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Pathogen burden, co-infection and major histocompatibility complex variability in the European badger (*Meles meles*)

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Abstract

Pathogen-mediated selection is thought to maintain the extreme diversity in the major histocompatibility complex (MHC) genes, operating through the heterozygote advantage, rare-allele advantage and fluctuating selection mechanisms. Heterozygote advantage (i.e. recognizing and binding a wider range of antigens than homozygotes) is expected to be more detectable when multiple pathogens are considered simultaneously. Here, we test whether MHC diversity in a wild population of European badgers (*Meles meles*) is driven by pathogen-mediated selection. We examined individual prevalence (infected or not), infection intensity and co-infection of 13 pathogens from a range of taxa and examined their relationships with MHC class I and class II variability. This population has a variable, but relatively low, number of MHC alleles and is infected by a variety of naturally occurring pathogens, making it very suitable for the investigation of MHC–pathogen relationships. We found associations between pathogen infections and specific MHC haplotypes and alleles. Co-infection status was not correlated with MHC heterozygosity, but there was evidence of heterozygote advantage against individual pathogen infections. This suggests that rare-allele advantages and/or fluctuating selection, and heterozygote advantage are probably the selective forces shaping MHC diversity in this species. We show stronger evidence for MHC associations with infection intensity than for prevalence and conclude that examining both pathogen prevalence and infection intensity is important. Moreover, examination of a large number and diversity of pathogens, and both MHC class I and II genes (which have different functions), provide an improved understanding of the mechanisms driving MHC diversity.

Keywords: evolutionary arms race, frequency-dependent selection, heterozygote advantage hypothesis, host–parasite co-evolution, pathogen-mediated selection, rare-allele advantage

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Introduction

The major histocompatibility complex (MHC) is a diverse gene family that plays a crucial role in the adaptive immune system (Hedrick 1994). MHC genes encode cell surface glycoproteins that are vital in both humoral and cell-mediated immune responses, as MHC molecules bind and present antigens to T cells and trigger an immune cascade (Swain 1983). Because of this essential role in the immune system, MHC genes are under constant selective pressures due to challenges

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from parasites and pathogens (hereafter, both are referred to as 'pathogens'; Jeffrey & Bangham 2000; Piertney & Oliver 2006). It is generally considered that balancing and diversifying selection, through an arms race between pathogens and hosts, maintains the extreme diversity in MHC genes. Other selection mechanisms, however, such as sexual selection or maternal–foetal interaction, could also contribute to the high diversity (Penn & Potts 1999).

Pathogen-mediated selection has been proposed to operate through a rare-allele advantage, heterozygote advantage and fluctuating selection (Spurgin & Richardson 2010). The rare-allele advantage hypothesis proposes that rare alleles within the population are likely to offer greater protection to pathogens than common alleles, and so have a selective advantage (Takahata & Nei 1990; Slade & McCallum 1992). Through a rare-allele advantage, specific pathogens drive a cyclic change in the frequency of MHC alleles, known as negative frequency-dependent selection. The heterozygote advantage hypothesis assumes that MHC heterozygous individuals are able to recognize and bind more antigens than homozygous individuals (Doherty & Zinkernagel 1975; Penn *et al.* 2002) and therefore trigger immune responses more effectively against both individual pathogens (Carrington *et al.* 1999; Penn *et al.* 2002; Worley *et al.* 2010) and co-infections (Hughes & Nei 1992; McClelland *et al.* 2003). We use 'heterozygote advantage' in the broad sense, including both dominance (i.e. on average heterozygotes exhibit higher fitness than the average fitness of homozygotes) and overdominance (i.e. heterozygotes exhibit a fitness advantage over the fittest homozygotes) (Hughes & Nei 1988; Penn *et al.* 2002). The fluctuating selection hypothesis proposes that spatial and temporal fluctuation in pathogen types, and their abundances, may maintain MHC diversity (Hedrick 2002; Spurgin & Richardson 2010). Pathogen fluctuations can alter the intensity of directional selection on MHC genes, and hence, different MHC alleles may be selected for at different points in time or space.

Most previous MHC–pathogen studies have reported associations between specific resistant/susceptible MHC alleles and pathogens (Fig. 1), for example in fish (Langefors *et al.* 2001), mammals (Harf & Sommer 2005; Meyer-lucht & Sommer 2005) and birds (Bonneaud *et al.* 2006; Loiseau *et al.* 2011) (reviewed in Table S1.1, Supporting information), and provide comparatively limited support to the heterozygote advantage hypothesis (but see Thursz *et al.* 1997; Penn *et al.* 2002; Froeschke & Sommer 2005; Oliver *et al.* 2009) (Fig. 1; Table S1.1, Supporting information). In well-studied model species, such as humans, mice and three-spined sticklebacks, both heterozygote advantage (dominance, overdominance or optimal) and MHC–pathogen associations have been reported (Penn & Potts 1999; McClelland *et al.* 2003; Wegner *et al.* 2003, 2004; Eizaguirre *et al.* 2012). The association between pathogens and specific alleles could potentially be due to frequency-dependent selection (Takahata & Nei 1990); however, there may also be a frequency-dependent component to heterozygote advantage (Apanius *et al.* 1997). Given that rare alleles occur mostly in heterozygous genotypes (Apanius *et al.* 1997), both rare-allele advantages and heterozygote advantages favour rare alleles and will also maintain large numbers of alleles, making it difficult to distinguish between them. The advantage of heterozygotes being able to recognize and bind a wider range of antigens may be beneficial with respect to both single pathogen infections (Carrington *et al.* 1999; Worley *et al.* 2010) and co-infection with multiple pathogens (Hughes & Nei 1992; McClelland *et al.* 2003). MHC heterozygotes may have an advantage due to being susceptible to fewer pathogens or fewer co-infections than homozygotes (McClelland *et al.* 2003). The comparatively limited evidence reported for heterozygote advantage could result from studies that only focused on single, or a few, pathogen taxa (most studies only examined three or fewer pathogens; faecal egg counts in some studies pooled all the gastrointestinal

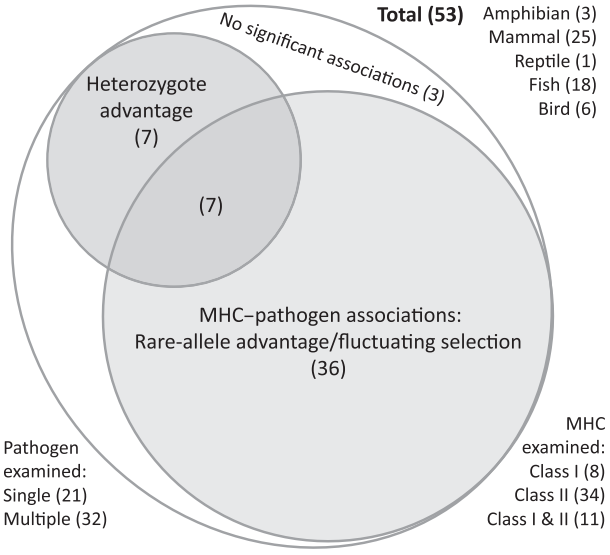

Fig. 1 Pathogen-mediated selection mechanisms in nonmodel species. The number of studies evidencing particular mechanism(s) is given in parentheses. Reported major histocompatibility complex allele/haplotype–pathogen associations are attributed to rare-allele advantage and/or fluctuating selection (Spurgin & Richardson 2010). The numbers of studies on different taxa, whether single or multiple pathogen(s) were examined, and the class of major histocompatibility complex (MHC) genes studied, are shown in parentheses. The MHC–pathogen studies in nonmodel species are given in Table S1.1 (supplementary materials S1).

pathogens together due to limitations in resolving power; see Table S1.1, Supporting information), limiting examination of the overall selection pressure to which animals are exposed. The relationship between pathogens and MHC can therefore be tested more effectively via a more comprehensive survey of pathogens—and ideally those occurring naturally in a wild population and to which individuals are actually susceptible.

