each year there were on average 2.31 ± 1.80 incidences (range: 0 – 6) of the bait returns showing two groups as having a very close tie that prevented them being assigned discrete territories. They were either classed as one 'super group' or in some cases there were too few returns for any discrete territory to be assigned to the group with confidence.

Capture – Mark – Recapture

Of the 2,237 different badgers caught in the study area between 1976 and 2009, 743 (33.2%) were categorized in terms of their movement patterns as they were captured five or more times over a period of three or more years. Of these individuals, 326 (43.9%) were classified as 'movers' and 417 (56.1%) as 'non-movers'. These values are very similar to those of Rogers *et al*. (1998) who quantified the same data set using 475 badgers that were trapped between 1978 and 1995 (Table 6.2). The majority of badgers that moved in the latest study were 'occasional movers' (n = 233, 71.7%), whereas 82 (25.2%) were 'permanent movers' and 10 (3.1%) were 'frequent movers'. Neither the additional 14 years of data nor the addition of a super-group category resulted in a different outcome to that reported by Rogers *et al*. (1998) (Table 6.2).

Table 6.2 Movement classifications assigned using capture-mark-capture data for badgers caught between, a) 1976 – 1995 (Rogers *et al*. 1998), b) 1976 – 2009 and c) 1976 – 2009, with the inclusion of the 'super group' re-classification (see Methods for category definitions).

	a) 1976 - 1995	**b) 1976 - 2009**	**c) 1976 – 2009 (inc. super group classification)**
# Badgers	475	743	743
'Non-Movers'	56.2%	56.1 %	58.0%
'Movers':	43.8 %	43.9 %	42.0 %
'Occasional Movers'	73.1 %	71.7 %	71.7 %
'Frequent Movers'	4.8 %	3.1 %	2.9 %
'Permanent Movers'	22.1 %	25.2 %	25.6 %

6.5 DISCUSSION

Using quantitative measures this study has shown that four methods available for the investigation of mammalian social organisation, movement patterns and contact rates provided comparable data. However, differences arose in the level of detail that was returned and hence conclusions that can be drawn, in particular, in relation to overall population connectivity. Those methods, such as radio-telemetry and proximity loggers, that have greater spatial and/or temporal resolution especially in relation to individual-

level behaviours were found to produce networks with a higher overall degree of population connectivity. This is due to intricate movement and contact patterns that occur on a day-to-day basis, including comparatively rare between-group encounters, only being consistently detected by such methods and only the proximity loggers detecting 'actual' contacts. Also, in the case of group-living animals, for which there may be an underlying within-group sub-structure, links between residents of the same social group may differ in strength and in some cases not all "group mates" may encounter each other. Thus, methods that assume shared group membership as a connection between individuals may over-estimate the strength of such connections for certain group-living species (like badgers), and in turn miss out on the finer scale detail that may underpin ecological, evolutionary and disease dynamics. Lower resolution methods such as baitmarking and capture-mark-recapture did, however, identify areas that may be of particular interest for more in-depth study, for example, where there is a greater level of connectivity between groups than would normally be expected. Here the findings are discussed in terms of reviewing the different methods, with their biological importance analysed and discussed in **Chapters 3-5**.

Results from baitmarking indicated a lower level of population connectivity compared to other methods. This may be because the data represents a 'snap-shot' of the population's behaviour, limited to the ten-day period over which the marked bait was fed. Thus, the probability of detecting an extra-group movement, even if they were comparatively frequent, is relatively low. Also, the level of extra-group movement may be underestimated as, for the most part, individuals are unlikely to be advertising their presence in the territories of neighbouring groups. That said however, there was a consistent low level of bait returns found in latrines within the territories of foreign social groups, which implies that extra-territorial movements may be occurring regularly if they can be detected over this relatively coarse level. Whilst the motivation behind leaving faeces in another group's territory is unclear, it is known that much information is conveyed between badgers through scent marking (Buesching, Stopka & Macdonald 2003). Thus, it seems likely that the individual is actively signalling, for example breeding status, to members of the other group (Palphramand & White 2007). It is important to note that scent marking can also take place without defecating (Stewart *et al*. 2002), so even this 'snap-shot' of information being provided, is just a partial picture. The method also relies on the ability to locate the faeces and the markers and thus may be subject to some degree of observer error. The primary value of baitmarking

is likely to be to provide the general pattern of spatial organisation among animal social groups, be that for ecological research or commercial mitigation strategies (Delahay *et al*. 2000b). However, it does provide a method of alerting us to areas where interesting movement patterns, contacts and social interactions may be occurring, for example, where the territories of two or more neighbouring groups or individuals appear to be overlapping or where the territory of a group cannot be assigned an MCP due to the inconclusive results from baitmarking. By flagging up such instances, methods that provide data with a greater spatial and temporal resolution could be used to investigate the more complex and intricate ways in which individual animals in a group utilise their available habitat and interact with conspecifics on a day-to-day basis.

I extended the capture-mark-recapture (CMR) study of Rogers *et al*. (1998) and found movement values to be very similar with the inclusion of more than ten years of additional data. In addition, the results were found to be similar with the inclusion of the 'super group' category. This is perhaps surprising as it would have been expected that the proportion of 'movers' in the population would decrease. This is because movement within what was previously classified as two separate territories would be classed as no movement under the supergroup classification due to the close ties between the groups and shared space utilisation. Whilst this method is likely to be accurate and reliable in terms of detecting permanent dispersal events, it does seem unlikely that non-permanent movements between groups have stayed at the same level over many years. The result most likely reflects the inability of the method to detect intricate movement patterns at a defined spatial scale. This is highlighted by the comparison of the individual-level network produced from the capture-mark-recapture data and that produced from the proximity logger data, with the latter showing a higher degree of population connectivity than the former. The capture-mark-recapture data did appear to overestimate the number of contacts between group members as the proximity loggers suggested some level of within group sub-structure where all individuals did not have an equal probability of encountering each other, as discussed below. However, the detection of this number of 'occasional movers' by such a coarse scale method suggests that there may be greater levels of contact and connectedness between badger social groups than is generally perceived and warrants further investigation. Again, capture-mark-recapture data cannot reveal the causes or motivation behind such movements. However, traps were placed at setts that were well within territorial boundaries so badgers caught here were not simply making foraging or prospecting incursions but

were more likely to be involved in some degree of social interaction. A limitation is that not all individuals in the population are equally 'trappable' and studies have shown that there are distinct 'trap-happy' and 'trap-shy' animals (Tuyttens *et al*. 1999). However, this may be less of a problem in other examples of CMR where resampling is easier, in particular, with studies that do not require physical recapture of the animal but rather a re-sighting event. A common example is the study of migratory birds marked with rings at one site that can then be read through binoculars when the birds arrive at their stopover/ staging and migration sites (Schaub *et al*. 2001). In other cases, such as marine mammals and whale sharks, initial capture may not even be necessary as individuals can be identified on sighting events or computer software can be used to identify individuals from photographs of their unique fin and/or fluke shapes or spot patterns (The Dolphin Project: Lapolla 2005; EUROPHLUKES: Evans 2003; ECOCEAN Whale Shark Photoidentification Library: Arzoumanian, Holmberg & Norman 2005). Ultimately, the important consideration in all CMR studies is the resampling effort, although it might be difficult to completely eradicate biases due to individual behaviours.

Tracking animals using radio-telemetry allows the study of individual-level movement patterns with a comparatively high spatial resolution. Comparison of the weighted networks produced from the proximity loggers that collected data over the whole year, and the radio-telemetry data from the same individuals that were collected four times for one-month periods spread across the year show them to be very similar in terms of both the individuals that they were connected to and also in the strength of the ties. Thus, although fewer data were collected using radio-telemetry the study did appear to provide a representative sample of badger behaviour. The limitation with radio-telemetry is likely to arise when it is necessary to sample above-ground movement and ranging patterns during the night, which is considerably more difficult than locating the animals when they are resting in their setts during the day. It is highly unlikely that the network produced from radio-telemetry data would detect the level of comparatively rare between-group contacts as detected by the proximity devices. Also, radio-tracking requires considerable effort to track a small number of animals for a limited amount of time, with the researchers being in close proximity to them and thus potentially influencing their behaviours. It should also be noted that the two networks may align so well due to the highly structured social and territorial system of the Eurasian badger in the UK. One could imagine that in less structured systems, such as social foragers with

their fission-fusion group dynamics, radio-telemetry would be much less accurate in inferring associations between individuals. Fission-fusion social systems, in which members of a social community form frequently changing subgroups, occur in a number of mammalian taxa including spotted hyenas *Crocuta crocuta* (Holekamp *et al*. 1997), bottlenose dolphins *Tursiops truncatus* (Lusseau *et al*. 2006), spider monkeys *Ateles spp.* (Symington 1990) and chimpanzees *Pan spp.* (Lehmann, Korstjens & Dunbar 2007). Whilst these animals may live in a tightly bonded group that defends a communal home range they regularly split into smaller groups that may change frequently in size and composition, perhaps to alleviate within-group competition. The group as a whole is rarely seen together in one place, hence radio-tracking a sub-sample of individuals in the smaller groups is unlikely to accurately describe their ties with all other group members.

