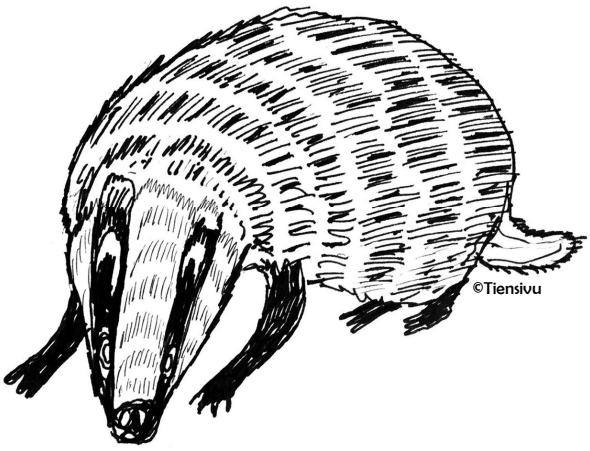
The genetic basis of variation in bovine tuberculosis infection, progression and diagnosis in a wild animal host



Submitted by

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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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Abstract

Parasites are ubiquitous in wild animal populations and have wide ranging effects on the health, fitness and eco-evolutionary dynamics of their host populations. To counter parasites, hosts have evolved a myriad of defence strategies, but individuals vary considerably in the efficacy of these strategies, and so, in their susceptibility to infection. While variation can generally be viewed as stemming from genetic and environmental effects, we currently have little knowledge of their relative importance in wild and unmanaged host populations. In this thesis, I use long-term mark-recapture data on a population of European badgers (*Meles meles*) to examine the genetic basis of variation in bovine tuberculosis infection and its progression. I first estimate a genetic pedigree and characterise variation in extra-group paternity in the population (Chapter 2). Then, adopting a pedigree-based quantitative genetic approach, I investigate the relative importance of genetic and social environmental sources of variation in bTB infection status (Chapter 3). Thirdly, I characterise associations between body weight and bTB infection and test for variation in host tolerance (Chapter 4). And finally, I examine the genetic basis of (co)variation in and among four diagnostic test responses, representing different aspects of host immune function (Chapter 5). Taken together, this work provides novel insight into the genetic architecture of bovine tuberculosis infection in a wild host species, and the evolutionary potential of immune traits in the wild.

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Suurimmat kiitokset kuuluvat kuitenkin ystävilleni ja etenkin perheelleni koti-Suomessa (tai milloin missäkin), joiden ansiosta olen aina saanut olla ja elää oman pääni mukaan – niin aion jatkossakin tehdä.

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Author's Declaration

With the exceptions stated below, I declare that the work contained in this thesis is my own and has not been submitted for any other degree or award.

My supervisors commented on earlier drafts of the thesis. Chapter 2 has undergone peer review. Jenni McDonald provided population size estimates and Andy Robertson group size data used in Chapter 2. Tom Houslay provided assistance with figures and Bayesian analyses, and Alastair Wilson assisted with statistical analyses throughout. Microsatellite genotyping was done, in part by myself, at the NERC Biomolecular Analysis Facility – Sheffield, where Deborah Dawson, Natalie dos Remedios and Gavin Horsburgh provided advice and support with genotype data. All trapping and sampling was performed and compiled by Animal and Plant Health Agency staff. Badger trapping and biological sample collection was carried out under license from the United Kingdom Home Office according to the Animals (Scientific Procedures) Act 1986.

Chapter 1

General Introduction

Parasites are ubiquitous in wild animal populations and have wide ranging consequences, often impacting the health and fitness of their hosts, causing disease, loss of fertility and sometimes death (Schmid-Hempel 2011). Parasites can also greatly impact host population dynamics on ecological and evolutionary scales, acting, for instance, as the driving force of sexual selection in many species (Sheldon & Verhulst 1996; Hatcher & Dunn 2011). As a consequence of these impacts on individuals and populations, understanding host-parasite interactions is critical for ecology, conservation and wildlife management, just as it is for management of health in humans and captive animals.

To counter the negative effects of parasites (including micro-organisms such as bacteria, viruses, protists, fungi), hosts have developed a range of defence strategies. The first line of defence will often be avoiding exposure to infection. One of the most obvious examples includes the feeling of 'disgust' in humans (Oaten et al. 2009), but animals exhibit a wide array of behaviours linked to avoidance of infection. For instance, they may avoid foraging in locations (Hutchings et al. 2002) or times (Folgarait & Gilbert 1999) of increased risk. Avoidance, even social ostracism, of infected individuals has also been observed in many social species, such as social lobsters (Behringer et al. 2006). However, avoidance is not a fool proof strategy and often exposure is inescapable. Thus, hosts have developed defences for preventing or limiting parasite entry given exposure. These can also include behaviours (e.g., grooming; Hart & Hart 2018) as well as physical and chemical barriers (e.g.

insect cuticles; Ortiz-Urquiza & Keyhani 2013). However, the primary defence against infections becoming established and causing disease, comes from the set of mechanisms that comprise the host's immune system.

The immune system can be viewed as comprising a number of distinct, but highly interconnected, arms. First, a distinction is generally made between innate and adaptive (acquired) immune responses. Innate responses are generally nonspecific, constitutive and rapid, taking minutes or hours (Schmid-Hempel 2011). Representing the first line of defence, these fast mechanisms (e.g. phagocytosis and inflammation) clear the majority of infections but can also cause damage to host tissue (e.g. oxidative stress, Bogdan et al. 2000). By contrast, adaptive immune responses are characterised by highly diverse specificity to parasites. They are mediated through exposure to antigens (foreign objects, parasite molecules) and become increasingly specific to ongoing infections through retention of immunological memory (Ahmed & Gray 1996). However, mounting a response is generally slow (relative to innate immune responses), and typically takes several days in mammals and longer in ectotherms (Whyte et al. 2007; Schmid-Hempel 2011). Though traditionally considered a feature of so-called 'higher' vertebrates, more recent studies have evidenced adaptive immune mechanisms in invertebrates (Sadd & Schmid-Hempel 2006; Roth et al. 2009). Both adaptive and innate immune responses are affected through humoral (non-cellular, soluble components in body fluids) and/or cell-mediated processes. In vertebrates, antibodies such as serum immunoglobulins (Ig) play a key role in the humoral response, recognising and neutralising antigens (Barclay, 2003), while a crucial function of immune cells, such as T helper cells, is the release of signalling proteins which help orchestrate responses against parasites (Hope et al. 2000; Frucht et al. 2001).

The immune mechanisms briefly outlined above are usually viewed as contributing to host 'resistance', broadly defined as the ability to prevent or limit infection (i.e. parasite growth; Boots et al. 2009). However, rather than trying to limit parasite growth directly, hosts can also show 'tolerance' to infection by minimising parasite-induced damage and limiting loss of fitness occurring (Simms, 2000; Medzhitov et al. 2012). Although tolerance is a well-established concept in plant ecology (Råberg et al., 2009), it has only been measured explicitly in a handful of animal studies (Råberg et al., 2007; Hayward et al., 2014b; Mazé-Guilmo et al., 2014). Perhaps unsurprisingly, the mechanisms underpinning tolerance in animal are not yet well-studied. They may again include behaviours since, for instance, tadpoles of *Hyla femoralis* have been found to minimise damage from encystment of larval trematode by deflecting infections to non-essential body sites (Sears et al., 2013). The majority of tolerance mechanisms are, however, thought to operate via immunological and physiological processes. Examples include tissue repair, neutralising parasite toxins and minimising pathology caused by the host's own immune responses ('immunopathology'; Graham et al., 2005a; Glass, 2012).

To date most work on host defence strategies in non-human animals has focused on resistance. This is particularly so in the veterinary sciences where increasing disease resistance in production animals is of great economic value (Morris, 2007). However, it is also true in an eco-evolutionary context where the prevailing view is that parasites should strongly select for increased host resistance. The evolution of resistance in the host population will then impose selection for counter-adaptations by parasites leading to a co-evolutionary 'arms race' (see Koskella 2018 and references therein). Numerous empirical studies in both laboratory and field systems have confirmed that hosts can and do

evolve resistance when challenged by pathogens (Janmaat et al. 2003; Bonneaud et al. 2011). Nonetheless, concerns about extrapolating laboratory results to natural populations can clearly arise. For instance, lab-based experiments lack ecological realism by using experimental inoculation of selected parasite strains (sometimes via transmission routes and/or at dosages that do not occur in nature). Experimental host populations are also often characterised by low genetic variability (Williams *et al*. 2005; Viney 2006; Corby-Harris *et al*. 2007) that may not be representative of wild populations. Finally, as already noted, the emerging importance of tolerance as a determinant of host fitness makes it clear that, in terms of evolutionary consequences of parasite infection, there is probably more to host defences than resistance alone.

A quantitative genetic approach to studying host defences in the wild

Parasites are generally expected to impose directional selection for increased effectiveness of host defence mechanism – including, but not limited to, resistance. However, since selection occurs when differential (relative) fitness arises from a causal dependence on individual phenotype (Morrissey et al., 2010), it can only occur if host populations harbour among-individual variation in defence traits. The importance of among-individual variation for population-level processes has become increasingly clear in recent years (e.g. Madritch & Hunter, 2002; Grist et al., 2014; Svanbäck et al. 2015), particularly with respect to our understanding of infection dynamics (Kramer-Schadt et al. 2009; VanderWaal & Ezenwa, 2016). We now know that within host populations some individuals are resistant to infection where others are not, and that some

infected individuals will rapidly clear parasites while others experience disease and even death. While the parasite itself undoubtedly influences the outcome of an infection (e.g. virulence of different strains; (Grech et al., 2006), heterogeneity in the magnitude and effectiveness of host defences is likely to stem from numerous intrinsic and extrinsic sources.

Among-individual variation in host defence phenotypes can generally be viewed as stemming from genetic and environmental (including social) effects. However, while genetic factors are well known to influence infection in humans and captive animal populations, we currently have little knowledge of their relative importance in wild populations. This is an important gap in our knowledge because, while among-individual variation is a prerequisite for selection, for an adaptive evolutionary response to occur there must also be a genetic basis to variation in any trait under selection (Falconer & Mackay 1996). The greater the variation that is explained by genes, the greater the potential response to selection will be. Thus, knowing what proportion of phenotypic variation has a genetic basis is crucial to our understanding of microevolutionary dynamics of traits. Quantitative genetics - the study of the genetic basis of complex (quantitative) traits – provides us with tools to determine this.

Quantitative genetic models were largely developed by plant and animal breeders in order to understand, predict, and optimise responses to artificial selection (Falconer & Mackay 1996). Crucially, even when the detailed genetic architecture of a trait is unknown, classical quantitative genetic approaches allow us to characterise patterns of phenotypic similarity among individuals of known relatedness (or relationship) and thus to infer levels of genetic variation underpinning traits. This statistical approach has been widely adopted by

evolutionary biologists to understand the effects of natural and sexual selection on traits and has generated a wealth of knowledge about the underlying causes of variation in phenotypes (Lynch & Walsh 1998). Though broadly applicable to any type of trait in any population, it is only in the last two decades that application of quantitative genetic analyses to data from wild populations has become widespread. This is thanks to the increasing availability of high volume, often long-term, individual-based data sets from wild animal populations (Clutton-Brock & Sheldon 2010), but also due to advances in molecular and statistical techniques (Kruuk et al., 2008). Molecular tools have been important because of the need to combine relatedness information with trait data in order to estimate genetic variance for phenotypic traits. While breeding experiments can be conducted in the laboratory, and pedigree data is often recorded in livestock, it is not generally easy to track relatedness among wild animals from observational data alone. However, molecular markers are now widely used to estimate relatedness and infer pedigree structures (Townsend & Jamieson, 2013; Bérénos et al., 2014). Statistical tools, notably a form of linear mixed effect model known as the 'animal model', have been equally important because data structures from wild populations are themselves usually complex (e.g. unbalanced family sizes, multiple generations, high levels of missing data) and so not well suited to classical methods for estimating genetic variance from controlled breeding experiments (e.g. ANOVA).

Estimating quantitative genetic parameters in the wild is also made challenging by the fact that populations under real-world conditions experience high levels of environmental heterogeneity. This in turn means that natural selection regimes are likely to be more variable and complex than those imposed artificially in selection experiments or breeding programs. For instance,

in contrast to most artificial selection experiments, natural selection is generally expected to act on multiple traits simultaneously, while changes in environmental parameters can impact trait expression (through plasticity), the phenotype-fitness relationship (i.e. selection) and potentially the genetic basis of variation itself. These processes mean that evolutionary outcomes may often differ greatly from the theoretical predictions of simple models that perform well under artificial conditions (Merilä et al., 2001; Morrissey et al., 2010).

Despite the inherent difficulty, if we hope to understand how phenotypes will evolve under natural selection, there is value in trying to estimate genetic variation in those same natural environments. To my knowledge, quantitative genetic techniques have only been used to examine host defences to specific parasites in two wild animal populations to date. Hayward et al. (2014a, 2014b) studied the heritability of and selection for resistance and tolerance against gastrointestinal nematodes in Soay sheep, while Mazé-Guilmo et al. (2014) found heritable variation in tolerance and resistance, and a genetic correlation between these traits, in a freshwater fish against an ectoparasite. In this thesis, I apply a quantitative genetic approach to investigate the genetic basis of variation in host defences in a third host organism, specifically a population of European badgers (*Meles meles*) naturally infected with bovine tuberculosis.

Study system: badgers and bovine tuberculosis

Bovine tuberculosis (bTB) is a chronic infectious disease of cattle caused by *Mycobacterium bovis*, a host-adapted bacterial strain in the *M. tuberculosis* species complex. In the United Kingdom (UK), it is a longstanding

socioeconomic burden on the livestock farming industry, costing the taxpayer an estimated £100 million a year (Defra, 2014) in addition to causing significant financial losses of individual farmers. Disease control in cattle - namely mandatory testing, slaughter of infected cattle, and herd movement restrictions - is complicated by low sensitivity of bTB diagnostic tests (tuberculin skin test; Roth *et al.* 2005) that leads to a proportion of infections going undetected. Additionally, current EU legislation prohibits vaccination of cattle (Council Directive 78/52/EEC) with the available BCG vaccine (which offers substantial, but incomplete protection; Wedlock et al. 2007) because vaccinated and infected animals cannot be distinguished with the tuberculin skin test. So, despite early successes in controlling the disease, incidence has steadily increased over the past 20 years, most notably in the southwest of England and in Wales (Proud 2006; Reynolds 2006).

One reason for the continued persistence of the disease in the UK is that the European badger (*Meles meles*) provides a wildlife reservoir for bTB. Badgers have been implicated in the transmission of bTB to cattle via several lines of investigation (Little et al. 1982; Krebs et al. 1998; Proud et al. 1998; Smith et al., 2006; Woodroffe et al. 2009), but perhaps the most extensive evidence comes from the Randomised Badger Culling Trial (RBCT). The RBCT was undertaken to quantify the effects of badger culling on bTB incidence in cattle (Bourne et al. 2007), and dynamic modelling of data suggested badgers could be responsible for up to 38% of bTB cases in cattle. Of these, an estimated 6% result from direct (badger-cattle) contact, while the rest are from onward transmission from infected cattle. However, these models show considerable uncertainty in their predictions (Donnelly & Nouvellet, 2013) and

managing bTB remains a highly contentious political issue, with government-implemented badger culls being met with both public and scientific opposition.

Relatively little is known about the dynamics of bTB infection in badgers. The UK-wide prevalence of bTB in badgers is estimated at 16.6%, but populations show both inter- and intra-regional, as well as temporal variation in infection level (with estimates ranging from 0 to 32%; (Allen et al., 2011). Populations can contain high levels of variation in infection and disease status, which is likely to be explained in part by local environmental conditions but may also be linked to among-individual differences in behaviour (e.g. aggression; (Jenkins et al., 2012), physiology and, intuitively, immune function. Nothing is currently known regarding the contribution of genetic factors to variation in bTB-related immune and infection phenotypes in wild badgers. Nonetheless, heritable variation has been found in studies of TB in both domestic animals and laboratory populations (Phillips et al., 2007; Schurr, 2011; Bermingham et al., 2014).

Pathogenesis of bTB in badgers

The primary route of infection in badgers is thought to be through inhalation of infectious aerosol, leading to chronic infection of the pulmonary system (though other organs can also be affected; (Gallagher et al., 1998; Murphy et al., 2010). Once in the respiratory system of the host, *M. bovis* gains entry through uptake by alveolar macrophages, leading to the formation of lesions (clusters of epithelioid cells surrounded by mild fibroblastic reaction) caused by a cell-mediated response by the hosts immune system (Corner et al., 2011). Infection severity varies widely from latency (infection without clinical signs and no visible lesions) to severe disease with generalised pathology, cachexia (wasting) and

death (Murphy et al. 2010). Cell-mediated immune responses play a major role in host defence against *M. bovis*, particularly during early stages of infection, with the main cells involved being macrophages and T-cells (Skinner et al., 2001). Humoral, antibody-mediated responses against *M. bovis* have also been observed in badgers, with MPB83 being the dominant antigen recognised by serum antibodies in infected badgers (Corner et al. 2011). However, the repertoire of *M. bovis*-specific antigens recognised by badgers appears limited compared to other host species (Corner et al. 2011).

The Woodchester Park badger study

This thesis investigates the genetic basis of among-individual variation in bTB susceptibility and progression in a wild population of badgers living in Woodchester Park, Gloucestershire, England. Woodchester Park lies in a steep-sided wooded valley surrounded by farmland and is home to a naturally infected population of badgers (200-300 animals). This population has been the subject of an ongoing mark-recapture study that was initiated in 1976 with the aim of studying the dynamics of *M. bovis* infection in badgers. Within the 11 km^2 study area, up to 45 social groups of badgers have been recorded at any time. Each group is associated with one or more underground dens (setts), and the number of social groups present has varied over time (as determined by bait marking; (Delahay et al., 2000a). However, the study area includes a core of 20-25 defined social groups that have consistent and continuous trapping records across the study period.

Sampling of badgers is done through quarterly 'trap-ups' at or around known active setts. At each trapping event, badgers are sampled for two

consecutive nights using peanut-baited steel mesh box traps, set after 4-8 days of pre-baiting. Trapped badgers are anaesthetized using a combination of ketamine, butorphanol and medetomidine (de Leeuw et al., 2004) before further sampling, while individuals caught previously in the same trap-up are not resampled. At first capture each animal is marked with a unique tattoo and blood samples or guard hairs are taken for microsatellite genotyping (Carpenter et al., 2005). The marking allows identity to be recorded at each subsequent recapture. Capture location, sex, age class (cub, yearling, adult), body weight, length, condition, and reproductive status are all recorded and clinical samples collected (described below). After recovery from anaesthesia, all badgers are then released at the point of capture. Since its initiation, the mark-recapture program at Woodchester Park has collected >15,000 observations of over 3,200 individual badgers. Because of this intensive sampling regime, birth and death dates are known for many individuals and we have a good understanding of individual life-histories as well as demographic features of the population.

At each (re)capture event, badgers are tested for bTB using several diagnostic procedures. First, clinical samples from a number of body sites (faeces, urine, tracheal aspirate, pus, bite wound swabs; Clifton-Hadley et al., 1993) are subjected to bacterial culture for *M. bovis*. Spoligotyping is used to identify any growth as *M. bovis* (Kamerbeek et al., 1997) and one or more positive results from among the body sites is considered indicative of current infection. Second, serological assays are used to test badger serum for IgM and IgG antibodies to *M. bovis*. These indicate activation of the humoral immune response and can thus indicate previous or current infection. Third, from 2006 onwards, an interferon-γ (IFN_γ, a signalling protein produced by T-cells) release assay has also been used to characterise cell-mediated immune activity. In this

two-part test, the production of IFN_γ in heparinised whole-blood culture is first stimulated with mycobacterial antigens (bovine tuberculin), and then quantified using sandwich ELISA, producing a continuous optical density (OD) measure (Dalley et al., 2008). Since infection by environmental mycobacteria from the *Mycobacterium avium* complex (MAC) can bias results of bovine tuberculin assays (Pollock et al., 2005), IFN_γ responses to avian tuberculin are simultaneously tested. Finally, in addition to testing for *M. bovis* specific responses, blood samples are also used to assay the cell-mediated immune response to pokeweed mitogen, a non-specific activator of humoral and cell-mediated responses (Janossy & Greaves 1972), as a putative marker of general immune function.

This long-term testing of the Woodchester Park population means that bovine TB infection is uniquely well-characterised in this badger population. Previous work has shown that individuals exhibit striking variation - both spatially and temporally - in the prevalence, progression, and severity of infection. For instance, (Delahay et al., 2001) showed that annual prevalence of bTB in a badger population in Woochester Park, Gloucestershire, varied between 10.3 and 17.7%, but was also strongly spatially aggregated (with higher incidence in the west of the study area). Interestingly, some social groups remained entirely test-negative for years, despite high rates of infection in neighbouring groups (Delahay et al. 2000b). Sexual dimorphism has also been documented, with males having a higher probability of infection, and suffering from faster disease progression and greater mortality than females (Graham et al., 2013; McDonald et al., 2014). The availability of individual-level ecological, genetic and epidemiological data now provides an opportunity to further characterise the drivers of variation among individuals.

Thesis overview

In this thesis I apply a quantitative genetic approach to investigate the drivers of variation in host defence traits and disease outcomes in a wild vertebrate population. I use bovine tuberculosis (bTB) infection in the Woodchester Park population of European badgers as a study system. By decomposing a phenotypic trait into variance components, it is possible to discern the relative importance of not just genetic, but also non-genetic factors (e.g. environmental, maternal, and social) contributing to observed phenotypic variation. Thus, quantitative genetics provides a useful tool for teasing apart the causal relationships between host, parasite, and environment. Furthermore, because adaptive evolution requires selection and heritable variation, quantifying the heritability of host defence traits *in situ* in wild populations is necessary to understand their evolutionary potential (and so the coevolutionary outcomes of host-parasite interactions). While it is clearly the case that (genetic) variation can also exist among parasites, with important fitness consequences for hosts (e.g. differential virulence among parasite strains), quantification of parasite traits is beyond the scope of the present thesis and is not investigated here. Instead I focus on variation within the host population. Specifically, this thesis aims to: i) estimate a genetic pedigree and characterise variation in extra-group paternity in the population, ii) investigate the relative importance of genetic and social environmental sources of variation in bTB infection status, iii) characterise associations between body weight and bTB infection and test for (genetic) variation in host tolerance, and iv) examine the genetic basis of (co)variation in and among four diagnostic test responses, representing different aspects of host immune function.

In **Chapter 2**, I use ecological and genetic data provided by the Animal and Plant Health Agency (APHA) to resolve a multigenerational pedigree for the Woodchester Park badger population, implementing molecular parentage assignment in a Bayesian framework. I then use the resulting pedigree to characterise the extent of extra-group paternity (EGP), occurring as a consequence of breeding excursions, and to test hypothesised drivers of variation of paternity distance (PD; estimated as the distance between the home sett of a cub and that of its father) at multiple levels. I test whether population density and sex ratio influence mean annual PD, and model cub-level PD and extra-group paternity to ask whether this varies consistently among social groups and/or parental individuals. This chapter therefore seeks to understand variation in propensity of adult badgers to engage in breeding excursions outside of their groups, both because it is biologically interesting, and – in the context of the overall thesis – because EGP has implications for subsequent quantitative genetic analyses. Specifically, (i) bTB infection is known *a priori* to vary among social groups (Delahay et al. 2000b); (ii) social groups are known to contain high levels of related individuals (Carpenter et al. 2005); and (iii) this population is not amenable to experimental manipulation designed to reduce gene-environment correlation (e.g. cross-fostering; (Kruuk & Hadfield, 2007). This means that EGP is likely to be important for allowing subsequent statistical partitioning of genetic from common environment (e.g., social group) effects on bTB related traits.

In **Chapter 3** I use the pedigree structure resolved in Chapter 2, together with a progressive categorisation of disease status that approximates bTB progression using diagnostic test results (from test negative to advanced stages of infection), to quantify the relative importance of genetic and environmental

effects. I find that while both disease progression and lifetime infection risk have significant estimates of heritability, more variation is explained by effects of group membership in time and space, and (for cubs) maternal identity. I then build on this work in **Chapter 4** by testing for among-individual, and genetic, variation in tolerance to bTB infection. To do this I use weight loss (and/or reduced growth) as a measure for infection severity and assume that the progressive bTB categorisation provides a valid proxy of pathogen load. I firstly confirm population-level average effects of bTB status on mean weight. Then adopting an individual 'reaction norm' approach in which individual tolerance is defined as the slope of an individual's relationship between weight and bTB status, I test for among-individual, and genetic, variation in tolerance. This chapter provides the first explicit evidence of variation in tolerance against bTB in a wild host species. Furthermore, I find evidence that tolerance is genetically variable, and thus evolvable in this population.

In **Chapter 5** I explore the genetic (co)variation among traits thought to capture mechanistically distinct (but potentially correlated) components of the immune system. Specifically, I analyse data from the four separate bTB and immune function diagnostic tests used on the Woodchester Park population. This chapter addresses the increasingly highlighted need in ecoimmunology (and evolutionary ecology more generally) for multivariate quantitative genetic studies to characterise the genetic relationships among traits and thereby address gaps in our understanding of trade-offs, evolutionary constraints and phenotypic integration. I firstly determine whether genetic factors contribute to among-individual differences in all traits and find heritable variation for all but the cell-mediated response. I then evaluate the among-trait phenotypic and genetic correlation structure among host immune responses and assess

evolutionary potential of the traits and the possibility of trade-offs to constrain responses to selection.

Finally, in **Chapter 6** I summarise the key findings from the preceding chapters and relate them to the broader context of understanding of host-parasite coevolutionary dynamics. I also briefly consider the potential implications of my work for the management of wildlife (and livestock) disease and highlight several future directions for research.

Chapter 2

Individual variation and the source-sink group dynamics of extra-group paternity in a social mammal

This chapter has been accepted for publication as: Marjamäki P. H., Dugdale H., Dawson D., McDonald R.A., Delahay R.J., Burke T.A., Wilson A.J. (in press) Individual variation and the source-sink group dynamics of extra-group paternity in a social mammal. *Behavioural Ecology*.

Abstract

Movement of individuals, or their genes, can influence eco-evolutionary processes in structured populations. We have limited understanding of the extent to which spatial behaviour varies among groups and individuals within populations. Here we use genetic pedigree reconstruction in a long-term study of European badgers (*Meles meles*) to characterise the extent of extra-group paternity, occurring as a consequence of breeding excursions, and to test hypothesised drivers of variation at multiple levels. We jointly estimate parentage and paternity distance (PD; distance between a cub's natal and its father's social group), and test whether population density and sex ratio influence mean annual PD. We also model cub-level PD and extra-group paternity (EGP) to test for variation among social groups and parental individuals. Mean PD varied among years but was not explained by population density or sex ratio. However, cub-level analysis shows strong effects of social group, and parental identities, with some parental individuals being consistently more likely to produce cubs with extra-group partners. Group effects were

partially explained by local sex ratio. There was also a strong negative correlation between maternal and paternal social group effects on cub paternity distance, indicating source-sink dynamics. Our analyses of paternity distance and EGP indicate variation in extra-group mating at multiple levels – among years, social groups and individuals. The latter in particular is a phenomenon seldom documented and suggests that gene flow among groups may be disproportionately mediated by a non-random subset of adults, emphasising the importance of the individual in driving eco-evolutionary dynamics.

Introduction

Movement of individuals and/or gametes influences the dynamics, persistence and genetic diversity of spatially structured populations (Ronce, 2007). Understanding movement is therefore crucial for wildlife conservation and management as it can determine species distributions (Holt, 2003), impact the vulnerability of populations to extinction (Thomas, 2000) and play an important role in the transmission of infections (Pope et al., 2007). Behaviours linked to 'dispersal', in the broadest sense of any movement with potential consequences for gene flow (Ronce, 2007), are widely viewed as adaptive, allowing individuals to escape from locally intense competition for resources or mates (Daniels & Walters, 2000; Matthysen, 2005), seek good or compatible genes in potential mating partners (Hamilton 1990; Zeh & Zeh 1996), or avoid inbreeding by leaving the vicinity of related individuals (Greenwood, 1980). However, as such movements carry risks as well as benefits, associated behaviours are likely to have evolved under the influence of multiple interacting factors that ultimately shape the balance of costs and benefits (Bowler & Benton 2005; Ronce 2007).

Some of the factors influencing the costs and benefits of movement and dispersal are well documented. For instance, sex (Clarke et al. 1997; Beirinckx et al. 2006; Rabasa & Gutie 2007), age (Dale et al. 2005; Bowler & Benton 2009; Kentie et al. 2014), and density (e.g. Matthysen 2005; Nowicki & Vrabec 2011) are common drivers of variation in many taxa, although density effects can themselves be scale-dependent (e.g. Marjamäki et al. 2013). However, in addition to demographic and ecological effects, it is also becoming apparent that populations can harbor among-individual variation in the tendency to disperse. Our understanding of what drives this variation within animal populations remains limited, although social interactions and behavioral

differences (e.g. “personality” variation in exploratory tendency) likely play an important role (e.g. Cote et al. 2010; Patrick et al. 2012; Weiß et al. 2016).

In this study, we employ an indirect approach to test for and investigate sources of variation in breeding excursions in a population of European badgers (*Meles meles*) in southwest England. Temporary excursions relating to mate acquisition are common in many populations but, while they will have important consequences for fine scale gene flow and genetic structure (e.g. among groups), temporary and short-term excursions can be difficult to observe directly. Nonetheless, in the absence of direct observation of movement, indirect inferences on breeding excursions can be made from genetic data. This can be done, for example, by characterising population genetic structure (or lack thereof; Wilson et al. 2004), or by detecting extra-pair or extra-group paternity (hereafter ‘EGP’), which is commonly seen in birds and mammals (Griffith et al. 2002; Isvaran & Clutton-Brock 2007). Combined with genetic pedigree analysis, the latter approach allows identification of those individuals engaging in, as well as resulting from, extra-group matings, enabling the drivers of among-individual variation to be investigated.

Badgers are a facultatively social species and form social groups at high densities through retention of offspring in natal groups (Kruuk & Parish 1982; da Silva et al. 1994). These social groups, ranging from 1 to 22 individuals of mixed age and sex, form discrete, defended territories containing several communal setts (underground dens). Badgers have a polygynandrous mating system where as many as seven males and females might breed within a social group annually (Dugdale et al. 2007). While within-population movement is common (e.g., detected in 44% of individuals studied by Rogers et al. 1998), the majority of movements between social groups are temporary, with

short-term movements tending to be predominantly between neighboring social groups (Rogers et al., 1998). High rates of EGP (up to 50% reported in high-density populations; Carpenter et al. 2005; Dugdale et al. 2007) are also consistent with an important role for breeding excursions in mediating gene flow, though whether EGP is mediated through transient contact between individuals, or temporary integration of individuals into social groups (or both) is not yet clear.

We use a long-term dataset on individually marked badgers from Woodchester Park (Gloucestershire, England) to reconstruct a genetic pedigree and indirectly estimate breeding excursions. We build on a previous parentage analysis of the population (Carpenter et al. 2005) to reconstruct a pedigree using a larger sample, more markers and more powerful parentage assignment methods. Crucially, for current purposes we adopt a Bayesian approach to pedigree analysis, which allows us to make better use of spatial and group membership information to improve the number of assigned relationships and our confidence in them (Hadfield et al. 2006). From this we simultaneously estimate both the pedigree structure and the mean distance between the father's social group and the cub's natal group (hereafter 'paternity distance') for each annual cohort. We first ask whether paternity distance varies among years as a function of population density and/or sex ratio, before using assigned parent-offspring relationships to test for among-individual (parent) variation in extra-group mating. Finally, noting that from a cub's perspective, EGP and non-zero paternity distance may reflect temporary excursions by either parent, we ask whether among-parent variation can be explained by known predictors of breeding behaviour in other systems, including intrinsic factors (e.g. age, body mass) and social group properties.