The interaction between pathogens and the MHC is also likely to be context dependent and only to emerge under particular environmental and disease exposure conditions (Bernatchez & Landry 2003; Sommer 2005; Piertney & Oliver 2006), furthering the benefits of examining the association between pathogens and MHC in natural populations. Here, we test the associations between thirteen pathogens and variation in both MHC class I and class II genes, in a population of wild European badgers (*Meles meles*; a medium-sized carnivore). Because of the analytical complications that arise from high allelic diversity (Richardson *et al.* 2005), a population with a relatively limited number of MHC alleles affords an ideal opportunity for identifying the ecological consequences resulting from MHC variation (Oliver *et al.* 2009), as MHC alleles can be assigned to loci and haplotypes can be inferred. In this badger population, only one DRB locus has been found to be polymorphic (See Fig. S2.1. Supporting information), with three putatively functional sequences (i.e. gene transcription confirmed, and no frameshift or premature stop codons were found; Sin *et al.* 2012c; Sin 2013). Although seven putatively functional MHC class I sequences were also identified, from at least two functional class I loci (Sin *et al.* 2012b), we believe only one locus to be polymorphic (See Fig. S2.1, Supporting information). As a consequence, this study system, with relatively low MHC diversity, provides a highly informative model for the study of MHC–pathogen relationships. MHC class II molecules present principally exogenous antigens, while class I present intracellular antigens (Bjorkman & Parham 1990; Hughes & Yeager 1998), although cross-presentation has also been identified (Heath & Carbone 2001; Ackerman & Cresswell 2004). We therefore predict that associations will occur primarily between, but not restricted to, class I alleles and intracellular pathogens, and class II alleles and extracellular pathogens. Only 11 of 53 studies on nonmodel species (reviewed in Table S1.1, Supporting information) have examined the effect of both MHC class I and class II genes simultaneously, despite the importance of including both classes for a comprehensive study of the MHC–pathogen system, in recognition of their different functions. Additionally, only three of these studies investigated correlations between multiple pathogens and both the MHC class I and II genes (supp. S1).

Badgers in this high-density population form social groups (Macdonald & Newman 2002; Macdonald *et al.* 2009), which use communal underground dens (termed setts), creating conditions potentially able to facilitate pathogen transmission. Pathogen transmission between groups is then promoted by traits such as a high rate of movement between groups (59% of 267 individuals were detected in more than one group; Macdonald *et al.* 2008), promiscuous mounting involving extra-group males (Dugdale *et al.* 2011) and a high rate of extra-group paternity (Carpenter *et al.* 2005; Dugdale *et al.* 2007). In addition, social interactions that involve contact between individuals that could spread pathogens are common, such as alloparental care (Dugdale *et al.* 2010), allogrooming (Stewart & Macdonald 2003; Johnson *et al.* 2004) and allomarking (which facilitates the exchange of subcaudal pouch bacteria between group members; Buesching *et al.* 2003; Sin *et al.* 2012a). European badgers are susceptible to a wide taxonomic range of pathogens: for example, viral [mustelid herpesvirus (MHV); King *et al.* 2004], bacterial (*Salmonella*; Wilson *et al.* 2003), protozoan [*Trypanosoma pestanai* (Macdonald *et al.* 1999); *Eimeria melis* and *Isospora melis* (Newman *et al.* 2001)], helminth (Torres *et al.* 2001) and invertebrate ectoparasites [*Paraceras melis*, *Trichodectes melis* and *Ixodes hexagonus* (Cox *et al.* 1999; San 2007)]. Some pathogens, such as *E. melis* in badgers, cause host morbidity and high juvenile mortality (Newman *et al.* 2001). Other pathogens, which cause only mild symptoms, may decrease host fitness, due to the cost of mounting an immune response, or through impairing the host's overall energy budgets while tolerating infection (Raberg *et al.* 2007)—potentially also increasing host vulnerability to other mortality factors (e.g. oxidative stress; van de Crommenacker *et al.* 2012; Bilham *et al.* 2013). To our knowledge, the unusually wide diversity of pathogens included here (13 including virus, bacteria, protozoa and ectoparasites) is unprecedented among studies of MHC–pathogen systems (Table S1.1, Supporting information).

Here, we investigate both pathogen prevalence (the presence/absence of pathogen) and infection intensity (pathogen quantity; Westerdahl *et al.* 2012) to advance a comprehensive understanding of how pathogen-mediated selection might drive badger MHC diversity. We first establish whether there are patterns in infectious status among hosts (e.g. by sex, age, weight/length ratio, standardized microsatellite heterozygosity, etc.). We then test whether (i) particular MHC haplotypes or alleles are associated with lower or higher pathogen burden (prevalence and infection intensity) and (ii) MHC heterozygotes exhibit a lower prevalence and/or intensity of individual pathogen infection and/or lower number of co-infecting pathogens than homozygotes. It

is difficult to disentangle the different pathogen-mediated selection mechanisms (Spurgin & Richardson 2010). If associations between specific pathogens and particular MHC alleles manifest, this would indicate a more substantial role for either rare-allele advantage or fluctuating selection. If associations are found between lower individual pathogen burden and/or number of co-infecting pathogens with MHC heterozygosity, then this would indicate a possible role for heterozygote advantage (Spurgin & Richardson 2010).

Materials and methods

Study population and sample collection

This study was conducted on a high-density badger population [36.4 ± 2.6 (SE) badgers/km^2; Macdonald *et al.* 2009] in Wytham Woods (a 6 km^2 deciduous woodland in Oxfordshire, UK; 51°46′26N, 1°19′19W). Seasonal trapping events have been undertaken since 1987 (Macdonald & Newman 2002), generally over 2 weeks in June (spring), September (summer), November (autumn) and January (winter) (Macdonald *et al.* 2009). Badgers were caught in mesh traps baited with peanuts, placed near the entrances of active setts (Macdonald & Newman 2002; Macdonald *et al.* 2009). Captured badgers were transported to a central handling facility and sedated by intramuscular injection of ketamine hydrochloride (McLaren *et al.* 2005). Upon first capture, all badgers were tattooed with a unique number on the left inguinal region for permanent individual identification. The sex, age-class [cub (<1 years old) or adult; see Macdonald *et al.* 2009; referred to as simply 'age' hereafter], weight (to the nearest 0.1 kg), body length (mm) and trapping location (social group affiliation) of each badger were recorded. Weight and body length were used to calculate a body condition index (weight/length ratio) (Macdonald *et al.* 2002).