Using the locational data collected from radio-telemetry studies, in addition to insights into movement patterns, studies have inferred patterns of indirect and direct contact measured using shared space use and proximity. Böhm *et al*. (2008) used radio-tracking data to quantify relative rates of proximity interaction in a population of badgers. However, due to the labour intensity of this method, 11 individuals were tracked in each season of the year, aiming for four nights comprising of six hours of tracking per individual per season. Contact between individuals was inferred when an animal's location was recorded every five minutes within a 50 m grid square (at the maximum resolution level), prompting the authors to conclude that between-group contacts were rare. However, we should perhaps exercise caution when interpreting such data as there is a possibility that a large proportion of these contacts were simply not detected due to the spatial resolution limitations of this method and the small sample size and relatively short study period.

Whilst outside the scope of this study, previous molecular genetic methods carried out on the Woodchester Park badger population have shown similar patterns to the more high-resolution methods, inferring that between-group contacts occur more frequently than is generally perceived. When carried out over a length of time this technique allows quantification of within-population dispersal patterns by comparing the fine-scale genetic structure of groups by means of genetic kinship measures and spatial distance, as demonstrated by Frantz *et al*. (2009). However, it should be noted that although extra-group paternity may be common, the incursions that lead to them may

still be comparatively rare. There are examples of samples being taken passively using snagged hair and faeces (Frantz *et al*. 2003, 2004), but samples provide only a snap-shot of behaviour and cannot be used to tease out intricate day-to-day patterns.

It goes without saying that the proximity loggers recorded a vast amount of information – more so than the other methods as they were continuously operational and provided data on 'actual' interactions. In the case of social group-living animals this may include both within and between-group contacts that are likely to differ significantly in their frequencies and durations. Between-group contacts may occur relatively infrequently and the probability of detecting them in the field, using, for example, radio-telemetry, is low but can be achieved using the proximity loggers. Thus, the data from such devices is likely to show a greater level of population connectivity, as demonstrated in this case study using the Eurasian badger, compared to other methods. Another advantage is the high-degree of temporal resolution in the data collected that allows specific questions to be addressed relating to the time of day that contacts are most likely to occur. This could be useful information when considering disease dynamics, and informing management practices.

Assessing the level of connectivity is highly informative both for addressing ecological and evolutionary-based questions, for example, those relating to sociality and information transfer and also for understanding the dynamics and transmission of disease. For example, in the case of the badger contact rates between animals from different social groups are likely to have a strong influence on how disease is spread both within the badger population and also to cattle. Modelling disease dynamics using lower levels of group connectivity derived from capture-mark-recapture or radio-telemetry data may provide misleading results and predictions. Proximity loggers have only recently become commercially available but have been widely employed in the study of disease dynamics in free-ranging animals (see references in Table 6.1). However, their utility in other biological contexts should not be undervalued as there is greater scope for these devices to be used in studies relating to behavioural ecology and sociobiology. Whilst at present proximity detection devices are perhaps the 'gold-standard' for recording interactions between individuals they do have a limited degree of spatial resolution. Whilst location can be inferred based on which animals are interacting, from using static devices at specific locations, or by conventional radio-

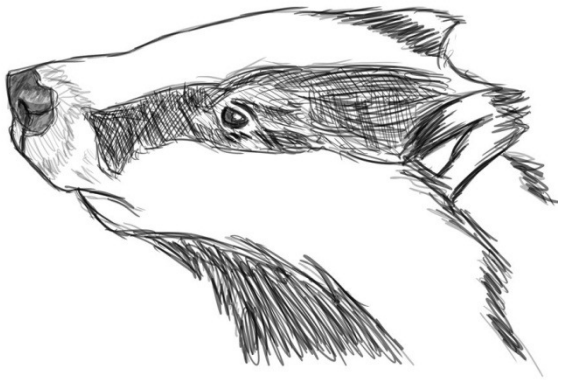
telemetry, if new generations of the devices incorporated a GPS function then the data collected would be unparalleled in terms of combined spatial and temporal resolution.

In terms of conclusions drawn from the data provided by the different methods employed there was a large degree of overall agreement at both the group and the individual-level. However, as the spatial and/or temporal resolution of the methods increased (from baitmarking to proximity loggers) more intricate patterns of movement were likely to be detected, in particular, comparatively rare between-group contacts, with associated implications for the overall level of population connectivity that is assigned. Also, these devices have allowed a within-group sub-structure to be identified showing that not all animals in a social group interact with each other to the same extent, as is assumed by some other methods, which may have implications for the prevalence of disease in the group. In the case of fission-fusion societies where individuals form frequently changing sub-groups, proximity loggers provide the only method to reliably describe continually changing association patterns. Current understanding of the social organisation of badgers is largely based on territorial delineation from baitmarking and radio-telemetry studies with estimates of interactions being inferred from home range overlap between individuals (Delahay *et al*. 2000b; Böhm *et al*. 2007), studies on the genetic variation within badger populations (Carpenter *et al*. 2005; Dugdale *et al*. 2008) and capture-mark-recapture studies (Rogers *et al*. 2008). Such methods have low resolution in comparison to the continuous data from the proximity loggers, so the general level of contact between social groups would have been underestimated. Capture-mark-recapture studies have quantified the degree of permanent dispersal between social groups but are likely to have underestimated the level of everyday background non-permanent movement and contact between groups. Radio-telemetry studies have detected both types of movement but due to the labour-intensity of this method both sample sizes and time periods have been limited, making it difficult to extrapolate findings to the population level. These limitations have implications for the understanding of how disease is spread through the population, and new values may be informative for parameterising a new generation of disease transmission models.

This study has shown that, in general terms, all of the methods investigated appear to provide biologically representative and meaningful data. Choosing the most appropriate will depend on the study species (e.g. whether they are readily observable or have a

stable social structure), the overall aims and budget of the investigation and the spatial/ temporal detail that is required. However, an important consideration for all methods is that a large enough representative sample of individuals in the population is used. This will maximise the chances of recording reliable data, detecting representative patterns of behaviour both in terms of individual differences and also population-level extrapolation and for allowing robust statistical assessment. In the case of some social animals this could include the detection of comparatively rare between-group and/or long range contacts/ dispersal events.

Chapter 7. General Discussion



7.1 OVERVIEW

In this thesis I have explored the space use and interaction patterns of Eurasian badgers (*Meles meles*) in a high-density population in the South-West of England using state-of-the-art technology. I have presented evidence from the field that badgers exhibit heterogeneities in space use and contact patterns at the social group, demographic and individual level, with patterns varying significantly across the seasons. The findings suggest that the social structure and associated behaviours of a badger population are likely to have a profound influence on bovine tuberculosis (bTB) disease dynamics and subsequently on management strategies and their outcomes. Thus, the unpredictable patterns of disease observed in some badger populations (e.g. Delahay *et al*. 2000a) are likely to reflect a complex mix of behavioural, social and spatial phenomenon. I found that behavioural differences across seasons in relation to demographics were one of the most predictable factors and appeared to be related to mating strategies and changes in the availability of food resources. After accounting for individual-level differences in relation to factors such as age and sex, considerable within-individual variation in space use and contact patterns remained. I also found evidence to suggest that those animals testing positive for bTB were consistently different in their behaviour both in terms of their space use and their interaction patterns with animals from their own, and from foreign social groups. Due to the limitations of bTB diagnostic tests at present (Chambers *et al*. 2009), the irregularity of capturing and testing study animals together with the intermittent nature of excretion in infected animals (which influences the likelihood of a culture-positive test result) it was difficult to draw a causative link for the observed differences in behaviour. It may be that the bTB test-positive individuals were behaving differently because they had disease, they may have contracted the infection due to their behaviours, or they could have been marginalised within the group by other members. This is a topic that merits further long-term study, and as our understanding of these individual-level causal links improves so too will our understanding of bTB disease dynamics and the effectiveness of potential management strategies. Whilst it is an appealing idea to be able to target ‘high-risk’ individuals for removal in a population in order to control disease spread, this is limited by the ability to detect these animals in the population. The non-predictable nature of badger behaviour may also pose problems for understanding how individuals will respond to social perturbation e.g. from culling practices and the proportion of a population that would require vaccination in order for it to be effective.

I began this thesis with a review of the different pathways for transmission of bTB within the badger population and their relative significance. The principal route of infection among badgers appears to be via the respiratory system, followed by infection from bite wounding (Cheeseman, Wilesmith & Stuart 1989). Thus, as identified in **Chapter 1**, rates of within and between-group contact are likely to exert a profound influence on the dynamics of the disease within a badger population and, in turn, a strong influence on the transmission of bTB to cattle. This is not to say, however, that badgers are solely responsible (or perhaps even highly responsible) for the maintenance of the disease in domestic cattle, with husbandry and movement practices playing their role, as discussed later. My personal interests lie more in the applied sciences, where research can be used to inform conservation strategies and/or management practices. Thus, whilst I have put emphasis on the implications for disease transmission and management I hope this has not detracted from the findings that are also interesting from a purely behaviourial point of view and have hopefully furthered the understanding of badger social organisation.

Having identified the importance of close direct contacts in the transmission of the disease, **Chapter 2** introduced the recently-developed technology, proximity loggers that provided me with the opportunity to quantitatively describe these contacts and study individual movement patterns using radio-telemetry. These devices, mounted onto leather collars, were employed in all aspects of this study. Devices for detecting interactions between free-ranging individuals are a rapidly evolving technology, with growing popularity across a range of disciplines having been deployed on species from sharks (Guttridge *et al*. 2010) to cattle (Böhm, Hutchings & White 2009). Proximity loggers manufactured by Sirtrack Tracking Solutions (Havelock, New Zealand) are increasingly being used to record interactions between animals and quantitatively describe their contact networks. Such information can be used to address important ecological and evolutionary questions, for example, relating to social structure and disease epidemiology. However, the accuracy and reliability of data collected by these devices was largely un-assessed prior to this investigation. In collaboration with a fellow researcher, using laboratory- and field-based studies I made five recommendations for the effective use of proximity loggers in the study of animal contact patterns. Sirtrack have expressed an interest in incorporating these recommendations into their instruction manual that accompanies the devices. In addition to quantifying inter and intra-species contact, proximity detection devices may

also be used to measure the presence or absence of animals in nest sites, dens and other places of interest, or simply to measure how often individual animals pass fixed points.