Methods

Study population & sampling

The badger population at Woodchester Park (51°42'35"N 2°16'42"W), Gloucestershire, UK, has been subject to an ongoing mark-recapture study since 1976. The study area is approximately 11 km^2 and consists of a steep-sided, wooded valley surrounded by farmland. Here we utilize data from a 30-year period from 1985 to 2014, for which badgers were trapped and sampled up to four times a year. Steel mesh box traps were deployed at active badger setts and set to catch for two consecutive nights after a period of 4-8 days of pre-baiting with peanuts. Trapped badgers were anaesthetized (de Leeuw et al. 2004) prior to examination and at first capture each individual received a unique identifier tattoo on their abdomen. Capture location, sex, age (if birth year known) or age class (adult, yearling, cub, based on size and tooth wear) and body weight were recorded (Delahay et al. 2013). Approximately 20-30 guard hairs were plucked and stored in 80% ethanol for microsatellite genotyping. After a recovery period, all badgers are released at the point of capture. The total trapping dataset is comprised of over 15,000 captures for 3,283 individuals. While most badgers are first caught as cubs or yearlings, 19% were first captured as adults and likely represent a minimum estimate of immigration into the population. Social group territorial boundaries were determined for each year of the study by bait marking (Delahay et al. 2000b). A total of 45 defined social groups were counted throughout the study period, but from 1996 onwards sampling was focussed on 20—25 groups only. Thus, the variation in the number of social groups reflects variation in both sampling effort through time and the configuration of social groups, which occasionally undergo fissions and

fusions (though territories are largely stable over time; Delahay et al. 2000a; Robertson et al. 2014). All work was carried out under licence from the UK Home Office and from Natural England.

DNA extraction & genotyping

Microsatellite data used for parentage analyses have been produced as part of the ongoing Woodchester Park study. For current purposes, we used existing published data (Carpenter et al. 2005) coupled with de novo genotyping at 6 loci described in Carpenter et al. (2003) and Lopez-Giraldez et al. (2007). In brief, individuals trapped between 1986 and 2002 have been genotyped with DNA extraction from hair samples according to protocols outlined in Carpenter et al. (2005), while samples between 2003 and 2014 were genotyped at the NERC Biomolecular Analysis Facility (University of Sheffield, UK) in batches across several time periods using the ammonium acetate extraction method described in Richardson et al. (2001). A minimum of 5 hairs with visible roots were used per individual.

Individuals have been genotyped at between 16 and 22 autosomal microsatellite loci, with slightly different, but overlapping subsets of markers used over the course of the project. We used a 2-µl Qiagen Multiplex PCR reaction (Qiagen Inc., Valencia, USA) and fluorescently-labelled primer sets, before separation of the amplicons on a 48-capillary ABI 3730 DNA Analyzer using Prism set D and a ROX size standard and genotype scoring using GENEMAPPER 3.7. Samples described in Carpenter et al. (2005) were genotyped at 16 loci (Mel 101-117; as described in Carpenter et al. 2003). An additional 6 loci were added to subsequent genotyping efforts (Mel 1, 10, 12, 14, 15 & 116; Carpenter et al. 2003, Lopez-Giraldez et al. 2007) though for 209

individuals born (or captured for the first time) after 2011, markers Mel 15 and 106 were not used. As genotyping has been done in batches over a number of years, samples have been cross-validated by retyping subsets of previously genotyped individuals (min. 15% of samples). This was used to calibrate allele sizes at each locus to ensure consistent scoring across time periods and different sequencers. After scoring genotypes, we tested for deviations from Hardy-Weinberg equilibrium (HWE) and linkage equilibrium (LD) for pairs of loci using 40 unrelated individuals (based on ML-Relate relatedness estimates <0.125) using Genepop 4.4.3 (Raymond & Rousset 1995). P-values for LD tests were corrected to account for multiple tests (false discovery rate; Benjamini & Hochberg 1995). No deviation from HWE (k = 22, alpha = 0.05) or LD (LD: k = 231, alpha = 0.05, adjusted p = 0.05-0.0002) were found. Null allele frequencies were estimated using CERVUS 3.0.7 (Marshall et al. 1998) and were <0.1 for all loci. Therefore, all loci were retained.

We also estimated mean allelic dropout (e1) and false allele rates (or stochastic sampling error, e2), using a random subset of individuals that were re-genotyped and analysed using PEDANT 1.0 (Johnson & Haydon, 2007) (Table S2.1). Overall, genotypes were available for 2,204 (out of 2,811) trapped individuals, at a mean (±standard deviation) of 16.1 (±5.1) loci per individual. Across loci the mean observed and expected heterozygosity were 0.56 (SD 0.15) and 0.61 (SD 0.13), respectively, and the mean number of alleles per locus was 4.85 (SD 1.47).

Parentage analysis

We conducted Bayesian parentage analysis for 1768 genotyped cubs trapped between 1986 and 2014 inclusive, using MasterBayes 2.54 (Hadfield et al.,

2006) in R 3.3.0 (R Development Core Team 2016). Relative to most wild birds and mammals in which molecular pedigree reconstruction has been applied, badgers present a particular challenge in that they are largely nocturnal and so difficult to observe. Furthermore, cubs remain underground for the first 12 weeks of life (Roper 2010), and alloparental care may occur at the sett (Dugdale et al. 2010). As such, while maternal identities can often be (reliably) inferred from observation in other species, this is not the case in badgers. In the absence of any known parents, life-history, spatial and genetic data were used simultaneously to assign paternity and maternity jointly for each cohort of cubs (n = 29) and estimate mean annual paternity distance. The final pedigree used in downstream analyses was then compiled based on parental assignments that met a minimum confidence threshold of 80%. For comparison, we also compiled a pedigree structure according to a stricter 95% confidence threshold.

Definition of candidate parents and use of spatial data

Parentage assignments were run for each annual cub cohort (n=29). Although neither parent can be determined by observation we follow the approach used in other systems (e.g. Walling et al. 2010; Nielsen et al. 2012) of applying a biologically informed set of criteria to define a non-excluded list of candidate parents for each cub. For each cohort, candidate mothers were restricted to females aged ≥2 years present in the cub's natal group (i.e. the group first captured in) in the year of birth, as females are sexually mature as yearlings and, due to delayed implantation (Yamaguchi et al. 2006), can first give birth as two-year olds. Males were considered candidate fathers (regardless of social group) if they were alive and ≥1 year of age 12 months before the cub was born, to account for delayed implantation. Individuals were designated as

belonging to a social group if they were caught within the territory of that group. Individuals recorded in multiple social groups were assigned joint membership to each; in years where individuals were not caught (but were known to be alive from subsequent captures), they were assigned to the social group(s) they were recorded in the preceding year. Only individuals caught as cubs or yearlings (i.e. those with known birth year) were included as offspring in parentage analysis, while badgers first caught as adults are likely to be immigrants and were included only as candidate parents. Since age data were incomplete for badgers that were not caught as cubs or yearlings (distinguishable from adults by size and tooth wear), we assumed adults of unknown age to be 2 years of age at first capture to prevent blanket exclusion from the set of candidate parents (note, this was for parentage assignment only, and assumed ages were not used in subsequent analyses described below). Similarly, where time of death was unknown, individuals were treated as being alive (for purposes of defining status as a potential candidate parent) for 1 year (cubs; Dugdale et al. 2007) or 3 years (adults; Carpenter et al. 2005) after their last capture. Individuals with missing sex or social group data were excluded.

In addition to microsatellite data, our parentage analyses also utilised geographical location data (main sett coordinates for each social group) for all offspring and candidate fathers. Inclusion of non-genetic data is expected to improve assignment where it provides additional information about the likelihood of parentage (Hadfield et al. 2006). For most cohorts (see below) we therefore used (Euclidean) “male distance” between the main sett of the candidate father’s social group and that of the cub’s natal group as a predictor of paternity, which yielded an estimate for each cohort (or year) of the mean paternity distance, i.e. distance between the main sett of the assigned father’s

social group and that in which the cub was born. Thus, paternity distance and parentage are jointly estimated from the data in a single analysis (i.e. it is not the case that distance effects on paternity likelihood are first estimated and imposed in a subsequent parentage assignment). Finally, we note that, while more complete genetic sampling of the population should result in greater parentage assignment success (all else being equal), the number of unsampled parents is estimated in a MasterBayes analysis, not specified *a priori* as an input parameter (as in some likelihood-based methods of parentage assignment). Here we have limited knowledge of the completeness of genetic sampling but certainly trapping does not sample all animals present on any given occasion. Quarterly recapture rates (i.e. across trapping sessions) are known to vary greatly across years, from 0.15-0.73 for females and from 0.20-0.78 for males (Graham et al. 2013). Approximately 19% of individuals are first trapped as adults, providing an upper bound estimate for the proportion of immigrants to the study area.

Parentage assignment settings and diagnostics

Markov chains were run separately for each year (i.e. cub cohort) for 2 million iterations, with a thinning rate of 100 and burn-in period of 500,000. Mismatch tolerance between cub and candidate parent was set to one. Tuning parameters were specified for each cohort to ensure that Metropolis–Hastings acceptance rates were within acceptable limits (0.2-0.5; Hadfield 2012). Per locus genotyping error (e1 and e2; Table S2.1) and allele frequencies calculated based on the full dataset were provided in the model specifications (as direct estimation of error rates by MasterBayes from the data, though possible in principle, is particularly computationally demanding; Hadfield 2012). The

presence of unsampled males (per population) and females (per social group) was also allowed for each cohort. Successive samples from the posterior distribution had low autocorrelation ($r < 0.10$) for estimates of unsampled males and paternity distance. Autocorrelation for unsampled females remained high (>0.10) for several cohorts, however, parentage assignments at ≥80% confidence for these cohorts did not differ when a fixed number of unsampled females (one per social group) was used, therefore all cohorts were retained.

In six of the 29 cohorts (1988, 1993, 2001, 2009, 2013 and 2014) inclusion of male distance as a predictor caused problems for the parentage assignment algorithm that we were unable to resolve. The reasons for this remain unknown but could include, for instance, undetected outliers or errors in the spatial data. For these cohorts, parentage assignment was therefore estimated without male distance as a predictor meaning no direct estimate of mean paternity distance was obtained. As including the distance variable is expected to increase confidence in assignments (Hadfield 2012), excluding this variable from pedigree models could affect the resulting parent assignments. In order to account for this, we reran a subset of cohorts (including 339 cubs) without male distance and compared assignments with and without paternity distance estimation. As expected, excluding male distance generally reduced the confidence assigned to a cub's most likely father, with the result that putative paternities were not assigned in 30 instances, when they had been with models utilising male distance. However, changes in most likely father were only observed for four cubs (out of 339). In all four cases, most likely candidate fathers failed to meet the 80% confidence threshold for assignment regardless of whether the male distance variable was included. Therefore, based on these comparisons, we expect fewer paternities will have been assigned for the six

cohorts where the distance variance could not be included, but consider it unlikely that the identity of the most likely father is sensitive to inclusion of male distance in many instances.

Analysis of breeding excursion proxies

We used the results of our pedigree analysis to extract and model variation in three response variables relating to extra-group paternity. First, we modelled among-cohort variation in mean paternity distance as estimated directly by MasterBayes (subsequently denoted PD_c). Second, for each cub with an assigned father, we extracted the individual paternity distance (denoted PD_i), and also defined a binary EGP variable (denoted EGP_i) according to whether the assigned father was from within (0) or outside (1) the cub's natal group. If a cub was assigned both within- and extra-group paternity by the same father (e.g. where a father was recorded in multiple social groups within a year), the cub was assumed to be within-group offspring. Both PD_i and EGP_i are defined for the cub (i) and non-zero values therefore reflect movements by the mother and/or the father beyond its own social group. We also note that these individual-level estimates are necessarily derived from an estimated pedigree and thus carry over error associated with parentage assignments to downstream analyses that is not readily accounted for. In this respect, we also note an unavoidable trade-off, regarding analyses of PD_i and EGP_i, between using assignments made at 80% confidence (increased samples size but higher error rate) or 95% confidence (reduced sample size but lower error rate). Here results from analyses are presented using the lower threshold but parallel analyses based on 95% confidence can be found in supplemental materials (Tables S6-S8). Overall, qualitative conclusions are consistent between

analyses based on the two thresholds. Note however that, since MasterBayes estimates a full posterior for PD_c, uncertainty in the annual mean paternity distances could be readily accounted for in our analysis of among-cohort variation.

Among-cohort variation in annual mean paternity distance

Our MasterBayes analyses generated estimated posterior distributions (15,000 values per cohort) of PD_c for 23 cohorts caught between 1986 and 2014 (Figure 1). As noted above, in six years (1988, 1993, 2001, 2009, 2013, 2014) inclusion of spatial data in the pedigree assignment step proved problematic so no estimates of PD_c are available. Using a simple multiple regression model of PD_c we tested whether total population size or population sex ratio, determined by dividing the number of males by total population size (as defined below), explained variation in mean paternity distance. We also included a (linear) effect of year to test for any systematic trend in PD_c across the study timeline. All three variables were mean centered to ease interpretation of the intercept (i.e. as predicted PD_c at mean population size, sex ratio and year). Because sampling effort for some social groups varied across years, proxies of total population size and population sex ratio values for each year were estimated using the POPAN model in the program MARK 8.2 (White & Burnham 1999) using capture data from 20 “core” social groups with consistent trapping efforts across all years. Graphical representation of annual mean estimates for population size and numbers of males and females can be found in Figure 1b. Badgers with missing sex information (n=2) were excluded from this analysis. In order to integrate across uncertainty in annual mean paternity distance estimation, our regression model was applied to the full posterior distributions of

PD_c for each cohort, allowing estimation of 95% credible intervals (CI) for the partial regression coefficients. These were considered significant if 95% CI did not span zero.

Among-individual and among-group variation in paternity distance and extra-group paternity

Using the program ASReml 3.0 (VSN International Ltd., Hemel Hempstead, UK), we fitted mixed effects models of PD_i (i.e. Euclidean paternity distance measured in meters), and EGP_i, a binary variable assigning the offspring of each male as either within (0) or extra (1) group. For both response variables, a Gaussian error structure was assumed but PD_i was natural log-transformed prior to analysis to reduce positive skew in residuals. While noting that the Gaussian assumption cannot be strictly true for bounded (ln PD_i) or binary (EGP_i) response variables, inspection of model residuals showed it to be a reasonable approximation here (Figure S2.2). We therefore chose this approach as being more pragmatic than, for instance, Bayesian implementation of generalised mixed models as it more readily allows inference on, and modelling of hypothesized covariance between, random effects (see below). Both variables were then scaled to standard deviation units (SDU) to ease interpretation of results.

For both response variables, models included fixed explanatory variables of maternal age, maternal body mass, maternal group size, and maternal social group sex ratio (as linear effects) and the corresponding paternal variables. Social group sizes (mean 6.40 SD ±3.60) reflect numbers of resident yearlings and adults (i.e. reproductively active individuals) in the cub's conception year,

where group residency is determined from capture records each year following Vicente et al. (2007). Social group sex ratios are calculated as the number of males divided by the total number of adult group members, representing the proportion of males in each group (mean 0.40 SD ±0.20). These measures exclude cubs and transient non-residents (based on criteria used by Vicente et al. 2007) caught within social group boundaries, but represent a baseline measure for the density of potential breeders encountered by individuals in their social group. Body mass was included to test for size-dependence of extra-group paternity and for individuals with more than one weight measurement within a year, the mean of these was used. Note that we also fitted the models using a standardised measure of body condition, the scaled mass index (SMI; Peig & Green 2009), in place of body mass. In principle, this might better account for sexual dimorphism and seasonal variation in body mass (Beirne et al 2015; Peig & Green, 2010). However, in practice, qualitative conclusions of the analyses were unaltered, and since use of SMI in place of body mass resulted in a 16% reduction in sample size, only the results of analyses using body mass are presented here (results for SMI analysis can be found in Tables S3-S5). Significance of fixed effects was determined using conditional Wald F-tests implemented in ASReml (with denominator degrees of freedom calculated following Kenward & Roger 1997).

Year (as a factor), maternal and paternal identities and maternal and paternal social group IDs were included as random effects in the models. This allowed us to partition variance in PD_i and EGP_i to assess the relative importance of individual and group level effects (conditional on fixed effects). We make the standard assumptions that random effects are normally distributed with means of zero and variances to be estimated. For ease of

interpretation, variance components were standardized to intraclass correlations (ICC) by dividing by phenotypic variance (determined as the sum of all variance components). ICC are thus interpretable as individual and group repeatabilities (R) for random effects relating to parental individuals and their social groups (Nakagawa & Schielzeth, 2010). In addition, we explicitly modelled a covariance term between the maternal and paternal social group identity effects. The strength and sign of this relationship is biologically informative since, for instance, if groups vary in EGP in a non sex-specific way we predict a positive covariance. Conversely, since cub natal and maternal social groups are the same, if EGP follows a source-sink dynamic with respect to genetic consequences (i.e. some groups are net importers of genes and some net exporters) we predict a negative relationship.

Statistical inference on random effects was by likelihood ratio test comparison of the full model to reduced formulations in which (co)variance components arising from the tested random effects were assumed absent. Twice the difference in log-likelihood between full and reduced models was assumed to have a χ^2- distribution, and we conservatively (see Visscher 2006) assume the degrees of freedom (DF) equal to the number of additional parameters in the full model.

The analyses described above were conducted using all available PD_i and EGP_i observations based on the 80% confidence threshold for parentage assignment. To assess sensitivity of results to this choice of confidence threshold, we repeated the analyses using only parentage assigned at 95% confidence. While the higher threshold should reduce 'measurement error' in PD_i and EGP_i arising from erroneous assignments, it also reduced sample size for analyses of these variables. Overall, conclusions regarding individual and

group-level variation remained broadly the same. Some inflation of variance components occurred in models using the higher threshold, and there were also some changes to the significance of fixed effects. Full results of these additional analyses are reported in the electronic supplement (Tables S2.6-S2.8) and commented on, where appropriate, below.

Results

Parentage analysis

In total, pedigree reconstruction resulted in 617 cubs being assigned at least one parent (35% of genotyped cubs included in the analyses), representing 29 cohorts and 6 generations (see Figure S2.1 for visual representation). Out of these, 556 (89%) cubs were assigned both parents, while 23 (4%) were assigned only a mother and 40 (7%) only a father. Overall, the 1,175 parental relationships (579 maternities and 596 paternities) were represented by 239 fathers and 278 mothers. Among these, half-sibship sizes (mean ±SD) varied from 1-11 (2.08 ±1.53) for mothers and 1-14 (2.49 ± 2.37) for fathers, with a total of 638 maternal and 1113 paternal sibships out of which 186 were full sibships. Additionally, 189 and 191 maternal grandmaternal and -paternal, as well as 155 and 161 paternal grandmaternal and -paternal links were present. Based on successful maternal assignments, mean litter size was 1.24 (range 1-3), which is slightly lower than previous reports for this and other populations (1.4-1.5; Carpenter et al. 2005; Dugdale et al. 2007; Annavi et al. 2014). Out of 101 litters of more than one cub, 23% (compared to a previous estimate of 16%; Carpenter et al. 2005) were multiple paternity litters, comprising 18 litters of n=2 and 4 of n=3 contributed to by two different fathers,

and one of n=3 with each cub assigned a different father. Parent-offspring assignments covered 37 social groups out of the 45 represented in the full database. Based on the parent-offspring assignments made, the mean rate of extra-group paternity over the 29 years was 37% (SD ±18.40). The relatively small proportion of assignments likely reflects the lack of strong prior information on maternity in badgers. Certainly, this greatly reduces power, and so the number of assignments, relative to paternity assignment when the mother is already known (Jones et al. 2010). Incomplete sampling of candidate parents is likely to be another contributing factor. The number of unsampled candidate parents estimated by MasterBayes varies considerably between cohorts with a median (range) of 0.82 (0.36-0.63) females per group, and 20.40 (5.13-239) males in the whole study area (Table S2.9). Out of the total parent-offspring assignments accepted at ≥80% confidence, 34% and 19% were assigned with ≥90% and ≥95% confidence, respectively.

Among-cohort variation in mean annual paternity distance

Across the 23 cohorts for which spatial data could be included in the parentage assignment, point estimates of PD_c obtained as the mean of the posterior distributions for each cohort varied from 173 m (95% CI, 93-275 m) to 608 m (95% CI, 270-1249 m) with a mean of 354 m (SE ±19.60) across cohorts. Despite relatively high uncertainty around some annual estimates, non-overlapping credible intervals for some pairwise comparisons indicate significant annual variation in PD_c (Figure 1a). However, this variation was not related to any of the explanatory variables (population size, sex ratio or year treated as a continuous variable to characterise any trend) tested in our multiple regression model (Table 1).

Table 1. Estimated effects of population size, sex ratio and cohort (year) on modal annual paternity distance (PD_c). Estimates are from multiple regression with uncertainty integrated over the full posteriors of annual PD_c (see main text). Predictors were mean centred for analysis.

	Estimate	95% credible interval
Intercept	332.43	319.90- 382.60
Population size†	0.36	-0.67 – 1.15
Sex ratio‡	-331.43	-1706.30 – 1743.66
Year	0.44	-7.81 – 4.74

† annual estimate of the number of badgers in Woodchester Park, based on 20 "core" social groups with consistent capture records

‡ calculated from annual population size estimates as the number of males divided by total population

Among-individual and among-group variation in paternity distance

Our mixed model analysis of PD_i indicated no significant effects of parental age, weight or group size (neither maternal nor paternal variables; Table 2). Maternal social group sex ratio, on the other hand, had a significant negative effect on paternity distance (Table 2), indicating that cubs from maternal social groups (i.e. cub's natal group) with a higher proportion of males have lower paternity distances on average. Paternal social group sex ratio showed the opposite trend, but the effect was not significant ($p>0.05$). Testing the random effects provided evidence of significant among-individual variation in PD_i for both mothers (among-mother repeatability, denoted $R_M = 0.16$ SE ±0.05, $\chi^2=40.29$, $p<0.001$) and fathers (among-father repeatability, denoted $R_P = 0.2$ SE ±0.06, $\chi^2=35.82$, $p<0.001$) (see Figure 2). Comparison of the full model fit to one in which maternal and paternal identity variance components were constrained to

be equal provided no significant evidence against the null hypothesis that mother and father explain equal variance in cub PD_i (χ^2=0.38, p=0.5). The random effect of year was estimated at c. 1% of the variance and was not significant.

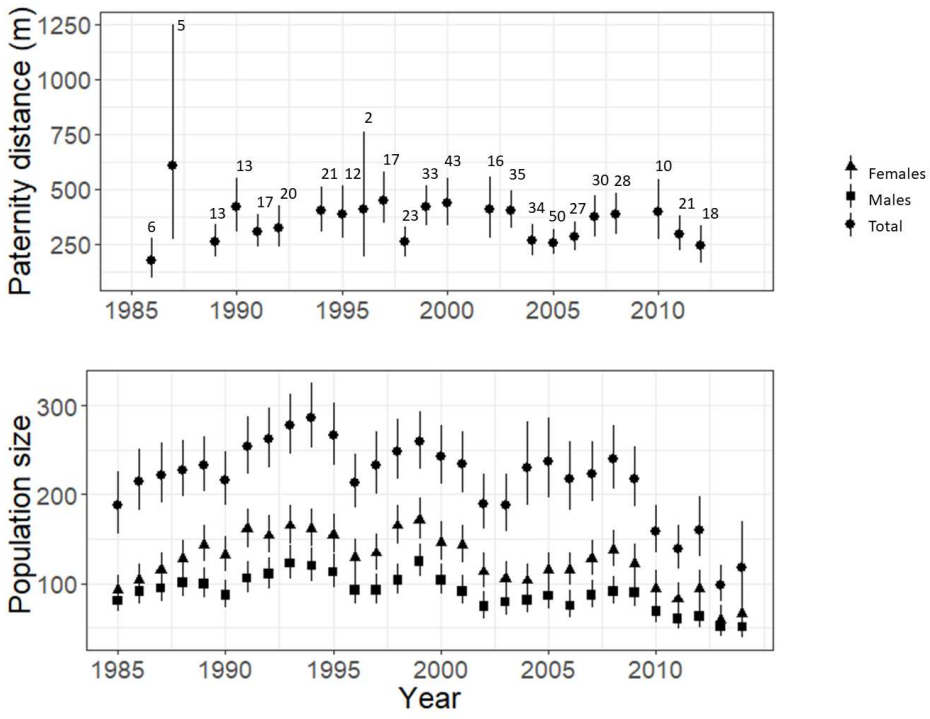

Figure 1. Top: Annual modal paternity distance (PD_c) estimated for each of 23 cohorts by MasterBayes (Hadfield et al. 2006) during pedigree reconstruction. Lines represent 95% credible intervals. Numbers above points represent the number of cubs assigned parentage in each year. **Bottom:** Total population size and number of males and females estimated in program MARK for each year of the study, based on 20 core social groups with consistent capture records. Bars represent standard errors.

Parental social group identities also explained significant variation in PD_i, with group level repeatabilities of R_{MSG}=0.25 (SE ±0.05; χ^2=58.2, p<0.001) and R_{PSG}=0.38 (SE ±0.06; χ^2=64.50, p<0.001), where MSG refers to maternal, and PSG to paternal social group (Figure 2). The difference in the proportion of variance in PD_i explained by PSG compared to that of MSG was marginally

non-significant (χ^2=3.43, p=0.06). There was a strong negative covariance between maternal and paternal group identity effects, which corresponds to a correlation (±SE) of $r_{MSG.PSG}$ = -0.99 (±0.03; χ^2=39.30, p<0.001; Figure 3c). Thus, social groups in which resident females (males) are more likely to mate with males (females) from further away are the same groups in which resident males (females) are less likely to mate with females (males) from further away. To visualise this pattern better, and the among-group variation in PD_i generally, we extracted the group level random effect predictions (best linear unbiased predictors or, BLUPs, see Table S2.2), which represent the predicted deviation of each (maternal and paternal) social group from the mean paternity distance, and overlaid them on a spatial map of the study area (Figure 3). This confirms that PSG with longer-than-average paternity distances, correspond to MSG with shorter-than-average paternity distances. Biologically, this is consistent with source-sink dynamics where some groups both retain resident male genes as well as attracting extra-group paternity, however, under the current methodology it is not possible to discern whether it is primarily driven by physical movement of males, females, or both. Note that while the sources of among-group variation are unknown, we highlight that estimates here are conditioned on group size and sex ratio, the latter having some effects as described above.

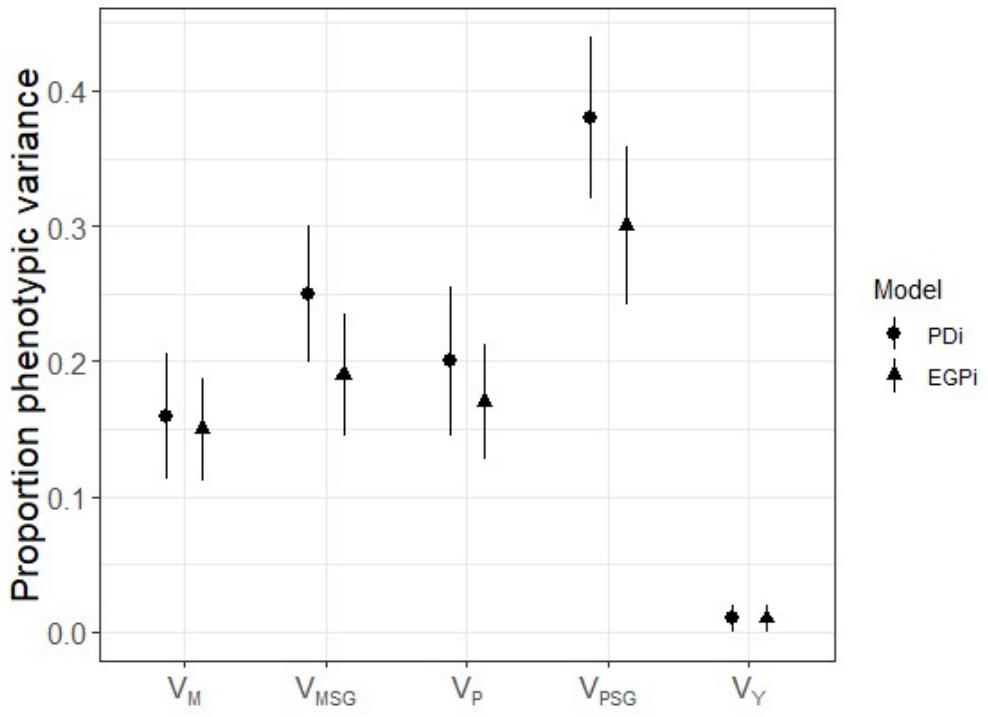

Figure 2. Estimated intra-class correlations (i.e. proportion of total phenotypic variance calculated by dividing each component by the sum of all variance components) for each random effect in models of PD_i and EGP_i. Bars represent standard errors. M and P denote maternal and paternal individuals, while MSG and PSG denote the corresponding maternal and paternal social groups.

Among-individual and among-group variation in extra-group paternity

Analysis of EGP_i yielded broadly similar insights to our model of PD_i, although paternal, as well as maternal, social group sex ratio had significant effects on extra-group paternity (Table 2). Similar to PD_i, the effect was negative for maternal, and positive for paternal group sex ratio. Thus, there is lower extra-group paternity among offspring in groups with higher male to female ratios. Other fixed effects were non-significant (Table 2). Maternal and paternal ID had significant repeatabilities ($R_M = 0.15 \pm 0.04$, $\chi^2=40.61$, $p<0.001$; $R_P = 0.17 \pm 0.04$, $\chi^2=35.34$, $p<0.001$) indicating consistent differences among individuals of both

sexes in their tendency to have offspring with extra-group partners (Figure 2). Social group level effects were also significant and again almost perfectly negatively correlated ($r_{MSG.PSG}$ = -0.99 SE ±0.03; Table 3, Figure 3). Differences in the amount of variance explained by maternal versus paternal identity, and MSG versus PSG were not significant, while year explained only a small (and non-significant) amount of variance in EGP_i (Table 3).

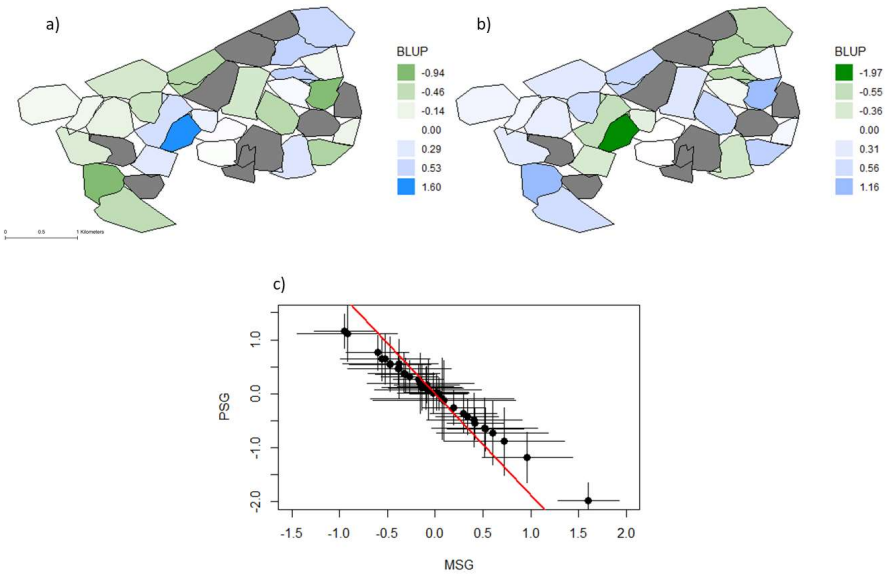

Figure 3. Spatial representation of **a)** maternal and **b)** paternal social group effects and **c)** the relationship between them. Effects are predicted from the mixed model of log-transformed PD_i (see main text) using best linear unbiased prediction (BLUP) while the spatial configuration of social group territories illustrated is derived from a bait marking survey in 1993 (when the maximum number of social groups were present). Six social groups included in current analyses are not shown on panels a) or b) due to missing bait-marking data, while grey shaded territories correspond to groups with no parentage assigned. Error bars in panel c) denote ± standard error and the regression line (red) slope is calculated directly from the model (co)variance estimates as $COV_{MSG.PSG}/V_{MSG.}$ MSG and PSG denote maternal and paternal social groups.