DNA samples were collected during sedation: ~100 guard hairs were plucked, and approximately 3 mL of blood was taken by jugular venipuncture using a vacutainer containing EDTA. Blood samples were stored at −20 °C and hair samples in 80% ethanol at room temperature. Faecal samples were collected following administration of an enema consisting of 7.5 mL warm soapy water per kilogram bodyweight (Newman *et al.* 2001). Faecal samples were preserved individually as two subsamples. The first subsample was preserved using 2.5% aqueous potassium dichromate ($K_2Cr_2O_7$) at 4 °C for later screening using the faecal flotation technique (Foreyt 2001), and the second subsample was stored at −20 °C until DNA isolation was performed. Blood and faecal samples used for pathogen screening were collected from individuals trapped June 2009–January 2010. The blood and hair samples for MHC genotyping were collected across years from 1987 to 2010.

Pathogen screening

Trypanosoma pestanai and MHV. DNA from 200 μL of whole blood samples was isolated using QIAamp DNA Blood kit (Qiagen, Hilden, Germany) and eluted in 100 μL ddH_2O. A quantitative real-time PCR (qPCR) approach was used to determine the prevalence and intensity of *Trypanosoma pestanai* [number of badgers (n_b) = 217; number of samples (n_s) = 360] and MHV (n_b = 218; n_s = 361). qPCR primers were designed for regions in the 18S rRNA gene from *T. pestanai* (forward: 5′-GTCCAGCGAATGAACGAAATTAA; reverse: 5′-AGGGCAGTTGTTCGTCAGAAG; PCR product size: 130 bp) using StepOne 2.1 [Applied Biosystems (ABI), Foster City, CA, USA]. Quantitative PCR was performed in a 20 μL reaction mix consisting of the following: 10 μL SYBR Green PCR Master Mix (ABI), 0.4 μL of each forward and reverse primer (200 nM), 4.2 μL of RNase-free water and 5 μL DNA sample.

For MHV, qPCR primers were designed for regions in the DNA polymerase gene (forward: 5′-GGAGAGTGCTGACCGATGGA; reverse: 5′-AAAAGCCTGGAATTGGATCAATAA; 150 bp) using StepOne 2.1 (ABI). Quantitative PCR was performed in a 20 μL reaction mix consisting of the following: 10 μL SYBR Green PCR Master Mix (ABI), 0.1 μL of each forward and reverse primer (50 nM), 4.8 μL of RNase-free water and 5 μL DNA sample.

Amplification and real-time fluorescence detection were performed with StepOnePlus PCR Systems (ABI). Each real-time PCR assay contained serial dilutions of 10^7 to 10^1 plasmid standards, which contained the PCR products amplified by each primer set (cloning methods are described in Sin *et al.* 2012a), to produce the calibration curve. All seven standards, samples and one negative control were run in triplicate on the same plate. The thermal cycling conditions were as follows: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. A melting curve analysis was included at the end to confirm the identity of the product. The number of pathogen DNA copies was calculated from the C_t values (cycle threshold) and standard curves and expressed as copies per microlitre of extracted DNA (Bell & Ranford-Cartwright 2002). The mean was taken for the triplicate values.

Enteric bacteria (Salmonella, Yersinia and Campylobacter). DNA from faecal samples was isolated using QIAamp DNA Stool kit (Qiagen), according to the manufacturer's instructions. A semiquantitative PCR approach was used to determine the prevalence and infection

intensity of *Salmonella*, *Yersinia* and *Campylobacter* (n_b = 99; n_s = 150). PCR primers were designed for regions in the *ompC* gene from *Salmonella* (forward: 5′-ATCGCTGACTTATGCAATCG; reverse: 5′-GTTGCT GATGTCCTTACCTTTAG; 355 bp), *pla* gene from *Yersinia* (forward: 5′-GCTTTATGACGCAGAAACAGGA; reverse: 5′-AACCAGCCTTTCACATTGAGGT; 270 bp; Woron *et al.* 2006) and 16S rRNA gene from *Campylobacter* (forward: 5′-GGATGACACTTTTCGGAG; reverse: 5′-AATTCCATCTGCCTCTCC; 246 bp; Rinttila *et al.* 2004). PCR was performed in a 10 μL reaction mix consisting of 0.6 μL of forward and reverse primer (100 μM), 2.7 μL ultrapure ddH_2O, 0.1 μL BSA (ABI), 200 μM of each dNTP, 1× PCR buffer (Qiagen), two units of HotStarTaq (Qiagen) and 1 μL of DNA. The thermal cycling conditions were as follows: initial denaturation at 95 °C for 15 min, followed by 38 cycles at 94 °C for 30 s, 63 °C (*Salmonella*) or 52 °C (*Campylobacter*) for 30 s and 72 °C for 30 s, with a final extension at 72 °C for 10 min. Touchdown PCR was performed for *Yersinia*; cycles started at 65 °C and dropped by 1 °C per cycle until the remaining 29 cycles continued at 55 °C. The reactions were performed in triplicate and the mean was taken. PCR products were resolved by electrophoresis on 1.5% agarose gel, visualized by ethidium bromide staining and analysed using GENETOOLS (SynGene).

Enteric parasites (Coccidia and helminths). The faecal flotation technique (Foreyt 2001) was used to assess the prevalence and intensity of gastrointestinal parasites present in faecal samples (n_b = 185; n_s = 275), including coccidia (*Eimeria melis* and *Isospora melis*) and nematodes (e.g. *Strongyloides* sp., *Capillaria* sp.). Following the quantitative methods of Newman *et al.* (2001), two slides per faecal sample were screened using microscopy for coccidia oocysts, helminth eggs and larvae and the mean intensity of infection (e.g. coccidian oocysts per gram of pelleted faecal material; Anwar *et al.* 2000) was then calculated. While absolute rate of infection can only be established by necropsy, assessing gastrointestinal parasitoses in live hosts from faecal oocyst/egg counts, as an indirect method, provides a reliable analogue (Seivwright *et al.* 2004). *Isospora melis*, *Capillaria* sp., *Strongyloides* sp. and other minor nematodes were only included in the co-infection analysis, but not in the individual pathogen analysis, because their prevalence and infection intensities were very low.

Ectoparasites (fleas, lice, and ticks). Ectoparasites were counted when handling sedated badgers (n_b = 226; n_s = 418). A standardized relative abundance index for badger fleas (*Paraceras melis*) was derived from counting fleas detected during a 20-s inspection of the badger's body (for full details of this method see Cox *et al.* 1999), turning the badger mid count, as disturbed fleas tend to run downwards relative to gravity and towards the posterior of the badger, following the direction of hair growth (Stewart & Macdonald 2003; Johnson *et al.* 2004). A standardized relative index of tick (*Ixodes hexagonus* and *Ixodes canisuga*) and host-specific lice (*Trichodectes melis*) abundance was derived from inspection of a 4 × 4 cm square of skin in the inguinal region, prone to infestation (see Cox *et al.* 1999).

Co-infection. Co-infection status (n = 120) was estimated from 90 individuals, with more than one co-infection status included for some individuals, from different trapping periods. The number of pathogens co-infecting each badger simultaneously was evaluated for those individuals for which screening was performed for all 13 pathogens (MHV, *Salmonella*, *Yersinia, Campylobacter*, *T. pestanai*, *Eimeria*, *Isospora*, *Capillaria*, *Strongyloides*, other nematodes, badger fleas, badger lice and ticks).