In **Chapter 3** I showed that variations in space use patterns correlated significantly with both demographic factors and disease status across seasons. In addition to sex and age effects that were likely to be related to mating behaviours, I found that bTB test-positive animals were consistently more likely to spend a greater proportion of their time using outlier, rather than main, setts throughout the year. After accounting for the individual-level factors above, significant variation between individuals still remained, which together with the demographic effects was found to be highly repeatable across seasons. I speculated that the differences in behaviour were related to an individual's movement and contact rate patterns, which set the scene for the subsequent chapters. This study provides novel support for the association between heterogeneity in the behaviour of individuals, whether this was influenced by demographics or potentially more intrinsic factors, and the transmission of disease.

In **Chapters 4 & 5** I presented the most comprehensive studies of contact patterns between badgers to date and further explored the idea of heterogeneities in behaviour. I began in **Chapter 4** by relating within and between-group contact patterns and social network position to demographic factors and explored the insights that this provided into badger social organisation. Contact rates and durations between individuals in the same social group were frequent, predominately occurring during the day time. On the whole, animals within a group were not found to interact assortatively in terms of age and sex class, but there were differences that could largely be related to mating behaviours. Younger individuals were found to contact a greater number of animals for longer, which may facilitate integration into the group. Adult females appeared to segregate themselves after giving birth, perhaps to reduce infanticide risks, with no evidence for allo-parental care from other group members. Between-group connections were largely nocturnal and driven by reproductive behaviour: contacts peaked in line with known breeding seasons and had higher probability of involving males and females, with males apparently initiating more of these contacts. I also found individually consistent differences in contact behaviour (both within and among social groups) that were independent of age and sex and that were highly repeatable across seasons. In addition, there was evidence of dispersal events and social group fusion involving prolonged diurnal interactions, suggesting social structure may be more

dynamic than often assumed. Standardised contact measures may mask some behavioural trends as my results suggest that not all animals in a group interact with others to the same extent and contacts are unlikely to occur every day. Thus, the use of social network analysis seems to be the most representative analytical method that can detect trends and account for non-independence of data points.

In **Chapter 5** I focused on the topical issue of how a badger's social network position and space use patterns may influence its risk of contracting and transmitting bTB. The findings of this chapter may have implications for our understanding of the manner in which disease is spread and for the effectiveness of proposed control strategies. I found that animals testing positive for bTB displayed different contact patterns in terms of both within and between-group interactions compared to those individuals that tested negative. My findings here were complimentary to those in **Chapter 3** with test-positive animals both using outlier setts to a greater extent, and interacting less frequently with the test-negative group members. The flow-betweenness scores of bTB test-positive animals were also found to be higher, suggesting that they encountered individuals from more than one other social group. This may make them influential in the spread of bTB through the population as a whole and potentially also onto cattle. However, as these test-positive individuals become more isolated from other group members, badger stable social organisation may be acting to limit the spread of bTB in the population as a whole. These findings raise questions for culling practices that have been shown to disrupt this social structure and hence may increase transmission rates to unpredictable levels. As in **Chapter 3**, we can only speculate as to the causal mechanism(s) underlying these results; did animals behave differently because of their infection status, did their intrinsic behaviours put them at a greater risk of contracting disease or were the differences driven by uninfected group members? At this time it is not possible to provide an affirmative answer due to the ability of infection to go undetected, particularly in the early stages. This is an area that would benefit from being pursued by future studies.

The thesis concluded in **Chapter 6** with a quantitative review of methods that are employed in the study of space use, movement and contact patterns in free-ranging animals. Here, using comparable quantitative data collected as part of a long-term field study of the high-density badger population at Woodchester Park, Gloucestershire, I compared four methods that are commonly used to study such behaviours: baitmarking,

capture-mark-recapture, radio-telemetry and proximity detection devices. I highlighted the strengths and weaknesses of each method, focusing on the level of data resolution that they provided at both the group- and the individual-level and the associated conclusions that were drawn. Whilst the different approaches were found to broadly agree with one another, proximity loggers stood out in terms of the continuous, high-resolution data that they collected remotely. Thus, choosing the most appropriate cost- and time-effective method (or combination of methods) will largely depend on the aims of the investigation, the study species and the degree of spatial and temporal resolution that is required of the data for the necessary conclusions to be drawn.

As I suspect is the case for most research endeavours, this work has generated as many (if not more) questions than it has answered and thus represents only a foundation of understanding in the study of contact rates and connectivity of badger populations upon which future work could build. In addition to the continued investigation of interactions between badgers, including on different populations across their geographical range, there are a number of topics outlined in the following discussion that may merit further study.

7.2 Badger Social Organisation

Eurasian badgers exhibit variation in their social organisation from pair- and group-living across their geographic range, which extends from the UK through mainland Europe and into Asia (Johnson, Macdonald & Dickman 2000). In the UK the majority of social groups contain from two-to-eight adults of mixed age and sex (although as many as 19 have been recorded; da Silva, Woodroffe & Macdonald 1993) and within these populations there were examples of badgers thriving in different habitats, such as, those that have successfully adapted to life in highly urbanised areas (e.g. Huck *et al*. 2008). There is still considerable debate surrounding the spatio-social structure of badger populations, its origins and the functional role of their sociality (reviewed in Roper 2010; **Chapter 4**). Eurasian badgers appear to be facultatively social, forming high-density groups in areas that are likely to be rich in food resources and mating opportunities but perhaps limited in the space available for the construction of new den sites. Badger setts are valuable resources that when maintained appear to last for centuries and, as they are energetically costly and time consuming to construct (Roper 1992b), there appear to be benefits to sharing a sett that is actively defended from

others. There are examples of other animals, such as the Red fox (*Vulpes vulpes*), that have a flexible social system comparable to that of badgers (Johnson *et al*. 2000), but the costs and benefits of natal philopatry vs. dispersal, e.g. the inheritance of breeding dominance, appear to be clearer in these animals compared to the badger.

In this thesis I have found the most significant correlates of studied social behaviours to be season and disease (discussed in the next section), with demographic factors playing comparatively minor roles at times. I found little evidence of cooperative behaviours, such as allo-parental care, which have been documented in other group-living mammals that show moderately high levels of genetic relatedness (e.g. Jennions & MacDonald 1994; Clutton-Brock *et al*. 2001). Rather, adult females appeared to segregate themselves from other group members after giving birth, perhaps to reduce aggression towards the cubs. Group-living appears to be costly for females with some degree of reproductive suppression experienced by subordinate individuals (Woodroffe & Macdonald 1995). Thus, to reduce this cost, females act to ensure that their young survive (for review in mammals see; Stockley & Bro-Jørgensen 2011). It would be interesting to further study patterns of allo-parental care exhibited by badger populations in other parts of their geographic range (e.g. in the multi-male/multi-female groups in Sweden) where selective pressures may be different, for example risk of predation from large carnivores (e.g. lynx *Lynx lynx*, and wolverines *Gulo gulo*). Whilst badgers in a group do display a level of relatedness, between-group contacts, particularly with those from neighbouring groups, were found to be comparatively frequent and most likely act to reduce inbreeding depression (Carpenter *et al*. 2005; Dugdale *et al*. 2008). The observed stable social organisation of high-density populations (e.g. Woodroffe *et al*. 2009) that also display low levels of dispersal may be related to a high level of habitat saturation with some populations approaching carrying capacity. Thus, dispersal in high-density populations may in fact prove to be costly and is not likely to be the favoured strategy when it appears that extraterritorial matings can provide the same reproductive advantages. However, in **Chapter 4** I presented evidence that suggests badger social structure may display some dynamic aspects with two social groups appearing to experience some degree of fusion, perhaps related to sex biases in the groups.

After taking into account individual-level differences that were attributable to factors such as age, sex and bTB status, significant variation remained in sett use and contact

patterns between individuals, which was consistent over seasons. The theoretical basis behind heterogeneity in the behavioural patterns of individuals in animal populations is well documented (see e.g. Bolnick *et al*. 2003), but it is not universally considered in wildlife studies. Indeed, whilst some studies refer to consistently expressed behavioural characteristics that can differ between individuals as 'personality', this is perhaps considered too tenuous a statement in other studies. However, there is amassing evidence for the presence of different categories of individuals in animal populations that display repeatable behaviours, for example, 'bolder' individuals that display more exploratory behaviours and/or initiate a greater number of contacts (reviewed in Bell, Hankison & Laskowski 2009). Indeed such traits may increase fitness measures of an individual, for example, greater mating success, particularly in males (e.g. Smith & Blumstein 2008), but can also incur a survival cost.