Table 2. Estimated fixed effect coefficients (standard error) and Wald F-tests from mixed models of log-transformed PD_i and EGP_i (see main text for details). Response variables were standardised into standard deviation units (SDU) prior to analysis. M and P denote maternal and paternal individuals, while MSG and PSG denote the corresponding maternal and paternal social groups. DF stands for degrees of freedom.

	Log(PD_i)				**EGP_i**			
	Estimate (SE)	**DF**	**F**	**P**	**Estimate (SE)**	**DF**	**F**	**P**
Intercept	-0.72 (0.15)	1, 214.7	24.56	**<0.001**	0.72 (0.14)	1, 226.9	74.97	**<0.001**
Age_M	-0.45 (0.15)	1, 533.1	0.09	0.76	-0.52 (0.15)	1, 534.0	0.12	0.73
Body $mass_M$†	-0.61 (0.13)	1, 302.8	0.22	0.63	-0.66 (0.13)	1, 304.1	0.26	0.61
Group $size_{MSG}$	0.94 (0.18)	1, 456.9	0.28	0.59	0.96 (0.18)	1, 443.0	0.29	0.59
Sex $ratio_{MSG}$‡	-0.74 (0.22)	1, 531.5	10.97	**<0.001**	-0.82 (0.22)	1, 524.2	13.55	**<0.001**
Age_P	0.28 (0.2)	1, 516.7	2.11	0.15	0.30 (0.2)	1, 517.3	2.4	0.12
Body $mass_P$†	-0.59 (0.12)	1, 213.4	0.25	0.62	-0.56 (0.19)	1, 215.0	0.23	0.64
Group $Size_{PSG}$	-0.12 (0.18)	1, 537.4	0.44	0.50	-0.12 (0.18)	1, 531.9	0.43	0.51
Sex $ratio_{PSG}$‡	0.43 (0.24)	1, 538.1	3.21	0.08	0.50 (0.24)	1, 536.0	4.48	**0.04**

Full models fitted for each response were $y \sim \mu + Age_M + Body\_Mass_M + Group\_size_{MSG} + Sex\_ratio_{MSG} + Age_P + Body\_Mass_P + Group\_size_{PSG} + Sex\_ratio_{PSG} + M + P + MSG + PSG + Year$ where italic font denotes random effects and y is either $log(PD_i)$ or EGP_i

† mean body mass for parental individuals with multiple weight measurements within year of cub's birth

‡calculated as number of males divided by group size where group size is males plus females

Table 3. Estimated (co)variance components (standard error) associated with random effects in mixed models of EGP_i and log-transformed PD_i. Statistical inference of random effects is by likelihood ratio test results (see main text for details). M and P denote maternal and paternal individuals, while MSG and PSG denote the corresponding maternal and paternal social groups.

	log(PD_i)				EGP_i			
	Variance (SE)	**df**	**χ^2_1**	**P**	**Variance (SE)**	**χ^2_1**	**df**	**P**
V_{year}	0.02 (0.02)	1	3.22	0.07	0.02 (0.03)	2.83	1	0.09
V_M†	0.26 (0.05)	1	40.29	**<0.001**	0.26 (0.06)	40.61	1	**<0.001**
V_P†	0.31 (0.06)	1	35.82	**<0.001**	0.31 (0.06)	35.34	1	**<0.001**
V_{MSG}‡	0.39 (0.15)	2	58.16	**<0.001**	0.34 (0.13)	55.00	2	**<0.001**
V_{PSG}‡	0.59 (0.21)	2	64.54	**<0.001**	0.54 (0.19)	62.91	2	**<0.001**
$COV_{MSG,PSG}$	*-0.48 (0.17)*	*1*	*39.33*	***<0.001***	*-0.43 (0.15)*	*36.84*	*1*	***<0.001***
V_R	0.32 (0.04)	-	-	-	0.32 (0.04)	-	-	-

† not significantly different from each other (logLRT, PD_i: $\chi^2 = 0.38$, p=0.5 EGP_i: $\chi^2 = 0.28$, p=0.6)

‡ not significantly different from each other (logLRT, PD_i: $\chi^2 = 3.43$, p=0.06, EGP_i: $\chi^2 = 3.68$, p=0.06)

Discussion

We examined variation in breeding excursions using pedigree-derived information on extra-group paternity and paternity distance in a wild population of badgers. We found evidence that cohort mean paternity distance (PD_c, the mean distance between the social groups of fathers and their cubs) varied among years. Contrary to our predictions, this among-cohort variation in PD_c was not explained by annual variation in population size or sex ratio, nor did we see any systematic temporal trend in paternity distance over the study period. However, individual (cub) level analyses showed significant among-parent (both mother and father) and among-social group variance in breeding excursions, with the latter contributed to (but not fully explained) by differences in group sex ratios. Below we discuss these findings in the context of the wider literature, focusing on their implications for ecological and evolutionary dynamics.

Among-cohort variation in average paternity distance

Our point estimates of PD_c varied considerably among years, suggesting temporal variation in the tendency of badgers to undertake breeding excursions. However, there was no systematic trend over time and cohort variation was not explained by changes in the size or sex ratio of the Woodchester Park population as a whole. A post hoc analysis of PD_i and EGP_i with population-level estimates included as additional predictors also revealed no significant effects of population size or sex ratio. Year-to-year variation in PD_c therefore remains unexplained at present, but could plausibly be linked to other variables such as weather conditions, relatedness and neighbouring group composition, all of which are known to influence movement, activity and dispersal in badgers (Annavi et al., 2014; Noonan et al., 2014), but which were not investigated here.

More generally, the absence of population size effects on PD_c contrasts somewhat with previous studies. In badgers and other species (e.g. Møller 1991; Mougeot 2004; Annavi et al. 2014), local density-dependence has been reported in rates of extra-group paternity – a pattern often linked to changes in mate guarding behaviour (e.g. Møller 1991; Kokko & Rankin 2006; Isvaran & Clutton-Brock 2007), though evidence for mate guarding in badgers is limited (Dugdale et al., 2007). Variation in movement distance has also been linked to population density in badgers (Frantz et al., 2010; Byrne et al., 2014) and is sensitive to local density reductions from culling (Tuyttens et al., 2000a; Tuyttens et al., 2000b; Pope et al., 2007). However, we note that paternity distance is considered a proxy for movements relating specifically to breeding excursions here. Certainly, the processes governing rates of breeding excursions may differ from those influencing other types of movement making direct comparisons difficult.

There are also several other explanations for the apparent discrepancy between our results and these previous findings. Firstly, it is possible that among-year density variation in the current study is not sufficient to reveal a density-dependent response, as Woodchester Park has one of the highest recorded densities (25 adults/km^2) of badgers throughout the species' range (Rogers et al. 1997) and the habitat may be saturated. However, population fluctuation over the period of this study suggests this is not the case, as population size increased in some years. Second, it is possible that the (overall) population density measure used here doesn't capture variation at the correct scale to reveal density-dependence. The latter appears to be the case for sex ratio, with temporal variation in population level PD_c not being predicted by population sex ratio, but local (i.e. group) sex ratios contributing to spatial

variation in EGP_i and PD_i defined at individual (cub) level (discussed further below). However, parallel local density effects (modelled as social group size effects) did not contribute to spatial variation in either EGP_i or PD_i. An additional consideration is the fact that the lack of a clear density-dependent pattern could conceivably be an artefact of the study scale, as high-density populations (such as Woodchester Park) typically involve sampling over smaller spatial areas and may therefore miss longer distance movement (Byrne et al. 2014). Finally, we note that the large proportion of unresolved parentage across the study period, as indicated by the relatively low number of parentage assignments (35% cubs assigned parent(s)), may well have resulted in a lack of power to distinguish density and sex ratio effects on cohort mean paternity distance.

Among-group variation in cub PD_i and EGP_i

Analysis of cub level proxies of (parental) breeding excursions revealed several important sources of variation. Parental social group sex ratios influenced both EGP_i and PD_i. Although we note that the effect of PSG sex ratio on PD_i was not statistically significant in the main analysis presented, it was significant when we refitted our model using only those paternity distances inferred from assignments at the 95% confidence threshold (see Table S2.6). Cubs had higher PD_i (on average) and were more likely to have an extra-group father if born into less male-biased social groups. Conversely, cubs born in groups with more male-biased sex ratios were more likely to be fathered by within-group males. These results are consistent with earlier analysis of trapping data in Woodchester Park in which Rogers et al. (1998) concluded that males preferentially move to groups with a higher proportion of females. Woodroffe et al., (1993) also found that the peak of these temporary excursions coincides, for

both males and females, with female oestrus while in the Wytham Wood (Oxfordshire, UK) badger population, while, similar to Woodchester Park, higher numbers of within-group males were associated with lower rates of EGP (Annavi et al., 2014). Taken together, these results are consistent with ongoing mate guarding by males (anti-kleptogamy hypothesis; Robertson et al. 2014) although they do not provide direct evidence. Although previous studies have thus emphasised the role of males in breeding excursions, we stress that our indirect inferences from paternity distance and extra-group paternity do not allow us to discriminate between male and female movements. Temporary excursions by both sexes are possible and our results could reflect important variation in female mating behavior in response to mate availability. For instance, females may be less inclined to seek extra-group matings in male-biased groups if they have greater choice of partners. Nevertheless, the relative importance of contributing factors (e.g. avoidance of male-male competition, female choice for extra-group males, inbreeding avoidance by either sex) is not clear (although see Annavi et al. 2014).

After accounting for sex ratio (and group size) effects, parental social group identities together account for more of the remaining variance in cub PD_i and EGP_i (63% and 49%, respectively) than any other variance component. Further, the strong negative correlation between maternal and paternal group identity effects in both models indicates that maternal groups that predispose to high paternity distance are the same as the paternal groups predisposed to low paternity distance. These social group identity effects are not readily explained as a simple consequence of, for example, (relative) distances between groups or edge-effects. In the former case, a positive correlation between maternal and paternal social groups would be present, while, in the latter, groups at the edges

of the study area would be expected to have below average PD_i. This is because we expect failure to assign paternity to cubs sired by unsampled males from outside the study area, such that edge effects are likely to cause downward bias in average PD_i and EGP_i for peripheral maternal groups. However, no such pattern is readily apparent in our analysis (see spatial maps of group effects on cub paternity distance in Figure 3).

Thus, while reiterating the earlier caveat that some long-distance movements may be missed by our analysis, among-group variation in cub paternity distance is not readily explained as an artefact here. Rather the emerging picture is one of source-sink dynamics, where some social groups are more 'attractive' than others thus both retaining and drawing in male genes. From the male's point of view this could signal variation in some unknown aspect of "quality" among females from different social groups, which itself may be mediated by spatial variation in resource availability (e.g. food, setts) that determine habitat preferences of females. Conversely, the observed pattern could reflect variation in female mating preferences if 'attractive' males are spatially clustered. Spatial variation in habitat quality has previously been linked to differences in group size across Woodchester Park (Delahay et al. 2006) and is certainly a plausible hypothesis for explaining among-group differences 'attractiveness', although variance explained by parental social group identities is estimated here conditional on a set of fixed effects including group size. Furthermore, group size itself was not a significant predictor of either response variable in the main analyses presented based on parentage assignments made at 80% confidence. However, using the more stringent assignments threshold of 95%, group sizes did have a significant effect. Given statistical support for group size effects is thus rather equivocal we draw no strong

conclusions about its role. However, at least in a qualitative sense it is worth pointing out that PD_i and EGP_i seem to increase with paternal group size and decrease with maternal group size.

Similar variation has been recorded in great cormorants (*Phalacrocorax carbo sinensis*), where Minias et al. (2016) found higher rates of extra-pair paternity in the periphery than in the centre of a nesting colony. This pattern was not explained by density but by variation in mate quality, as indicated by nest site location. Habitat structure has also been shown to influence rates of extra-pair paternity, for instance, in blue-footed boobies (*Sula nebouxii*), by restricting movements within the colony (Ramos et al., 2014). Although our results, as well as results from previous studies (Carpenter et al., 2005; Rogers et al., 1998), suggest that movement in this population is focused around neighbouring social groups, with an average PD_C of 358 m and a nearest neighbour distance between social group main setts of 355 m (SD 84) m, habitat structure *per se* is unlikely to influence movement in this population, spatial structuring (particularly of females) instead being mediated by resource availability (da Silva et al., 1994; Delahay et al. 2006).

Among-individual variation in cub PD_i and EGP_i

In addition to social group effects, we found that there was repeatable variation among both mothers and fathers for cub PD_i and EGP_i. The most parsimonious interpretation of these results is that there is among-individual variation, in both sexes, for breeding behavior. This interpretation is in line with trapping-based inferences for the Woodchester Park badger population (Rogers et al., 1998), as well as studies of other taxa. For instance, Whittingham et al., (2006) found the proportion of extra-pair young produced to be highly repeatable for female

tree swallows (*Tachycineta bicolor;* intra-class correlation, r= 0.83). In coal tits (*Parus ater*), the proportion of extra-pair young showed repeatability in both sexes among the same social pairing (r=0.33 and 0.47 for males and females respectively; Dietrich et al. 2004). Conversely, breeding excursions were found not to be a repeatable behaviour in female roe deer (*Capreolus capreolus*; Debeffe et al., 2014). Among-individual differences in other dispersal and exploratory behaviours have also been recorded for spiders (Bonte et al., 2009; Johnson et al., 2015), fish (Harrison et al., 2015), amphibians (Cosentino & Droney, 2016) and birds (Reid et al. 2011a; Patrick et al. 2012; Grist et al. 2014). Thus, among-individual variance in PD_i and EGP_i could be linked to both reproductive decision making (i.e., individuals varying in their propensity/ability to seek or obtain extra-group matings), and more general exploratory traits influencing encounter rates between badgers from different groups. Regardless, a further aspect of our analysis worth noting is that similar levels of variation in cub PD_i and EGP_i were explained by maternal and paternal identities. Thus, whether gene flow from breeding excursions is being mediated primarily by variation in movement *per se,* or by reproductive decision making, both sexes appear to have an equal impact.

Our analyses have not clearly identified the underlying source(s) of among-individual variance in (parental) mating behaviour. Neither size nor age (of either parent) significantly predict PD_i and EGP_i in the main analyses, although we note that using the 95% confidence pedigree the positive effects of paternal age on both response variables are statistically significant (Table S2.4). This suggests that older males tend to produce more extra-group offspring and make longer breeding excursions (or mate with females that do), though this conclusion remains tentative. In a broader sense, among-individual

variation will reflect the fact that individuals experience different environmental conditions (e.g. maternal effects, food availability, social status) even within groups and years (which were both modelled separately), although genetic variation may also be present. Dispersal distance has been shown to be heritable in a free-living population of great tits (*Parus major*; h^2= 0.15 SE ± 0.01; Korsten et al. 2013), as has EGP rate in in female, but not male, song sparrows (*Melospiza melodia*; Reid et al. 2011a&b). It is, therefore, possible that the among-individual variance found here has a partial genetic basis. In fact, the pedigree will facilitate testing this, although it would best be achieved through quantitative genetic modelling of independently obtained trapping data.

Conclusions

We have used a genetic pedigree to characterise variation in paternity distance and extra-group paternity in a high-density badger population. We show there to be variation among years and social groups, but also among-parental individuals (both mothers and fathers) within groups. Although effects of social group sex ratio (and potentially group size and paternal age) were detected, in general this variation is not readily explained by life-history and social correlates. Among-group variation appears to follow a pattern of source-sink dynamics, suggesting that some social groups are more attractive to extra-group partners than others, though levels of among-parental variation in our metrics were similar across the sexes. Not readily explained by age or body size, it is possible that genes as well as individual-specific (rather than group level) environmental factors contribute to among-individual variation although this remains to be tested. Individual-level differences can have important consequences for many ecological and evolutionary processes, and our results

highlight the fact that individuals can vary consistently in their mating behavior. Together these results emphasise the importance of including individual-level variation in evolutionary models of animal movement and mating behavior, as well as management and conservation measures.

Chapter 3

The relative importance of social, maternal and additive genetic effects on *Mycobacterium bovis* infection status of European badgers (*Meles meles*)

Abstract

Within host populations, individuals can vary in their susceptibility to infection by parasites and in rates of disease progression once infected. Though mediated through differences in behaviour, resistance or tolerance, variation in disease outcomes ultimately stems from both genetic and environmental (including social) factors. Despite obvious implications for evolutionary, ecological and epidemiological dynamics of disease traits, the relative importance of these factors has rarely been quantified in wild animal hosts. Here, we use a long-term capture-mark-recapture study of group-living European badgers (*Meles meles*) to characterise genetic and social environmental sources of variation in bovine tuberculosis (bTB) infection status. Quantitative genetic analyses of individual variation in disease progression and lifetime infection risk yielded significant, but low, estimates of heritability. Of more importance were the effects of social group membership (in time and space), and maternal effects were an important source of variation in cub infection status. Thus, while genes do contribute to among-individual variation in this population, rapid evolution of host defence strategies under presumed parasite-mediated selection is unlikely. Conversely, our results lend further support to the view that social and early-life

environments are important drivers of dynamics of bTB infection in badger populations specifically, and of disease traits in wild hosts more generally.

Introduction

Pathogens and parasites are key drivers of the ecological and evolutionary dynamics of their host populations (Schmid-Hempel 2011). To counter them, hosts have evolved a myriad of defence strategies that include behavioural avoidance of infection (Behringer et al.2006), immune responses that limit parasite growth (resistance; Rigby et al.2002), and repair of parasite-induced damage to minimise costs of infection (tolerance; Medzhitov et al. 2012). However, within host populations, there can be considerable variation among individuals in these traits which, in turn, leads to differences in susceptibility to infection and disease progression. The importance of among-individual variation for population-level processes has become increasingly clear in recent years (e.g. Madritch & Hunter, 2002; Grist et al., 2014; Svanbäck et al. 2015), particularly with respect to our understanding of infection dynamics (Kramer-Schadt et al. 2009; VanderWaal & Ezenwa, 2016). Nonetheless, while variation can generally be viewed as stemming from genetic and environmental (including social) effects, we currently have little knowledge of their relative importance in wild and unmanaged host populations where environmental factors can exert considerable influence on infection dynamics.

From an evolutionary point of view, parasites (in which we include pathogenic bacteria, viruses, fungi and protozoa) are expected to select for improved host defences. However, any response to selection is contingent on the presence of genetic variance in the host. A partial genetic basis of variation in host defence strategies against infectious disease is well-established in humans, model organisms, and livestock studies (Morris, 2007; Yan et al. 2006; Breitling et al., 2008). For instance, selective breeding for resistance to specific pathogens is important to agriculture and aquaculture (Stear et al. 2001; Yáñez

et al. 2014). In addition to enabling host selection responses, genetic variation among individuals may also impact pathogen emergence and prevalence by modifying transmission dynamics (Yates et al., 2006; Doeschl-Wilson et al., 2011) and determining susceptible host availability. Thus, among-host genetic variation can influence the population-level dynamics of infection through multiple routes (Nath et al. 2008; Lough et al. 2015). However, at present relatively little is known about the extent of genetic variation in disease susceptibility in wild host populations, in large part due to the difficulties of obtaining appropriate immunological data coupled to genetic information over multiple generations. In wild bird populations, several quantitative genetic studies have investigated genetic variation in immune response traits with findings ranging from an apparent absence of genetic effects (Pitala et al. 2007) to evidence that immune function is moderately heritable (Bonneaud et al. 2009; Kim et al. 2013). In other studies, genetic variation in both resistance and tolerance to an ectoparasite has been reported in wild dace (*Leuciscus leuciscus*; Blanchet et al. 2010; Mazé-Guilmo et al. , 2014), while quantitative analyses of helminth infections in Soay sheep (*Ovis aries*) revealed genetic variation in host resistance but not tolerance (Hayward et al., 2014a; Hayward et al., 2014b). There is also growing evidence that consistent differences in behavioural processes likely to influence infection risk (e.g. dispersal, sociability; Barber & Dingemanse, 2010) are heritable in natural populations (Korsten et al. 2013; Petelle et al. 2015). However, whether this represents an important source of genetic variation for infection status or disease progression remains to be determined.

If studies to date have yielded mixed conclusions about the importance of genetic variation in disease traits in wild animal hosts, then a corollary of this

is that we also have limited understanding of how environmental factors contribute to among-host variation. Abiotic factors (e.g. rainfall, seasonality) play an important role in shaping disease dynamics at the population level (Altizer et al. 2006), as do biotic environmental influences such as the distribution and social structure of host populations. Social effects, broadly defined as influences of phenotype arising from interactions with conspecifics, are associated with heterogeneity in disease dynamics; on the one hand, transmission of pathogens within groups of closely interacting individuals represents a major cost of group living (Kappeler et al. 2015), while on the other hand, close-knit groups with limited among-group contact can inhibit the spread of disease over larger scales (Carter et al. 2007). In many cases social effects will also be age-specific. For example, mature individuals can be exposed to higher infection risks from inter-sexual contacts (e.g., sexually transmitted infections; Rhule et al.2010).

In species with parental care, mothers (and/or fathers) are another source of social effect likely to be age (or stage) specific. In birds and mammals, for instance, offspring immunocompetence in early life is strongly linked to the transfer of maternal antibodies (Grindstaff et al. 2003; Grindstaff et al. 2006). The protection afforded by maternal antibodies to offspring is, however, temporary, lasting from a few days to months (Grindstaff et al. 2003) and subsiding with the beginning of antibody production by the offspring's own immune system. More general environmental conditions experienced by the mother (e.g. food availability) can have knock-on effects on offspring immune development and disease resistance (Karell et al. 2008; Garbutt et al. 2014). Regardless of the mechanism, maternal effects present in early life will tend to decline with age, as the maternal phenotype typically has less opportunity to

influence the offspring after the cessation of maternal care. This pattern has been well documented for other trait types (e.g., growth, morphology, life history; Wilson et al. 2005b; Houde et al., 2015; Falica et al. 2017), but does not mean adult phenotypes can be assumed to be free from maternal influence (see e.g., Bonne et al 2015).

Here, we seek to examine the relative importance of genetic and social environmental (including maternal) sources of variation in *Mycobacterium bovis* (the cause of bovine tuberculosis; bTB) infection status in a wild population of European badgers (*Meles meles*). Badgers are an important wildlife reservoir for bTB infection, a longstanding socioeconomic burden on the livestock industry and tax payers in the UK (Defra, 2014). Some of the drivers of disease in badgers have now been documented. For instance, sexual dimorphism occurs, with bTB infection probability, disease progression and mortality risk all being higher in males (Graham et al., 2013; McDonald 2014). Age effects have also been observed (Graham et al., 2013; McDonald et al., 2014; Beirne et al. 2016), while at the population-level, bTB incidence and prevalence exhibit seasonal variation (incidence being highest in spring and prevalence peaking in autumn; Delahay et al. 2013). However, the relative importance of social environmental and genetic effects on bTB status has not been effectively characterised in part because kin-biased social groups make these potential sources of variation difficult to disentangle in the absence of experimental approaches.

Badgers are facultatively social, forming groups in medium to high density populations but adopting a more solitary lifestyle when living at low density (Roper 2010). At the level of the population, natal philopatry and territorial defence limit mixing of animals among groups, which in turn is

expected to reduce disease transmission among social groups (Delahay et al. 2000b) while at the same time being associated with relatively high within-group transmission rates. Social group structure should thus drive spatial clustering of bTB, and high among-group variation in disease status, relative to that found within-groups, has been previously reported (Delahay et al. 2000b). However, genetic data suggest alternative explanations may also have merit. Parentage analysis shows that among-group breeding dispersal is limited (though, crucially for current purposes, does occur; Chapter 2; Marjamäki et al. in press), leading to greater relatedness within than among groups (Dugdale et al., 2008). Recent work has also shown that bTB infection risk for cubs is increased by the presence of closely related infected adults (including but not limited to mothers) within the natal group (Benton et al. 2016). Spatial heterogeneity in host disease status is consistent with within-group (and by extension kin-biased) social interactions impacting infection risk, maternal effects, and/or genetic variation in one or more host defence strategies. These alternative explanations are in no sense mutually exclusive.

While it is therefore clear that infection status can be influenced by numerous factors at multiple scales, the long-term life-history and genetic pedigree data from the Woodchester Park study affords a rare opportunity to assess their relative importance in a wild vertebrate system. We adopt a quantitative genetic animal model approach to decompose the variance in bTB infection status into its component parts and examine the relative contributions of genetic and environmental factors. We employ a progressive categorisation of disease status (based on live diagnostic test results) that approximates bTB progression from test negative to advanced stages of infection (Graham et al. 2013). We ask: i) whether variation in host bTB infection status has a detectable

genetic basis; ii) what are the relative contributions of genetic and social (group and/or maternal) effects on the observed variation in host bTB status; and, iii) do the relative contributions of genetic and social effects on bTB status vary in relation to host age?

Methods

Study site and sampling

The badger population under study at Woodchester Park (Gloucestershire, UK) has comprised approximately 200–300 badgers that have been the subjects of an ongoing capture-mark-recapture study, initiated in 1976. The study area, approximately 11 km^2, consists of a steep-sided wooded valley surrounded by farmland and is located in an area where bTB infection is endemic in cattle and wildlife. Badger dens, or setts, in the study area have been the subject of trapping operations up to four times a year for two consecutive nights using peanut-baited steel mesh box traps, set after 4–8 days of pre-baiting. Trapped badgers were anaesthetized (de Leeuw et al. 2004) and their capture location, sex and age class (cub, adult) are then recorded. Individuals caught as cubs (distinguished from adults by size, pelage and toothwear; (Delahay et al. 2013) could then be accurately aged at subsequent captures. The bTB infection status of captured badgers was determined by bacterial culture of clinical samples (faeces, urine, sputum, pus, bite wound swabs; (Clifton-Hadley et al. 1993), and a serological test for the presence of *M. bovis* antibodies (Brock Elisa used 1982 to 2006 (Goodger et al., 1994) and BrockTB Stat-Pak test used 2006 to 2014 (Chambers et al., 2008)). Guard hairs were taken for DNA extraction and subsequent microsatellite genotyping (see below). After a recovery period, all

badgers were released at the point of capture. Social group boundaries were determined for each year of the study by bait marking (Delahay et al. 2000a). Overall, the mark-recapture dataset used here contained 15,252 observations on 3239 individual badgers between 1976 and 2014.

Microsatellite genotyping and parentage analysis

The procedures for microsatellite genotyping and parentage analysis are described in detail in Marjamäki et al. (in press) (Chapter 2). Briefly, DNA was extracted from hair samples using either the protocol outlined in Carpenter et al. (2005), or an ammonium acetate extraction method (Richardson et al. 2001). We used a minimum of five hair follicles with visible roots per individual for extraction. Individuals were genotyped using a minimum of 16 (Carpenter et al. 2005) and maximum of 22 fluorescently labelled autosomal microsatellite markers. We used a 2 µl Qiagen Multiplex PCR reaction (Qiagen Inc., Valencia, USA), before separation of the amplicons on an ABI 3730 DNA Anazlyer and genotype scoring using GENEMAPPER 3.7. Microsatellite genotypes and spatial data were then used for Bayesian parentage analysis performed using the R (R Core Team 2016) package MasterBayes (Hadfield et al. 2006). Markov chains were run separately for each year (i.e. cub cohort) for 2 million iterations, with a thinning rate of 100 and burn-in period of 500,000. Tuning parameters were specified for each cohort to ensure that the Metropolis–Hastings acceptance rates were within acceptable limits (0.2–0.5; Hadfield 2017). The presence of unsampled males (per population) and females (per social group) was also allowed for each cohort. Assignments were accepted and used in downstream analyses when a confidence threshold of 80% was met, resulting

in a total of 1175 parentage assignments (579 maternities and 596 paternities). A total of 617 cubs were assigned at least one parent (35% of genotyped cubs included in the analyses), and out of these, 556 (89%) cubs were assigned both parents. The pedigree information is therefore far from complete, which may have some implications for our analyses (discussed further below). We note that, in contrast to comparable long-term avian and mammalian studies, maternal identities cannot be determined by observation in badgers owing to their nocturnal and subterranean habits. Thus, pedigree analysis is more challenging because maternities must be estimated simultaneously with paternities based on genetic and spatial data.

Infection status

Based on diagnostic test results from samples taken at each capture event, badgers were assigned to one of four bTB infection status categories on an ordinal scale following Graham et al. (2013). Individuals that returned negative results for both the serological test and bacterial culture were classified as test-negative (N) and assumed to be free of infection. Badgers that tested positive for blood antibodies but had negative culture results were assigned test-positive (P) status, indicating recent exposure to *M. bovis.* This classification potentially also includes individuals that have acquired immunity from a previous infection, though there is currently no evidence for acquired immunity to bTB in badgers. Positive test results could also indicate cross-reaction or presence of maternal antibodies (Maas et al., 2013; but see Tomlinson et al, 2012). To account for this possibility, and to reduce the error caused by false positive test results, a single test-positive followed by only negative results was considered a false

positive and classified as N. Badgers that tested positive for the presence of *M. bovis* by bacterial culture were assigned as either one-site (O) or multi-site (M) excretors, based on the number of sampled body sites that tested positive at each capture. Although culture has relatively low sensitivity as a diagnostic test, a positive result is a strong indicator of established bTB infection (see Drewe et al. 2010). These two categories are therefore considered to represent more advanced, infectious disease states.

Based on models of bTB immunopathogenesis in badgers (Mahmood et al., 1987; Lesellier et al., 2008), we assume these four categories can reasonably be ordered to reflect the progression of bTB within a host and converted them to a numerical score (N=0, P=1, O=2, M=3) to be used as a pseudo-continuous response variable in our quantitative genetic models. Finally, to further reduce the impact of the limited performance of the diagnostic tests, we elected to make the score progressive so that once a badger reaches a certain status, it can no longer return to a lower categorisation. As serological tests were performed using two different platforms (Brock Elisa 1982 -2005, BrockTB Stat-Pak 2006-2014) we reran the presented models (see 'Statistical Analyses') on two subsets of the data where only Brock Elisa or Stat-Pak was used.

Statistical analyses

We performed variance decomposition of two response variables. Firstly, we used the multisite bTB status score described above that ranges from 0–3 (hereafter denoted as bTB_{multi}) for which individuals have repeated observations, one for each capture event in the database. Secondly, we define

a binary “lifetime” bTB status score ($bTB_{lifetime}$) for which individuals have a single observation of either 0 (if they did not test positive for bTB during their entire recorded lifetime) or 1 (if they did). Both response variables were analysed using an animal model approach (Wilson et al., 2010), in which an individual’s breeding value – the additive effect of its genotype on phenotype (relative to the population mean) – is included as a random effect. This allows estimation of the (additive) genetic variance V_A in addition to variance components attributable to other specified random effects. Fixed effects were also included in models of both response variables (as described below) to control for known sources of variance. These effects are not directly relevant to the current hypotheses and so are not discussed in detail (but see Supplemental Tables S3.4-S3.5 for a full presentation of parameter estimates and statistical inference).

Multisite bTB status

First, we analysed bTB_{multi} on the observed (0–3) scale using ASReml 4.0 (VSN International) with an assumption of Gaussian residuals. The assumption must necessarily be violated given the definition of the response variable and so statistical inference should be treated with some caution. Nonetheless, inspection of model residuals suggests it is not an unreasonable assumption (i.e. residuals show unimodal distributions with a strong central tendency) and so we view this as a pragmatically sensible decision. Significance of fixed effects was determined using conditional Wald F-tests, while statistical inference on random effects was by likelihood ratio test (LRT) comparison of the full model to reduced formulations in which the tested random effect was

omitted. Twice the difference in log-likelihood between the full and reduced models was assumed to have a χ^2 distribution, and following Visscher (2006), we assumed the test statistic to be asymptotically distributed as an equal mix of χ^2_0 and χ^2_1 (denoted as $\chi^2_{0,1}$).