MHC and microsatellite genotyping

Genomic DNA was isolated using the GFX Genomic Blood DNA Purification Kit (Amersham Biosciences, Little Chalfont, UK), following the scalable method in the manufacturer's protocol, or from a minimum of 20 hairs with visible follicles, using a Chelex protocol (Walsh *et al.* 1991). We used published primers to amplify exon 3 and exon 2 regions (Sin *et al.* 2012b,c) that encode the antigen-binding domain in MHC class I and class II DRB genes, respectively. Previous studies of the badger MHC indicated the presence of at least two class I loci and two class II DRB loci, with seven and four putatively functional sequences identified by transcription analysis, respectively (Sin *et al.* 2012b,c). The MHC sequences were separated by reference strand-mediated conformation analysis (RSCA) following Sin (2013), in which each 'RSCA allele' was confirmed to be an individual, putatively functional, sequence. Regenotyping of 10% of the samples corroborated consistent results as the first genotyping. We used the number of alleles per individual as a measure of MHC heterozygosity across multiple loci (Richardson *et al.* 2005; Westerdahl *et al.* 2005). 'Heterozygosity' hereafter refers to the allele diversity exhibited in class I and class II genes. Allele frequencies of *Meme-DRB*01*, *-DRB*03* and *-DRB*04* were 75.7%, 18.0% and 6.6%, respectively, in 1141 individuals. Allele frequencies of *Meme-MHCI*01*, *-MHCI*02*, *-MHCI*03*, *-MHCI*04* and *-MHCI*07* were 15.7%, 6.0%, 27.4%, 50.0% and 0.9%, respectively, in 1117 individuals. *Meme-DRB*02* and *Meme-MHCI*05* were present in all individuals, and *Meme-MHCI*06* was only present with *Meme-MHCI*07* (see Sin 2013), and they were thus excluded from the analyses.

The polymorphic locus of the MHC class I and MHC class II DRB (Supp. 3) holds five class I (*Meme-MHCI*01*, *-MHCI*02*, *-MHCI*03*, *-MHCI*04* and *-MHCI*07*) and three class II DRB (*Meme-DRB*01*, *-DRB*03* and *-DRB*04*) alleles. These loci are in linkage disequilibrium ($P < 0.01$; GENEPOP 4.2; Raymond & Rousset 1995). MHC class II–class I haplotypes were included in the analysis and were calculated using parentage data by assuming Mendelian inheritance. Eight haplotypes were identified. The frequency of haplotypes in 205 individuals included in the analysis was as follows: *DRB*04–MHCI*02*, 1.5%; *DRB*04–MHCI*01*, 4.1%; *DRB*03–MHCI*03*, 18.5%; *DRB*01–MHCI*04*, 54.4%; *DRB*01–MHCI*02*, 3.7%; *DRB*01–MHCI*01*, 7.6%; *DRB*01–MHCI*03*, 8.3%; and *DRB*01–MHCI*07*, 2.0%. The sampling size for haplotype analyses was smaller than that for MHC allele analyses (see Pathogen screening section), due to the availability of parentage data, and is given in supp. S4. 'Haplotype heterozygosity' hereafter refers to the heterozygosity at the haplotype level.

To measure neutral variation, to control for potential confounding effects of demographic processes on MHC structure (Spurgin & Richardson 2010), we genotyped all individuals at up to 35 microsatellite loci (detailed in Dugdale *et al.* 2007; Annavi *et al.* 2011, 2014) and estimated the standardized multilocus heterozygosity of each individual (Coltman *et al.* 1999; Annavi 2013; Annavi *et al.* 2014). We genotyped each individual for a mean of 34.0 loci (95% confidence interval = 33.8–34.1; range = 18–35). Fourteen badgers had no DNA left after the initial set of 22 microsatellites had been genotyped, so for these, we based the analysis on the 18–22 available loci.

Data analysis

Multimodel inference. Because a multitude of factors influence pathogenic infection in the wild, we employed linear mixed models, which allow the inclusion of multiple explanatory variables and random effects (Paterson *et al.* 1998; Oliver *et al.* 2009). We used multimodel inference to establish which explanatory variables had an effect, averaged over all plausible models (Burnham & Anderson 2002; Anderson 2008; Symonds & Moussalli 2011). Analyses were performed using the packages LME4 0.999375-42 (Bates & Maechler 2009), MUMIN v1.7.7 (Barton 2009) and AICCMODAVG v1.25 (Mazerolle 2011) in R 2.15.0 (R Development Core Team 2011). We conducted an initial exploration of our data to ascertain their distribution and spread and to identify outliers and examine relationships between variables (Zuur *et al.* 2009). The infection intensities of MHV, *Salmonella*, *Yersinia*, *Campylobacter*, *T. pestanai*, *Eimeria* and lice were $\log_{10}$ (intensity + 1) transformed, to correct for heterogeneity of variance.

To reduce the number of factors included in a single model, multimodel inference was conducted in two steps. In step one, we tested whether different life-history factors affected the (i) prevalence (the presence/absence) of a particular pathogen, (ii) pathogen infection intensity (infected individuals only, as the absence of infection can be due to nonexposure or resistance) or (iii) number of pathogens co-infecting a badger simultaneously. We built linear mixed models (LMM: noncount intensity data) and generalized linear mixed models [GLMM: prevalence (binomial error, logit link), and co-infection or ectoparasite counts (Poisson error, log link)]. The starting models included five fixed effects: three categorical [age-class (cub or adult), sex and season] and two continuous effects (weight/length ratio and standardized microsatellite heterozygosity). The number of fleas was also included in the *T. pestanai* infection model, as *P. melis* is its vector (Lizundia *et al.* 2011). We controlled for individuals with multiple samples and for individuals from the same group by including individual identity and social group identity as random effects, respectively. Model selection was based on Akaike's information criterion corrected for sample size (AICc; Akaike 1973). Models that are more plausible have lower AICc value. Multimodel inference (Burnham & Anderson 2002) was performed for models with ΔAICc < 7 (Burnham *et al.* 2011). Significant variables were retained in a reduced model for use in step 2.

In step two, we investigated whether pathogen prevalence, pathogen infection intensity and co-infection were related to (i) the presence of specific MHC haplotypes and (ii) MHC haplotype heterozygosity. We included haplotype presence or haplotype heterozygosity as fixed effects in the reduced model. Eight haplotypes (see MHC and microsatellite genotyping section) were included in the reduced model. In addition, analyses using both MHC class I and class II alleles instead of haplotypes were also performed because these have different immunological functions. We used variance inflation factors (VIF) to assess which explanatory variables were collinear, and these VIF values showed which alleles should be retained in the analyses (i.e. VIF values <3; Zuur *et al.* 2009). Consequently, we included eight alleles (three class II DRB: *Meme-DRB*01*, *-DRB*03* and *-DRB*04*, Sin *et al.* 2012b; five class I: *Meme-MHCI*01*, *-MHCI*02*, *-MHCI*03*, *-MHCI*04* and *-MHCI*07*, Sin *et al.* 2012a) in the reduced model. Model averaged parameter estimates and parameter estimates with shrinkage (i.e. parameter estimates set to zero in models that did not include the parameter) are reported. The unconditional standard errors (a conser-

vative measure, as it accounts for model uncertainty) and 95% confidence intervals (Anderson 2008) of parameter estimates are also reported, to allow model uncertainty to be included in both the model evaluation and the derivation of parameter estimates. The relative importance of a parameter was defined as the sum of Akaike weights (where the Akaike weight of each model is its relative likelihood [exp{−0.5*ΔAICc}] divided by the sum of Akaike weights of all models) for all models (ΔAICc < 7) including the predictor (Burnham & Anderson 2002). The parameter with the largest sum was inferred to be the most influential.