7.3 Disease Transmission

Infectious diseases can be transmitted indirectly through environmental contamination with parasite-laden material such as bedding or faeces or directly through interactions (reviewed in **Chapter 1**). My studies focussed on the route of direct pathogen transmission when in close proximity as the high incidence of pulmonary infection found during *post mortem* examinations of bTB-infected badgers suggests that infectious aerosols may be a primary route of badger-to-badger transmission (e.g. Clifton-Hadley, Wilesmith & Stuart 1993). When parasite transmission is a function of direct contacts, then prevalence is likely to increase with group size or population density (McCallum, Barlow & Hone 2001). However, this has not been shown to be the case with bTB in badgers. This could be because indirect transmission plays a more significant role than is generally perceived or because heterogeneities in host behaviour across time and space have a strong influence on how disease is transmitted and who becomes infected (e.g. Drewe 2009). Heterogeneities in behavioural patterns (e.g. space use and contact rates) of individuals in animal populations have potentially important ecological, evolutionary and conservation implications (Bolnick *et al*. 2003). In this thesis I have drawn attention to differences between age and sex classes and found that they largely appear to correlate with mating behaviours and social processes at different times of the year. In addition, there is much interest into the existence of different strategies and behaviours exhibited in a population, with the notion of animal 'personalities'; behaviour that varies among individuals, but is consistent across time

and/or contexts within individuals (Stamps & Groothuis 2010). However, to explore this further would require further exploration and quantification of individual heterogeneities.

The role of animal behaviour in the transmission of parasites and pathogens has long been recognised as influencing both the exposure to infectious agents and also individual susceptibility to infection once exposed. Hawley & Altizer (2011) propose that these two mechanisms may act in concert in natural populations, whereby individuals who are most exposed to infectious agents or have the most contact with conspecifics are also the most susceptible or infectious. Thus, animal behaviour may serve as a key link between these two primary steps generating new infections in a host population. In **Chapter 5** I showed that individuals testing positive for bTB interacted significantly less with their negative counterparts than would be expected by chance and less than the test-negative animals interacted with each other. At the same time, these test-positive individuals encountered animals from a greater number of different social groups compared to the others. Thus, whilst there appear to be animals displaying different behaviours that may be more influential in disease transmission (positive co-variation), it also appears that the relatively stable social organisation of high-density badger populations can act to limit the spread of bTB through the population (negative co-variation). However, due to bTB test intervals and the ability of infection to go undetected, particularly in the early stages of the disease, it was not possible to investigate a causal link between differences in the behaviours of positive and negative badgers as we cannot determine at what point in time they had become infected. Thus, we can only speculate as to whether intrinsic behaviours of an individual had influenced its risk of contracting disease, whether infection was affecting an animal's behaviours, or whether differences were driven by avoidance measures from other group members. Future longer-term studies should aim to quantify individual-level behaviours before becoming infected with bTB in order to compare them with those exhibited post-infection, which will allow 'cause and effect' to be established.

7.4 Other potentially important factors

The second and third stages in the process of infectious disease transmission are the successful transfer of infectious agents from the infected to the susceptible animal and the subsequent development of the disease. This is likely to be strongly influenced by

individual-level factors that affect an individual's susceptibility to contracting the disease. I have demonstrated that whilst differences in the behaviours of individuals in a population may exert a strong influence on how disease is transmitted between animals, there were still patterns of infection that could not be explained. Whilst infected individuals in a group did interact significantly less with negative group members there were still opportunities for them to transmit disease to other members. In the historical data from Woodchester Park there do not appear to be incidences where all group members test positive for bTB at the same time (although the insensitive nature of the diagnostic tests should be taken into consideration). This may be related to some intrinsic characteristics of the individual, with a number of potentially important and interacting factors that may influence an animal's risk of becoming infected by a pathogenic agent and this developing into disease. The discipline of ecological immunology examines the underlying causes of variation in immune function between individuals or populations (see Schulenburg *et al*. 2009; Hawley & Altizer 2011). One suggestion is that disease resistance constitutes a costly investment that must be traded off with other traits such as reproduction, sexual ornamentation and dispersal.

In vertebrates, immune system functioning (and its effectiveness) depends on the innate availability of particular major histocompatibility (MHC) alleles and also previous exposure to parasites (Woelfing *et al*. 2009). This can vary between individuals and can also be influenced by a number of other factors, for example, stress and senescence. Stress in wild animals can be induced by a multitude of factors, for example, human disturbance, severe weather, food restrictions or exposure to contaminants or parasites. Stressors can cause the release of adrenal glucocorticoids (GC), primarily cortisol or corticosterone from the adrenal glands of the animal (Norris 1996). The cost of elevated GCs is well documented, with effects including a decrease in overall health and reduced individual growth, changes in metabolism, delayed repair of tissues or healing and immunosuppression, resulting in an increased incidence of disease in these animals (e.g. Acevedo-Whitehouse & Duffus 2009). It would be informative to assess baseline variation in cortisol levels within both undisturbed badger populations and also those that have been subject to management practices. This could be achieved non-invasively from hair or faecal samples (e.g. von der Ohe & Servheen 2002), as capturing the animals is likely to elicit an immediate stress-response that could obscure the results.

When immunological and behavioural effects co-occur they may interact additively or synergistically. For example, observed patterns of sex-biased infection may be related to the influence of testosterone on disease susceptibility and spread (Zuk & McKean 1996). In addition to having important effects on transmission-relevant behaviours such as contact rates and aggressiveness, testosterone has also been shown to have effects at the immunological level. In **Chapter 4** I presented evidence to suggest that male badgers may be driving some of the between-group contacts and current studies (J. Graham and A. Tomlinson, *unpublished data*) have also found that males may have higher bTB-related mortality than females. There are a number of possible explanations for this. For example males may be more likely to contract disease due to their ranging patterns, and indeed radio-telemetry studies have suggested that males have larger home ranges (e.g. Tuyttens *et al*. 2000). Alternatively, it could be related to the mechanism of infection. For example, males have been found to have a greater incidence and number of bite wounds (Delahay *et al*. 2006a) and transmission in this way may result in disease progressing more rapidly possibly due to haematogenous spread (Clifton-Hadley, Wilesmith & Stuart 1993). Finally, there are a number of examples in the literature of male vertebrates being more susceptible to disease owing to high levels of testosterone, which may suppress the immune system (Zuk & McKean 1996). It may also be informative for studies to address the effect of senescence (the decrease in somatic or reproductive investment with age) on both disease susceptibility and how the age at death is related to the age of first infection, i.e. whether older badgers that contract disease have higher mortality rates than those that contract it at an earlier age and are perhaps able to up-regulate their metabolisms to combat the infection.

7.5 Management Strategies

The cost of bTB to both the farming industry and the taxpayer has increased rapidly in recent years, reaching unsustainable levels (DEFRA 2010; Butler, Lobley & Winter 2010). However, the complex issue of management strategies for both badgers and cattle has complicated remedial action, and has yet to result in a sustained reduction in bTB in cattle. It does seem however, in light of the present government's proposal for a badger cull, that more practical measures will soon be carried out (although this is likely to be a gradual year-by-year increase), whether the cull goes ahead or whether opposition causes a shift in focus to vaccination programmes and more husbandry measures. The development of successful approaches to the management of disease in

wild populations will require careful consideration of the entire host community (although whether this could be practically achieved is debatable), of the economic dimensions and of the practical challenges of successfully implementing any intervention measures (Delahay, Smith & Hutchings 2009). It is generally accepted that the best approach to managing the bTB situation is likely to be a holistic one that targets both cattle and wildlife. Such an integrated approach will comprise of multiple options that may range from culling and vaccination to preventative measures such as bio-security on farms.

Culling is frequently used as a tool for the control of diseases in wildlife populations (Wobeser 1994). It operates on the basis that disease may be eliminated if host numbers are reduced below a certain threshold required for persistence of the infection (Anderson 1991). Whilst the action of culling alone can be surrounded by controversy, this is further exemplified in the case of the badger as scientific evidence does not fully support this approach for reducing bTB levels in cattle. Indeed, in **Chapter 5** I also showed that badger social organisation may have a role to play in limiting disease transmission in the population. Thus, if it is disrupted, consequences may include an increase in disease prevalence in both badgers and cattle. Whilst early studies provided compelling evidence that comprehensive, localised badger culling operations reduced disease in cattle (Clifton-Hadley *et al*. 1995), they did not incorporate sufficient experimental controls and did not investigate the potential detrimental effects of culling in adjoining areas. This was remedied in the Randomised Badger Culling Trial (RBCT; for details see: Independent Scientific Group (ISG) Report; Bourne *et al*. 2007) and whilst their findings were also published in peer-reviewed journals the conclusions have not been universally accepted/ adopted. The RBCT showed that culling badgers may cause spatial perturbations within the population and disrupt social cohesion (Tuyttens *et al*., 2000; Woodroffe *et al*. 2006). This may result in increased immigration into culled areas, disruption of territoriality, increased ranging by individuals and mixing of social groups, which can persist for many years (reviewed in Carter *et al*. 2007). Such movements are likely to increase contacts and also the probability of disease transmission amongst badgers and between cattle and badgers, although the full extent of these consequences is unclear. As mentioned previously, stress and disturbance to an animal can act to reduce their immuno-competence. Thus, in addition to possible increases in contact rates, if animals are experiencing some degree of immune-suppression induced by culling then they may be more susceptible to contracting

disease. In the final report from the RBCT, the ISG recommended a range of policy options focussing on more stringent cattle control measures and concluded that 'badger culling cannot meaningfully contribute to the future control of cattle TB in Britain' (Bourne *et al*. 2007). However, this opinion is not universally accepted and plans are being considered at present for the removal of badgers in areas where the incidence of bTB in cattle is high and persistent. As transmission rates do not directly correlate with group size or population density, being confounded by host behaviour (Woodroffe *et al*. 2009; **Chapters 3 & 5**), simply reducing population size or density alone may be an ineffective management option in the case of badgers and bTB.