Sex, season (spring = Mar–May, summer= Jun–Aug, autumn= Sep–Nov, winter = Dec–Feb), and the linear, quadratic and cubic effects of age, were included as fixed predictors. Age was zero-centred to the mean (known) age of adult records (4.06 years) in the data set. Random effects included the additive genetic merit, a permanent environment effect, maternal identity, social group, year of observation, and a social-group-by-year interaction. These random effects are assumed to be drawn from distributions with zero means and variances – to be estimated – of V_A, V_{PE}, V_M, V_{SG}, V_Y and V_{SGxY}, respectively. The group by year interaction was included since, if bTB infection varies through time and among groups, then it seems plausible that the worst impacted groups could vary among years. Permanent environment effects were added to account for sources of among-individual variance not otherwise explicitly modelled. We note that progressive categorisation of bTB status may cause an upward bias in the amount of variance explained by individual identity (i.e., 'measurement error' will contribute to both V_{PE} and V_R here) so we limit biological interpretation of these generic 'environmental' variances. To ease interpretation of results, bTB_{multi} was scaled to standard deviation units (giving it an observed variance of 1) prior to model fitting. Thus, for example V_A can actually be interpreted as an estimate of the heritability unconditional of fixed effects. However, estimated variance components were also divided by phenotypic variance V_P, calculated as the sum of estimated variance components, to obtain intra-class correlations (ICC), conditional on fixed effects

(Wilson 2018), giving the proportion of variance in bTB status explained by each component. After removing individuals with missing social group information (n= 293), analyses were run using 14,846 observations of 2946 individuals.

To further disentangle the relative importance of maternal and additive genetic effects, we ran a variation of the above model ('Model 1') where maternal effects were restricted to cubs ('Model 2'). Comparisons between these two models (Models 1 and 2) allows us to determine whether the previously reported effects of relatedness on bTB infection (Benton et al. 2016) stem from additive genetic or maternal (genetic and environmental) effects, or a combination of the two. Additionally, it allows us to examine whether the relative importance of V_A and V_M changes with age. Since Models 1 and 2 have the same number of parameters, significance testing through LRT was not possible and model fit was determined based on Akaike Information Criterion (AIC; Akaike 1974). Heritability and intra-class correlations for Model 2 variance components were calculated separately for adults and cubs, as V_M in this model contributes to total phenotypic variance in cubs only (i.e., $V_{Pc} = V_A+V_G+V_Y+V_{GxY}+V_{PE}+V_M+V_R$, while for adults $V_{Pa} = V_A+V_G+V_Y+V_{GxY}+V_{PE}+V_R$, where V_R is residual variance). We also fitted Model 1 separately on adult and cub data. These age-specific models added relatively little further insight, so we do not present results in full below (but see electronic supplement Tables S3.6-S3.7 and brief comments in the discussion).

Lifetime bTB status

Using the binary $bTB_{lifetime}$ metric as the response variable, we fitted a Bayesian generalised linear mixed model (GLMM) using the package MCMCglmm

(Hadfield 2010) in the R environment. The data set contained 3239 observations of $bTB_{lifetime}$ of which 59% were zero. Sex and a linear effect of age at last capture were included as fixed effects, along with quadratic and cubic effects of age at last capture. Age at last capture was zero-centred to the mean (known) age of adult records (2.12 years) in the data set. The additive genetic merit, maternal ID, natal group (i.e. first group a cub is recorded at), and birth year, were included as random effects. We also included a natal-group-by-birth-year effect by creating a new factor 'group-birth year' which groups individuals based on both their natal group and birth year. We used a parameter expanded prior distribution on one degree of freedom for random effects while residual variance was fixed to 1 (de Villemereuille 2012). We also attempted to fit the model using a scaled Fisher prior, however, we struggled to obtain satisfactory convergence in our dataset, so results are not presented. Fixed effects had normally distributed diffuse priors. After initial tests, the Markov chain was run on family "categorical" (see electronic supplement tables S8-S9 for threshold model) for 7005000 iterations with a thinning interval and burn-in period of 5000 iterations each to ensure convergence and low autocorrelation (<0.1) between saved values. This resulted in 1,400 samples, which were used to calculate posterior modes and 95% credible intervals for the fixed effects and variance components on the latent scale. To enable more intuitive biological interpretation, estimated variance components on the latent scale were transformed to the corresponding ICC values (including heritability, h^2) on the observed using the functions 'QGparams' and 'QGicc' from the R package 'QGglmm' and the model 'binom1.logit' (de Villemereuil 2017).

Results

For the pseudo-continuous variable bTB_{multi} estimated variance components were very similar for Models 1 and 2, with only V_M differing notably between the two (Figure 1). However, the preferred model based on AIC comparison was that in which maternal effects were restricted to cubs (Model 2: AIC= 4987.87 vs. Model 1: AIC= 5049.73). Likelihood ratio tests of variance components under Model 2 indicate the presence of significant additive genetic, social group, and maternal contributions to among-individual variance in infection status (Table 1). bTB_{multi} has an estimated heritability (conditional on fixed effects) of 5% in both adults and cubs (Table 1; note ICC are not identical between age classes as V_M contributes to V_{Pc} but not V_{Pa}). Though the heritability is low, the additive genetic variance is nonetheless significantly greater than zero (LRT, $\chi^2_{0,1}$ =5.06, P=0.01). Overall, social group effects account for considerably more variation in bTB status than additive genetic effects.

Social group and social group x year effects explain a combined 13% and 10% of variation in bTB_{multi} in adults and cubs, respectively. Most of the among-group variance was partitioned into V_{SGxYc} (11% and 9%, respectively) indicating that the social groups themselves vary considerably in their effect on individual infection status between years. There was also evidence of significant among-year differences in bTB status that were independent of social group (with V_Y accounting for 8% and 7% of the variance in adults and cubs respectively; Table 1). For cubs, maternal effects explained a further 14% of variance in bTB_{multi} under Model 2, indicating that cubs born to the same mother share similarities in phenotype over and above those attributable to genetic and social group effects.

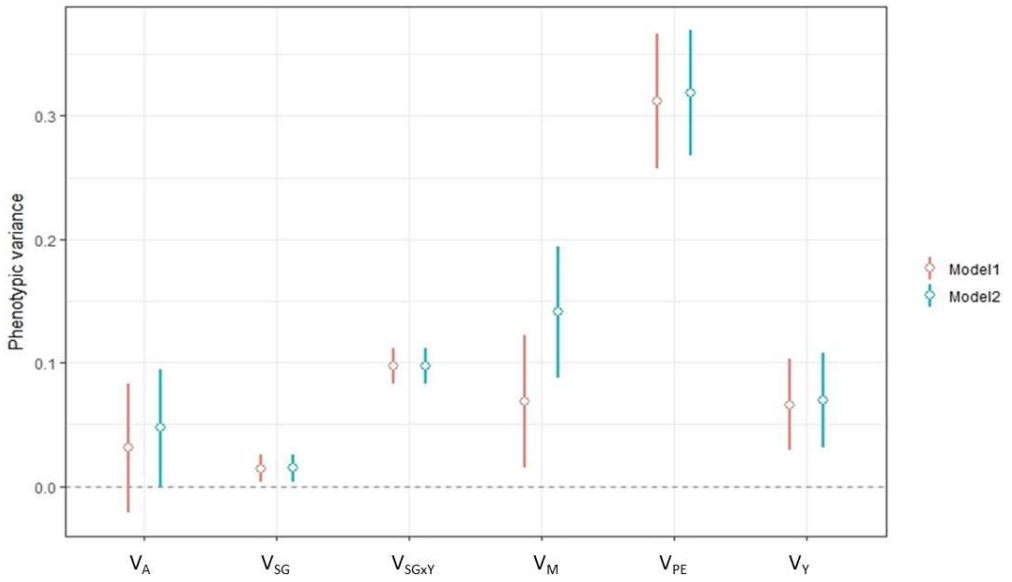

Figure 1. Comparison of variance component estimates from alternative bTB_{multi} models. In Model 1, maternal effects (V_M) were estimated for adults and cubs, whereas in Model 2 they were restricted to cubs only. All other variance components (additive genetic [V_A], social group [V_{SG}], year [V_Y], social group by year[V_{SGxY}], permanent environment [V_{PE}]) were estimated across age groups in both models. Lines represent 95% confidence intervals.

Results from modelling $bTB_{lifetime}$ yielded qualitatively similar biological conclusions for the most part (Table 2). On the observed scale h^2 (95% credible interval) was estimated as 7% ($3.9x10^{-6}$ – 0.13), with natal group and natal group x birth year effects explaining 4% (0.01 – 0.08) and 14% (0.09 – 0.23) of observed variance, respectively. Birth year accounted for 14% of variance in $bTB_{lifetime}$. While the Bayesian analysis does not lend itself to statistical inference in the frequentist sense, we note that posterior modes are distinct from zero for all estimated variance components (liability scale) except V_M for which the observed variance in $bTB_{lifetime}$ was only 0.01 (Supplemental Figure S3.1). We therefore conclude that maternal effects have a minimal influence on $bTB_{lifetime}$ (Table 2). Results for the subset analyses where only results from either Brock ELISA or StatPak were used to infer bTB status were overall

consistent with the full dataset analyses and are not discussed below but can be found in supplementary files (Table S3.1-S3.3).

Table 1. Log-likelihood ratio test results for variance components from bTB_{multi} model and corresponding intraclass correlation (ICC) estimates with standard errors in parentheses. Parameter estimates are from Model 2 in which maternal effect variance (V_M) is restricted to cubs only (denoted with subscript c). Consequently, ICC estimates are given for cubs ($_c$) and adults ($_a$) separately by dividing each variance component by the sum of the variance components (V_P) such that $V_{Pa} = V_A + V_{SG} + V_Y + V_{SGxY} + V_{PE} + V_R$ while $V_{Pc} = V_A + V_{Mc} + V_{SG} + V_Y + V_{SGxY} + V_{PE} + V_R$, where additive genetic = V_A, social group = V_{SG}, year = V_Y, social group by year = V_{SGxY}, permanent environment = V_{PE} and maternal effects = V_M

	$\chi^2_{0,1}$	P	ICC (SE)	
			Adult	**Cub**
$\mathbf{V_A}$	5.06	0.01	0.052 (0.027)	0.045 (0.023)
$\mathbf{V_{SG}}$	25.88	<0.001	0.016 (0.006)	0.014 (0.005)
$\mathbf{V_Y}$	122.3	<0.001	0.078 (0.020)	0.067 (0.018)
$\mathbf{V_{SGxY}}$	673.12	<0.001	0.109 (0.008)	0.094 (0.007)
$\mathbf{V_{PE}}$	304.24	<0.001	0.355 (0.029)	0.307 (0.026)
$\mathbf{V_{Mc}}$	70.18	<0.001	-	0.136 (0.023)
$\mathbf{V_R}$	-	-	0.389 (0.012)	0.335 (0.013)

Table 2. Posterior means for variance components of $bTB_{lifetime}$ and their corresponding intraclass correlation (ICC) estimates run on family "categorical". Estimates relate to the observed scale and 95% credible intervals are shown in parentheses.

	Mean	**ICC**
$\mathbf{V_A}$	0.014 ($7.6x10^{-7}$- 0.028)	0.069 ($3.9x10^{-6}$ – 0.134)
$\mathbf{V_M}$	0.002 ($1.8x10^{-10}$- 0.009)	0.009 ($8.2x10^{-10}$ – 0.042)
$\mathbf{V_{NG}}$	0.008 (0.003 - 0.017)	0.040 (0.013 – 0.079)
$\mathbf{V_{BY}}$	0.027 (0.016 - 0.046)	0.138 (0.093 – 0.229)
$\mathbf{V_{NGxBY}}$	0.019 (0.013 - 0.031)	0.094 (0.062 – 0.146)

Discussion

We have examined bovine tuberculosis (bTB) infection status in a naturally infected population of European badgers to ask whether, and to what extent, social (i.e. group, maternal) and genetic effects contribute to variation among individuals. Using two measures of bTB infection status (bTB_{multi}, a progressive measure of disease at each capture event and $bTB_{lifetime}$, a binary lifetime infection score), animal model analyses support the presence of a small, but statistically significant, heritable component of infection status. However, our results also indicate that more variation in bTB status is due to the environment provided by social group and, at least for bTB_{multi} in cubs, the mother. Although results are somewhat equivocal (see below), overall our analyses suggest maternal effects on bTB infection status are largely limited to early life.

Genetic variation in bTB status and progression

Variation in both the progression of bTB (as measured by bTB_{multi}) and the lifetime risk of infection have a partial genetic basis, with heritabilities of 5% and 7% respectively. Thus, in the Woodchester Park badger population, most variation in bTB infection status arises from environmental effects, a situation that also seems to generally hold in cattle where estimates of h^2 for bTB resistance range from 0.06 to 0.18 (Allen et al. 2010). Interestingly, after experimental infection with *M. bovis*, h^2 of bTB resistance was estimated at 0.49 in one population of farmed red deer (*Cervus elaphus*; Mackintosh et al., 2000), a species that may also play an important role as a wildlife reservoir for the disease (Vicente et al. 2006; Delahay et al., 2007). There are few heritability estimates for other diseases in wild vertebrate populations and so

(unsurprisingly) there is currently little emergent consensus on the importance of standing genetic variation. However, a number of studies have found moderate additive genetic variation for host defence traits, including resistance to strongyle nematodes in feral sheep (inferred from nematode-specific antibody titers; Hayward et al., 2014a) and both resistance and tolerance to copepod parasites in a freshwater cyprinid fish (Mazé-Guilmo al. 2014). Conversely, experimental studies provided no evidence for a significant influence of host genotype on cell-mediated immune responses in house martins (*Delichon urbica*; (Christe et al. 2000) and house wrens (Sakaluk et al. 2014). Interestingly, Ardia and Rice (2006) reached similar conclusions in two populations of tree swallows (*Tachycineta bicolor*) but actually found evidence for moderate heritability of immune function in a third (reported as h^2 =0.42 (0.27-0.51)).

Practical constraints in quantifying bacterial infection loads in a wild host meant that we have used live diagnostic test results (serological assays) and a qualitative presence/absence measure of parasite burden, both with imperfect sensitivities, to infer disease status and progression. It is thus the case that our lifetime and multi-site measures of bTB status and progression represent the outcome of multiple contributing traits and processes (e.g., behavioural exposure risk, resistance, tolerance). These underlying processes may themselves differ in their extent of genetic control. However, regardless of how the observed bTB progression and lifetime infection status scores are determined, the implication of their low heritabilities is that any natural selection acting has limited scope to affect an evolutionary response in this host population. Though we do not have formal estimates of contemporary selection, it is certainly possible that the observed, low levels of additive variance reflect

historically strong selection (i.e., if advantageous alleles go to fixation and deleterious ones are purged then variance is reduced; Fisher 1930). However, recent studies show that badger reproductive success does not appear to be impacted by disease (Tomlinson et al. 2013; McDonald et al. 2016), suggesting that the (presumed) costs of infection are mediated through survival. Indeed, badgers (particularly males) infected with bTB do show increased mortality rates (Graham et al. 2013; McDonald et al. 2016).

In interpreting our heritability estimates, it should be noted that the serological tests and bacterial culture used to define phenotypes have relatively low levels of sensitivity, meaning that some proportion of truly infected animals will not have been correctly identified as such. Phenotypic scores may also be affected by intermittent excretion and latent periods characteristic of bTB infection (Clifton-Hadley et al. 1993; Gallagher et al. 1998) while seropositive test results can indicate recent recovery from, or acquired immunity to, infection (Maas et al. 2013), though whether this is the case in badgers is currently unknown. Thus, both bTB status response variables used in our analyses are likely to be subject to non-trivial variance from measurement error. This will be partitioned into residual and, in the case of bTB_{multi}, the generic permanent environment variances and so may well contribute to the low heritabilities estimated. If so, improved confidence in assigning individual infection status (e.g. use of probabilistic approaches to incorporate full test histories, Buzdugan et al., 2016) may be needed if we hope to gain greater resolution on genetic factors predisposing to disease. We note however, that high measurement error will impact all ICC estimates and so cannot explain the low importance of genetic factors relative to other modelled effects (see below).

Social effects explain more variation than genetic factors

While significant additive genetic effects were detected, social environment effects attributable to (natal) social group and, at least for cubs, maternal identity collectively explained considerably more variation in bTB status. Spatial clustering of infection at the social group level has been reported previously in badger populations, with some groups in the Woodchester Park population remaining test-negative for long periods (Delahay et al., 2000b; Vicente et al. 2007). We note that social group identity coincides with main sett location in the study area, so it is possible that group effects are driven by spatial heterogeneity in the habitat rather than social effects per se. However, the finding that most group effects were year-specific strongly suggests otherwise (since group composition varies on a year-to-year basis whereas location is fixed). Year effects were also detected independently of social group. These findings are consistent with an observed increase in bTB prevalence and incidence in Woodchester Park over the study period (Delahay et al., 2013). They also corroborate previous studies that suggest the importance of social processes (but which did not control for potentially confounding genetic or maternal effects). For instance, social network analyses have revealed evidence suggesting a positive association between bTB infection and levels of extra-group contact (Weber et al. 2013; Silk et al. 2018) . Extra-group contacts may include temporary excursions for breeding purposes, the rates of which have recently been found to vary among social groups (Chapter 2, Marjamäki et al. in press). Seasonal variation in bTB incidence (accounted for in our models by the fixed effect structure) has also been shown to correlate with peaks of within-group social contact (Silk et al., 2017), although indirect transmission

(e.g. via environmental contamination of communal latrines and setts) can also occur (Courtenay et al. 2006; King et al. 2015).

Maternal effects were most important in explaining variation in individual bTB status among badger cubs in the present study. Though widely observed for life-history, reproductive and growth traits, the relative importance of maternal effects for disease risk is less well documented (but see e.g., Hall & Ebert, 2012; Seppälä & Langeloh, 2016). However, in Soay sheep maternal effects on offspring parasite load appear, at least in part, to occur through maternal age and parasite load (Hayward et al., 2010). In that population, and in some domestic sheep, quantitative genetic analyses support a substantial contribution of maternal genetic effects to nematode resistance (Coltman et al., 2001; Stear et al., 2001). As noted above, our data are not informative for specific mechanism(s), although similarity among maternal siblings (over and above that attributable to additive genetic and social group effects) could arise from maternal provisioning of antibodies, variation in maternal infection status, or differential contact time with cubs. Second order mechanisms are also possible, for instance if maternally influenced nutritional status has consequences for cub immune response. Our results also do not suggest a genetic basis to the maternal effects detected (i.e. contribution of maternal genotype on offspring phenotype above and beyond direct inheritance), although we acknowledge that incomplete pedigree data limit our inference in this regard (discussed below). If present, maternal genetic effects will impact rates (and potentially directions) of selection response in offspring traits (McAdam et al. 2014), so the above conclusion that bTB status has limited evolvability may be contingent on the maternal effects detected being of principally environmental origin.

Maternal effects on offspring phenotype often decline with age, but whether this is the case for bTB status in Woodchester Park badgers is not completely clear from our analyses. On the one hand maternal effects on $bTB_{lifetime}$ were absent and the best supported model for bTB_{multi} was one in which maternal effects were restricted to cubs (i.e. Model 2). On the other hand, fitting a model including maternal effects to cub versus adult specific bTB_{multi} data sets yielded very similar point estimates of additive and maternal variances for cubs and adults (Table S3.7 in supplemental materials). Taken together, we view these results as suggestive, but not conclusive, evidence for a declining role of maternal effects with age, a pattern rarely documented for disease traits (but see e.g., (Clark et al., 2014). Regardless of this uncertainty, our results lend further support to previous studies highlighting the importance of early-life environment to bTB infection (Tomlinson et al. 2013), and suggest that the observed relationship between cub infection risk and the presence of infected relatives (Benton et al. 2016) could be driven by both maternal and additive genetic effects.

Implications of genotype-(social) environment correlation

As noted earlier, the preponderance of within-group paternities in the Woodchester Park population (63% within-group vs 37% extra-group paternity, Chapter 2; Marjamäki et al. in press) means that genetic relatedness will be, on average, greater for pairs of individuals that share a (natal) social group environment than for pairs that do not. Similarly, siblings necessarily share a maternal environment. The population is thus characterised by a 'genotype-environment correlation' that cannot easily be disentangled. Since experimental

approaches (e.g. cross-fostering; Kruuk & Hadfield, 2007) are not appropriate in this or similar systems, we have taken the conservative (with respect to estimation of h^2) approach of simultaneously modelling additive genetic, maternal and social group (including group x year) effects. Failure to model common environment effects, including mothers and shared habitat use by relatives is a well-known potential source of upward bias in h^2 estimates (e.g. Wilson et al. 2005a; Stopher et al., 2012; Regan et al. 2015). In our study, omitting maternal effects resulted in only modest increases in heritability estimates (e.g., from 3 to 4% under Model 1), but additional omission of social group and group by year effects led to larger increases (from 3% to 9 and 12%, respectively). This illustrates the point that wider modelling of shared (social) environmental effects in space (e.g., Stopher et al. 2012; Regan et al. 2015) and time, may be important for quantitative genetic analyses of longitudinal field-based data. However, accurate separation of correlated genetic and environmental effects necessarily depends on data structure and quality. Here incomplete parentage data is likely to have produced errors in the pedigree (e.g. unrecognised relatedness among true siblings) even in the unlikely event that all parentage assignments made are correct. Although pedigree error will usually downwardly bias the estimation of h^2 (Morrissey et al. 2007) the consequences are not readily predicted here given the social group structure and the fact that maternal and paternal identities are both uncertain.

Conclusions

The long-term study of the Woodchester Park badger population provides a unique and valuable opportunity to investigate the factors driving among-

individual variation in bTB infection status. We have found that genetic factors play a small, but significant role in structuring variation in bTB infection status. However, it is clear that social influences arising from group membership (in space and time) and maternal effects (in cubs) play a greater role. Genetic and social effects may influence observed bTB infection status through multiple pathways, including effects on infection risk (e.g. through behavioural traits), resistance, and/or its ability to limit damage caused (tolerance). Though not mutually exclusive, resistance and tolerance in particular are predicted to have very different consequences for parasite fitness; by limiting parasite growth, resistance will negatively impact parasite fitness, while tolerance can, in fact, promote parasite fitness by increasing the period over which transmission can occur. Given the implications of individual variation in infectiousness for the long-term persistence of parasites (Kramer-Schadt et al. 2009) and microevolutionary dynamics of both host and parasite (Best et al. 2008), determining whether genetic and environmental determinants of bTB status operate through resistance, tolerance, or both should be a useful – if empirically challenging – priority.

Chapter 4

Heterogeneity in tolerance of *Mycobacterium bovis* infection in a naturally infected population of European badgers (*Meles meles*)

Abstract

In order to defend themselves against parasites, hosts can either *resist* infection by preventing or limiting parasite growth or *tolerate* it by minimising parasite-induced damage. Understanding the occurrence, causes, and consequences of variation in both resistance and tolerance is important as they can jointly and separately determine host fitness and thereby affect population dynamics on ecological and evolutionary scales. While variation in host resistance is well characterised, little is currently known about the causes and consequences of heterogeneity in host tolerance. We used long-term data on individually marked European badgers (*Meles meles*) to investigate associations between body weight and infection by *Mycobacterium bovis*, the causative agent of bovine tuberculosis (bTB). Our analyses reveal significant among-individual variation in tolerance, which we measure as the slope of a reaction norm describing the change in body weight with progression in bTB infection status, assigned from live diagnostic test results. This provides the first explicit demonstrations of variation in tolerance of tuberculosis in a wild host species. Furthermore, despite the *a priori* prediction that positive selection should rapidly erode genetic variance, we find that among-individual variance in tolerance is primarily attributable to additive genetic effects. Our results add to a growing literature that supports the idea of tolerance as a key host defence strategy alongside

resistance and suggest that it may be a key component of host-parasite coevolution.

Introduction

Among hosts, differences in parasite infection and disease progression stem from multiple processes. One mechanism is heterogeneity in host defence strategies against challenges from parasites (including bacteria, viruses, protists and fungi). Two broad categories of defence strategy are commonly considered; hosts can 'resist' infection by preventing or limiting pathogen growth (Boots, 2009) and/or they can 'tolerate' infection by limiting and mitigating damage caused by the parasite, by repairing damaged tissue for example (Simms 2000; Medzhitov et al. 2012). Understanding the occurrence, causes, and consequences of variation in both resistance and tolerance is important, as they can jointly and separately determine host fitness, and thus impact population dynamics on ecological and evolutionary scales (Boots et al., 2009; Martin et al., 2006). However, while there is a large body of literature on the epidemiological, ecological and evolutionary implications of variation in host resistance, the recognition of tolerance as a distinct aspect of host defence is more recent (though well-established in plant immunology studies; Baucom & de Roode 2011).

The extent to which tolerance, as opposed to resistance, contributes to host defence strategies in animal populations is predicted to have important ecological and (co)evolutionary consequences. For instance, host resistance negatively affects the fitness of a parasite, limiting its growth and prevalence in the host population. This in turn can drive negative frequency dependent selection on resistance phenotypes and thus maintenance of among-host genetic variance (Miller et al, 2005). Conversely, tolerance tends to promote the persistence and spread of infection through a host population by improving host

survival. This generates a positive feedback loop; increased parasite prevalence selects for increased tolerance in the host population which in turn results in a reduction of genetic variance as alleles increasing tolerance rapidly go to fixation in the host population (Best et al., 2008). Despite this theoretical prediction, genetic variation in tolerance has been widely observed in plants (e.g. Kover & Schaal 2002; Koskela et al. 2002) and has been reported in animals under laboratory conditions (Råberg et al. 2007; Vincent & Sharp 2014). However, tolerance in wild animal host populations has seldom been investigated (but for exceptions see Blanchet et al. 2010; Mazé-Guilmo et al. 2014; Hayward et al. 2014b). As a consequence, we have little understanding of whether tolerance varies among individuals and, if so, whether genetic as well as environmental factors contribute to the apparent variation.

In this study we use long-term data on individually marked badgers from the Woodchester Park study population (Gloucestershire, United Kingdom) to characterise among-individual variation in tolerance to infection by *Mycobacterium bovis*, the causative agent of bovine tuberculosis (bTB). Badgers are considered an important wildlife reservoir for transmission of *M. bovis* to cattle in the UK, where bTB continues to be a major disease challenge for the livestock industry (Donnelly & Nouvellet 2013; Defra 2014). The primary route of infection in badgers is thought to be through inhalation of infected aerosol, leading to chronic infection of the pulmonary system, though other organs can also be affected (Gallagher et al., 1998; Murphy et al., 2010). Interestingly, progression of infection and presenting symptoms are very variable among individuals. In most badgers, infection remains latent (i.e. bacteria are effectively contained) while in others it can progress, sometimes rapidly, to generalised disease (Murphy et al., 2010). As a consequence, some

infected badgers reproduce successfully and survive for years (Clifton-Hadley et al. 1993; Tomlinson et al. 2013) while others do not. Although sex is one important determining factor – males suffer from higher rates of infection, disease progression and mortality than females (Graham et al., 2013; McDonald et al., 2014) – there is also considerable heterogeneity among individuals. This is likely to arise, at least in part, from variation in the mechanism and effectiveness of host defence strategies in the population, a notion supported by recent quantitative genetic analysis indicating heritable variation for bTB infection status (measured from diagnostic test results) and lifetime infection risk (a putative proxy for resistance; Chapter 3).

Here we build on our previous study, by testing for (genetic) variation in tolerance of *M. bovis* infection. To do so we use repeated capture records of marked individuals and characterise tolerance as the changes in individual weight with the progression of bTB infection status. Body weight is an important trait contributing to reproductive success and survival in the European badger (Macdonald & Newman, 2002; Macdonald et al., 1995), and thus weight loss (or reduced weight gain) is used here as a proxy for the impact of infection on fitness. This follows the approach of Hayward et al. (2014b) who similarly used weight change with increasing parasite burden to investigate tolerance of helminth infections in Soay sheep (*Ovis aries*). Importantly for current purposes, bTB infection is generally associated with a reduction in weight and body condition in badgers (Clifton-Hadley et al. 1993; Gallagher & Clifton-Hadley 2000), although males and females show somewhat different patterns. Tomlinson et al. (2013) observed a gradual decline in body mass from initial test-positive status in females, whereas for males marked weight loss occurred only at the more advanced stages of disseminated disease. We first seek to

confirm the previously reported population-level effects of bTB status on mean weight (while controlling statistically for other known or hypothesised sources of variation in weight). We then test for among-individual variation in tolerance adopting a 'reaction norm' approach in which individual tolerance is conceptualised as the slope of an individual's relationship between weight and infection status. Individuals that are more tolerant of bTB are, therefore, identified as those that experience less severe infection-related weight loss (i.e. have shallower slopes). Finally, using a pedigree structure based on genetic parentage analysis (described in Chapter 2; Marjamäki et al. in press) we ask whether genetic variance in tolerance contributes to heterogeneity of disease outcomes in this population.

Methods

Study system & sampling

Woodchester Park, Gloucestershire, UK is the site of a long-term capture-mark-recapture study of a naturally infected population of 200-300 badgers. The dataset used here contains 14846 records of 2946 individual badgers captured between 1976 and 2014. The study area of approximately 11 km^2 consists of a steep-sided wooded valley surrounded by farmland. Within this area, up to 45 badger social groups are resident, each occupying a territory containing several underground dens (setts). Group territorial boundaries are determined annually by bait marking (Delahay, et al., 2000a). The majority of records come from a core of 20-25 social groups with consistent and continuous trapping records throughout the study period.

Badgers are trapped in the vicinity of their setts up to four times a year for two consecutive nights using steel mesh box traps baited with peanuts. They are transported to a central location for examination and sampling before being released at the point of capture. Sampling of badgers is carried out under anaesthesia (de Leeuw et al. 2004) and at first capture each animal is marked with a unique tattoo. At each subsequent capture event, the sex, age class (cub, yearling, adult), body weight, length, condition, and capture location are recorded. Most badgers are first caught as cubs or yearlings (distinguished from adults by size and toothwear; Delahay et al. 2013) and can therefore be aged accurately at each subsequent capture. The infection status of captured badgers is determined at each capture event using bacterial culture of clinical samples (faeces, urine, tracheal aspirate, pus, bite wound swabs; Clifton-Hadley et al 1993), and a serological test for the presence of *M. bovis* antibodies (Brock Elisa used 1982 to 2006; Goodger et al. 1994 and BrockTB Stat-Pak test used 2006 to 2014; Chambers et al. 2008). Based on these test results, badgers are assigned a bTB status of test-negative (0), seropositive (positive serological test only; 1), excretor (positive culture from a single body site sampled; 2) or multisite excretor (positive culture from >1 body site; 3). This progressive categorisation approximates the progression of infection from uninfected to exposed, to more advanced stages of disseminated infection (Lesellier et al., 2008; Mahmood et al., 1987), though we acknowledge that we expect it to do so imperfectly (due for example to varying diagnostic test sensitivities and specificities (Drewe et al., 2010), or potential recovery from infection; see Chapter 3 for further discussion).

Since 1986, guard hairs have been taken from each individual badger at first capture for DNA extraction and subsequent microsatellite genotyping using

a minimum of 16 (Carpenter et al., 2005) and maximum of 22 fluorescently labelled autosomal microsatellite markers (Marjamäki et al. in press). Based on this genetic information, a six-generation genetic pedigree structure was recently estimated by Marjamäki et al. (in press), using the R (R Core Team 2016) package MasterBayes (Hadfield et al., 2006). In contrast to most avian and mammalian systems, badger maternal identities cannot be determined from direct observation as cubs spend most time pre-weaning out of sight and underground. The requirement to simultaneously estimate maternities and paternities from genetic data alone, reduces the ability to assign parentage with high confidence. Consequently, the available pedigree is far from complete. Nevertheless, including those parent-offspring assignments where a candidate parent was specified in at least 80% of the samples of the posterior distributions of the pedigree, results in a structure containing 1175 parentage assignments (579 maternities and 596 paternities), with 617 offspring individuals assigned at least one parent and 556 of those assigned both parents. For full details of the badger pedigree, see Marjamäki et al. (in press).