In instances where an effect was found between MHC heterozygosity and either pathogen prevalence or infection intensity, which could potentially to be due to association between MHC heterozygosity and the presence of a specific allele, we present the Kendall rank correlation coefficient (τ) between MHC heterozygosity and allele presence.

Results

Trypanosoma pestanai. There was significant association between prevalence and presence of specific haplotype, where individuals with *DRB*01–MHCI*03* haplotype had lower *Trypanosoma pestanai* infection prevalence (Fig. 2; Table S4.1, Supporting information). High-class I genes heterozygosity was associated with low *T. pestanai* prevalence (Fig. S3.1 and Table S5.1, Supporting information). The correlation between class I gene heterozygosity and allele presence was highest for *Meme-MHCI*03* (0.42, $P < 0.001$), and correlations for *Meme-MHCI*01*, *02*, *04* and *07* were 0.17 ($P < 0.01$), 0.19 ($P < 0.001$), 0.15 ($P < 0.01$) and 0.38 ($P < 0.001$), respectively, in the *T. pestanai* data set. *T. pestanai* prevalence was higher in adults than in cubs and lower in females than in males (Table S5.1, Supporting information). Flea load was associated positively with *T. pestanai* prevalence.

Intensity of *T. pestanai* infection was lower in adults and in females than in cubs and males, respectively. Badgers with lower standardized microsatellite heterozygosity exhibited lower infection intensity (Table S5.2, Supporting information). The intensity of infection with *T. pestanai* was not associated with the MHC haplotypes/alleles or MHC heterozygosity analysed (Fig. 3).

Mustelid herpesvirus

Mustelid herpesvirus was detected in nearly all blood samples (354/361 = 98.1%), and thus, only infection intensity was analysed. Adults had lower MHV intensity than cubs, and weight/length ratio associated negatively with MHV infection intensity (Table S5.3, Supporting information). Seasonally, badgers exhibited lower MHV intensities in winter than in spring. The presence of all MHC class II–class I haplotypes with the allele *Meme-DRB*01* exhibited higher MHV infection intensity (Fig. 3; Table S4.3, Supporting information),

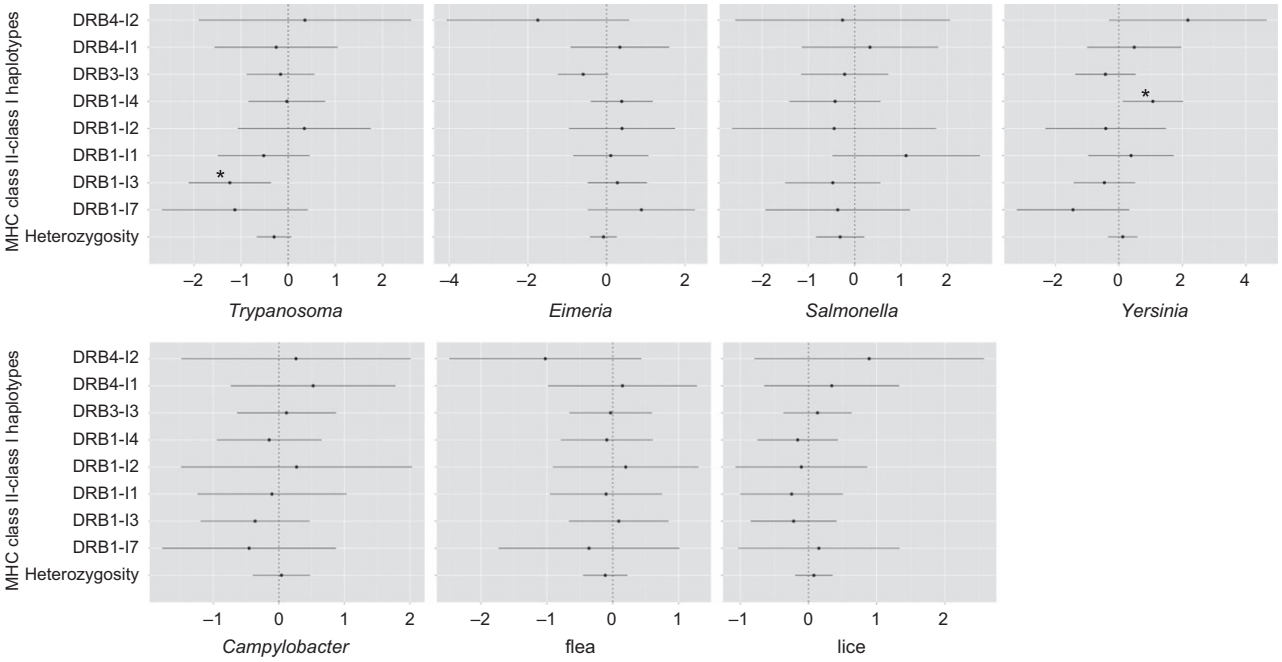

Fig. 2 Model averaged parameter estimates and their 95% confidence intervals for the nine major histocompatibility complex (MHC) predictors (the presence/absence of MHC class II–class I haplotypes, haplotype heterozygosity) associated with the prevalence of seven pathogens. *A parameter with a significant effect.

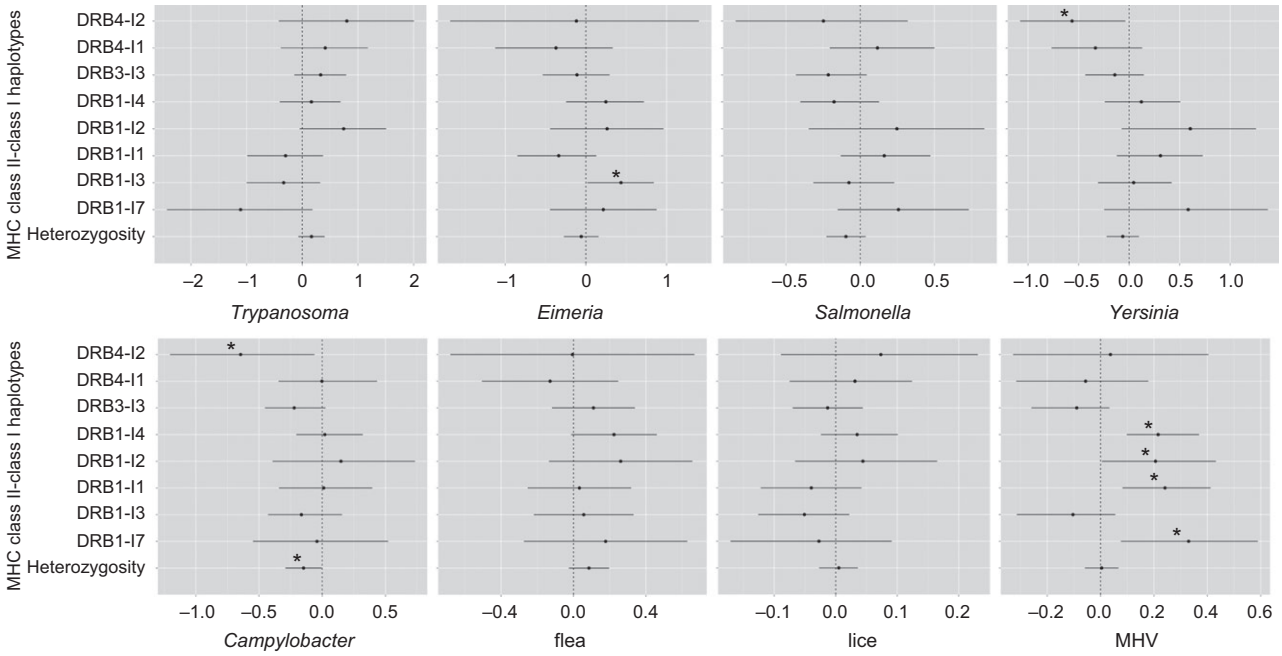

Fig. 3 Model averaged parameter estimates and their 95% confidence intervals for the nine major histocompatibility complex (MHC) predictors (the presence/absence of MHC class II–class I haplotypes, haplotype heterozygosity) associated with the infection intensity of eight pathogens. * A parameter with a significant effect.