If a cull were to be successful and reduce bTB levels in cattle it would require substantial reductions in badger numbers whilst minimising the effects of perturbation. One way to limit these effects would be the use of a vaccination against the disease, although there are currently no data on the extent to which badger vaccination might influence bTB incidence in cattle. The vaccination of badgers and cattle is an attractive disease control option and works by reducing the onward transmission risk of the disease, with minimal effects on the animals themselves and their social structure. However, the development of an effective vaccine, with an appropriate strategy for its delivery, licensing and commercial availability all pose technical and regulatory challenges (Wilson, Carter & Delahay, 2011). Options for badgers include capture and manual delivery, for instance by injection or oral delivery in a bait. The human TB vaccine, Bacille Calmette-Guérin (BCG), is presently the only candidate and has been shown to be safe for use in badgers (Lesellier *et al*. 2006) and also effective when delivered as a live vaccine (Chambers *et al*. 2011). The UK Government began using the licensed injectable vaccine in the Badger Vaccine Deployment Project in July 2010 (BVDP; http://www.defra.gov.uk/fera/bvdp) to provide valuable information on the true costs and practicalities of large-scale vaccine deployment. Whilst the scope of the project was reduced from six target areas to just one, it is still working to provide training for lay-vaccinators with the National Trust, Wildlife Trusts and some people in the farming community having received training to vaccinate badgers on their land. Ultimately, delivery of a vaccination via oral bait appears to hold the best long-term prospects for deployment to badgers over a wide area (Delahay *et al*. 2003) and studies are currently underway to investigate ways of maximising uptake rates and effectiveness. However, it is likely to be a number of years before a licensed oral vaccine is available for wide-scale use.

Bio-security is an important consideration and has been shown to be highly effective in preventing badger visits to farm buildings and thus may potentially reduce the risk of bTB transmission from badgers to cattle (Tolhurst *et al.* 2008; Ward, Judge & Delahay 2010). However, cost, time and a lack of evidence that these measures reduce the risk of TB in cattle are a deterrent for farmers. A core part of bTB management strategies should also be based on minimising contact between herds (e.g. pre-movement checks etc.). The need for such measures was highlighted earlier this year when evidence emerged that some cattle farmers in the South-West and Midlands may have been illegally swapping cattle ear tags, retaining TB-positive animals in their herds and sending less productive animals to slaughter in their place (http://www.defra.gov.uk/news/2011/03/31/cattle-bovine-tb). This could have adverse effects, increasing the risk of spread of bTB to other herds in the country especially if the animals are moved long distances, and also to susceptible wildlife hosts such as badgers. Animal Health responded to this, and in April 2011 measures were put into place to tag and keep a sample of DNA from test-positive cattle for cross-checking before slaughter. Also, modern ways of life and farming methods may have a role to play in the maintenance of bTB in Britain. As the intensity of farming increases and animals are kept in confined, unnatural and/or stressful conditions they are likely to become weak and more susceptible to disease. Indeed, in recent decades there have also been major incidences with other catastrophic livestock diseases such as bovine spongiform encephalopathy (BSE) and foot and mouth disease (FMD). Matthews *et al*. (2006) found evidence that a reduced risk of bTB was associated with the management of farmland in ways favourable to wildlife conservation. Thus, for farming to remain viable, practices should also become more sustainable, with customers also being educated in the long-term benefits of buying those products that have been responsibly farmed.

Over the course of the three years that I have been carrying out this PhD investigation, management strategies have continually been changing, with a strong influence from the government. It has been interesting to follow how policies change according to public opinion, politics and science, and inevitably it is difficult to strike an even balance. It would be naïve to think that management plans could be changed based on evidence presented in this thesis. However, I would hope that points I have raised, especially concerning the importance of certain individuals, of badger social organisation and of

outlier setts to bTB transmission dynamics are mentioned and considered when discussing viable strategies for long-term management of the disease.

7.6 CONCLUSION

The work presented here brings us a step further to understanding badger social and spatial organisation and the influence that it may have on the transmission of bTB within the badger population. It builds on the work of Goodman (2007) and Böhm, Hutchings & White (2009) to provide a more complete understanding of contact rate patterns in high-density Eurasian badger populations naturally infected with bTB. However, we still lack an understanding of the causation of many of the findings. As the technology is advancing, so we can build-up long-term data sets for contact patterns, which will not only address our unanswered questions but also provide new insights into others, for example, relating to baseline dispersal and natural mortality rates. The eventual incorporation of a GPS unit into the proximity logger devices (with advances for GPS signal detection in woodland) will provide unparalleled data with both high temporal and spatial resolution. The findings of this study should be applicable to other investigations that are considering the development and importance of heterogeneities in animal behaviour at the individual, demographic and/or population level.

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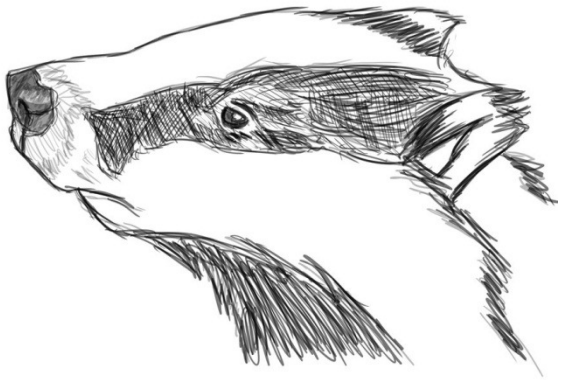

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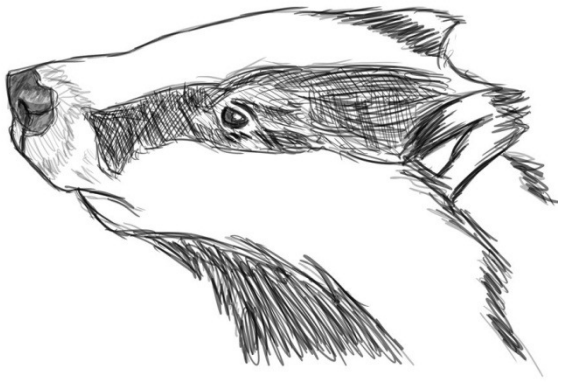
APPENDICES



APPENDIX A

The following is a guide to the R functions that accompany **Chapter 2** and were used in the preparation of data for **Chapters 4, 5 & 6**.

Raw Data

Both functions assume data are in the following format. Column names have to be as listed; otherwise the functions will throw an error. Column order must also be preserved.

Variable Descriptions

Record: unique record id logged by the loggers

Encounter: unique code of the other collar encountered

Date: calendar date of contact. Must be in the format 'dd/mm/yyyy'.

Time is the start time of the contact. Must be in format 'HH:MM:SS'

Length: Duration, in seconds, of the contact.

record	encounter	date	time	length
240	31	21/05/2009	03:48:57	1
241	31	21/05/2009	03:50:12	1
242	31	21/05/2009	03:51:28	1
243	31	21/05/2009	03:56:54	1
244	31	21/05/2009	03:58:14	673
245	31	21/05/2009	04:15:15	354
246	31	21/05/2009	04:43:00	1

‘Contactweld’ Function

Function designed to combine broken contacts that fall within a user-specified time limit into one contiguous record.

Step by Step Guide

1. Read the data table into R in the above raw data format.

2. The ‘contactweld’ function takes two arguments. Firstly the name of the dataframe containing the raw data, and then the time difference (in minutes) that will decide if records will be combined.

R Code:

```
newdata<-contactweld(dataframe,5)
```

In general, if *t* minutes are specified, records of length equal to, or less, than *t**60 seconds will be combined. Therefore, if record *x+1* starts within *t* minutes of record *x* ending, these two will be combined. If record *x+2* also starts within *t* minutes of *x+1* ending, all three will be combined. In this case the ‘start’ time of record *x* will be taken as the start, and the ‘end’ time of record *x+2* taken as the end, and the time difference between these two times calculated.

Output:

Columns 1-5 of the output will be exactly the same as the input dataframe (Raw Data), which may be useful for error checking. The new column ‘contact’ is the corrected contact time between two individuals. There is also an ‘end’ time column which one might find useful, but this is a combined date and time object so if it is exported to a spreadsheet editor one might wish to split the columns so that the date and time can be manipulated separately.

Troubleshooting:

If the function throws an error, it is most likely one of two things: Either the column names aren't exactly as above, or the date is in a format that R the function doesn't recognize.

'Matrixbuild' Function

Builds a symmetrical association matrix from raw proximity logger data.

Matrix options include specifying a matrix of Total Contact Duration or Total Frequency of Contact Events, as well as being able to only select records falling within a specified timeframe (e.g. only records from 5pm to 10pm). Matrices built by this function can be fed directly into social networking software like UCINET (http://www.analytictech.com/ucinet/) and SOCPROG (http://myweb.dal.ca/hwhitehe/social.htm).

The function automatically selects the MAXIMUM datum recorded between pairs of individuals. This can occur because two loggers within the threshold proximity should theoretically record the same duration of contact, but often will not. In cases where this discrepancy occurs, the largest value will always be the one to appear in the output matrix. The matrix will also be symmetrical in that the lower half will be a perfect reflection (duplicate) of the upper half.

N.B. This function incorporates the 'contactweld' function listed above, and so 'contactweld' must be loaded in order for 'matrixbuild' to work. It is best to familiarize yourself with the contactweld function prior to using 'matrixbuild'.

Step-by-Step Guide:

1. Set working directory where the individual data files are located.
   The function assumes each individual has its own .txt data file and that all these files are stored in the same folder. Names of data files must start with the unique animal ID, but may contain suffixes. '008p.txt' and '008pWinter.txt' are both fine.