Statistical analyses

We investigated the association between body weight and bTB infection status at the population level (i.e. average tolerance) and tested for among-individual and genetic variance in weight and tolerance using a series of nested linear mixed effects models described in full below and fitted in ASReml 4.0 (VSN International). All models had a common fixed effect structure but differed in their random effects. Significance of fixed effects was determined using Wald F-tests, while statistical inference on random effects was by likelihood ratio tests (LRT). Twice the difference in log-likelihood between full and reduced models

was assumed to have a χ^2 distribution with the degrees of freedom equal to the number of additional parameters in the more complex model. However, following Visscher (2006), we assume the test statistic to be asymptotically distributed as an equal mix of χ^2_0 and χ^2_1 (denoted as $\chi^2_{0,1}$) where a single variance component was being tested. We also use Akaike Information Criterion (AIC) as an additional guide for model comparison. The response variable (body weight) was scaled to standard deviation units prior to analysis to ease interpretation of estimated variance components, which can thus be interpreted as the proportion of (observed) variance attributable to a given effect. However, we also obtained intra-class correlations (ICC) conditional on fixed effects (Wilson, 2018), as the ratio of each random effect variance to the conditional phenotypic variance (V_P), calculated as the sum of the variance component estimates.

Population-level association between body weight and bTB status

First, we fitted a model of body weight that included the fixed effects of sex, age, age^2, sex x age and sex x age^2 (Model 1). This was to capture sex specific changes in mean weight with age. Badgers show sexual dimorphism in body size; males growing faster to become heavier (on average) as yearlings, with an average difference of 1.2 kg between adult (≥ 2yr) male and female badgers caught in Woodchester Park. In addition, senescence in body weight is observed in older animals, occurring more rapidly in males (Beirne et al., 2015). Marked seasonal fluctuations in body weight are also observed in this population and so season was included (as a four-level factor), as was the observed age at last capture for each individual. The latter was to reduce any

possible bias from selective disappearance (Bouwhuis et al., 2009). Finally, Model 1 included a fixed effect of bTB status (as a four-level factor to avoid assuming a linear relationship) and a sex-by-bTB status interaction term to test for sex-specific negative effects of infection status on mean weight.

Random effects were included in Model 1 both to prevent pseudo-replication in fixed effect inference and to partition remaining variance around the (fixed effect) mean. Individual identity was included (to account for the repeated observations on individuals), as was social group identity, year of observation, and a social group-by-year term. These latter effects were included to account for temporal and spatial heterogeneity in the environment and reduce the risk of genetic parameters being biased by common-environment effects in subsequent models (described below). Since maternal effects on mammalian body weight are widespread, we also included a random effect of maternal identity on records for cubs (<1yr) only. This decision was based on preliminary models (not reported here) that found significant maternal variance on cub weight, but not when records from ages were analysed simultaneously. Note that as a consequence of this decision, ICC (for all models) will differ between cubs and adults (≥1yr), since V_P includes the maternal variance (V_M) in cubs only. We make the standard assumption that all random effects (and residuals) are drawn from Gaussian distributions with means of zero and variances to be estimated, and that residuals are uncorrelated across observations.

Among-individual variance in tolerance

Having established a negative relationship between bTB status and body weight (see results), Model 1 was used as a null model against which to test for among-individual variation in tolerance. Tolerance was defined as the slope of the reaction norm for body weight across bTB status (i.e. range tolerance). This was done using a first order random regression formulation, following several recent studies (e.g. Råberg et al. 2007; Baucom & De Roode 2011; Hayward et al. 2014b), by adding an interaction between individual identity and bTB status (as a continuous covariate; Model 2). Note that individual reaction norm slopes (i.e. tolerances) were allowed to covary with individual intercepts (interpretable as individual effects on weight at bTB status = 0).

Genetic variance for weight and tolerance

In order to estimate the heritability (h^2) of body weight, Model 1 was extended into a quantitative genetic 'animal model' (Model 3), which uses population genetic pedigree information to estimate the effect of relatedness on phenotypic similarity. This is achieved by including the additive genetic merit of an individual as a random effect, thereby decomposing the variance arising from individual effects into distinct additive genetic (V_A) and permanent environment (V_{PE}) components. Comparison of Models 1 and 3 thus provides a test for genetic variance in body weight. In Model 4 we assume the additive genetic effect is constant with bTB status, but the permanent environment effect was modelled as a random regression (as per Model 2). Finally, we decomposed among-individual variance in tolerance, by fitting both additive genetic and permanent environment effects as first order random regressions of bTB status

simultaneously (Model 5). Comparison of Models 4 and 5 thus provides a test for genetic variance in tolerance of body weight to bTB status. Results show high level of consistency among models. For clarity, we present population-level results from Model 1 and individual-level results from Model 5 below.

Results

Model 1 provided evidence for the previously described seasonal effects (badgers being heaviest in autumn and lightest in spring/summer), and for sex-specific patterns in mean weight with age (Table 1). Though not directly relevant to current hypotheses, model predictions of body weight across age show the expected sexual dimorphism in growth and adult size, as well as late-life declines in mean weight consistent with senescence (Beirne et al., 2015). Conditional on other fixed effects, bTB status (fitted as a factor) also has a significant effect on body weight, although the bTB status x sex interaction term was marginally non-significant (Table 1). Thus, in contrast to previous studies, we find no strong statistical support for sex-specificity of bTB effects on weight per se. There is a general pattern of decreasing weight with increasing bTB status, with multisite excretors (bTB status = 3) weighing the least, on average (Figure 1). However, somewhat counterintuitively, test-positive badgers (bTB status =1) are predicted to be heavier on average than test-negative badgers (bTB status = 0).

Table 1. Fixed effect estimates (standard errors) and associated inference from conditional Wald F-tests from Model 1 testing population-level associations between body weight and bTB status. DF= degrees of freedom P-values in bold denote significance at α=0.05

Model Term (reference level)	Effect (SE)	F	DF	P
Intercept	1.303 (0.518)	4.61	1, 5712.3	0.032
Age	0.508 (0.009)	6614.61	1, 13519.7	**<0.001**
Age^2	-0.045 (0.001)	4392.31	1, 13780.8	**<0.001**
Sex (NA)		35.25	2, 3391.9	**<0.001**
Female	1.001 (0.514)			
Male	0.949 (0.517)			
Season (Spring)		2244.13	3, 12294.3	**<0.001**
Winter	0.707 (0.019)			
Autumn	0.850 (0.017)			
Summer	-0.0003 (0.016)			
TB status (NA)		30.87	4, 2753.8	**<0.001**
0	-0.175 (0.084)			
1	0.005 (0.060)			
2	-0.233 (0.071)			
3	-0.350 (0.084)			
Age last capture	-0.006 (0.004)	2.09	1, 2389.0	0.149
Sex x Age (Female)		149.64	1, 14240.2	**<0.001**
Male	0.160 (0.013)			
Sex x Age^2 (Female)		105.95	1, 13744.5	**<0.001**
Male	-0.016 (0.002)			
TB status x Sex (Female)		2.23	4, 13121.1	0.063
0.Male	0.134 (0.065)			
1.Male	0.194 (0.074)			
2.Male	0.193 (0.095)			
3.Male	0.032 (0.004)			

Table 2. Estimated variances (V), covariances (COV) and intraclass correlations (ICC) from Models 1 testing associations between body weight and bTB status (0-3) and and Model 5 a random regression of body weight on bTB status including additive genetic effects. Both models included the same fixed effects. Subscripts denote source of variance (Y=year, SG=social group, M= maternal, I=individual, A=additive genetic, PE =permanent environment, R=residual) and, for Model 5 differentiate random regression intercepts (i) and slopes (s). ICC are evaluated at bTB status =0 under Model 5 and differ between cubs and adults for both models as maternal effects are restricted to cubs. Standard errors are shown in parentheses.

	Random effect	Estimate (SE)	Cub ICC (SE)	Adult ICC (SE)
Model 1				
	$\mathbf{V_Y}$	0.018 (0.006)	0.022 (0.007)	0.031 (0.010)
	$\mathbf{V_{SG}}$	0.051 (0.014)	0.062 (0.016)	0.086 (0.022)
	$\mathbf{V_{YxSG}}$	0.065 (0.005)	0.079 (0.007)	0.110 (0.010)
	$\mathbf{V_M}$	0.176 (0.028)	0.215 (0.027)	-
	$\mathbf{V_I}$	0.232 (0.010)	0.283 (0.014)	0.299 (0.034)
	$\mathbf{V_R}$	0.280 (0.014)	0.341 (0.014)	0.475 (0.026)
Model 5				
	$\mathbf{V_Y}$	0.019 (0.006)	0.021 (0.007)	0.028 (0.009)
	$\mathbf{V_{SG}}$	0.049 (0.014)	0.055 (0.015)	0.074 (0.019)
	$\mathbf{V_{YxSG}}$	0.063 (0.005)	0.072 (0.006)	0.096 (0.008)
	$\mathbf{V_M}$	0.224 (0.032)	0.252 (0.028)	-
	$\mathbf{V_{Pei}}$	0.036 (0.016)	0.041 (0.018)	0.055 (0.024)
	$\mathbf{COV_{PE(i,s)}}$	0.000 (-)[1]	0.000 (-)[1]	0.000 (-)[1]
	$\mathbf{V_{Pes}}$	0.000 (-)[1]	-	-
	$\mathbf{V_{Ai}}$	0.222 (0.021)	0.250 (0.023)	0.335 (0.029)
	$\mathbf{COV_{A(i,s)}}$	-0.044 (0.010)	-	-
	$\mathbf{V_{As}}$	0.041 (0.008)	-	-
	$\mathbf{V_R}$	0.274 (0.004)	-	-

[1]The estimate of V_{PEs} was bound to (almost) zero and no standard error is estimated. In this situation the slope-intercept covariance is also bound and the corresponding correlation undefined.

All random effects specified in Model 1 were statistically significant, based on likelihood ratio test comparison to reduced models. Body weight varies among individuals ($\chi^2_{0,1}$= 2121.622, p<0.001) with estimated ICCs (i.e. repeatabilities) of 0.28 (0.01) and 0.30 (0.03) in cubs and adults respectively (conditional on fixed effects; Table 2). Significant effects of social group ($\chi^2_{0,1}$= 279.100, p<0.001), year ($\chi^2_{0,1}$= 285.660, p<0.001), and group-by-year ($\chi^2_{0,1}$= 673.662, p<0.001) are relatively small (Table 2) but in sum account for 16 % and 23 % of variance in cub and adult weights, respectively. A significant effect of maternal identity on cub weight ($\chi^2_{0,1}$= 158.048, p<0.001) is more important, explaining 22 % of body weight variation in this age group.

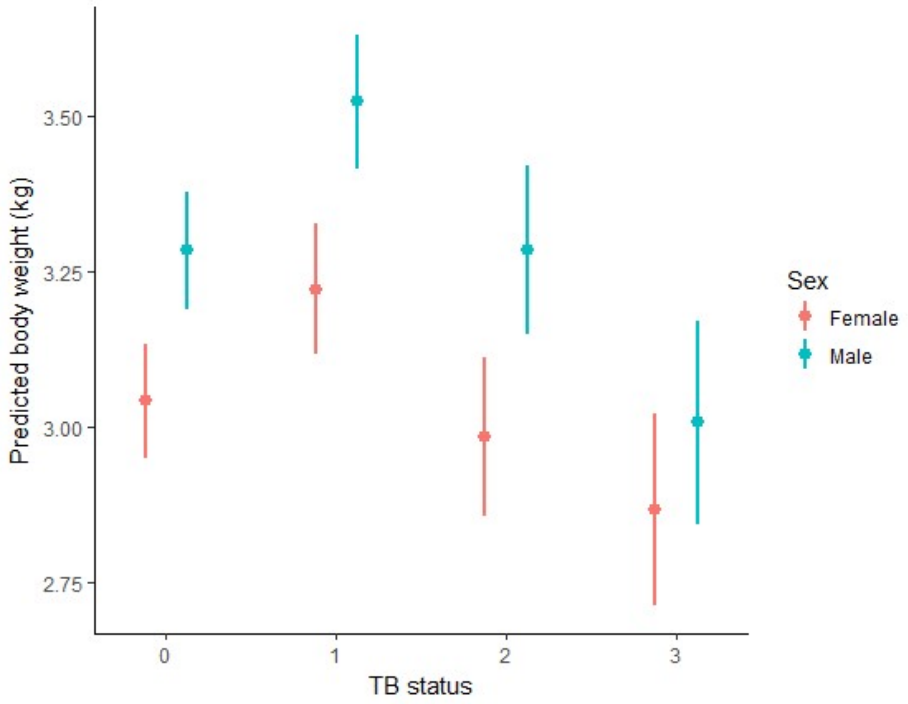

Figure 1. Predicted effect of bTB status on mean body weight in male and female badgers of average age. TB status 0 = test negative, 1 = test positive, 2 = excretor, 3 = multisite excretor. Bars represent 95% confidence intervals and predictions are from Model 1.

The simple animal model (Model 3) was a better fit to the data than Model 1 (Table 3) supporting the presence of additive genetic variance in weight. Comparison of models with more complex decompositions of among-individual variance portioned in Model 1 also provided evidence for variance in the individual slopes of body weight on bTB status, indicating among-individual variance in tolerance is present (Model 2 and 4). Furthermore, Model 5 was a significant improvement on Model 4 (Table 3) and was the preferred model overall (based on AIC), from which we conclude that variation in tolerance has a partial genetic basis. In fact, under Model 5, all variation in tolerance was partitioned into the additive genetic, as opposed to the permanent environment, component (Table 2).

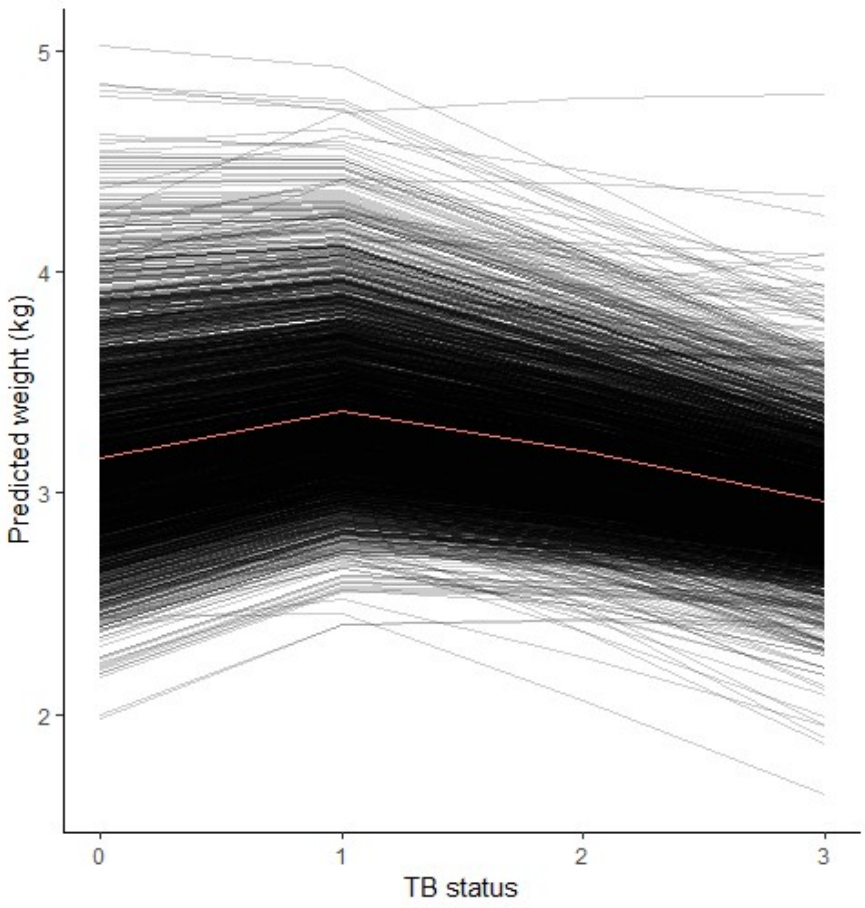

Figure 2. Predicted body weight with changing bTB status (0 test -ve, 1 test +ve, 2 excretor, 3 multisite excretor) showing the population mean (red line) and individual reaction norms (black). Individual reaction norms incorporate additive and permanent environment deviations from the population average pattern in intercept (weight at bTB=0) and slope (tolerance) as predicted under Model 5.

(Co)variance components estimated under Model 5 indicate body weight heritabilities of 0.25 (0.02) and 0.34 (0.03) for cubs and adults, respectively, at bTB status = 0 while the genetic correlation (standard error) between reaction norm intercepts (i) and slope (s) was -0.46 (0.08). This indicates that genotypes predisposing to heavier weight in test negative individuals also predispose to reduced tolerance (i.e. more weight loss with increasing bTB status). This pattern is seen in the predicted individual reaction norms (Figure 2) and leads to a reduction in genetic variance for weight at higher bTB status scores. Note, however, that the genetic correlation between intercept (weight at bTB status =0) and slope (tolerance) is significantly different from -1 (comparison to constrained formulation of Model 5 in which a correlation of -1 is imposed; χ^2_1=4.564, p=0.03). This indicates that some genetic variation in tolerance is independent of weight. In other words, genetic variance in tolerance cannot be fully explained as a consequence of (genetically) heavier individuals simply having more weight to lose.

Finally, we note that with the covariance matrix of random effects constrained to be positive definite, the estimate of the permanent environment slope (V_{PEs}) was bound to (effectively) zero. This precludes biological interpretation of the permanent environment intercept-slope covariance and, interpreting V_{PEs} as zero, means that the corresponding intercept-slope correlation is undefined.

Table 3. Comparison of models 1-5 of body weight. All models contain the same fixed effects (sex, age, age^2, season, bTB status, age at last capture, sex x age, sex x age^2, sex x bTB status) and random effects (social group, year, group x year and maternal identity), but differ in the decomposition of among-individual variance. V =variance, COV=covariance, I=individual, PE= permanent environment, A= additive genetic, i= intercept, s= slope, LogL= log-likelihood, AIC = Akaike Information Criterion, DF= degrees of freedom.

Model	Variance partition	LogL	AIC	Comparison	Tests for	χ^2	DF	P
1	V_I	-826.36	1664.72	-	-	-		-
2	$[V_{Ii,}\ COV_{I(i,s)},V_{Is}]$	-798.86	1613.71	1 vs 2	Variance in tolerance	55.00	2	<0.001
3	$V_{PE} + V_A$	-799.11	1612.21	1 vs 3	Genetic variance in weight	54.5	1	<0.001
4	$[V_{PEi,}\ COV_{PE(i,s)},V_{PEs}] + V_A$	-772.89	1563.77	-	-	-	-	-
5	$[V_{PEi,}\ COV_{PE(i,s)},V_{PEs}] + [V_{Ai,}\ COV_{A(i,s)},V_{As}]$	-767.02	1556.03	4 vs 5	Genetic variance in tolerance	11.74	2	<0.01

Discussion

We have identified evidence for significant among-individual variation in the slope of the regression of host body weight on bovine tuberculosis infection status. We view this as the first explicit evidence of variation in tolerance against bovine tuberculosis in a wild host species. Furthermore, we found heritable variation in both body weight and in the rate of weight loss with the progression of infection status. The latter indicates that our proxy for tolerance (reaction norm slopes) has a significant additive genetic basis of variation. Given an expectation of positive selection, increased tolerance is therefore expected to evolve, bringing consequences for the dynamics of disease in this host. In what follows, we first highlight features of the population-level patterns of variation in weight and discuss the eco-evolutionary implications of (genetic) variance in tolerance, while noting some important assumptions and caveats underpinning our interpretation.

With respect to variation in the population mean, the effects revealed by our current analyses are largely consistent with previous studies of weight in this, and other, badger populations. Seasonal fluctuations followed expected patterns, and likely result from temporal variation in weather conditions and food abundance (Delahay et al., 2006; Macdonald et al., 2010). Similarly, sexual dimorphism and age effects estimated here match previously noted patterns of sex-specific growth and senescence reported for this population (Rogers 1997; Beirne et al. 2015), which are also common to other mammals (Clutton-Brock & Isvaran, 2007; Douhard et al., 2017). We also found evidence that bTB infection tends, overall, to reduce weight in the Woodchester Park badgers. Weight loss is a common symptom of tuberculosis, in humans and other animal hosts, particularly during late-stage infection (Macallan 1999; Lisle et al. 2002;

Tomlinson et al. 2013). Our study found that animals in the most advanced disease status category (i.e. multisite excretors, bTB status =3) were lightest on average (conditional on other effects in the model), but somewhat counterintuitively, that test-positive badgers (bTB status =1) were heavier on average than test-negative individuals (bTB status =0). This appears to be driven by cubs and yearlings (the most abundant age classes in the data) as a post hoc analysis limited to adults (i.e. ages ≥ 2) predicts sequential declines with advancing bTB status from a mean weight that is highest in test-negative individuals (results not shown). The overall pattern could potentially be an artefact of data structure (e.g., there are few cubs with high bTB scores) or may indicate some underlying (but as yet unknown) age-related variation in responses to infection. In contrast to Tomlinson et al. (2013), using the current, more extensive data set and, we did not detect statistically significant sex-dependence of the bTB status effects on body weight. Although we note the interaction term was marginally non-significant, model predictions suggest both sexes exhibit a qualitatively similar pattern of gradual weight loss from first testing positive to disseminated disease. This contrasts somewhat with the earlier conclusion that males maintain weight till late-stage infection (Tomlinson et al. 2013).

While bTB infection causes weight loss on average, our analyses also provide evidence of significant among-individual variation in reaction norm slopes and intercepts. Furthermore, our pedigree-based quantitative genetic analyses show this variation is largely underpinned by genetic factors. Among-individual variation in reaction norm intercept is unsurprising since this can be interpreted biologically as variation in weight at bTB status =0. Thus, under Model 5, weight has an estimated repeatability of approximately 39% in test-

negative adults (conditional on fixed effects), of which most (33.5%) was partitioned as heritability. In cubs, additive genetic and maternal effects each explained approximately 25% of body weight at bTB status =0. Maternal effects on cub weight have not previously been reported in badgers, but these results add to the increasingly well-documented importance of maternal provisioning for offspring growth in wild mammals (e.g. Wilson et al. 2005b; Dantzer et al. 2013; English et al. 2014). Of greater importance in the current context is the finding of among-individual, and genetic variation in the degree of weight loss with increasing bTB status. Although there are some important caveats to note (see below), we interpret this as evidence that badgers in the Woodchester Park population vary (genetically) in their tolerance of *M. bovis* infection. Our results add to a growing literature that supports a key host defence role for tolerance alongside resistance (Restif & Koella, 2004; Råberg et al., 2009a; Hayward et al., 2014a).

Variation in tolerance could arise through numerous pathways. Speculatively, these could include, for instance, individual differences in efficacy of tissue repair and anti-toxin pathways (Råberg et al., 2009; Glass, 2012;). Stress-related changes in immune function can also be mediated by reproductive or social status, and intra-specific competition (Hawley et al., 2006; Sapolsky, 2005; Theodorou et al., 2007) while habitat quality could impact tolerance via effects on host diet and nutritional status (Clough et al. 2016; Kutzer & Armitage 2016b). Although the current study is not informative for the specific pathways involved, it does indicate that heterogeneity in tolerance to *M. bovis* infection in badgers appears to have a largely genetic basis. This runs counter to some theoretical predictions. Specifically, if tolerance improves host survival (mortality tolerance) it should positively impact parasite fitness (Best et

al. 2008). This is because increasing host longevity without limiting parasite growth (as resistance does) prolongs the period over which transmission can occur, thereby increasing parasite persistence. This leads to a positive feedback loop whereby increased parasite prevalence selects for increased host tolerance which increases prevalence further, with an expectation that genetic variance in tolerance will be rapidly lost from the population (Roy & Kirchner, 2000).

Empirical support for the prediction that genetic variance in tolerance will largely be absent is mixed, as our results highlight. For example, in Soay sheep tolerance against helminth infection was found to be under positive selection through lifetime reproductive success but not genetically variable (Hayward et al. 2014b). Conversely, genetic variation in tolerance has been found in plants (Kover & Schaal, 2002;Du et al., 2008) and in several recent animal hosts studied under laboratory and field conditions (Råberg et al. 2007; Mazé-Guilmo et al. 2014; Lough et al. 2017; but see Lefèvre et al. 2011). Together with our results, these latter studies therefore raise the question of how (genetic) variation in host tolerance is maintained. One general possibility is that tolerance mitigates parasite-induced costs to fecundity (sterility tolerance) rather than survival. In which case, tolerance will not necessarily increase parasite fitness and the positive feedback loop is broken, which could allow genetic polymorphism (and thus variance) in host tolerance to persist (Best et al., 2008). However, while body weight is linked to both reproductive success (Woodroffe, 1995; Woodroffe & MacDonald, 1995; Dugdale et al., 2011) and survival (MacDonald & Newman 2002) in badgers, population-level reproduction and recruitment measures appear largely unaffected by bTB infection in Woodchester Park (McDonald et al., 2016; Tomlinson et al., 2013). In contrast,

bTB-induced reductions in survival are readily detected (McDonald et al. 2014, 2016).

Trade-offs in resource allocation among immune and/or life-history traits have also been invoked as a general explanation for the maintenance of genetic heterogeneity in tolerance. Several studies have reported a negative correlation between measures of resistance and tolerance (Fineblum & Rausher 1995; Miller et al. 2005; Råberg et al. 2007), suggesting investment in one defence strategy could sometimes come at the cost of the other (but see Mauricio et al. 1997). In the Woodchester Park badger population, our recent analysis of bTB status provides putative support for genetic variance in resistance, although we note that obtaining unbiased measures of resistance without experimental manipulation is problematic (see below), limiting our ability to test for this trade-off here. Trade-offs could also occur among immune responses to different parasites and/or strains of the same parasite. For instance, helminth infection, which is common in badgers (Torres et al., 2001; Sin et al., 2014), can alter the severity of microparasite-induced disease (Furze et al. 2006; Graham et al., 2005b), while hosts may also vary in their response to different strains of the same parasite (Morrison et al., 2010). Scrutiny of coinfections and variation within parasite populations could therefore provide key insights into maintenance of host tolerance variation.

As with all analyses, there are a number of caveats to the conclusions drawn from this study. Most crucial among them is our quantification of tolerance. While the reaction norm approach adopted here has clear advantages over other approaches to characterising tolerance variance (e.g. point tolerance; Kutzer & Armitage 2016a) it is also true that weight loss is a non-specific symptom. Thus, we cannot be certain that what we are measuring

is exclusively representative of the host's ability to mitigate infection costs. Our finding that heavier individuals (at bTB status =0) tend to exhibit lower tolerance could, at least in part, be driven by the fact that hosts in better condition (i.e. heavier badgers) have "farther to fall". Nonetheless, the correlation between test-negative weight (intercept) and tolerance (slope) being significantly greater than -1 indicates that some variation in tolerance is (statistically) independent of initial weight.

Similarly, because obtaining direct measurements of parasite load was not possible here, we used bTB status (inferred from live diagnostic test results) as a proxy. This necessarily assumes a linear relationship between parasite load and bTB status which may be too simplistic. Although bacterial culture (used here to assign badgers 'excretor' status) could feasibly provide a direct measure of parasite burden, data were only available as a qualitative (presence/absence) measure in the current study. Additionally, low sensitivity of bacterial culture means that many true infections are likely to be missed, leading to expected bias in tolerance estimates based on this one test alone (Drewe et al., 2010). Overall, previous studies suggest the bTB status categorisation used here is a reasonable approximation of disease progression (Mahmood et al., 1987; Lesellier et al., 2008) and, as direct measurements of parasite load are not practicable here (or in most wild systems), we view these simplifying assumptions as justifiable. Nonetheless, results must be interpreted accordingly.

Conclusions

In summary, we found significant among-individual variation in tolerance to infection by *M. bovis*, measured as the rate of weight loss with increasing infection status, in a naturally infected population of badgers. Pedigree-based analysis reveals this variation can largely be ascribed to genetic factors. This is despite the theoretical prediction that positive feedback between selection on host and parasite should lead to rapid fixation of the most tolerant genotypes in a host population. Our results provide the first evidence for tolerance against bovine tuberculosis in a wild host and add to a growing body of work documenting among-individual variation in tolerance in natural host populations. Further research into the mechanisms underpinning tolerance, together with investigation of selective processes hypothesised to maintain (genetic) variation are needed to determine the importance of tolerance for host-parasite coevolutionary dynamics.

Chapter 5

Genetic correlations among responses to bovine tuberculosis diagnostic tests in European badgers (*Meles meles*)

Abstract

Knowledge of the causes and consequences of variation in host responses to infection is crucial for understanding host-parasite co-evolutionary dynamics as variation among individuals is a prerequisite for natural selection, while a genetic basis to such variation is required for evolution. Quantification of this host heterogeneity depends on appropriate measures of host immune responses. However, the complexity of the immune system and environmental variability limit extrapolation based on single measures, which has led to calls to integrate multiple immune measures into immune phenotypes in order to better characterise host variation and its evolutionary implications. This need arises because natural selection rarely operates on single traits in isolation, and it is the genetic correlation structure among traits that shapes (and sometimes constrains) the multivariate selection responses. Yet few studies have attempted to characterise the genetic associations among different measures of immunity and infection status in wild systems. Here, we take a quantitative genetic approach to investigate the phenotypic and genetic correlation structure among four measures of host immune responses to infection; nonspecific immune function, antibody- and cell-mediated responses and bacterial load. Using longitudinal data from a population of European badgers (*Meles meles*) infected with *Mycobacterium bovis* we find significant correlation structure

among the traits; phenotypic correlations were positive among *M. bovis*-specific traits, and both antibody production and parasite load were negatively correlated with nonspecific immune function, a qualitative pattern indicating general immune function positively (though weakly) predicts an individual's ability to fight bTB infection. Genetic correlations among traits were relatively weak and we suggest that while heritable traits are not completely free to evolve independent of each other, there is no compelling evidence for trade-offs that might constrain their evolution.

Introduction

Parasites are ubiquitous in wild animal populations and have wide ranging consequences. Infectious disease outbreaks can sometimes pose a threat to the persistence of wildlife populations (Daszak et al. 2000), while wild hosts can also act as important reservoirs for pathogens that impact human and livestock health (Allen et al., 2011; McDaniel et al., 2014). From an evolutionary perspective, parasites negatively impact the fitness of an infected host, and can therefore be an important source of selection, and hence driver of host evolutionary dynamics. Though behavioural strategies to limit exposure to parasites are clearly important (Sarabian et al., 2018), the suite of defence strategies comprising host immune function provides striking evidence of evolutionary adaptation to parasite challenge. However, populations typically contain heterogeneity in host immune response and so infection outcomes; some individuals are resistant to infection where others are susceptible, some infected individuals may rapidly clear parasites, others experience disease and even death. Variation among individuals is a prerequisite for natural selection, while a (partial) genetic basis to such variation is required for evolution (Falconer & Mackay 1996). Understanding the causes and consequences of variation in host responses to infection is therefore likely to provide key insight into host-parasite co-evolutionary dynamics. However, quantification of host heterogeneity depends on appropriate measures of host immune responses.