except for the haplotype with the allele *Meme-MHCI*03*, which showed a negative effect on MHV infection (Fig. S3.2, Supporting information). The presence of *Meme-DRB*01* and *Meme-MHCI*03* associated with the intensity of MHV infection (Fig. S3.2 and Table S5.3, Supporting information). Individuals with *Meme-DRB*01* exhibited higher MHV intensity and individuals with *Meme-MHCI*03* had lower MHV intensity. MHV infection intensity was not associated with MHC heterozygosity (Fig. 3).

Enteric parasites

Badgers with the *DRB*01–MHCI*03* haplotype presented higher *Eimeria* infection intensity (Fig. 3). Adults exhibited lower *Eimeria* prevalence and intensity than did cubs (Tables S5.4 and S5.5, Supporting information). Badgers in summer and autumn presented lower *Eimeria* infection intensity than those in spring (Table S5.5, Supporting information).

Enteric bacteria

The prevalence of *Salmonella* was lower in adults than in cubs (Table S5.6, Supporting information). More individuals were infected by *Salmonella* in summer and autumn than in spring (Table S5.6, Supporting information). *Salmonella* infection intensity was associated negatively with standardized microsatellite heterozygosity (Table S5.7, Supporting information). *Salmonella* infection was not associated with specific MHC haplotypes/alleles or MHC heterozygosity (Figs 2 and 3; Figs S3.1 and 3.2, Supporting information).

The prevalence of *Yersinia* was higher in summer and autumn than in spring (Table S5.8, Supporting information). Higher *Yersinia* prevalence was found in individuals with the *DRB*01–MHCI*04* haplotype, which was the only haplotype included in the analyses that included *Meme-MHCI*04* (Fig. 2). Individuals with *Meme-MHCI*04* were also more likely to be infected by *Yersinia* (Fig. S3.1, Supporting information). The infection intensity of *Yersinia* was lower for individuals with the *DRB*04–MHCI*02* haplotype (Fig. 3). Individuals with *Meme-DRB*01* showed higher infection intensity and individuals with *Meme-DRB*04* lower infection intensity (Figs S3.2 and S3; Table S5.9, Supporting information). *Yersinia* infection was not associated with MHC heterozygosity (Figs 2 and 3).

None of the tested explanatory variables correlated with *Campylobacter* prevalence (Fig. 2; Table S4.10, Supporting information). Infection intensity of *Campylobacter* was lower in summer than spring (Table S5.11, Supporting information). Individuals with the *DRB*04–MHCI*02* haplotype had lower *Campylobacter* infection intensity (Fig. 3). Badgers with high haplotype heterozygosity and heterozygosity of the class II DRB genes exhibited lower *Campylobacter* infection intensity (Figs 3 and S3.2, Supporting information). In this *Campylobacter*

data set, the correlations of class II DRB gene heterozygosity with *Meme-DRB*01, 03* and *04* were 0.14 ($P > 0.05$), 0.62 ($P < 0.001$) and 0.37 ($P < 0.001$), respectively.

Ectoparasites

The prevalence of fleas was greater for adults than for cubs and associated negatively with weight/length ratio (Table S5.12, Supporting information). Flea prevalence was higher in summer and autumn than in spring. Flea intensity was higher in summer and lower in winter than in spring (Table S5.13, Supporting information). Neither flea prevalence nor intensity was associated with specific MHC haplotypes/alleles or MHC heterozygosity (Figs 2 and 3).

The prevalence of lice was associated negatively with weight/length ratio (Table S5.14, Supporting information). Lice prevalence was higher in summer than in spring and higher for males than for females. Lice infection intensity was associated negatively with weight/length ratio (Table S5.15, Supporting information). Females had lower lice intensity than males. Lice infection was not associated with specific MHC haplotypes/alleles or MHC heterozygosity (Figs 2 and 3).

Co-infection

Individual badgers were simultaneously co-infected with between two and eleven different pathogens (mean = 6.0, SE ±0.075). Co-infection was not associated with the presence/absence of any individual MHC haplotypes/alleles or MHC heterozygosity (Figs 4 and S3.3, Supporting information), and there was no association between the number of co-infecting species and any of the tested explanatory variables (Table S5.16, Supporting information). Excluding ectoparasites, which are subject to grooming and thus removal by the host species, we re-examined the number of co-infecting internal pathogens (range = 2–9, mean = 4.4, SE ±0.065) and found no association with the explanatory variables.

Discussion

Both MHC heterozygote advantage and associations between specific pathogens and particular MHC haplotypes and alleles were evidenced for this population. By examining infection intensity, we were able to identify haplotypes and alleles that provided quantitative, as well as qualitative, resistance (Westerdahl *et al.* 2012), thus providing a more complete picture of MHC–pathogen associations. The associations between MHC class II–class I haplotypes and specific pathogens

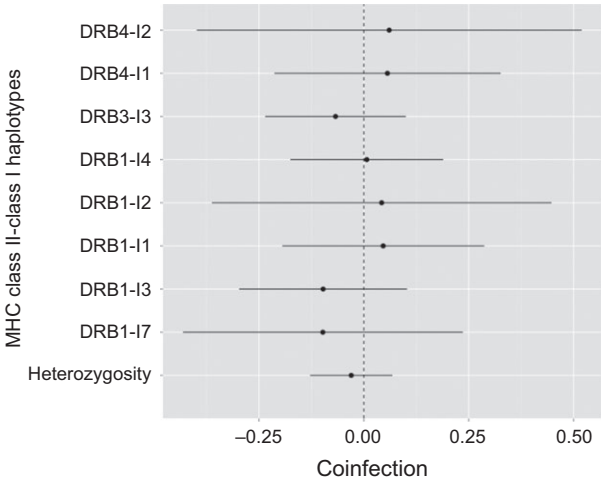

Fig. 4 Model averaged parameter estimates and their 95% confidence intervals for the nine major histocompatibility complex (MHC) predictors (the presence/absence of MHC class II–class I haplotypes, haplotype heterozygosity) associated with co-infection by 13 pathogens.

were, in most cases, due to the presence of a resistant or susceptible allele. For instance, all MHC haplotypes with *Meme-DRB*01* were associated with higher MHV infection intensity, except for the haplotype that carried the allele *Meme-MHCI*03* (which was associated with lower MHV infection intensity). Similarly, *MHCI*04,* and the only haplotype (*DRB*01–MHCI*04*) that carried it, was associated with higher infection prevalence of *Yersinia*; both haplotype *DRB*04–MHCI*02* and the *DRB*04* allele were associated with lower *Yersinia* infection intensity. In a few cases, however, haplotypes, but not allele presence, were associated with infection. These associations between certain MHC haplotypes/alleles and pathogen resistance or susceptibility concord with the rare-allele advantage hypothesis (Takahata & Nei 1990). This predicts that MHC alleles that have been subject to recent pathogen evasion will render the host more susceptible and become less frequent, and rare or new alleles will provide resistance to newly mutated pathogens and increase in frequency. The change in allele frequencies over time for the alleles associated with pathogen resistance/susceptibility could strengthen the support for the rare-allele advantage hypothesis. In addition to host–pathogen co-evolution and cyclical change in allele frequencies, the presence of susceptibility alleles could also be due to simultaneous resistance to one type of pathogen and susceptibility to others (Penn & Potts 1999; Kubinak *et al.* 2012; Kamath *et al.* 2014). For example, the haplotype *DRB*01–MHCI*03* was associated with lower *Trypanosoma pestanai* prevalence and higher *Eimeria* intensity.