2. Make a vector in R containing a list of all the filenames. Each one has to be in quotes, but you don't need the file extension (like .txt) e.g.

R Code:

```
listofanimals<-c('008pwinter','019psummer','010y','011y', '016p','018p','020y'…)
```

3. Make a 2-column dataframe containing unique collar IDs (see 'Raw Data' section) in the first column, and the unique animal ID in the second column. The function will use these data to match the logged ID in the raw data to the individual wearing that collar. The IDs in column 2 will be the IDs that appear in the final contact matrix and MUST match the IDs found at the start of the list of filenames in 'listofanimals'(see above).

   This dataframe should contain information on ALL individuals/collars that have been deployed. This is because you may read in data files for 30 individuals, but these files may contain contact information from >30 proximity loggers. Such situations arise when loggers are deployed but lost/never retrieved. These individuals must be represented here so that they appear in the final matrix.

Example table of unique proximity logger IDs (ID) and the corresponding unique animal ID (Individuals). Column headings can be named arbitrarily, as long as the column order is preserved.

ID	Individual
1	008p
2	067y
3	045p
4	050y
5	063p
6	043w
7	082w
8	062p
9	015p
10	012b

The dataframe above can be constructed in R with the following code. However large tables are more efficiently loaded directly from an external table.

R code:

```
collarids<-data.frame(ID=seq(10),Individual=c('033p','067y','045p'…))
```

4. Then, simply pass the vector of filenames and the dataframe of IDs to the 'matrixbuild' function:

R Code:

```
newmatrix<-matrixbuild(listofanimals,collarids)
```

5. Firstly, the function will ask for the number of characters required to extract the animal IDs from the filenames. Thus, if the file was called '008pwinter', entering '4' will extract '008p', which matches the 'Individual' in row 1 of the 'collarid' dataframe. It is important to always make sure that the IDs can be matched in this fashion.

N.B. If unique animal IDs (column 2) are of differing length (e.g. "Jake","Bob","Jo") it is advised to pad the filenames with trailing underscores ("Jake","Bob_","Jo__") so that all IDs are extracted correctly with a single argument for number of characters.

6. The function will prompt you with the name of the working directory from where the datafiles will be loaded. If the directory is correct, enter 'Y' or 'y'. If you enter 'N' or 'n' the function will terminate so that you can change the working directory.
7. If you entered 'Y', the function will tell you how many datafiles were loaded successfully. This should be the same as the number of individuals in the 'listofanimals' vector that was passes to the function.
8. You will then be prompted for the threshold number of minutes to be used by the 'contactweld' function to join broken contacts together (e.g. 5 minutes). So if you enter '5' (no quotes) the function will run the contactweld function for each of the datafiles in turn using this threshold.

NB. If you do not wish to use the contactweld function (i.e. build the contact matrix directly from the raw data, simply enter '0' (zero)

9. An output will inform you that the contactweld function is running for each of the *n* data files. Depending on the number and size of raw data files, this may take some time (1-2 minutes).

R Output:

[1] Running contactweld Function for 37 datafiles using threshold of 5 minutes

10. You will be asked if you wish to subset the data based on the time of the records. If you specify 'N', the function will move onto Step 12, otherwise you will be asked for the start and end time of the specified time window, in 24h format (e.g. 17 for 5pm, or 10 for 10am). Note that currently the function only supports subsetting to units of 1 hr i.e. not to the resolution of minutes.
11. Output will confirm the time window which you have specified e.g. if one specified '10' for start time and '12' for end time, output will show:

R Output:

[1] Subsetting Records to Those Between 10:00 and 12:00

12. At this step you can specify the metric you desire: either 'D' for total summed contact duration between individuals, or 'F' for frequency (count) of contact events, irrespective of duration. These metrics are calculated AFTER the 'contactweld' function has run.
13. Output will then inform you of the dimensions of the matrix being built. If some loggers have not been retrieved, the dimensions will typically be larger than the number of datafiles initially supplied.

R Output:

[1] Building 44 x 44 Matrix...

14. Assuming no errors, you will be informed that the matrix has been built successfully, as well as the largest contact (frequency or duration, depending on specified option)

R Output:

[1] Matrix Built Successfully...Longest Contact is 812.3 minutes

15. If you have specified time subsetting, it is possible that some of the *n* files read into R may contain no records in the specified window. If this is the case, you will be informed of a) the number of files with no records and b) the IDs of the individuals:

R Output:

[1] N.B. 2 datafiles have no records in the specified timeframe
[1] Animal IDs 013p,032y have no contacts

16. You will then be prompted for the name of the output file. Entering 'Matrix1' (no quotes), will create a file called Matrix1.csv in the working directory that can then be opened with a spreadsheet editor. The matrix will also be available to manipulate in R, as long as it is 'assigned' using '<-' e.g. in this example an object called 'newmatrix' will be created in R.

R Input:

```
newmatrix<-matrixbuild(listofanimals,collarids)
```

APPENDIX B

The following is the R code that accompanies **Chapter 2** and was used in the preparation of data for **Chapters 4, 5 & 6** – see **Appendix A** for a user guide. It is best imported into the R code editor 'Tinn-R' (http://sciviews.org/Tinn-R/).

#a) **'Contactweld' Function**

```
contactweld<-function(dataframe,threshold){
        if(missing(threshold)) stop("Please Enter Value for threshold")
contact1<-dataframe

#If threshold is zero, copy 'length' over to contact and leave data unchanged, else run
function
if(threshold==0){

        contact1$contact<-contact1$length
        contact1
        } else {
#Sort out time formatting
stamp<-paste(contact1[,3],contact1[,4])

start<-strptime(stamp,format="%d/%m/%Y %H:%M:%S",tz="GMT")
which(is.na(start))

#Add duration of contact to start time contact - gaps between these are more informative
end<-contact1$length+start
contact2<-cbind(contact1,start,end)

#Order dataframe based encounter ID, then date then date/time
contact2<-contact2[order(contact2$encounter,contact2$start),]

#Set threshold value (seconds) for time difference in contacts
t60<-threshold*60+1

#Calculate difftime for all adjacent rows in dataframe
#Difference is from end time row x and start time row x+1
bottom<-seq(1:(nrow(contact2)-1))
top<-seq(2,nrow(contact2),1)
diffs<-difftime(contact2$start[top],contact2$end[bottom],units="secs")
```

```
diffs2<-c(0,diffs)
contact3<-cbind(contact2,diffs2)


#Does ID in row x match ID in x-1 & is time difference < threshold?
#Means rows with TRUE are part of the record above, rows with FALSE are either a
new badger (encounter) or
#the record started more than "threshold" minutes after the previous record ended
matches<-contact3[top,2]==contact3[bottom,2] & contact3[top,8]<t60

#Add matches. First record will always be false as nothing precedes it
contact4<-cbind(contact3,(c(FALSE,matches)))

#Size of dataframe = last row index
size<-nrow(contact4)


#If FALSE, newstart gets the start time in the same row (new record)
#If TRUE, must take start time for the previous row - which will now be in 'newstart' on
the previous row
newstart<-rep(0,size)
for (k in 1:size){
if(contact4[k,9]==FALSE){newstart[k]<-contact4$start[k]} else {newstart[k]<-
newstart[(k-1)]}
}

#Convert newstart back to times and dates
#R uses 1st Jan 1970 as baseline
newstart<-as.POSIXlt(newstart,origin='1970-01-01')
contact5<-cbind(contact4,newstart)


#Work out contact duration for every row between 'end' time and 'newstart'
contact<-difftime(contact5$end,contact5$newstart)
contact6<-cbind(contact5,contact)

#Need code to remove repeated observations, based on boundaries between logical
operators
#If FALSE is followed by FALSE, this is a standalone record (i.e. not the start of a run
of trues), so keep it
#If TRUE followed by FALSE, this is the last in a run of a chain of contacts, so keep
#If FALSE then TRUE, this is the start of a chain, and we only want the end of the
chain, so discard
#Everything else (like TRUE followed by TRUE) is the middle of a chain, so discard
keepcode<-rep(0,size)
for (h in 1:(size-1)){
```

```
        if (contact6[h,9]==FALSE & contact6[(h+1),9]==FALSE){keepcode[h]<-1}
else if (contact6[h,9]==TRUE & contact6[(h+1),9]==FALSE){keepcode[h]<-1} else if
(contact6[h,9]==FALSE & contact6[(h+1),9]==TRUE){keepcode[h]<-0} else
{keepcode[h]<-0}
        }

#Separate code for last value as cannot have "last row + 1"
#Will always have to keep it, as it will either be false or the end of a run of trues
keepcode[size]<-1


contact7<-cbind(contact6,keepcode)

#Remove '0' values that are duplicates
contact8<-subset(contact7,keepcode==1)
contact8

}

}
```