Most of our understanding of host heterogeneity comes from experimental studies conducted in laboratory models and livestock systems. Such studies have found heritable variation in a number of immune responses and susceptibility to disease (Ham & Yang, 1996; Morris, 2007; Gunia et al.,

2015). It is logistically and ethically challenging to utilise similar experimental infection approaches in wild animal hosts, though it is sometimes possible to bring wild-caught hosts into the laboratory for this purpose (e.g. Diegel et al., 2002; Bonneaud et al., 2018). Experimentally clearing infections by treating wild animals is also a useful approach to understand the impacts of infection on host fitness (Craig, Jones et al., 2009; Jolles & Ezenwa, 2015). Nonetheless, there is also considerable interest in directly measuring host immune function in unmanipulated populations, with three broad approaches being used by ecoimmunologists. First, and common, has been the measurement nonspecific immune activity in wild animals using non-infectious immunostimulants such as lipopolysaccharides (LPS; e.g. Moret & Schmid-Hempel 2000; Bonneaud et al. 2003) or phytohaemagglutinin (PHA; e.g.(Chakarov et al., 2017). Such generic immune challenges are thought to provide a measure of 'immunocompetence', with higher responsiveness indicating the overall 'strength' of an individual's immune system and its ability to fight disease. Second, more recent studies have utilised assays of parasite-specific immune responses, which may be targeted to examine different arms of immune system (e.g. humoral vs cell-mediated responses). For example, parasite-specific antibody responses have been used to study helminth infections in wild mice (Clerc et al., 2018) and Soay sheep (Hayward et al. 2014a). Thirdly, quantification of parasite burden may also be used to measure infection status from which inferences about host immune function can be made (subject to assumptions) (e.g. Graham et al., 2013). Typically, high parasite loads are presumed to indicate low resistance. It is worth noting perhaps that in many cases parasite loads themselves may also be indirectly inferred. For instance, faecal egg counts are a widely used proxy of

gut helminth load in ecological studies (e.g., Debeffe et al., 2016) but may not always be perfectly correlated with worm burden.

Variable environments may impact infection probability, immune function and/or fitness consequences of infection via multiple routes (Prokkola et al., 2013;Garbutt et al., 2014; Patterson et al., 2017). Furthermore, the complexity of the immune system means that relationships among different measures of immune function and response may not always be straightforward to predict. Although perhaps tempting to conceptualise variation in immune function as lying along an axis of performance from low to high, this may sometimes be too simplistic. For instance, the need to allocate limited resources to defence may result in trade-offs between i) immune function and life history (Van Der Most et al., 2011); ii) different defence strategies (e.g., resistance versus tolerance; (Restif & Koella, 2004); and/or different components of the immune system (e.g. investment in type 1 T helper cell (Th1) mediated immunity against intracellular parasites versus Th2 mediated humoral responses against extracellular parasites; Habig et al. 2015). The fitness consequences of variation in immune function, mediated for example via differential disease progression and symptom severity may also depend on environmental conditions (Lazzaro & Little, 2009). In this respect it is also worth noting that an implicit assumption of many studies using immunocompetence measures and parasite-specific antibody assays is that high responsiveness should lead to high fitness. However, given the costs of mounting an immune response (e.g. energetic, or resulting immunopathology), individuals with the strongest immune responses are not necessarily the fittest (Viney et al., 2005). Nor will a low parasite load necessarily mean high (relative) fitness in populations where individuals differ in tolerance.

Uncertain, and sometimes contrasting, predictions for relationships among immune traits have led to increasing calls for the use multiple measures to characterise immune phenotypic variation (Adamo, 2004; Martin et al., 2006b; Hawley & Altizer, 2011). Practically, this will sometimes help to 'triangulate the truth' if, for instance, the goal is to determine individual infection states and diagnostic tests have high error rates (i.e. low sensitivity and/or specificity, e.g. Buzdugan et al. 2016). However, from an evolutionary standpoint, the key advantage is that it becomes possible to characterise the genetic relationships among different immune traits (provided data on among-host relatedness can also be obtained; (Kruuk, 2004). In this sense, calls to integrate multiple immune measures into immune phenotypes converge with a wider recognition of the need for multivariate studies in evolutionary ecology (Blows 2007; Wilson et al. 2008). This need arises because natural selection rarely operates on single traits in isolation, and it is the genetic correlation structure among traits that shapes (and sometimes constrains) the multivariate selection responses (Blows & Walsh 2009). Thus, for instance, if resource allocation trade-offs among immune traits really are present and act as evolutionary constraints, we predict that there will be negative genetic correlations between them (with traits defined so as to be under positive selection; Kruuk et al., 2008) even if total phenotypic correlations are positive (e.g., due to environmentally driven heterogeneity in resource acquisition; van Noordwijk & de Jong, 1986).

Few studies to date have attempted to characterise the genetic associations among different measures of immunity and infection status in wild systems. Here, we attempt to do this, taking a quantitative genetic approach to investigate the correlation structure among different measures of host immune

responses to infection, using longitudinal data from a population of European badgers (*Meles meles*) infected with *Mycobacterium bovis* (cause of bovine tuberculosis; bTB). This unmanaged population has been subject to consistent live testing for bTB using a number of diagnostic tests and provides a unique opportunity to explore the genetics underpinning immune phenotypic variation in the wild. Using a pedigree reconstructed from genetic data (Chapter 2; Marjamäki et al. in press) and a progressive infection status categorisation ('bTB status'; Graham et al 2013) we have previously found evidence for genetic variation in disease state (Chapter 3) and tolerance to infection (inferred from weight loss with increasing bTB status; Chapter 4).

In the current study we examine the genetic basis of (co)variation in, and among, four diagnostic test responses, two of which have been used to assign bTB status. These four diagnostic traits represent different, but putatively correlated, aspects of host immune function and infection status: general immunocompetence (pokeweed mitogen response), bTB-specific humoral (blood antibody assay) and cell-mediated responses (interferon gamma; IFNγ assay) and parasite burden (bacterial culture). Our objectives are firstly to determine whether genetic factors contribute to among-individual differences in all traits and secondly to evaluate the among-trait genetic correlation structure. The latter will allow us to determine whether different immune traits are free to evolve independently of each other and, if not, whether there is potential for trade-offs among traits to act as evolutionary constraints.

Methods

Study system & data collection

Woodchester Park contains a naturally infected population of badgers, comprising 200-300 animals at any one time, which are the subjects of an ongoing mark-recapture study. The study area (ca. 11 km^2) consists of a steep-sided wooded valley surrounded by farmland. Within the study area up to 45 social groups have been recorded, each associated with one or more underground dens (setts). The number of social groups (as determined by bait marking; Delahay et al. 2000a) varies among years, but the study area includes a core of 20-25 social groups with relatively consistent and continuous trapping records across the study period. In total, the mark-recapture dataset used here contains 15252 observations of 3239 individual badgers sampled between 1976 and 2014. Full details of trapping procedures are presented elsewhere (Delahay et al. 2013) but, in brief, badgers are trapped at or around setts up to four times a year for two consecutive nights using peanut-baited steel mesh box traps. Once captured, the identity, sex, age class (cub, yearling, adult), body weight (KG), and capture location of each marked badger is recorded. Badgers caught more than once during a trap-up are only sampled once. Most animals are first caught as either cubs or yearlings (distinguished from adults by size and toothwear; Delahay et al. 2013) allowing determination of age at all subsequent captures. Sampling is carried out under anaesthesia (MacKintosh *et al*. 1976; de Leeuw *et al*. 2004), with guard hairs, blood and clinical samples taken for genotyping and bTB diagnostics (see below). Badgers are then released at the point of capture after recovery from anaesthesia.

Diagnostic tests and designation of bTB infection status

Following each capture event, badgers are tested for bTB using several diagnostic procedures (Table 1). First, clinical samples from a number of body sites (faeces, urine, tracheal aspirate, pus, bite wound swabs; Clifton-Hadley *et al.* 1993) are subjected to bacterial culture for *M. bovis*. Spoligotyping is used to identify any growth as *M. bovis* (Kamerbeek et al. 1997) and one or more positive results from among the body sites is considered indicative of current infection. This 'CULTURE' status is used in our analyses as a binary response variable in which *M. bovis* is detected as present (1) or absent (0). Second, serological assays are used to test badger serum for IgM and IgG antibodies to *M. bovis*, which indicate activation of the humoral immune response and can thus indicate previous or current infection. Between 1982 and 2005 these serological tests were done using an enzyme-linked immunosorbent assay (ELISA) Goodger et al., 1994) referred to as Brock-ELISA. This was replaced in 2006 with a lateral flow immunoassay (referred to as BrockTB Stat-Pak; Chambers et al., 2008). However, for present analyses these two serological assays are combined into a single response variable, 'BROCK' indicating either presence (1) or absence (0) of antibodies. Third, from 2006 onwards, an interferon-γ (IFN_γ) release assay has also been used to test cell-mediated immunity. In this two-part test, the production of IFN_γ in heparinised whole-blood culture is first stimulated with mycobacterial antigens (bovine tuberculin), and then quantified using sandwich ELISA, producing a continuous optical density (OD) measure (Dalley et al. 2008). Since infection by environmental mycobacteria from the *Mycobacterium avium* complex (MAC) can bias results of bovine tuberculin assays (Pollock et al. 2005), IFN_γ responses to avian

tuberculin are simultaneously tested. The OD measure from the avian stimulus is then subtracted from that obtained with the bovine tuberculin stimulus to yield a putatively *M. bovis* specific test measure that we refer to as 'B-A'. Finally, in addition to testing for *M. bovis* specific responses, blood samples are also used to assay the cell-mediated immune response to pokeweed mitogen ('PWM'), This non-specific activator of cell-mediated and humoral responses (Janossy & Greaves 1972) used here as a putative test of generalised immune function. We include this data to characterise the strength of the (genetic) correlation between general immune function and bTB-specific immune responses (see below). All assays are conducted in duplicate and the mean of these two used in analyses.

Due to the low and variable sensitivities of the different diagnostic tests (Table 1) they have been used in combination to assign a bTB infection status ('bTB status') for previous studies of this population (Graham et al. 2013). This progressive infection status categorisation runs from 0-3 and is determined as follows. At each capture, individuals testing negative to both serological test and culture are assigned test-negative status (0). Badgers with a positive serological test but negative bacterial culture are considered test-positive (1). Badgers testing positive to bacterial culture are assigned a bTB status of either 2 (if a positive test comes from a single body site) or 3 (if positive cultures are obtained from multiple body sites). This categorisation is progressive and unidirectional such that once a badger reaches a certain status category it can no longer be assigned a lower one. We have previously shown this summary variable to be heritable in the Woodchester Park population (Chapter 3).

Table 1. Response variables used in analyses, including a summary of the diagnostic tests underpinning each and the years in which each test has been applied. Sensitivity (false negative rate) and specificity (false positive rate) estimates from Greenwald et al. 2003, Buzdugan et al. 2016 (and references therein).

Variable	Diagnostic test	Years applied	Description	Sensitivity	Specificity
CULTURE	Bacterial culture	1976-present	Tests presence/absence of *M. bovis* bacilli in sampled tissue/excreta	8%	100%
BROCK	Brock Elisa	1982-2005	Tests presence/absence of *M. bovis* antibodies in blood serum (humoral response). Enzyme linked immunosorbent assay.	47%	89–94%
	BrockTB Stat-Pak	2006-2014	Tests presence/absence of *M. bovis* antibodies in blood serum (humoral response). Lateral flow immunosorbent assay	49-78%	93%
B-A	Interferon-γ	2006-present	Detection of IFN_γ in whole-blood cultures (cell-mediated immune response). Measured as the difference in continuous optical density of IFN*γ* production between stimulus by *M. bovis* and *M. avium.*	52-85%	93.6%
PWM	Interferon-γ	2006-present	Detection of IFN_γ in whole-blood cultures (cell-mediated immune response). Measured as the continuous optical density of IFN*γ* production in response to challenge with pokeweed mitogen. Used as generic measure of immune responsiveness.	-	-

Overview of statistical analyses

In order to characterise genetic variation in, and associations among, response variables from the different diagnostic tests, we fitted a number of univariate and multivariate pedigree-based 'animal models' (Wilson et al. 2010). Since our primary interest is in estimating the multivariate genetic correlation structure (as opposed to statistical inference) we took the pragmatic decision to model traits using restricted maximum likelihood (REML) under an assumption of (multivariate) residual normality. All models were thus fitted using ASReml 4.0 (VSN International) with response variables scaled to standard deviation units prior to analysis. This approach was taken because it yields valid genetic correlation estimates on the observed data scale, while greatly improving ease of model fitting relative to the alternatives (e.g., building multivariate generalised animal models with parameterisation by MCMC). However, since the assumption of residual normality is necessarily violated for binomial and ordinal response variables (BROCK, CULTURE, bTB status), all statistical inferences presented should be treated as provisional. We note that previous analysis by MCMC does support a genetic basis of variation in a binary 'lifetime bTB status' in the population (Chapter 3).

For present purposes, and reiterating the above caveat, we provisionally test the significance of variance and covariance terms associated with model random effects by assuming that twice the difference in log-likelihood between full and reduced models has a χ^2 distribution with the degrees of freedom equal to the number of additional parameters in the more complex model. Following Visscher (2006), we assume an equal mix of χ^2_0 and χ^2_1 (denoted as $\chi^2_{0,1}$) for the specific case that a single variance component is being tested. We also use Akaike Information Criterion (AIC) as an additional guide for comparison of non-

nested models, and the approximate standard errors (SE) as a guide to significance of individual correlation estimates (i.e., we consider a correlation nominally significant at $\alpha=0.05$ if $|r|/SE > 1.96$).

Animal models utilised a pedigree structure recently estimated for the Woodchester Park population using microsatellite genotype data and a Bayesian pedigree reconstruction method implemented in the MasterBayes library (Hadfield et al. 2006) for R (R Core Team 2016). This analysis and the resultant pedigree structure is described in Marjamäki et al. (in press). The pedigree has a maximum depth of six-generations and includes all parent-offspring assignments where a candidate parent was specified in at least 80% of the samples of the posterior distributions of the pedigree. In total 617 individuals captured as cubs are assigned at least one parent (35% of genotyped cubs), of which 556 have both parents assigned.

Univariate analyses

We first ran four univariate animal models to test for and estimate the heritability of each diagnostic response variable (BROCK, CULTURE, B-A, PWM). We have previously shown that heritable variation exists in the progressive bTB status (denoted bTB_{multi} in Chapter 3; Marjamäki et al. in press). However, bTB_{multi} is also subject to environmental effects arising from year, social group, and – in cubs – maternal effects, while mean phenotype also varies with sex, season and age. To maintain consistency across traits we therefore elected to include a common set of fixed and random effects for all traits. These were fixed effects of sex, season (a four-level factor denoting Spring, Summer, Autumn or Winter), and linear, quadratic and cubic age (zero-centred to the mean age (4.06 years) of adult records). To account for the use of two different serological tests, an additional fixed effect of 'type' was included in the BROCK model to

control for any difference in mean test results between Brock ELISA and Stat-Pak.

Random effects included the individual breeding values (i.e. additive effect of an individual's genotype on phenotype relative to the population mean), as well as a permanent environment effect of individual identity (to account for non-genetic sources of among individual variation given the repeated measures), and a factor defined by the interactions of social group and year of sampling. (Note that in our previous modelling of bTB_{multi} we partitioned the latter into separate random effects of social group, year, and group x year but these are not of specific interest here and collapsing to a single factor facilitates model fitting by reducing the number of parameters). As in the bTB_{multi} model we also included a random effect of maternal identity but restricted to records on cubs only. Since failure to adequately model maternal effects can lead to upward bias in genetic parameters (Wilson et al., 2005a), we validated this decision by refitting univariate models with maternal effects included on all ages. Full results of these latter models are not shown as AIC comparison confirmed that the preferred model formulation was with cub-restricted maternal effects in all cases (ΔAIC of 937.271, 194.360, 110.820, 167.170 for BROCK, CULTURE, B-A, PWM respectively). Random effects are assumed to be drawn from distributions with zero means and variances of V_A (additive genetic), V_{PE} (permanent environment), V_M (maternal), and V_{SGxY} (social group x year) to be estimated. For each response variable, the estimated variance components were divided by total phenotypic variance (V_P), calculated as the sum of estimated variance components, to obtain heritabilities and other intra-class correlations (ICC) conditional on fixed effects (Wilson 2018). This was done separately for adults and cubs, as V_M in these models contributed to total

phenotypic variance in cubs only (i.e., $V_P = V_A+V_{GxY}+V_{PE}+V_M+V_R$, while for adults $V_P = V_A+V_{GxY}+V_{PE}+V_R$, where V_R is residual variance).

Multivariate analyses

We next estimated phenotypic and genetic correlation structures among the four scaled diagnostic response variables using multivariate models. First, we simply estimated the total phenotypic correlation structure among observed (scaled) data by fitting all four traits in a multivariate model with no fixed or random effects (except for trait specific means). This yields an estimate of the phenotypic covariance matrix among observed traits (which we denote $\mathbf{P_O}$) which was rescaled to give the corresponding correlations (noting that between traits x and y, $r = COV_{x,y}/(V_x.V_y)^{0.5}$). We then refitted this model with fixed effects (as described above) included on all traits to determine the total phenotypic covariance matrix conditional on fixed effects (**P**). Note that $\mathbf{P_O}$ and **P** simply describe the total covariance/correlation among traits at the level of the observation (capture event) before and after conditioning on the fixed effects. Assuming BROCK, CULTURE, B-A are all indicative of bTB infection, we expect uniformly positive correlation structure among these traits at this level. However, if PWM is a useful measure of general immunocompetence, and if this in turn negatively influences the probability of bTB infection, then we would expect covariances between PWM and the other three traits to be negative.

We then added the full complement of random effects (as described for univariate models), including the additive genetic merit, to the model. This allowed us to estimate the among-trait genetic covariance matrix **G** and the corresponding pairwise genetic correlations (r_G). The estimate of V_A was bound

to zero in the univariate model of B-A suggesting that there is no heritable variation in this trait (see Results). This trait was consequently dropped from the multivariate animal model to facilitate convergence. Note that previous work has suggested responses to diagnostic tests may be size (or condition) dependent (Waring) and we have shown that size (measured as weight) is itself heritable (see Chapter 4). We therefore also estimated a 'conditional' **G** matrix which we denote $\mathbf{G}_{|WT}$ to determine whether the effect of weight on genetic correlations among diagnostic tests influences our conclusions in any way. This was done by adding weight as an additional response to the multivariate animal model (with all fixed and random effects as specified above). This yields an estimate of the genetic covariance between weight and each of the other traits, which can be used to calculate $\mathbf{G}_{|WT}$ (following equations presented in Hansen and Houle 2008).

Finally, we also ran bivariate animal models to estimate r_G between bTB_{multi} and BROCK, CULTURE and PWM (but not B-A as it lacked detectable heritable variation in the univariate model). Bivariate models were used because inclusion of bTB_{multi} as a response in the full multivariate model resulted in convergence problems. This is likely because the bTB_{multi} phenotype is defined by the combination of BROCK and CULTURE.

Results

Univariate animal models indicated the presence of statistically significant additive genetic variance for BROCK, CULTURE and PWM (all $p<0.001$ based on likelihood ratio tests; Table 2). Corresponding estimates of adult heritability (SE) on the observed scale are; $h^2_{BROCK}= 0.091$ (0.024), $h^2_{CULTURE}= 0.096$

(0.022) and h^2_{PWM}= 0.138 (0.036) (Table 3). However, no additive genetic variance was detected for B-A, the estimate being bound to zero (Table 3). We therefore conclude that there is a lack of heritable variation for this trait in the Woodchester Park population. Permanent environment, social group-by-year effects, contribute nominally significant variance to all traits, as do maternal effects on cubs (Table 2). These findings differ somewhat with previously documented social and environmental effects on bTB incidence and progression (see analysis of bTB_{multi} presented in Chapter 3), which could reflect the slight differences in random effects (i.e. exclusion of social group and year from the current models). Since our primary focus here is on the genetic basis of trait (co)variation we do not discuss these results further. Nor are fixed effect estimates directly relevant to present aims (but see supplemental table 1 for a full presentation).

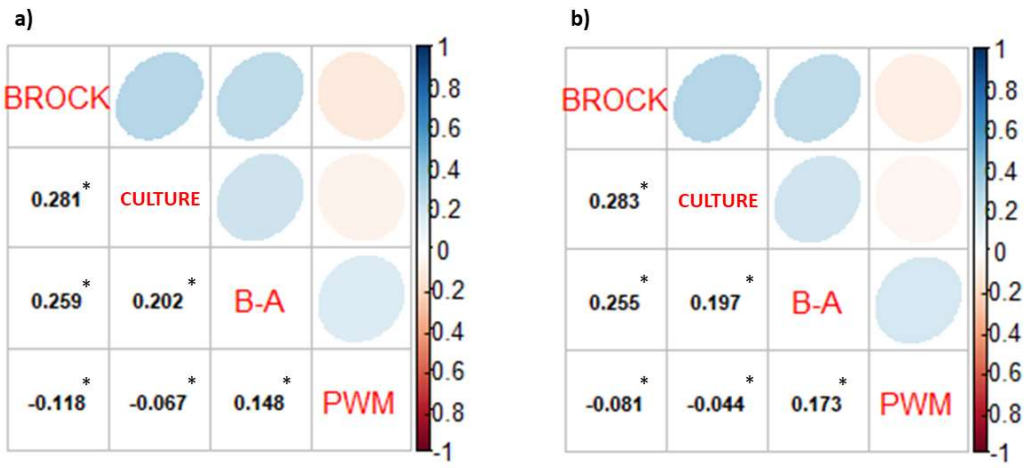

Figure 1. Raw phenotypic correlations **a)** and correlations conditional on fixed effects **b)** represented in numerical form below diagonal, and graphically above diagonal. * denotes statistical significance as inferred from standard errors (|r|/SE >1.96).

Multivariate models revealed that the total phenotypic correlation structure among BROCK, CULTURE and B-A is positive as predicted. Furthermore, phenotypic correlations between BROCK and CULTURE, and PWM are negative (Figure 1). Counter to our prediction, however, B-A was positively correlated with PWM. Overall, there is evidence of significant covariance structure among the observed traits (likelihood ratio comparison between the unconstrained multivariate model and a simplified model in which all covariances are constrained to zero; χ^2_6= 4849.06, P<0.001) and pairwise correlations among the observed traits are all nominally significant (based on|r|/SE >1.96; Figure 1a). However, despite apparent statistical significance, the correlations are also uniformly weak (Figure 1a), ranging in magnitude from a maximum of r=0.28 between BROCK and CULTURE, to a minimum of r=-0.07 between PWM and BROCK. Furthermore, these phenotypic correlations are largely unaffected by conditioning on fixed effects of sex, season and age at capture (Figure 1b).

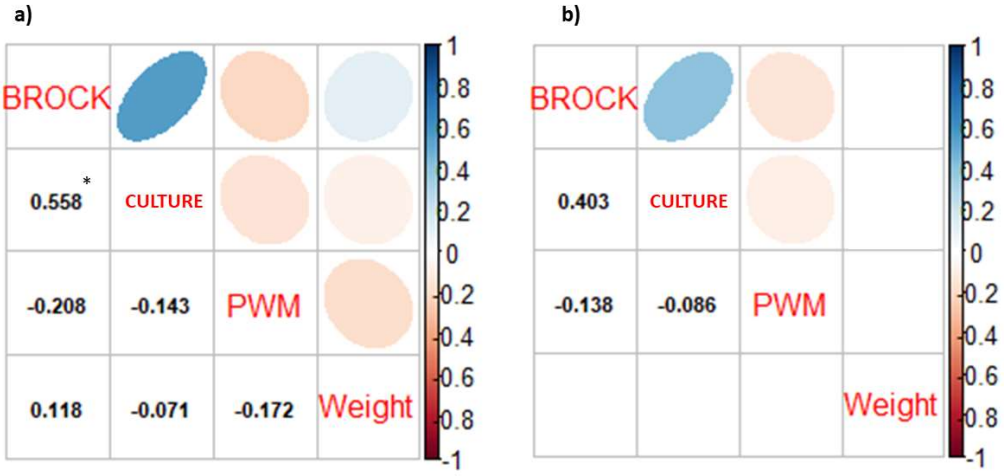

Figure 2. Genetic correlations among diagnostic tests from multivariate animal model (B-A excluded due to lack of genetic variance). **a)** shows r_G **b)** shows $r_{G|WT}$. * denotes statistical significance as inferred from standard errors (|r|/SE >1.96).

The genetic correlation structure among diagnostic traits (as estimated from the multivariate animal model including weight) was also similar to the phenotypic structure (though r_G estimates between B-A and other traits were not estimated; Figure 2). Overall, there was evidence of significant genetic covariance structure in **G** based on likelihood ratio comparison between the unconstrained multivariate model and a simplified model in which all genetic covariances were constrained to zero (χ^2_6=13.08, p=0.042). Examining the pairwise correlations, a positive (and nominally significant) correlation was estimated between BROCK and CULTURE (r_G=0.558 (0.119)) indicating that antibody production is genetically associated with *M. bovis* infection. PWM was weakly (and non-significantly) negatively correlated with both BROCK and CULTURE (r_G= -0.208 (0.199) and -0.143 (0.227), respectively). Inclusion of weight allowed us to confirm previous analyses showing size to be heritable, with V_A as estimated in the multivariate animal model equating to heritabilities (SE) of h^2=0.29 (0.021) and 0.28 (0.029) for weight in cubs and adults. However, we detected no strong (or significant) pairwise genetic correlations between the diagnostic traits and body weight. Consequently, it is unsurprising that the correlation structure among diagnostic traits in **G** is almost unchanged by conditioning on weight to determine $\mathbf{G}_{|WT}$ (Figure 2b).

Table 2. Variance component estimates (standard error) and likelihood ratio tests of random effect from univariate models for each diagnostic test variable; BROCK = serological test for bTB specific antibody production, CULTURE = bacterial culture of *M.bovis*, B-A = IFNγ production in response to bovine tuberculin minus IFNγ production in response to avian tuberculin, PWM = IFNγ production in response to pokeweed mitogen. V=variance. Subscripts denote source of variance; SGxY = social group by year, M = maternal, PE = permanent environment, A = additive genetic, R = residual

	BROCK			CULTURE			B-A			PWM		
	Estimate (SE)	**$\chi^2_{0,1}$**	**p**	**Estimate (SE)**	**$\chi^2_{0,1}$**	**p**	**Estimate (SE)**	**$\chi^2_{0,1}$**	**P**	**Estimate (SE)**	**$\chi^2_{0,1}$**	**P**
V_{SGxY}	0.301 (0.017)	6155.80	<0.001	0.133 (0.009)	1272.38	<0.001	0.223 (0.026)	1056.56	<0.001	0.347 (0.037)	2978.81	<0.001
V_M	0.472 (0.052)	961.78	<0.001	0.192 (0.028)	194.34	<0.001	0.712 (0.121)	598.78	<0.001	0.225 (0.050)	167.25	<0.001
V_{PE}	0.350 (0.028)	320.92	<0.001	0.149 (0.022)	68.88	<0.001	0.577 (0.039)	112.84	<0.001	0.116 (0.029)	25.41	<0.001
V_A	0.105 (0.028)	24.56	<0.001	0.105 (0.024)	14.50	<0.001	0.000 (-)*	0	0.500	0.125 (0.034)	19.97	<0.001
V_R	0.399 (0.003)	-	-	0.705 (0.005)	-	-	0.437 (0.007)	-	-	0.322 (0.005)	-	-

*Variance component estimate bound to zero so no standard error is estimated

Table 3. Intraclass correlations (standard error) for cubs and adults calculated as the ratio of each variance components to total phenotypic variance (V_P). Note ICC differ between age classes since V_M contributes to V_P in cubs but not adults. BROCK = serological test for bTB specific antibody production, CULTURE = bacterial culture of *M.bovis*, B-A = IFNγ production in response to bovine tuberculin minus IFNγ production in response to avian tuberculin, PWM = IFNγ production in response to pokeweed mitogen.

	BROCK		CULTURE		B-A		PWM	
	Cub ICC	**Adult ICC**	**Cub ICC**	**Adult ICC**	**Cub ICC**	**Adult ICC**	**Cub ICC**	**Adult ICC**
Social group x year	0.185 (0.010)	0.261 (0.011)	0.103 (0.007)	0.122 (0.008)	0.115 (0.013)	0.180 (0.018)	0.298 (0.026)	0.381 (0.026)
Maternal	0.290 (0.023)	-	0.150 (0.018)	-	0.365 (0.040)	-	0.218 (0.034)	-
Permanent environment	0.215 (0.018)	0.303 (0.024)	0.116 (0.017)	0.137 (0.020)	0.296 (0.023)	0.466 (0.020)	0.099 (0.025)	0.127 (0.032)
Heritability	0.064 (0.017)	0.091 (0.024)	0.082 (0.018)	0.096 (0.022)	0.000 (-)*	0.000 (-)*	0.108 (0.029)	0.138 (0.036)

* Additive genetic variance component estimate bound to zero, so no standard error is estimated on corresponding heritabilities

Finally, bivariate animals model yielded significant positive estimates of r_G between the progressive bTB status (bTB_{multi}) and both BROCK (r_G =0.913 (0.114)) and CULTURE (r_G=0.856 (0.055); Table 4). This is not surprising as bTB status is assigned based on the results of these two diagnostic traits. The genetic correlation between PWM and bTB status was moderately negative though not statistically significant (r_G = -0.413 (0.272)).

Table 4. Estimated genetic correlations (r_G) between diagnostic test traits and the progressive bTB infection status score (bTB_{multi}). Estimates (with standard errors) are from bivariate animal models and likelihood ratio tests against a null hypothesis of r_G=0 are shown.

	r_G (SE)	**X^2_1**	**P**
BROCK	0.913 (0.114)	15.960	<0.001
CULTURE	0.856 (0.055)	13.980	<0.001
PWM	-0.413 (0.272)	2.600	0.106

Discussion

Using a quantitative genetic approach, we have characterised the contribution of genetic factors to variation in, and covariation among, four diagnostic measures of host immune response and bTB infection status in the Woodchester Park badger population. We detected a small, but significant, heritable component of variation in three of these; bTB-specific antibody production (BROCK), parasite load (CULTURE) and general immune function (PWM). However, we found no evidence that genetic factors contribute to variance in cell-mediated immune response against bTB (as measured by B-A). There is also significant correlation structure among the traits. Phenotypic correlations were positive among bTB specific traits, and both BROCK and CULTURE were negatively correlated with PWM, a qualitative pattern that means general immune function positively (though weakly) predicts an individual's ability to fight bTB infection. Except between BROCK and CULTURE, genetic correlations among traits were relatively weak and we suggest that while heritable traits are not completely independent of each other, there is no compelling evidence for trade-offs.

The finding of heritable variation in bTB-specific antibody production (BROCK) and parasite load (CULTURE) corroborates our previous conclusion that genes do contribute to variance in immune function and disease state (Chapter 3). We have previously identified a partial genetic basis for variation in a progressive bTB status (bTB_{multi}) and in lifetime infection risk, the latter providing a putative proxy of resistance (Chapter 3). We have also found evidence of heritable variation in tolerance to bTB infection (Chapter 4). Here we add further to this picture, by showing that general immune function (PWM) is also genetically variable. However, in all cases estimated heritabilities are low

and (social) environment factors, including maternal effects for cubs, explain more variance than genetics. Whether this finding is more generally true for immune traits in wild populations is difficult to assess at present as comparable studies remain limited in number and taxonomic scope. In birds, studies of response to PHA skin test (a widely used field-assay of non-specific immune function) have produced variable results, with no (or very little) genetic variation detected in some cases (Kilpismaa et al. 2007; Pitala et al., 2007; Sakaluk et al., 2014) and moderately high heritabilities reported in others (e.g. 0.46,Bonneaud et al., 2009; 0.38, Drobniak et al., 2010). Published heritability estimates for parasite-specific immune responses and infection status are even scarcer, but those that do exist are low. For example, in Soay sheep, faecal egg count and strongyle-specific antibody titres used as proxies of resistance yield estimates of h^2= 0.11-0.14 (Coltman et al. 2001; Hayward et al. 2014a).

Thus, while standing genetic variance is present in Woodchester badgers, its importance as a source of host heterogeneity in immune traits should not be overstated. We also note that there was no detectable genetic variance for cell-mediated responses to bTB as measured by the IFNγ assay (B-A). This could potentially be an artefact of the diagnostic measure if the component traits 'B' (optical density of response to bovine tuberculin) and 'A' (response to avian tuberculin) are themselves heritable and positively genetically correlated. This follows mathematically because, for any two variables x and y, $V_{x-y} = V_x - 2COV_{x,y} + V_y$. However, additional *post hoc* univariate animal models detected no genetic variance in either optical density measure contributing to the B-A diagnostic (results not shown). Although speculative, the lack of genetic variation in the bTB-specific cell-mediated response could be indicative of past selection pressures. Since production of

IFNγ is critical to the effectiveness of cell-mediated immune responses against intracellular parasites such as *M. bovis* (Skinner et al. 2001), strong directional selection could have led to the trait becoming fixed in the population. Indeed, the same argument may also explain the low heritabilities of other traits; if *M. bovis* has imposed strong directional selection on them, standing genetic variance may have been eroded. We have no direct evidence for this however, and note also that low values of h^2 can equally reflect high levels of environmental variance rather than an absolute lack of V_A.