Both rare-allele advantages and fluctuating selection can lead to associations between pathogens and specific MHC alleles. Selection for rare MHC alleles through host–pathogen co-evolution has been shown to be a stronger driving force than heterozygote advantage for MHC diversity (Borghans *et al.* 2004). The substantial number of MHC–pathogen associations reported in the literature (Fig. 1) could, therefore, be due to the rare-allele advantage providing the principal selective pressure maintaining MHC diversity. Conversely, fluctuating selection could produce the same effect on MHC–pathogen associations, and it is very difficult to separate the two nonexclusive mechanisms (Spurgin & Richardson 2010).

The pathogen–MHC allele associations that we discovered were identified while controlling for genome-wide heterozygosity and were, therefore, not by-products of confounding effects of demographic processes on MHC structure (Spurgin & Richardson 2010). We identified associations with microsatellite heterozygosity only for *T. pestanai* and *Salmonella* infection intensities. *Salmonella* intensity was lower for individuals with higher microsatellite heterozygosity, consistent with heterozygosity–fitness correlations (Hansson & Westerberg 2002; Annavi *et al.* 2014). Individuals with lower microsatellite heterozygosity had lower *T. pestanai* intensity, however, perhaps due to the disruption of locally adapted genes.

MHC class II molecules principally bind exogenous antigens and are only expressed on antigen-presenting cells, such as B cells and macrophages (Hughes & Yeager 1998). MHC class I molecules are responsible primarily for intracellular antigen binding and are expressed on the surface of all nucleated somatic cells (Bjorkman & Parham 1990), while cross-presentation also allows class I molecules to process exogenous antigens (Heath & Carbone 2001; Ackerman & Cresswell 2004). We found that class I alleles were associated with both an intracellular pathogen (i.e. MHV) and an extracellular pathogen (i.e. *Yersinia*); potentially attributable to cross-presentation of exogenous antigens by MHC class I molecules (Heath & Carbone 2001; Ackerman & Cresswell 2004). Class II DRB alleles were associated with an extracellular pathogen (i.e. *Yersinia*) and an intracellular pathogen (i.e. MHV). Linkage between the DRB alleles and other immunity-related genes (Sasazuki *et al.* 1983) might explain the association of a DRB allele with MHV.

The majority of MHC–pathogen studies have considered only one, or a few pathogens (Table S1.1, Supporting information), but heterozygous advantages may better be detected by assessing a larger number and diversity of pathogens (e.g. Wegner *et al.* 2003; Oliver *et al.* 2009). Indeed, a heterozygosity advantage was more often detected when co-infection was examined, than when it was not examined (i.e. 3/9 co-infection studies found this vs. 11/44 studies that did not examine co-infection; Table S1.1, Supporting information). Despite examining thirteen pathogenic species from a wide variety of taxa, we found no support for MHC heterozygosity being associated with fewer co-infecting pathogens. An MHC heterozygote advantage can work against single pathogen infection if heterozygous individuals recognize and present more antigens originating from that individual pathogen. We found a heterozygote advantage of class I genes for *T. pestanai* prevalence and of class II DRB genes and haplotype for *Campylobacter* infection intensity. The heterozygosity advantage identified in this study was, however, less common than MHC–pathogen associations. This resonated with the results of our literature review of other MHC–pathogen systems, which report that the rare-allele advantage/fluctuating selection is more common ($n = 43$) than the heterozygosity advantage ($n = 14$) (Fig. 1). There was an association between MHC heterozygosity and the presence of a specific allele [for *T. pestanai* prevalence, the correlation between class I heterozygosity and *Meme-MHCI*03* was 0.42 ($P < 0.001$); for *Campylobacter* intensity, the correlation between class II DRB heterozygosity and *Meme-DRB*03* was 0.62 ($P < 0.001$)]. These alleles, however, were not associated significantly with lower *T. pestanai* prevalence and *Campylobacter* intensity (Figs S3.1 and 3.2, Supporting information). This indicates that the heterozygote advantage we identified was not caused by the effect of specific alleles, nor specific haplotypes, because the haplotypes that associated with *T. pestanai* prevalence or *Campylobacter* intensity were not correlated with MHC heterozygosity more than with other haplotypes (supp. S6). These findings again highlight the complexities of disentangling different pathogen-mediated selection mechanisms (Spurgin & Richardson 2010), as there could be a rare-allele advantage component to heterozygote advantage (Apanius *et al.* 1997); that is, different mechanisms are not mutually exclusive.

Factors other than the presence of MHC alleles were also associated with the burden of different pathogen species. Flea (*Paraceras melis*) number correlated positively with *T. pestanai* prevalence, consistent with *P. melis* being the vector of *T. pestanai* in European badgers (Lizundia *et al.* 2011). Adults exhibited a higher prevalence of *T. pestanai* than did cubs; probably due to the higher prevalence of fleas and thus continuing lifetime exposure to the risk of infection. The co-evolution of vector and pathogen has been well studied in tick-borne rickettsiae, showing how arthropod vectors are often more important in the long-term maintenance

of arthropod-borne pathogens than the vertebrate host (Azad & Beard 1998).

Age-class also associated with certain other pathogens: compared with adults, cubs exhibited higher MHV intensity, prevalence of *Salmonella*, and prevalence and intensity of *Eimeria.* The higher burden of *Eimeria* in cubs is consistent with trends observed by Anwar *et al.* (2000) and Newman *et al.* (2001). The higher intensity of MHV in cubs must be contracted through vertical transmission (Jones 2001; Corey & Wald 2009), because they do not reach sexual maturity and risk sexual transmission until they are at least 1 year old. Higher pathogen load in juveniles compared with adults is a common pattern observed in host–pathogen assemblages (e.g. Gregory *et al.* 1992; Dawson & Bortolotti 1999; Sol *et al.* 2003). According to the 'immunity' hypothesis, cubs are still undergoing acquired immunological development of T cells, B cells and dendritic cells (Adkins 1999; Marshall-Clarke *et al.* 2000; Dakic *et al.* 2004) and are consequently more susceptible to pathogens than are adults (Sol *et al.* 2003). The effect for MHC association might be underestimated if cubs die because of high pathogen load (e.g. coccidia infection), prior to being first caught at around 16 weeks of age. Among those badgers surviving long enough to be trapped, however, we have a comprehensive representation of alleles, because on average 83% of the population is caught per year (Macdonald *et al.* 2009) and 95% of the intertrap intervals were within 525 days (n = 6193) (Dugdale *et al.* 2007).