#b) **'Matrixbuild' Function**

```
matrixbuild<-function(idfiles,collarids){

chars<-readline('Select number of letters of filenames to use to match individual IDs.
e.g. 4 to select 008p from 008pWinter...')
ids<-substr(idfiles,1,chars)
nids=length(ids)

collarframe<-data.frame(ID=ids)
collarframe$collar<-collarids[,1][match(collarframe[,1],collarids[,2])]

#Throw error if there are any NAs
if(length(which(is.na(collarframe$collar)>0))) stop("Not All Collar IDs Matched")

#Read In datafiles
work<-getwd()

filesyes<-readline((paste("Data will be loaded from",work,"...Is this
correct?...(Y/N)...")))

if(filesyes %in% c("n","N","no","No")) {stop("Function terminated")} else if (!filesyes
%in% c("Y","y","yes","Yes"))
{stop("Function terminated")} else {
```

```
#Read in Datafiles
#Suppress Errors with 'try'
#If dataframe not found, throw an error and return name of file causing issues
for (k in 1:nids){
try(eval(parse(text=paste("t",k,"<-
read.table('",work,"/",idfiles[k],".txt',header=T)",sep=""))),silent=TRUE)
eval(parse(text=paste("if(class(try(nrow(t",k,")<1,silent=TRUE)) == 'try-error') stop
('Problem with Datafile ",idfiles[k]," : Check for Spaces in
File',call.=FALSE)",sep="")))
}

print(paste(nids,"datafiles loaded"),quote=FALSE)

##Run Contactweld Function for Each Datafile

cwthreshold<-readline("Enter Number of Minutes for 'contactweld' Function....")

#If non-zero argument passed to cwthreshold, run contactweld, otherwise just pass all
raw data t[k]s to b[k]s
if (cwthreshold !=0){
        print(paste("Running contactweld Function for",nids,"datafiles using threshold
of",cwthreshold,"minutes"),quote=FALSE)
   } else {
                        print(paste("Contactweld Function not employed. Matrix will be
built from raw data..."),quote=FALSE)
                        }

for(k in 1:nids){
try(eval(parse(text=paste("b",k,"<-
contactweld(t",k,",",cwthreshold,")",sep=""))),silent=TRUE)
eval(parse(text=paste("if(class(try(nrow(b",k,")<1,silent=TRUE)) == 'try-error') stop
('Badger Function for ",idfiles[k]," Has Failed.',call.=FALSE)",sep="")))
                }


######
#Day and Night Start
#######


timeyes<-readline("Do You Want to Subset Data Based on Time?...(Y/N)...")

if(timeyes %in% c("Y","y","yes","Yes")){
```

```
recstart<-readline("Enter Start Hour in 24h Clock Format (e.g. 17 for 5pm or 8 for
8am)...")
recend<-readline("Enter End Hour in 24h Clock Format (e.g. 17 for 5pm or 8 for
8am)...")

if(recstart==recend){stop("Error: Start and End Time Cannot Be Identical")}

recstartinfo<-paste(recstart,":00",sep="")
recendinfo<-paste(recend,":00",sep="")

print(paste("Subsetting Records to Those
Between",recstartinfo,"and",recendinfo),quote=FALSE)

#####
#Subsetting Based on Hours
######

#Calculate Hour of Time
for (k in 1:nids){
eval(parse(text=paste("framehour<-as.POSIXlt(b",k,"$newstart)",sep="")))
eval(parse(text=paste("b",k,"$hour<-framehour$hour",sep="")))
}

##########
#Subset to only records after start or before end
###########
#If recend is less than recstart, times crosses midnight and slightly different code
needed

if (as.numeric(recend)<as.numeric(recstart)){
        for (k in 1:nids){
        eval(parse(text=paste("bsub",k,"<-
b",k,"[which(b",k,"$hour>=",as.numeric(recstart),"|b",k,"$hour<",as.numeric(recend),")
,]",sep="")))
        }
        } else {
for (k in 1:nids){
        eval(parse(text=paste("bsub",k,"<-
b",k,"[which(b",k,"$hour>=",as.numeric(recstart),"&b",k,"$hour<",as.numeric(recend),
"),]",sep="")))
        }
        }

#Store vecotr of row dimensions
rowdims<-numeric(nids)
for (k in 1:nids){
```

```
try(eval(parse(text=paste("rowdims[",k,"]<-nrow(bsub",k,")",sep=""))),silent=TRUE)
}
recstoremove<-which(rowdims==0)

#Removing Datafiles if Some Badgers Have No Records in The Alotted Time
#KLUDGE: those files that have zero records are replaced with the original dataframes,
but all contact lengths are set to zero
if(length(recstoremove)>0){

        for (j in 1:length(recstoremove)){
        eval(parse(text=paste("bsub",recstoremove[j],"<-b",recstoremove[j],sep="")))
        eval(parse(text=paste("bsub",recstoremove[j],"$contact<-0",sep="")))
        }

        }

#Restore to b's from bsub's
for (k in 1:nids){
        eval(parse(text=paste("b",k,"<-bsub",k,sep="")))
        }

}


##########
#Common Code Irrespective of Time Subsetting
##########

#Match Collar Numbers to Animal IDs for alternative pivotable
for (k in 1:nids){
eval(parse(text=paste("b",k,"$idencounter<-
collarids[,2][match(b",k,"$encounter,collarids[,1])]",sep="")))
}


#######
#Pivot Table Calcs
#######

#Now contains an option to have either the total contact duration between badgers (D)
or the Count (F) or separate contact events per badgers

freq.or.duration<-readline("Enter required metric: 'D' for Total Duration of Contacts, or
'F' for frequency (number) of Contacts...")

#Duration Stuff
```

```
if(freq.or.duration %in% c('D','d')){
#Sum of Contacts (pivot-table equivalent) (currently set to badger id not collar id)
for(k in 1:nids){
eval(parse(text=paste("b",k,"d<-
aggregate(contact~idencounter,FUN=sum,data=b",k,")",sep="")))
                }} else if(freq.or.duration %in% c('F','f'))
                 {

                #Frequency stuff
                #Extra line of code to reformat b[k]d's to be identical to the duration
output

                        for(k in 1:nids){
                        eval(parse(text=paste("b",k,"dtemp<-
with(b",k,",data.frame(table(idencounter)))",sep="")))
                        eval(parse(text=paste("b",k,"d<-
subset(b",k,"dtemp,b",k,"dtemp[,2]>0)",sep="")))
                        eval(parse(text=paste("colnames(b",k,"d)[2]<-'contact'",sep="")))
                                                                }} else {stop("Input
for Metric Not Recognised: Choose from 'F' or 'D'")}



#Collar Numbers
collars<-collarframe$collar

#Work out which collars are present from the encounter histories in the dataframes
idsequence<-seq(nids)
eval(parse(text=paste("anim<-
c(",paste("as.character(b",idsequence,"d[,1])",sep="",collapse=","),")")))

#Add vector of collar IDs inputted and work out unique, then order sequentially
anim2<-unique(c(anim,as.character(collarframe[,1])))
animpresent<-anim2[order(anim2)]


########################################

#Make a list of containing all the badger data
blist<-paste("list(",paste("b",idsequence,"d",collapse=",",sep=""),")")
idlist<-eval(parse(text=blist))

#Work out where in the list of present badgers the collar id's are
#These are the columns of the matrix that will be filled
col.positions<-which(animpresent %in% collarframe[,1])
newcols<-data.frame(anim=animpresent[col.positions],cols=col.positions)
```

```
#Blank matrix
nsamp<-length(animpresent)

mat<-matrix(0,nrow=nsamp,ncol=nsamp)
rownames(mat)<-animpresent
colnames(mat)<-animpresent

print(paste("Building",length(animpresent),"x",length(animpresent),"Matrix..."),quote=
FALSE)

#Flesh out contact matrices
#Number of columns to fill = number of collars worth of data
nind<-length(collars)
for (k in 1:nind){
mat[,col.positions[k]]<-idlist[[k]][,2][match(animpresent,idlist[[k]][,1],nomatch=NA)]
}

#Working out max value between 'identical' pairs of contacts
mat2<-mat
mat2[which(is.na(mat2))]<-0

mat2l<-mat2
mat2u<-mat2
mat2u[lower.tri(mat2u)]<-NA
mat2l[upper.tri(mat2l)]<-NA


#Blank matrix to take max values
matblank<-mat2

#Calc max
for(i in 1:nsamp){
        for(j in 1:nsamp){
                matblank[i,j]<-max(mat2l[i,j],mat2u[j,i])
                }}



#Symmetrise matrix
mb2<-matblank
matblank[is.na(matblank)]<-0

for(i in 1:nsamp){
        for(j in 1:nsamp){
                mb2[j,i]<-max(matblank[i,j],matblank[j,i])
```

```
                }}

if(freq.or.duration %in% c('D','d')){
print(paste("Matrix Built Successfully...Longest Contact
is",round(max(mb2)/60,dig=2),"minutes"),quote=FALSE)
} else {
        print(paste("Matrix Built Successfully...Largest Number of Contact Events With
a Single Individual is",round(max(mb2)/60,dig=2)),quote=FALSE)
        }

#Tell User How Many Individuals Were Lost Due To Data/Time Subsetting
if(timeyes %in% c("Y","Yes","y","yes")){
        print(paste("N.B.",length(recstoremove),"datafiles have no records in the
specified timeframe"),quote=FALSE)

        if(length(recstoremove)>0){
                                print(paste("Animal
IDs",paste(ids[recstoremove],collapse=","),"have no contacts"),quote=FALSE)

                }
        }

filnam<-readline("Enter Name of Output File...")
filnam2<-paste(filnam,".csv",sep="")
write.csv(mb2,filnam2,row.names=TRUE)

        }
mb2
}
```

#END

APPENDIX C

Table A.C.1. Demographic and geographical information for study groups in 2009 and *2010*.