Our multivariate analyses provided evidence for significant phenotypic and genetic correlation structure among the immune traits. The positive correlations among bTB-specific responses are broadly consistent with the previous conclusion that seropositivity is more likely in badgers at advanced stages of infection (characterised by excretion of bacteria and thus positive culture tests; Buzdugan et al. 2016). The moderately strong positive genetic correlation between BROCK (i.e. presence of bTB-specific antibodies) and bacterial CULTURE indicates these traits likely have a shared genetic basis of variation and suggests that high antibody levels are more indicative of current infection than of expected resistance to infection. This follows since, in the latter case, negative correlations between antibodies and pathogen loads would be expected (Graham et al. 2011).

We found only weak phenotypic relationships between bTB specific cell-mediated immune responses (B-A) and BROCK and CULTURE (r=0.259 and 0.202, respectively). This was initially surprising given that cell-mediated immune response, measured by IFNγ production, has been found to predict future (6 to 24 months) seropositivity and excretion of *M. bovis* (Buzdugan et al. 2016). However, the total phenotypic correlations estimated in the current

analysis are within a single sampling 'time step'. Thus, it seems the phenotypic correlations among traits differ within, versus across, sampling events, which implies that the temporal structure of response differs among the various components of the immune system over the course of an infection (Buzdugan et al 2016). Given that B-A is not heritable, genetic correlations between cell-mediated immune response and the other traits (including the bTB_{multi}) were not be estimated. We note that significant positive genetic correlations were detected between both BROCK and CULTURE and bTB_{multi}. However, this is an almost inevitable consequence of trait definition so provides little additional biological insight.

From an evolutionary standpoint, the presence of at least some genetic covariance structure among heritable traits mean that immune response has some potential to evolve under selection. However, rapid evolution is unlikely given low heritabilities, and the traits considered are not free to evolve completely independently. Nonetheless, we find no suggestion that trade-offs among components of the immune system will constrain adaptation. In fact, negative genetic (and phenotypic) correlations between bTB-specific traits and responses to pokeweed mitogen provide some, albeit limited, evidence that higher general immune responsiveness is associated with lower risk of bTB infection. Thus, our results are largely consistent with the position, commonly assumed in ecoimmunological studies, that using non-infectious stimulants (e.g. PHA, PWM) to assay general immunocompetence can provide information about the ability of individuals (or genotypes) to fight specific infections (Sheldong & Verhulst 1996). However, these negative associations are weak (and none of the genetic correlations are significantly less than zero when considered individually). Furthermore, the pattern is imperfect since, at the

phenotypic level PWM is weakly (but significantly) positively correlated with B-A. This could be indicative differential investment in cell-mediated (B-A, PWM) versus humoral responses (BROCK), but it is difficult to make firm inferences from what is a weak pattern.

Beyond the present study, attempts to estimate phenotypic correlations among components of immune response in wild populations have produced a mixed picture, ranging from positive to negative correlations (e.g. Møller et al. 2001, Buchanan et al. 2003; Faivre et al. 2003). Corresponding genetic correlation estimates are scarce for wild populations, and even relatively rare in livestock and laboratory model systems (but see (Lambrechts et al., 2004; Okamura et al., 2016). Consequently, there is no emergent empirical consensus yet as to whether trade-offs among immune traits are widespread, or whether variation among individuals and/or genotypes lies more along a single high-low axis of immune function competence or 'quality' (*sensu* Wilson and Nussey 2010). A simple, but slightly trite, conclusion would be that different patterns arise for methodological and/or taxonomical reasons; the majority of field-studies having used experimental stimulation (i.e. subcutaneous injections) in avian species (but see e.g. Nussey et al., 2014). In fact, both experimental and observational studies have reported an apparent lack of (phenotypic) association among immune response traits in bird and mammal hosts (Martin et al., 2006a; Matson et al., 2006; Arriero et al., 2017). Such a scenario could easily arise if genetically based trade-offs among costly immune traits do exist but are masked by environmentally driven heterogeneity in resource acquisition (van Noordwijk and de Jong 1986). Such effects are unlikely in Woodchester Park badgers, as we directly estimate the genetic correlation structure itself and

show it to be similar to the phenotypic structure. Nor is genetic correlation structure notably changed by conditioning on body weight.

In summary, we find evidence that bTB-specific and general immune function traits are genetically variable in the Woodchester Park population of badgers, and that there is correlation structure among them arising, in part, from genetic effects. These conclusions must be tempered by acknowledgement of analytical limitations arising from phenotypic data structure (e.g., the proportion of infected badgers is low overall) and volume (e.g., IFNγ testing only commenced in 2006), and the incomplete pedigree data (which likely, but not inevitably, will lead to downward bias in h^2 estimates; Morrissey et al., 2007). We also reiterate that assuming (multivariate) residual normality in our models means all statistical inferences should be treated as provisional. These caveats notwithstanding, the findings here are; i) that genetic variation is present, but is a much less important driver of immune response variation than temporal, spatial and social (including maternal) factors; and ii) that there is genetic covariance present (notably between bTB-specific antibody production and parasite load), but no support for genetically determined trade-offs among the different arms of the immune response assayed. The extent to which these patterns generalise to other wild host-parasite systems is unknown, and we suggest that more genetic studies of multivariate phenotypes are urgently needed if we hope to understand how immune response evolves under selection in wild populations.

Chapter 6

General discussion

Parasites, such as *Mycobacterium bovis* (cause of bTB), are expected to impose directional selection on defence traits in host populations through their detrimental effects on fitness (Schmid-Hempel 2011). Among-individual variation in defence traits is a prerequisite for selection, but in order for hosts to evolve, there must also be a genetic basis to this variation. Thus, knowing what proportion of phenotypic variation has a genetic basis is crucial to our understanding of microevolutionary dynamics of host-parasite interactions. However, while genetic factors are well known to influence infection in humans and captive animal populations (Morris, 2007; Yan et al. 2006; Breitling et al., 2008), we currently have little knowledge of their relative importance in wild populations. The central aim of this thesis was to address this gap, by characterise the genetic basis of variation in bovine tuberculosis infection and its progression in a wild host and key wildlife reservoir for the disease – the European badger. In this final chapter, I first summarise first the key findings and conclusions of each chapter. I then reflect on the implications of this work for (i) understanding eco-evolutionary dynamics of host-parasite in this system and more generally, and (ii) management of bovine tuberculosis more specifically. Finally, I conclude with by briefly highlighting several promising avenues for future work.

Summary of main findings

In **Chapter 2**, I used the wealth of ecological and genetic data produced by the four-decade long mark-recapture study at Woodchester Park to build a multigenerational pedigree for the population. This represented a crucial first methodological step to understanding genetic architecture of bTB infection, but also provided an opportunity to explore variation in extra-group paternity in the population. Analysing the annual mean paternity distance (PD), generated simultaneously with the pedigree, I found among-cohort variation was present suggesting that the tendency of adults to engage in short term breeding excursions is not completely stable through time. However, this temporal variation was not explained by changes in population density or sex ratio, factors that are widely hypothesised to drive movement for breeding purposes.

In contrast, modelling cub-level variation in PD and extra-group paternity (EGP) was more successful in identifying source of variation, with strong effects of social group, and parental identity detected. With respect to the former, I found a strong negative correlation between maternal and paternal social group effects on cub paternity distance, indicating that maternal groups that predispose to high paternity distance are the same as the paternal groups predisposed to low paternity distance. This pattern is not readily explained as a simple consequence of, for example, (relative) distances between groups or edge-effects, but instead suggests source-sink dynamics, with some social groups both retaining and 'drawing in' male genes. With respect to the latter, both paternal and maternal identity effects mean that some individuals are consistently more likely to produce cubs with extra-group partners. The behavioural mechanisms underpinning this remain to be resolved, which represents a formidable empirical challenge given the shy nature of badgers

makes observational studies difficult. For instance, relatively little is known about female excursions. Do mothers that produce more extra-group sired offspring do so because of a greater tendency to leave the group territory to find partners, or because of a greater tendency to accept intruding males from outside? At present we do not know.

However, what the analysis does demonstrate, is variation in extra-group mating at multiple levels – among years, social groups and individuals. The latter in particular is a phenomenon seldom documented and emphasises the importance of considering the individual in driving eco-evolutionary dynamics. It also suggests among-individual differences in traits related to movement (dispersal, exploration) that may well have implications for conspecific encounter rates and thereby disease transmission (Weber et al., 2013). Crucially, in the context of the wider thesis, the resolved pedigree structure suggests moderate levels of EGP (an estimated 37% across the study period) that serve to break up (social) environment – genetic correlation, providing a data structure in which we could reasonably hope to statistically partition genetic from common environment effects on immune function traits.

In **Chapter 3**, I sought to examine the relative importance of genetic and social environmental (including maternal) sources of variation in bTB infection status, using a previously described progressive score assigned at each capture event based on diagnostic tests. Quantitative genetic analysis of this metric of disease progression, and of a simplified (binary) proxy of lifetime infection risk yielded evidence of significant additive genetic variance, providing the first estimates for heritability of bTB infection in a wild host. However, despite nominal significance, levels of additive genetic variance are low relative to modelled sources of environmental variation (social group and, for cubs,

maternal). Thus, while I showed that genes do contribute to among-individual variation in this population, rapid evolution of host defence strategies from standing genetic variance under (presumed) parasite-mediated selection seems unlikely. Instead, the results lend further support to the view that social and early-life environments are important drivers of dynamics of bTB infection in badgers. However, due to the scarcity of heritability estimates for disease traits in wild vertebrate populations there remains little consensus on the importance of standing genetic variation.

Variation in bTB infection status as measured in Chapter 3 could result from numerous pathways involved in immune response. With some assumptions (e.g. that individuals are equally exposed to *Mycobacterium bovis*), variation in infection risk can be interpreted as reflecting among-individual and/or genotype differences in 'resistance' – the ability of a host to prevent infection by limiting parasite growth or entry. However, the fitness consequences of infection will also depend on host tolerance – the ability to limit damage caused by the parasite. Though not mutually exclusive, these two host conceptually distinct defence strategies are predicted to have very different consequences for parasite fitness, and as a consequence, for co-evolutionary dynamics. By limiting parasite growth, host resistance will negatively impact parasite fitness while tolerance can, in fact, promote parasite fitness by increasing the period over which transmission to a new host can occur (*Miller et al. 2006; Boots et al. 2009)*. Given the divergent implications of resistance and tolerance on host ecological and evolutionary dynamics, determining whether genetic and environmental determinants of infection status operate through resistance, tolerance, or both, should advance our understanding of bTB dynamics.

There is a large literature on the epidemiological, ecological and evolutionary implications of variation in resistance, even if estimates of genetic variance remain scarce for wild hosts. By comparison, variation in tolerance as a distinct aspect of host defence has been less well characterised. I attempted to bridge this gap in **Chapter 4**, by testing for (genetic) variation in tolerance of *M. bovis* infection. To do this I adopted a 'reaction norm' approach in which individual tolerance is conceptualised as the slope of an individual's relationship between weight and infection status. Weight was chosen in part because we know bTB causes weight loss but also for pragmatic reasons as there are no records of bTB specific symptoms for the population. The analyses revealed significant among-individual variation in tolerance to bTB and also provides the first explicit demonstrations of heritable variation underpinning this in any wild host species. In fact, the finding that among-individual variation in tolerance is primarily attributable to additive genetic effects is counter to *a priori* expectations. Specifically, I had predicted that positive selection would rapidly erode genetic variance – such that variation in tolerance would, if present, be principally attributable to environmental factors. This result leads to the question of what maintains genetic variance, assuming of course that it really is under strong positive selection in the host population. Others have suggested that the mode through which tolerance operates (e.g., by mitigating costs on survival versus fecundity), and/or trade-offs with resistance (or life-history traits) could allow genetic variation to persist in populations. However, whether such mechanisms are at play in the Woodchester badger population remains to be determined.

Finally, **Chapter 5** seeks to characterise genetic variance in traits that can be tied more mechanistically to distinct arms of the immune response. At

the same time, in this chapter I was also looking to overcome a central limitation to many studies of evolutionary ecology, the focus on single trait. In fact, natural selection rarely operates on single traits in isolation, but rather on multivariate phenotypes comprised of suites (potentially) correlated traits. Importantly, multivariate selection responses are shaped (and sometimes constrained) by the extent to which correlations structure is determined by genetic factors (Morrissey et al. 2010). While this could certainly be the case for host immune responses which are generated through multiple distinct pathways, few studies to date have attempted to characterise the genetic associations among aspects of immune function and/or infection status in wild systems.

In this chapter I estimated the genetic basis of (co)variation in, and among, four diagnostic test responses, two of which are used to assign the progressive bTB status used in previous chapters. The four diagnostic traits represent different aspects of host immune function and infection status: general immunocompetence (PWM), bTB-specific humoral (BROCK) and cell-mediated responses (B-A), and parasite burden (CULTURE). Of these detected a small, but significant, heritable component of variation in all but the cell-mediated response measured with the interferon gamma assay (B-A). I also found significant phenotypic correlation structure (within a sampling event) that was partially attributable to genetic effects. Correlations were positive among bTB specific traits, and both BROCK and CULTURE were negatively correlated with PWM. Thus, the signs of the correlations (both phenotypic and genetic) are broadly consistent with the premise that assays of general immune function (here PWM) positively predict an individual's ability to fight a specific infection (here bTB). Thus, traits are not completely free to evolve independently under selection, but, I found no support for trade-offs among them.

Eco-evolutionary implications

In this thesis I find evidence for genetic variance contributing to among-individual heterogeneity in both resistance and tolerance of Woodchester badgers to infection by *Mycobacterium bovis*. Broadly speaking, genetic heterogeneity in resistance – putatively captured here by multisite and lifetime bTB status scores - is consistent with theoretical predictions. Commonly models assume negative frequency-dependent selection arises because resistant traits are costly. As resistance spreads in a host population parasite abundance will be reduced (Boots et al. 2009). This in turn weakens selection because the benefits of resistance decline relative to the costs. Less easy to explain is why genetic variance for tolerance should persist in a host population; the simplest prediction being that alleles favourable for tolerance should rapidly go to fixation while deleterious alleles are purged (Best et al. 2008). This is because of a positive feedback loop in the strength of selection; increasing tolerance in a host population should increase parasite fitness (and so abundance), further strengthening selection for tolerance. In this context, the result that detectable heterogeneity in tolerance is primarily due to genetic rather than non-genetic effects is surprising, and potentially suggestive of some unknown costs to tolerance. These may occur if, for instance, there are trade-offs between tolerance and other host defences or components of life-history (Best et al. 2008).

So, what can be said about the likely eco-evolutionary dynamics of TB-related traits in this population? Based on the presence of heritable variation there is some potential for resistance and tolerance - and the immune traits underpinning them - to evolve. However, this potential is limited in as much as the amount of genetic variation present is low (at least relative to non-genetic

variation arising from social, spatial and temporal effects). Notwithstanding the above argument for frequency dependent selection on resistance, low levels of standing genetic variance are consistent with parasites asserting consistent, strong directional selection on host defences in populations where they are endemic. Interestingly, however there are some well documented instances of resistance and/or tolerance evolving rapidly in wild vertebrates during epizootic events where parasites invade previously naïve host population (e.g. *Mycoplasma gallisepticum* in house finches; Bonneaud et al. 2018). One possibility then is that host defences can evolve rapidly from standing genetic variation in response to novel selection imposed by an invading parasite, but over time, further adaptation to endemic infections becomes increasingly dependent on *de novo* mutations. If this is the case then, substantial genetic variance for host defences may be a relatively transient phenomenon in wild populations.

While I have focussed here on genetic (co)variation a more complete understanding of the evolutionary dynamics of host defence strategies clearly requires quantitative estimates of selection. We know that bTB infection causes disease that ultimately reduced survival and reproduction in badgers, so it is probably reasonable to assume positive selection on host defences. But, how strong is this selection? Does it vary among groups or through time? And how much variance in relative fitness (which sets the opportunity for selection) is caused by infection dynamics relative to, for instance, variation arising from sexual selection or stochastic mortality events (e.g. road kills). Answering these questions requires a proxy of individual fitness such as lifetime reproductive success (LRS), usually defined as the number of offspring produced by an individual. While LRS inferred from pedigree structures is widely used for

selection analyses in wild vertebrates (Pemberton, 2008), pedigree data for Woodchester is sufficiently incomplete (35% cubs assigned parents) that LRS estimates are likely to be rather inaccurate and imprecise. Likewise, death dates are unknown for most individuals in the population and emigration from the study area certainly occurs, making estimation through survival or longevity alone similarly difficult.

Practically, overcoming these limitations will likely require future increases in genotyping and/or capture effort. While challenging logistically and financially, robust estimates of individual fitness would allow us to build on the current results by: (i) generating selection gradients on (multivariate) immune response allowing us to generate evolutionary predictions (e.g. using the multivariate breeders equation); (ii) formally test for the widely hypothesised fitness trade-offs between tolerance and resistance (Best et al. 2008), and (iii) look for evidence that investment in immune function really is costly (e.g. in terms of future reproduction or survival).

Potential impacts on management of bTB in badgers and other hosts

As well as influencing eco-evolutionary dynamics, heritable variation in host defence strategies may have potential implications for the management of bTB. In cattle, genetic variation for bTB is found among- and within-breeds but appears to be present at a low level, just as we find in the Woodchester badgers. Thus, while breeding for resistance has been generally suggested as a viable option for managing some livestock diseases (Stear et al. 2001; Yáñez et al. 2014), it may have limited application in the case of reducing bTB in cattle. More generally, artificial selection for resistance can sometimes be complicated

by genetic correlations that impose trade-offs with production traits; for instance, in dairy cattle increased resistance to mastitis correlated with reductions in milk yield (Heringstad et al. 2000). Interestingly, if genetic variance in tolerance is also widespread in livestock, it may provide a useful alternative for disease management in livestock, as by definition it limits damage to 'fitness' which should mean improvements to both welfare and production traits. However, in the particular case of bTB in cattle this is not a viable option given that the disease is notifiable and infected animals are legally required to be slaughtered. Given the risk to human health risk for those working with cattle, a move towards tolerance of bTB infection in cattle - in any sense of the word - seems unlikely.

From the perspective of managing disease in cattle, if genetic variance facilitates evolution of resistance in badgers this would be advantageous. Lower rate of infection in the wildlife host population should, all else being equal, mean reduced transmission to livestock. Conversely, genetic variance in tolerance could detrimental consequences. If tolerance can spread under selection, bTB incidence in badgers is expected to rise and thus increased transmission to cattle is likely. The heterogeneity detected also raises interesting questions regarding the general role of more tolerant individuals and genotypes in the epidemiology and pathogenesis of bTB in badgers and other hosts. Particularly, the potential links between tolerance and so-called 'superspreaders', a term describing individuals that contribute disproportionately to the transmission and spread of disease through a population (Lloyd- Smith et al., 2005). In Woodchester Park, superspreading appears to operate both through heightened bacterial excretion and behavioural differences affecting social contacts among individuals (McDonald et al, 2018). The links between these

three mechanisms present an interesting avenue of investigation. Tolerance mechanisms could, at least in part, also explain the preponderance of latent infection observed in mycobacterial infections.

However, as noted above, attempting to predict eco-evolutionary dynamics based solely on heritable variation and an untested assumption of strong directional selection is problematic. Furthermore, while genetic variation is vital for evolutionary responses, it is clearly not the most important source of heterogeneity in bTB infection or progression for Woodchester Park badgers. Ultimately, this provides some grounds for cautious optimism in the sense that managing environmental effects, no matter how challenging, is surely more tractable that attempting to manage the genetic make-up of a wild population. For instance, social effects appear to play a significant role in bTB infection, and while intervention strategies to date are (arguably) counterproductive in this respect (e.g. culling causing social disruption that increase movement and thus bTB transmission; Carter et al., 2007) it is possible that other manipulations targeting social structure could be beneficial. However, probably the most promising short-term avenue for control in badgers is vaccination (Chambers et al., 2011).

Finally, a major challenge to understanding and managing bTB – both in wildlife and livestock – continues to be the relatively low sensitivity of available diagnostic tests. In my thesis, this adds some nuance to the interpretation of phenotypic correlations reported among immune response traits in Chapter 5. For instance, taken at face value the relatively low phenotypic correlation between measures of humoral and cell-mediated immune responses within capture event suggests these two components of the immune response are not strongly coupled in their expression (at least within a time point). An alternative

possibility is that in fact they are, but that the correlation is low because of one or both diagnostic tests is subject to high levels of measurement error (and errors are uncorrelated across tests). In this respect it is notable, for instance that between CULTURE and BROCK the genetic correlation (which should be robust to such effects providing test errors are not themselves linked to genotype) is double the phenotypic correlation. Thus, while not directly connected to the genetic questions at the core of this thesis, an important task for management strategies going forward should be to better determine the reliability and sensitivity of the tests being used and consider how best to use them for reliable inference of individual status. Increasing the breadth of immune tests even further could add biological understanding but may – or may not - improve accurate determination of a specific individual's infection status. An alternative strategy may be to increasing sampling effort with existing tests (or even a subset of them) to obtain more repeat measures on individuals (over short time periods) which might be averaged to improve accuracy.

Future directions and concluding remarks

The natural world is multivariate, and host immune function is a prime example of the complexity underlying many phenotypic traits. Multivariate approaches provide the necessary tools for examining associations between immune traits and fitness, by extension enabling the strength and direction of selection on traits, and thus their full evolutionary potential, to be assessed. Multivariate quantitative genetics is also well-suited suited to answer outstanding questions in ecoimmunology. For instance, it is increasingly recognised that coinfection is

the norm rather than the exception in natural environments and can lead to complex within-host interactions that augment infection outcomes (Bordes et al. 2011), yet relatively few studies of wild host populations consider multiple infections (but see e.g. Clerc et al.). Multivariate approach would allow the phenotypic and genetic associations among different parasites, host responses and fitness to be characterised. Coinfection with gastrointestinal helminths, commonly found in badgers (Sin et al. 2014), may be of particular relevance to understanding the dynamics of bTB infection as they have been shown can lead to suppression of key anti-TB immune defences (Ezenwa et al., 2010).

While I highlight the need for more (multivariate) studies of immune traits in the wild, the availability of longitudinal data and pedigree information remains a major limitation for many populations. Going forward, however, advances in genomics and declining costs of sequencing are likely to provide access to methods and study systems that were once unfeasible for wild populations. Crucially, this will enable quantitative genetics studies in large populations on individuals that are not closely related or whose relationships are unknown. Advances in genomics (and other 'omics) technologies will also greatly facilitate exploration of immune markers and their underlying genetic in non-model species and enable better detection of parasites in the wild. Combined with the detailed mechanistic, molecular and physiological understanding of host and parasite biology provided by laboratory studies, these advances promise to advance our understanding greatly. Ultimately, however, more multivariate approaches will be necessary to disentangle this complexity and gain more realistic evolutionary insight of host-parasite interactions.

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Supplementary Tables and Figures for Chapter 2

Table S2.1. Per locus mean allelic dropout (e1) and false allele or stochastic sampling error rates (e2), estimated using PEDANT 1.0 (Johnson & Haydon 2007) using 209 individuals for which repeat genotypes were available. Loci for which estimated error was zero, and those for which estimation was not possible (Mel15 & 106) due to lack of repeat genotypes, the default rate of 0.005 was used (Hadfield 2012).

Locus	E1	95% CI	E2	95% CI2
Mel1	0.03	0.005-0.08	0.006	0.0006-0.02
Mel10	0.03	0.005-0.1	0	0-0.02
Mel12	0.1	0.07-0.2	0.07	0.05-0.1
Mel14	0.02	0.006-0.04	0.03	0.01-0.04
Mel15	0.005	-	0.005	-
Mel101	0.1	0.03-0.2	0.02	0.002-0.06
Mel102	0.02	0.006-0.05	0	0-0.009
Mel103	0.02	0.0009-0.07	0.03	0.006-0.06
Mel104	0.03	0.008-0.08	0.01	0.001-0.04
Mel105	0.03	0.01-0.05	0.05	0.03-0.07
Mel106	0.005	-	0.005	-
Mel107	0.01	0.002-0.05	0	0-0.007
Mel108	0.01	0.003-0.04	0	0-0.007
Mel109	0.07	0.04-0.1	0.08	0.05-0.1
Mel110	0.02	0.003-0.05	0.004	0.00008-0.02
Mel111	0.08	0.04-0.1	0.04	0.01-0.07
Mel112	0.006	0-0.03	0.003	0.00006-0.02
Mel113	0.06	0.02-0.1	0.02	0.005-0.06
Mel114	0.05	0.004-0.2	0	0-0.06
Mel115	0.02	0.004-0.04	0.006	0.0005-0.02
Mel116	0.1	0.05-0.3	0.2	0.002-0.07
Mel117	0.009	0.001-0.03	0	0-0.01

Table S2.2. Best linear unbiased predictor (BLUP) values (represent the predicted deviation of each (maternal and paternal) social group from the mean paternity distance) and standard errors for each maternal (M) and paternal (P) social group extracted from the PD_i model. Values represent the predicted deviation of each social group from the mean. Groups with missing data had no parentage assignments, therefore BLUPs were not estimated. Results are on the log-transformed scale with untransformed PD in meters.

Social group	$BLUP_P$ (SE)	$BLUP_M$ (SE)
Arthurs	0.31 (0.29)	-0.27 (0.25)
Atcombe West	-0.09 (0.75)	0.07 (0.62)
Atcombe Corner	1.12 (0.53)	-0.92 (0.43)
Bamboo	0.08 (0.56)	-0.07 (0.46)
Beech	0.26 (0.26)	-0.17 (0.22)
Bungalow	-0.63 (0.55)	0.52 (0.45)
Cedar	0.35 (0.31)	-0.31 (0.26)
Cole Park	-0.88 (0.63)	0.72 (0.52)
Colliers Wood	0.03 (0.3)	0.01 (0.28)
Convent	-	-
Dark Wood	-	-0.38(0.45)
Dingle	-0.73 (0.53)	0.59 (0.43)
Field Farm	0.55 (0.50)	-0.47 (0.42)
Gully	-	-
Hedge	-0.36 (0.35)	0.30 (0.29)
Hogarths	-	-
Holly Wood	0.41 (0.41)	0.41 (0.41)
Honeywell	0.65 (0.41)	-0.56 (0.34)
Inchbrook	0.12 (0.42)	-0.14 (0.35)
Jacks Mirey	1.16 (0.32)	-0.95 (0.27)
Kennel	-0.003 (0.30)	0.036 (0.25)
Larch	0.14 (0.29)	-0.10 (0.25)
Listers	-0.73 (0.59)	0.59 (0.48)
Nettle	0.64 (0.47)	-0.52 (0.39)
Old Oak	0.38 (0.37)	-0.32 (0.31)
Park Mill	0.11 (0.39)	-0.09 (0.33)
Peglars	0.02 (0.37)	-0.02 (0.31)
Septic Tank	0.56 (0.28)	-0.38 (0.24)
Thistle Wood Bank	-	-
Top Sett	-1.97 (0.32)	1.60 (0.26)
West	0.17 (0.34)	-0.09 (0.28)
Windsor Edge	0.76 (0.33)	-0.60 (0.28)
Wood Farm	-0.42 (0.33)	0.34 (0.27)
Wych Elm	-0.25 (0.32)	0.19(0.26)
Yew	-0.55 (0.29)	0.42 (0.25)

Table S2.3. Reanalysis of PDi and EGPi using standardised body mass index (SMI) in place of body mass. Response variables were standardised into standard deviation units (SDU) prior to analysis. M and P denote maternal and paternal individuals, while MSG and PSG denote the corresponding maternal and paternal social groups.

	Log(PD_i)				**EGP_i**			
	Estimate (SE)	**DF**	**F**	**P**	**Estimate (SE)**	**DF**	**F**	**P**
Intercept	0.73 (0.16)	1, 284.6	20.47	**<0.001**	0.74 (0.16)	1, 297.7	21.01	**<0.001**
Age_M	-0.009 (0.01)	1, 539.9	0.55	0.46	0.01 (0.01)	1, 543.3	0.64	0.42
SMI_M†	0.009 (0.01)	1, 333.3	0.78	0.38	0.009 (0.01)	1, 336.9	0.69	0.41
$Group\_size_{MSG}$	0.007 (0.02)	1, 461.4	0.19	0.66	0.008 (0.02)	1, 446.9	0.20	0.66
Sex_ratio_{MSG} ‡	-0.71 (0.22)	1, 533.4	10.28	**<0.001**	-0.79 (0.22)	1, 526.5	12.74	**<0.001**
Age_P	0.03 (0.02)	1, 506.6	2.24	0.14	0.03 (0.02)	1, 507.9	2.54	0.11
SMI_P†	-0.02 (0.01)	1, 247.7	1.15	0.29	-0.02 (0.01)	1, 249.9	1.11	0.29
$Group.Size_{PSG}$	-0.02 (0.02)	1, 538.2	0.69	0.41	-0.02 (0.02)	1, 532.4	0.68	**0.04**
Sex_ratio_{PSG} ‡	0.42 (0.24)	1, 539.6	3.06	0.08	0.50 (0.24)	1, 537.1	4.29	**<0.001**

†mean body mass for parental individuals with multiple weight measurements within cub's birth year

‡calculated as number of males divided by group size where group size is males plus females

Full models fitted for each response were $y \sim \mu + Age_M + SMI_M + Group\_size_{MSG} + Sex\_ratio_{MSG} + Age_P + SMI_P + Group\_size_{PSG} + Sex\_ratio_{PSG} + M + P + MSG + PSG + Year$ where italic font denotes random effects and y is either $\log(PD_i)$ or EGP_i

Table S2.4. Estimated (co)variance components (standard error) associated with random effects in mixed models of EGP_i and log-transformed PD_i, reanalysed using using standardised body mass index (SMI) in place of body mass. Statistical inference of random effects is by likelihood ratio test results (see main text for details). M and P denote maternal and paternal individuals, while MSG and PSG denote the corresponding maternal and paternal social groups.

	log(PD_i)				EGP_i			
	Variance (SE)	**df**	**χ^2_1**	**P**	**Variance (SE)**	**Df**	**χ^2_1**	**P**
V_{year}	0.06 (0.02)	1	3.76	**0.05**	0.02 (0.01)	1	3.20	0.07
V_M†	0.25 (0.05)	1	40.74	**<0.001**	0.26 (0.05)	1	40.91	**<0.001**
V_P†	0.31 (0.06)	1	35.22	**<0.001**	0.31 (0.06)	1	34.71	**<0.001**
V_{MSG}‡	0.41 (0.15)	1	20.64	**<0.001**	0.35 (0.13)	1	19.92	**<0.001**
V_{PSG}‡	0.60 (0.21)	1	26.57	**<0.001**	0.54 (0.19)	1	27.5	**<0.001**
$COV_{MSG,PSG}$	*-0.49 (0.17)*	*1*	*39.84*	**<0.001**	*-0.44 (0.15)*	*1*	*37.05*	**<0.001**
V_R	0.32 (0.04)	-	-	-	0.32 (0.03)	-	-	-

† not significantly different from each other (logLRT, PD_i: $\chi^2_1 = 0.22$, p=0. 0.64; EGP_i: $\chi^2_1 = 0.30$, p=0.59)

‡ not significantly different from each other (logLRT, PD_i: $\chi^2_1 = 3.73$, p= 0.05; EGP_i: $\chi^2_1 = 3.69$, p=0.05)

Table S2.5. Repeatabilities (R) of variance components from reanalyses of EGP_i and log-transformed PD_i, reanalysed using standardised body mass index (SMI) in place of body mass. R calculated as variance component/sum of all variance components. Values for $COR_{MSG,PSG}$ are correlation coefficients. M and P denote maternal and paternal individuals, while MSG and PSG denote the corresponding maternal and paternal social groups.

	log(PD_i)	EGP_i
	R (SE)	**R (SE)**
R_{year}	0.01(0.008)	0.009 (0.008)
R_M[a]	0.13 (0.04)	0.14 (0.04)
R_P[a]	0.16 (0.04)	0.17 (0.04)
R_{MSG}[b]	0.22 (0.05)	0.20 (0.05)
R_{PSG}[b]	0.31 (0.06)	0.30 (0.06)
$COR_{MSG,PSG}$	*-0.99 (0.03)*	*-0.99 (0.03)*
R_R	0.17 (0.04)	0.18 (0.04)

Table S2.6. Estimated fixed effect coefficients (standard error) and Wald F-tests from mixed models from reanalysis on log-PD_i and EGP_i using the 95% confidence pedigree, where only those parent assignments that met a 95% confidence threshold were included. Response variables were standardised into standard deviation units (SDU) prior to analysis. M and P denote maternal and paternal individuals, while MSG and PSG denote the corresponding maternal and paternal social groups.