Social Group	Adults/Sub-Adults			Cubs			No. of Main setts	No. of outliers	Territory size (m)
	Male	Female	**Total**	Male	Female	**Total**			
Beech	3/3	3/3	**12**	2	0	**2**	2	4	294156
	4/1	*4/0*	***9***	*1*	*0*	***1***			*246844*
Breakheart	0/0	3/0	**3**	0	1	**1**	1	3	192652
	1/0	*2/1*	***4***	*1*	*0*	***1***			*331212*
Cedar	1/3	4/1	**9**	5	1	**6**	2	3	562878 [a]
	0/4	*1/1*	***6***	*0*	*0*	***0***			*338889*
Kennel	3/1	1/4	**9**	0	0	**0**	2	5	366069
	5/0	2/1	***8***	*0*	*0*	***0***			*213561*
Larch	1/2	6/1	**10**	2	2	**4**	1	8	236043
	1/2	*5/0*	***8***	*0*	*0*	***0***			*379543*
Septic Tank	1/2	0/1	**4**	0	0	**0**	1	6	562878 [a]
	0/3	*2/0*	***5***	*0*	*1*	***1***			*741340* [c]
Top	3/2	2/0	**7**	1	1	**2**	1	7	484357 [b]
	4/1	*2/1*	***8***	*0*	*1*	***1***			*256134*
West	1/2	2/0	**5**	2	0	**2**	1	4	440535
	1/2	*3/0*	***6***	*0*	*0*	***0***			*406589*
Wych Elm	1/3	2/1	**7**	0	0	**0**	2	4	280750
	3/0	*5/0*	***8***	*0*	*0*	***0***			*179483*
Yew	2/0	3/2	**7**	1	0	**1**	2	7	484357 [b]
	4/0	*5/0*	***9***	*0*	*0*	***0***			*741340* [c]

[a,b,c] denote groups that had close ties and their individual territories could not be defined based on baitmarking alone.

APPENDIX D

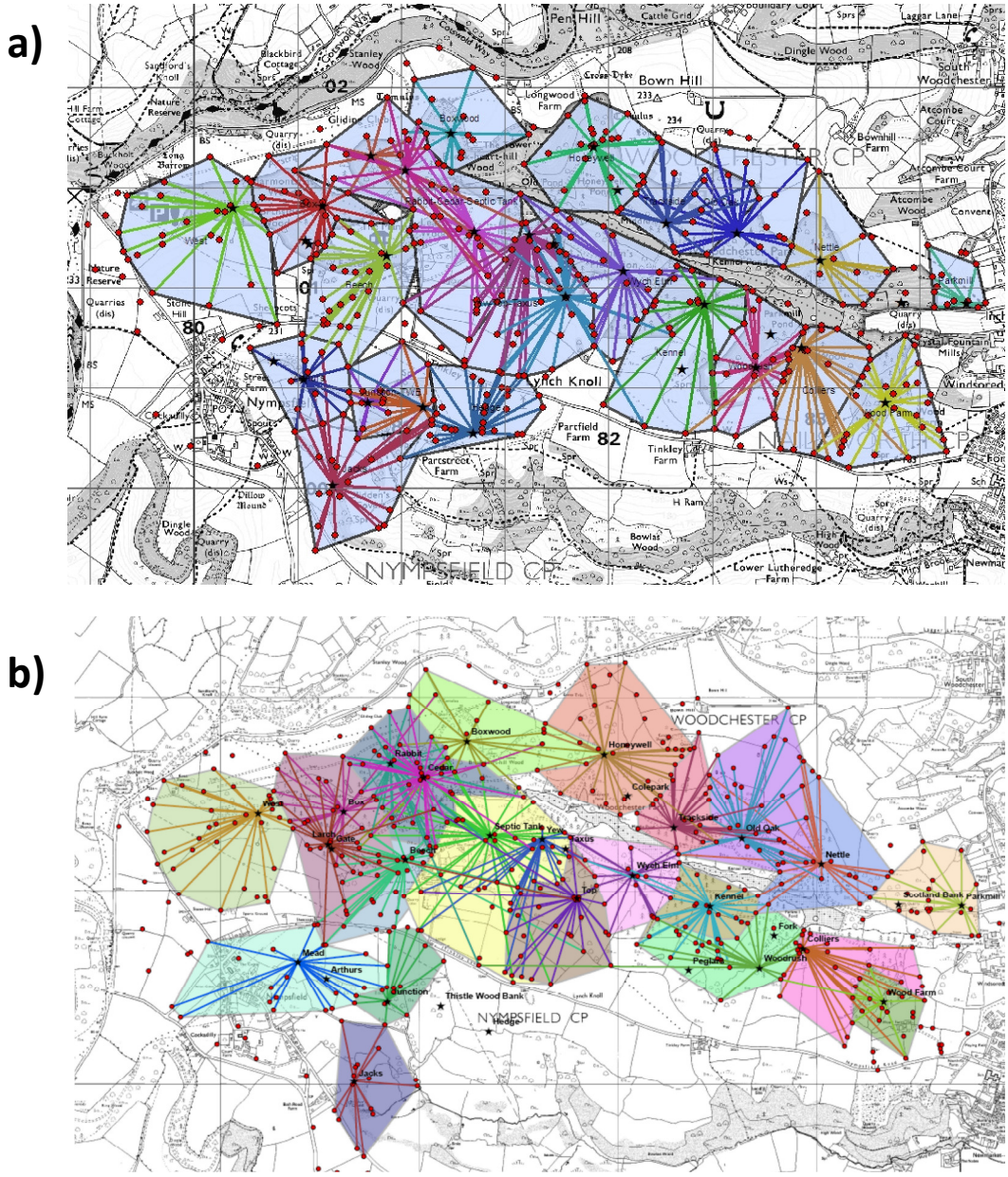


Figure A.D.1. Baitmarking maps for a) 2009 and b) 2010. Each group was fed bait laced with different coloured indigestible plastic beads. The lines (spokes) radiate from the main sett where bait was fed, to the latrines (red circles) where marked droppings were found. MCPs represent the extent of each social group's territory.

APPENDIX E

Table A.E.1. The numbers of collars deployed on different demographic classes across social groups in the Woodchester Park high-density badger population.

Social Group	**Adults**			**Sub-Adults**			**Total No. of Collared Animals**
	Male	Female	Total	Male	Female	Total	
Beech	1	2	3	3	3	6	9
Cedar	0	3	3	0	0	0	3
Kennel	1	1	2	0	4	4	6
Larch	1	2	3	2	0	2	5
Septic Tank	1	0	1	2	1	3	4
Top/Yew	4	4	8	2	2	4	12
Wych Elm	1	2	3	3	1	4	7
West	1	2	3	2	0	2	5
							51

APPENDIX F

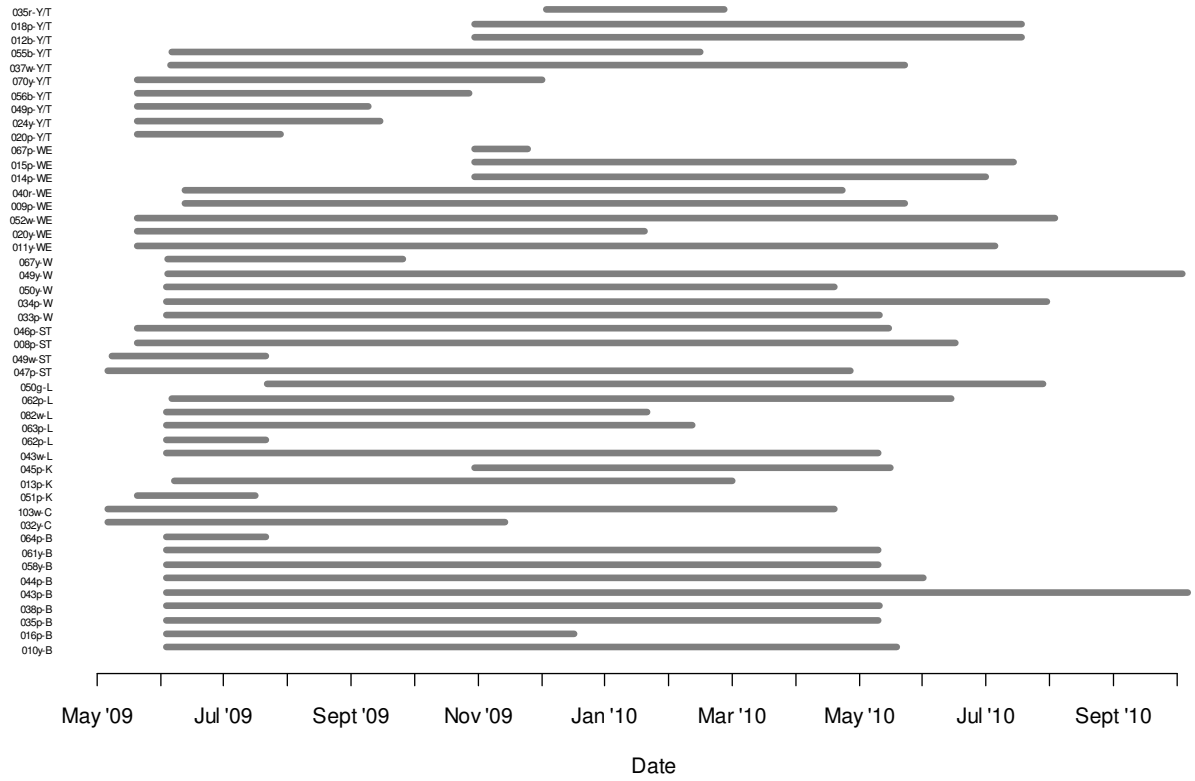


Figure A.F.1. Length of proximity logger deployment. Collars were retrieved in summer 2010, but some fell off and were located in the field prior to this.

‘Badger hates Society, and invitations, and dinner, and all that sort of thing.’

– Kenneth Grahame, *The Wind in the Willows*