	Log(PD_i)				**EGP_i**			
	Estimate (SE)	**DF**	**F**	**P**	**Estimate (SE)**	**DF**	**F**	**P**
Intercept	0.62 (0.23)	1, 94.3	7.22	**<0.01**	0.64 (0.23)	1, 92.7	7.51	**<0.01**
Age_M	-0.01 (0.008)	1, 115.9	0.01	0.91	0.002 (0.007)	1, 115.5	0.09	0.76
Body $mass_M$†	-0.04 (0.026)	1, 219.1	2.01	0.16	-0.04 (0.03)	1, 220.0	2.09	0.15
$Group\_size_{MSG}$	0.12 (0.01)	1, 96.0	127.59	**<0.001**	0.13 (0.01)	1, 88.3	185.62	**<0.001**
Sex_ratio_{MSG} ‡	-3.29 (0.14)	1, 142.5	535.52	**<0.001**	-3.65 (0.13)	1, 135.1	805.31	**<0.001**
Age_P	0.03 (0.008)	1, 112.0	19.16	**<0.001**	0.03 (0.007)	1, 112.9	18.67	**<0.001**
Body $mass_P$†	-0.02 (0.03)	1, 156.8	0.71	0.40	-0.02 (0.03)	1, 160.7	0.66	0.42
$Group.Size_{PSG}$	-0.08 (0.01)	1, 97.0	28.90	**<0.001**	-0.09 (0.01)	1, 89.4	52.63	**<0.001**
Sex_ratio_{PSG} ‡	2.84 (0.17)	1, 160.2	287.70	**<0.001**	3.22 (0.15)	1, 149.8	446.25	**<0.001**

†mean body mass for parental individuals with multiple weight measurements within cub's birth year

‡calculated as number of males divided by group size where group size is males plus females

Full models fitted for each response were $y \sim \mu + Age_M + Body\_Mass_M + Group\_size_{MSG} + Sex\_ratio_{MSG} + Age_P + Body\_Mass_P + Group\_size_{PSG} + Sex\_ratio_{PSG} + M + P + MSG + PSG + Year$ where italic font denotes random effects and y is either log(PD_i) or EGP_i

Table S2.7. Estimated (co)variance components (standard error) associated with random effects in mixed models of EGP_i and log-transformed PD_i, reanalysed using 95% confidence pedigree. Statistical inference of random effects is by likelihood ratio test results (see main text for details). M and P denote maternal and paternal individuals, while MSG and PSG denote the corresponding maternal and paternal social groups.

	log(PD_i)				**EGP_i**			
	Variance (SE)	**df**	**χ^2_1**	**P**	**Variance (SE)**	**Df**	**χ^2_1**	**P**
V_{year}	0.04 (0.02)	1	45.88	**<0.001**	0.04 (0.02)	1	60.89	**<0.001**
V_M†	1.86 (0.23)	1	115.12	**<0.001**	1.94 (0.24)	1	131.15	**<0.001**
V_P†	1.80 (0.25)	1	98.34	**<0.001**	1.93 (0.26)	1	114.94	**<0.001**
V_{MSG}‡	2.21 (0.71)	1	41.93	**<0.001**	2.13 (0.68)	1	55.91	**<0.001**
V_{PSG}‡	2.22 (0.71)	1	197.85	**<0.001**	2.16 (0.69)	1	80.31	**<0.001**
$COV_{MSG,PSG}$	*-2.04 (0.66)*	*1*	*37.27*	**<0.001**	*-1.96 (0.64)*	*1*	*35.58*	**<0.001**
V_R	0.005 (0.0008)	-	-	-	0.004 (0.0006)	-	-	-

†not significantly different from each other (logLRT, PD_i: $\chi^2_1 = 0.038$, p=0.85; EGP_i: $\chi^2_1 = 0.002$, p=0.96)

‡ not significantly different from each other (logLRT, PD_i: $\chi^2_1 = 0$, p= 1; EGP_i: $\chi^2_1 = 0.006$, p=0.94)

Table S2.8. Repeatabilities (R) of variance components from reanalyses of EGP_i and log-transformed PD_i, reanalysed using 95% confidence pedigree. R calculated as variance component/sum of all variance components. Values for $COV_{MSG,PSG}$ are correlation coefficients. M and P denote maternal and paternal individuals, while MSG and PSG denote the corresponding maternal and paternal social groups.

	log(PD_i)	**EGP_i**
	R (SE)	**R (SE)**
R_{year}	0.005(0.002)	0.004 (0.002)
R_M[a]	0.23 (0.04)	0.24 (0.04)
R_P[a]	0.22 (0.04)	0.24 (0.04)
R_{MSG}[b]	0.27 (0.05)	0.26 (0.05)
R_{PSG}[b]	0.27 (0.05)	0.26 (0.05)
$COR_{MSG,PSG}$	*-0.92 (0.05)*	*-0.92 (0.05)*
R_R	0.0007 (0.0002)	0.0005 (0.05)

Table S2.9. Posterior mean (credible intervals) estimates of unsampled males and females per cohort estimated in MasterBayes simultaneously with parentage and paternity distance. Values for unsampled males represent population-level estimates, while number of unsampled females was estimated per social group.

Year	Unsampled males	Unsampled females
1986	11.827 (0.407-43.445)	1.2651 (0.316-3.474)
1987	35.622 (6.093-89.752)	0.819 (0.1945-1.958)
1988	26.1864 (0.805-96.173)	0.975 (0.122-2.801)
1989	6.401 (0.250-21.541)	0.548 (0.044-1.626)
1990	10.764 (0.676-32.665)	0.803 (0.054-2.465)
1991	16.404 (1.693-44.149)	2.314 (0.960-4.439)
1992	37.147 (6.335-90.084)	0.380 (0.011-1.269)
1993	20.403 (0.6008-68.087)	0.843 (0.123-2.136)
1994	12.696 (0.680-39.903)	0.359 (0.009-1.341)
1995	40.303 (5.744-102.097)	6.283 (2.062-12.629)
1996	239.383 (32.810-812.610)	2.370 (0.091-7.561)
1997	16.930 (2.090-43.140)	0.980 (0.100-2.640)
1998	47.200 (17.15-89.200	0.540 (0.110-1.250)
1999	35.000 (10.390-71.470)	0.650 (0.160-1.400)
2000	35.081 (12.290-68.310)	0.799 (0.239-1.76)
2001	28.868 (12.280-50.640)	0.604 (0.226-1.174)
2002	55.9474 (12.230-132.450)	0.428 (0.010-1.610)
2003	24.937 (5.672-55.067)	0.517 (0.066-1.312)
2004	20.150 (2.961-49.365)	0.705 (0.142-1.695)
2005	10.192 (1.035-26.744)	0.919 (0.313-1.850)
2006	5.129 (0.208-17.236)	2.653 (1.141-4.951)
2007	6.859 (0.389-20.929)	1.363 (0.403 -2.915)
2008	18.010 (4.195-39.820)	1.896 (0.661-3.836)
2009	16.416 (5.726-31.224)	0.702 (0.210-1.462)
2010	18.812 (0.970-57.216)	2.353 (0.482-6.379)
2011	6.698 (0.168-23.674)	1.3234 (0.308-2.703)
2012	49.145 (13.680-105.520)	0.739 (0.153-1.795)
2013	50.206 (21.790-88.780)	0.614 (0.167-1.338)
2014	111.922 (49.660-217.92)	2.225 (1.000-4.137)

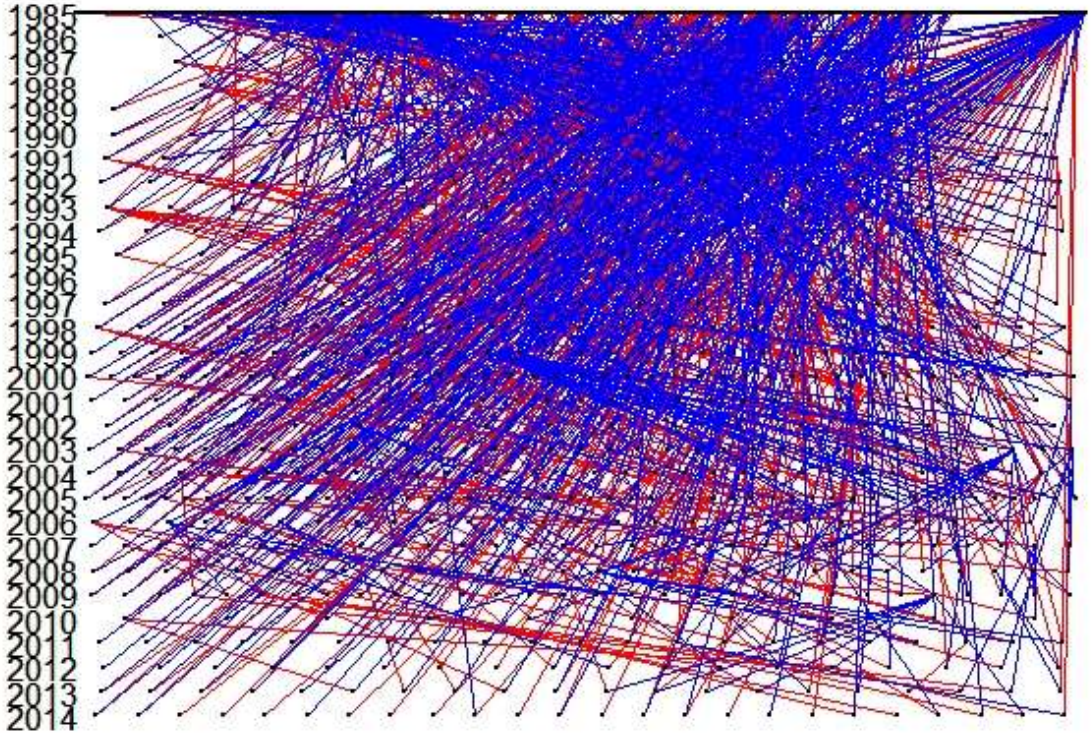


Figure S2.1. Inferred pedigree structure for 29 cohorts showing maternal assignments in red, paternal in blue and individuals as dots. Reconstructed pedigree has a maximum depth of six generations and contains 579 maternal-cub and 596 paternal-cub links, 186 full sibships, 452 maternal half-sibs, and 927 paternal half sibs.

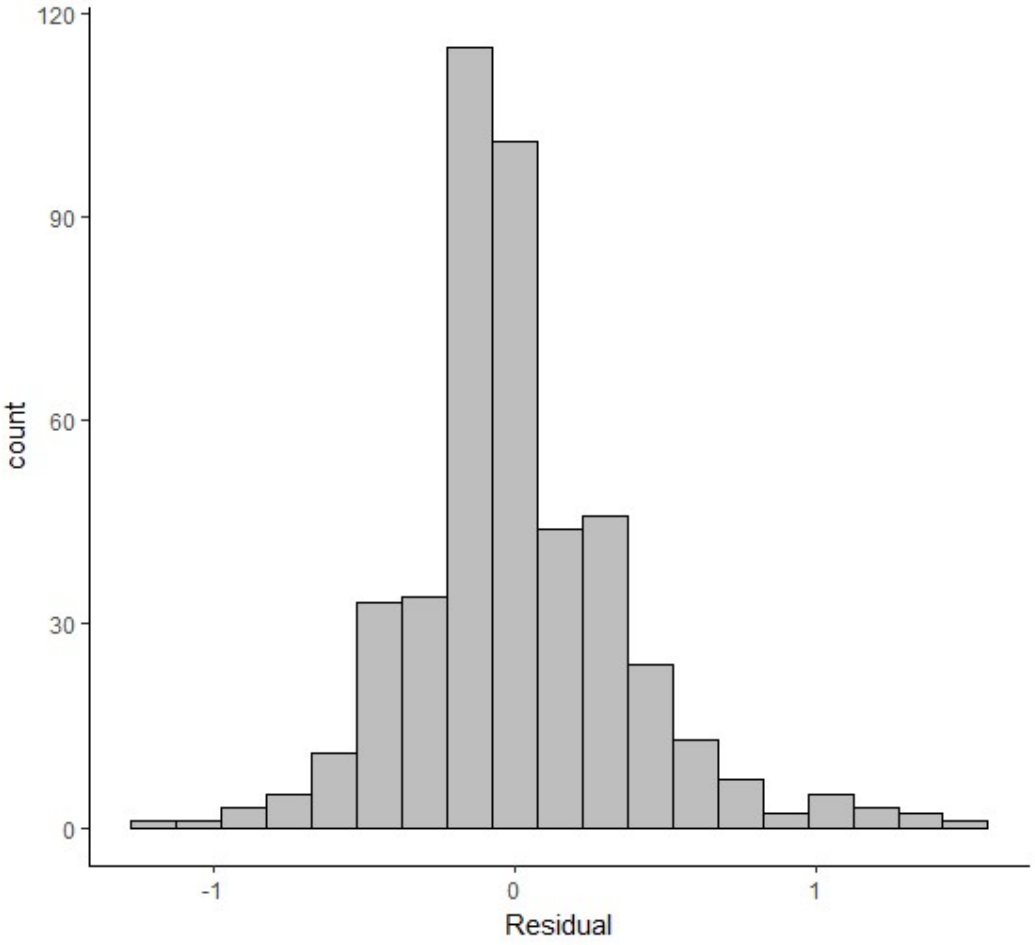


Figure S2.2. Histogram of model residuals for binary EGP_i (0/1) run in ASReml 3.0 with a Gaussian error structure.

Supplementary Tables and Figures for Chapter 3

Table S3.1. Estimates (standard error) and Wald F-test results of bTB_{multi} analysis run on subsets where test-negative/-positive (0/1) status is determined using only Brock ELISA (dataset spanning 1982 -2005) or BrockTB StatPak (dataset spanning 2011-2014) assay. DF = degrees of freedom; SE = standard error; Bold p-values are significant at α = 0.05.

	Brock ELISA				**BrockTB StatPak**			
	Estimate (SE)	**DF**	**F**	**p-value**	**Estimate (SE)**	**DF**	**F**	**p-value**
Intercept	0.585 (0.503)	1, 4556.4	1.43	0.232	0.957 (0.080)	1, 16.6	212.37	**<0.001**
Sex.male	-0.061 (0.501)	2, 2986.8	0.42	0.657	-0.074 (0.066)	1, 602.1	1.26	0.262
Sex.female	-	-	-	-	-	-	-	-
Scaled age	0.067 (0.007)	1, 10374.1	102.9	**<0.001**	0.013 (0.017)	1, 1714.0	60.49	0.4533
Scaled age^2	-0.009 (0.001)	1, 10382.9	16.23	**<0.001**	-0.022 (0.003)	1, 2324.0	69.58	**<0.001**
Scaled age^3	0.001 (0.0003)	1, 10319.0	0.3	**0.005**	0.003 (0.001)	1, 2518.5	16.27	**<0.001**

Table S3.1 continued…

Season.spring	0.025 (0.026)	3, 9833.2	8.9	**<0.001**	-	3, 2149.3	24.44	**<0.001**
Season.winter	-	-	-	-	0.166 (0.041)	-	-	**-**
Season.autumn	0.089 (0.021)	-	-	-	0.228 (0.035)	-	-	-
Season.summer	0.036 (0.019)	-	-	-	0.008 (0.034)	-	-	-

Model structure: bTB_{multi}~ sex + season + scaled_age + scaled_age^2 + scaled_age^3 a_i + *ID* + *maternal ID* + *group* + *year* + *group.year* where terms in italics represent random effects, a_i denotes additive genetic merit of individual *i*, and *group.year* the interaction between social group and observation year

Table S3.2. Log-likelihood ratio test results for variance components from bTB_{multi} model (standard error) run on subsets where test-negative/-positive (0/1) status is determined using only Brock ELISA test (dataset spanning 1982 -2005). Parameter estimates are from Model 2 in which maternal effect variance (V_M) is restricted to cubs only (denoted with subscript c). Consequently, ICC estimates are given separately for cubs and adults separately by dividing each variance component by the sum of the variance components (V_P) such that $V_{Pa} = V_A + V_G + V_Y + V_{GY} + V_{PE} + V_R$ while $V_{Pc} = V_A + V_{Mc} + V_G + V_Y + V_{GxY} + V_{PE} + V_R$.

	$\chi^2_{0,1}$	P	ICC (SE)	
			Adult	**Cub**
V_A	13.42	<0.001	0.110 (0.009)	0.093 (0.009)
V_{SG}	19.7	<0.001	0.019 (0.008)	0.017 (0.007)
V_Y	36.94	<0.001	0.031 (0.011)	0.026 (0.02)
V_{SGxY}	519.72	<0.001	0.111 (0.009)	0.09 (0.010)
V_{PE}	174.5	<0.001	0.308 (0.035)	0.262 (0.031)
V_{Mc}	43.84	<0.001	NA	0.147 (0.030)
V_R	-	-	0.420 (0.011)	0.358 (0.015)

Table S3.3. Log-likelihood ratio test results for variance components from bTB_{multi} model (standard error) run on subsets where test-negative/-positive (0/1) status is determined using only Stat Pak assay (dataset spanning 2011-2014). Parameter estimates are from Model 2 in which maternal effect variance (V_M) is restricted to cubs only (denoted with subscript c). Consequently, ICC estimates are given separately for cubs and adults separately by dividing each variance component by the sum of the variance components (V_P) such that $V_{Pa} = V_A + V_G + V_Y + V_{GY} + V_{PE} + V_R$ while $V_{Pc} = V_A + V_{Mc} + V_G + V_Y + V_{GxY} + V_{PE} + V_R$.

	$\chi^2_{0,1}$	P	ICC (SE)	
			Adult	**Cub**
V_A	0.322	0.285	0.031 (0.061)	0.026 (0.052)
V_{SG}	6.456	<0.001	0.017 (0.011)	0.015 (0.009)
V_Y	11.028	<0.001	0.022 (0.014)	0.019 (0.012)
V_{SGxY}	44.98	<0.01	0.043 (0.009)	0.036 (0.007)
V_{PE}	100.388	<0.001	0.578 (0.626)	0.493 (0.058)
V_{Mc}	31.31	<0.001	NA	0.147 (0.039)
V_R	-	-	0.309 (0.016)	0.264 (0.018)

Table S3.4. Effects of sex, season and scaled age on multisite bTB status and lifetime bTB risk. Coefficient estimates and Wald F-test results for the bTB_{multi} (scaled to standard deviation units) models where maternal effects are estimated for all ages (Model 1) and where they are restricted to cubs (Model 2). Spring is reference level for season; SE = standard error; DF = degrees of freedom.

	Model 1				Model 2			
	Estimate (SE)	**DF**	**F**	**p**	**Estimate (SE)**	**DF**	**F**	**p**
Intercept	0.74 (0.49)	1, 5228.4	2.16	0.14	0.72 (0.5)	1, 5493.2	2.00	0.16
Sex.male	-0.08 (0.49)	2, 3560.6	0.59	0.55	-0.06 (0.49)	2, 3613.8	0.51	0.6
Sex.female	-0.11 (0.49)	-	-	-	-0.09 (0.49)	-	-	-
Scaled age	0.05 (0.006)	1, 13805.3	76.83	<0.001	0.05 (0.006)	1, 13751.8	84.52	<0.001
Scaled age^2	-0.01 (0.001)	1, 13500.6	135.94	<0.001	-0.01 (0.001)	1, 12478.0	117.07	<0.001
Scaled age^3	0.001 (0.0002)	1, 12762.3	23.02	<0.001	0.001 (0.001)	1, 12712.9	18.01	<0.001
Season.winter	0.02 (0.02)	3, 12216.5	15.71	<0.001	0.02 (0.02)	3, 12051.9	16.08	<0.001
Season.autumn	0.09 (0.02)	-	-	-	0.09 (0.02)	-	-	-
Season.summer	0.01 (0.02)	-	-	-	0.02 (0.02)	-	-	-

Table S3.5. Effects of sex, season and scaled age on multisite bTB status and lifetime bTB risk. Mean estimates of the posterior density for fixed effects in the $bTB_{lifetime}$ categorical model. Statistical significance was assessed based on 95% credible intervals (in parentheses) and pMCMC.

	Mean	pMCMC
Intercept	-1.899 (-2.730 – -1.130)	**<0.001**
Sex.male	0.506 (0.173 – 0.849)	**<0.001**
Scaled.age	0.589 (0.447 – 0.743)	**<0.001**
Scaled.age^{2} †	-0.134 (-0.193 – -0.084)	**<0.001**
Scaled.age^{3} †	0.009 (0.003 – 0.014)	**<0.01**

†age at last capture

Table S3.6. Estimates and Wald F-test results of bTB_{multi} analyses run separately on adult (including yearlings) and cub data. Standard error = standard error; DF = degrees of freedom. In an attempt to examine changes in the importance of social and genetic effects with age, we ran the bTB_{multi} model on adult and cub data separately, in addition to the models described in the main text. Overall, these models give little evidence regarding age-related differences in maternal or additive genetic effects, and the qualitative conclusions regarding variation in bTB_{multi} are largely in line with the main results. The most notable differences between these analyses and the main results are the non-significant heritabilities and a difference in the amount of variance explained by the group-by-year term between adults and cubs. The former is likely a result of the loss of power due to reduced sample size (adults: 10123 obs. for 2044 individuals, cubs: 4723 obs. for 2407 individuals), while the latter likely reflects differences in data structure between cubs and adults. Namely, individual badgers only ever experience a single year as cubs, while adult badgers experience several. Therefore, year specific spatial effects (group by year) will likely be inflated for cubs when compared to adults.

	Adults[a]				**Cubs[b]**			
	Estimate (SE)	**DF**	**F**	**p**	**Estimate (SE)**	**DF**	**F**	**p**
Intercept	0.58 (0.06)	1, 45.3	99.5	<0.001	0.12 (0.5)	1, 2624.0	0.20	0.65
Sex.male	-	-	-	-	-0.03 (0.5)	2, 2347.1	0.17	0.85
Sex.female	-0.08 (0.06)	1, 1792.7	4.97	**0.03**	-0.01 (0.5)	-	-	-
Scaled age	0.06 (0.006)	1, 9081.8	102.9	**<0.001**	-	-	-	-
Scaled age^2	-0.01 (0.001)	1, 8855.6	16.23	**<0.001**	-	-	-	-
Scaled age^3	0.0002 (0.0003)	1, 8417.4	0.3	0.58	-	-	-	-

Table S3.6 continued…

Season.spring	0.04 (0.02)	3, 7980.9	8.9	**<0.001**	-	-	-	-
Season.winter	-	-	-	-	0.82 (0.04)	3, 3098.2	89.71	**<0.001**
Season.autumn	0.09 (0.02)	-	-	-	0.4 (0.04)	-	-	-
Season.summer	0.05 (0.02)	-	-	-	0.15 (0.04)	-	-	-

[a] Model structure: bTB_{multi}~ sex + season + scaled_age + scaled_age^2 + scaled_age^3 a_i + *ID* + *maternal ID* + *group* + *year* + *group.year* where terms in italics represent random effects, a_i denotes additive genetic merit of individual *i*, and *group.year* the interaction between social group and observation year

[b]Model structure: bTB_{multi}~ sex + season a_i + *ID* + *maternal ID* + *group* + *year* + *group.year* where terms in italics represent random effects, a_i denotes additive genetic merit of individual *i*, and *group.year* the interaction between social group and observation year

Table S3.7. Variance estimates (standard error), log-likelihood ratio test results for variance components and corresponding intraclass correlations (ICC) values from bTB_{multi} model run separately for adults and cubs.

	Adults				Cubs			
	Estimate	**$\chi^2_{0,1}$**	**p-value**	**ICC (SE)**	**Estimate**	**$\chi^2_{0,1}$**	**p-value**	**ICC (SE)**
V_A	0.04 (0.04)	0.94	0.33	0.05 (0.03)	0.01 (0.03)	0.1	0.75	0.01 (0.04)
V_G	0.02 (0.006)	20.24	**<0.001**	0.02 (0.007)	0.02 (0.01)	19.7	**<0.001**	0.03 (0.01)
V_Y	0.08 (0.02)	98.9	**<0.001**	0.08 (0.02)	0.06 (0.02)	50.44	**<0.001**	0.07 (0.02)
V_{GxY}	0.09 (0.008)	451.06	**<0.001**	0.09 (0.008)	0.14 (0.02)	86.62	**<0.001**	0.15 (0.02)
V_{PE}	0.44 (0.05)	103.62	**<0.001**	0.46 (0.05)	0.32 (0.04)	63.84	**<0.001**	0.36 (0.04)
V_M	0.1 (0.05)	5.96	**0.01**	0.10 (0.05)	0.08 (0.01)	5.04	**0.02**	0.09 (0.04)
V_R	0.27 (0.005)	-	-	0.29 (0.01)	0.34 (0.01)	-	-	0.38 (0.02)

Table S3.8. Threshold model for lifetime bTB risk ($bTB_{lifetime}$) using a χ^2 prior. Posterior mean estimates for fixed effects $bTB_{lifetime}$ threshold model. Statistical significance was assessed based on 95%? credible intervals (in parentheses) and pMCMC.

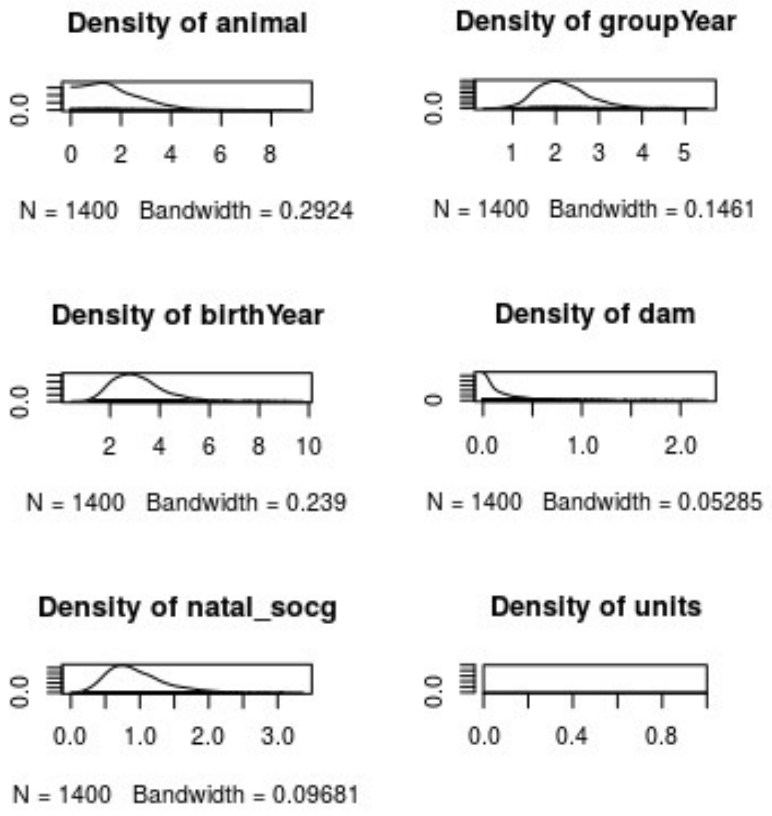
	Mean	pMCMC
Intercept	-1.084 (-1.633 – -0.546)	**<0.001**
Sex.male	0.280 (0.081 – 0.474)	**<0.01**
Scaled.age	0.332 (0.233 – 0.453)	**<0.001**
Scaled.age^{2} †	-0.075 (-0.111 – -0.043)	**<0.001**
Scaled.age^{3} †	0.005 (0.002 – 0.008)	**<0.001**

†age at last capture

Table S3.9. Posterior means for variance components from the threshold model of $bTB_{lifetime}$ and their corresponding intraclass correlation (ICC) estimates. Estimates relate to the observed scale and 95% credible intervals are shown in parentheses.

	Mean	ICC
$\mathbf{V_A}$	0.016 (0.001- 0.031)	0.072 (0.006 – 0.136)
$\mathbf{V_M}$	0.002 ($4.7x10^{-11}$- 0.006)	0.008 ($2.1x10^{-10}$ – 0.029)
$\mathbf{V_{NG}}$	0.005 (0.002 - 0.011)	0.025 (0.007 – 0.048)
$\mathbf{V_{BY}}$	0.019 (0.011 - 0.034)	0.09 (0.048 – 0.147)
$\mathbf{V_{NGxBY}}$	0.013 (0.008 - 0.019)	0.057 (0.035 – 0.087)

Figure S3.1. Posterior density plots of means for variance components from $bTB_{lifetime}$ model



Supplementary Tables and Figures for Chapter 5

Table S5.1. Fixed effects estimates (standard error) and Wald F-test results for univariate models of each diagnostic test. BROCK = response to bTB specific antibodies (0/1), CULTURE = bacterial culture of *M. bovis* (present/absent), B-A= IFNγ production in response to bovine tuberculin minus IFNγ production in response to avian tuberculin, PWM = IFNγ production in response to pokeweed mitogen. All models contained the same fixed (below) and random effects (maternal ID, individual ID, social group x year, additive genetic merit)

	BROCK			**CULTURE**			**B-A**			**PWM**		
	Estimate	**F**	**P**	**Estimate**	**F**	**P**	**Estimate**	**F**	**P**	**Estimate**	**F**	**P**
Intercept	0.532 (0.039)	467.52	<0.001	0.315 (0.570)	0.30	0.584	0.511 (0.063)	55.43	<0.001	1.261 (0.058)	567.35	<0.001
Sex (Female)		4.00	0.046	0.003 (0.570)	1.32	0.266		0.72	0.395		12.43	**<0.001**
Male	0.056 (0.277)			0.040 (0.570)			-0.058 (0.068)			-0.158 (0.045)		
Age	0.036 (0.004)	80.46	**<0.001**	0.035 (0.005)	54.88	**<0.001**	0.063 (0.014)	19.91	**<0.001**	-0.071 (0.012)	38.17	**<0.001**
Age^2	-0.006 (0.001)	91.79	**<0.001**	-0.006 (0.001)	57.72	**<0.001**	-0.0003 (0.002)	0.02	0.890	0.014 (0.002)	55.54	**<0.001**
Age^3	0.001 (0.0002)	31.49	**<0.001**	0.0004 (0.0002)	3.53	0.060	-0.0002 (0.0006)	0.16	0.688	-0.0007 (0.0005)	2.56	0.109
Season (Autumn)		26.87	**<0.001**		2.11	0.097		10.24	**<0.001**		158.02	**<0.001**
Spring	-0.002 (0.012)			0.008 (0.015)			-0.131 (0.027)			0.140 (0.023)		

Table S5.1 continued…

Summer	-0.051 (0.008)			-0.010 (0.010)			-0.089 (0.211)			-0.056 (0.018)		
Winter	-0.087 (0.012)			-0.031 (0.015)			-0.073 (0.027)			0.430 (0.235)		
Type (StatPak)		0.32	0.569		-	-						
Brock ELISA	0.021 (0.036)	-	-	-	-	-	-	-	-	-	-	-