Badgers with a higher body condition index (weight/length ratio), that is badgers in better condition, exhibited lower flea and lice prevalence and lower MHV and lice intensity. Individuals in better body condition might more readily allocate resources to combating pathogens (Macdonald *et al.* 2002; Montes *et al.* 2011). Alternatively, individuals with a higher severity of infection might suffer compromised metabolic budgets, losing condition as a consequence. For example, when juvenile gerbils (*Gerbillus andersoni*) are parasitized by natural levels of fleas, infected individuals lose body mass faster and gain body mass slower than uninfected individuals (Hawlena *et al.* 2006b), leading to poorer survival (Hawlena *et al.* 2006a). Costs of infection include direct pathogen-related morbidity (e.g. nutrient costs or tissue damage), the costs of mounting an immune response and the costs of any resultant immunopathology (Viney *et al.* 2005). The assumption is that the energy and resources allocated to an immune response are traded off between the immune system and other biological processes, such as growth and reproduction (Viney *et al.* 2005). For example, exposure to lipopolysaccharide in the house sparrow (*Passer domestics*) leads to decreased activity, body mass, feeding rate and reproductive success (Bonneaud *et al.* 2003). Variation in pathogen burdens can thus impact on host health and survival traits, which highlights the importance of having advantageous alleles in order to mount the most effective immune response, enhancing host fitness (Brouwer *et al.* 2010).

Conclusion

Our study provides evidence for the association between specific MHC haplotypes/alleles and particular pathogens tested in a wild mammal population. We found no evidence for an MHC heterozygote advantage in relation to co-infection status, but did find evidence of an MHC heterozygote advantage in relation to single pathogen infection. This suggests that rare-allele advantage and/or fluctuating selection, and heterozygote advantage are major selective forces shaping MHC diversity in this species. We stress, however, that these findings do not preclude other selection mechanisms, such as mating preferences or postcopulatory mate choice, and apply only to those pathogens investigated here. Future studies are needed to evaluate the importance of these pathogen-mediated and sexual selection mechanisms in MHC evolution in this and other MHC systems.

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Y.W.S., H.L.D., C.N. and DWM planned the study. D.W.M. obtained long-term project funding. Y.W.S., G.A. and C.N. conducted the field research as part of D.W.M.'s long-term field study. The laboratory work was conducted in T.A.B.'s laboratory, where Y.W.S. genotyped the MHC loci and screened the pathogens,

and G.A. and H.L.D. genotyped the microsatellite loci. Y.W.S. counted the enteric parasites. Y.W.S. analysed the data and created the first draft of the work, to which all the authors then contributed.

Data accessibility

MHC data, standardized microsatellite heterozygosity data and pathogen data are available via Dryad (doi: 10.5061/dryad.6408r).

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1.1 Summary of MHC and pathogen studies on non-model species.

Fig. S2.1 The three MHC class II DRB haplotypes and five MHC class I haplotypes in the study population of European badgers (*Meles meles*), based on putatively functional sequences.

Fig. S3.1 Model averaged parameter estimates and their 95% confidence intervals for the 10 MHC predictors (presence/absence of five MHC class I and three MHC class II genes, class I and class II heterozygosity) associated with the prevalence of seven pathogens.

Fig. S3.2 Model averaged parameter estimates and their 95% confidence intervals for the 10 MHC predictors (presence/absence of MHC genes, class I and class II heterozygosity) associated with the infection intensity of eight pathogens.

Fig. S3.3 Model averaged parameter estimates and their 95% confidence intervals for the 10 MHC predictors (presence/absence of MHC genes, class I and class II heterozygosity) associated with the co-infection by 13 pathogens.

Table S4.1 The association of *Trypanosoma pestanai* infection prevalence (presence or absence) in badgers (number of badgers [n_b] = 198; number of samples [n_s] = 333) with (a) presence or absence of MHC class II-class I haplotypes and (b) haplotype heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S4.2 The association of *Trypanosoma pestanai* infection intensity in badgers (n_b = 198; n_s = 333) with (a) presence or absence of MHC class II-class I haplotypes and (b) haplotype heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S4.3 The association of Mustelid herpes virus infection intensity in badgers (n_b = 198; n_s = 333) with (a) presence or absence of MHC class II-class I haplotypes and (b) haplotype heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S4.4 The association of *Eimeria* infection prevalence (presence or absence) in badgers (n_b = 170; n_s = 258) with (a) presence or absence of MHC class II-class I haplotypes and (b) haplotype heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S4.5 The association of *Eimeria* infection intensity in badgers (n_b = 170; n_s = 258) with (a) presence or absence of MHC class II-class I haplotypes and (b) haplotype heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S4.6 The association of *Salmonella* infection prevalence (presence or absence) in badgers (n_b = 88; n_s = 137) with (a) presence or absence of MHC class II-class I haplotypes and (b) haplotype heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S4.7 The association of *Salmonella* infection intensity in badgers (n_b = 88; n_s = 137) with (a) presence or absence of MHC class II-class I haplotypes and (b) haplotype heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S4.8 The association of *Yersinia* infection prevalence (presence or absence) in badgers (n_b = 88; n_s = 137) with (a) presence or absence of MHC class II-class I haplotypes and (b) haplotype heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S4.9 The association *Yersinia* infection intensity in badgers (n_b = 88; n_s = 137) with (a) presence or absence of MHC class II-class I haplotypes and (b) haplotype heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S4.10 The association of *Campylobacter* infection prevalence (presence or absence) in badgers (n_b = 88; n_s = 137) with (a) presence or absence of MHC class II-class I haplotypes and (b) haplotype heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S4.11 The association of *Campylobacter* infection intensity in badgers (n_b = 88; n_s = 137) with (a) presence or absence of MHC class II-class I haplotypes and (b) haplotype heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals

and relative importance, after model averaging (models with ΔAICc < 7).

Table S4.12 The association of fleas infection prevalence (presence or absence) in badgers (n_b = 205; n_s = 388) with (a) presence or absence of MHC class II-class I haplotypes and (b) haplotype heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S4.13 The association of fleas infection intensity in badgers (n_b = 205; n_s = 388) with (a) presence or absence of MHC class II-class I haplotypes and (b) haplotype heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S4.14 The association of lice infection prevalence (presence or absence) in badgers (n_b = 205; n_s = 388) with (a) presence or absence of MHC class II-class I haplotypes and (b) haplotype heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S4.15 The association of lice infection intensity in badgers (n_b = 205; n_s = 388) with (a) presence or absence of MHC class II-class I haplotypes and (b) haplotype heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S4.16 The association of co-infection status in badgers (n_b = 79; n_s = 108) with (a) presence or absence of MHC class II-class I haplotypes and (b) haplotype heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S5.1 The association of *Trypanosoma pestanai* infection prevalence (presence or absence) in badgers with (a) presence or absence of alleles and (b) MHC genes heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S5.2 The association of *Trypanosoma pestanai* infection intensity in badgers with (a) presence or absence of alleles and (b) MHC genes heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S5.3 The association of Mustelid herpes virus infection intensity in badgers with (a) presence or absence of alleles and (b) MHC genes heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S5.4 The association of *Eimeria* infection prevalence (presence or absence) in badgers with (a) presence or absence of alleles and (b) MHC genes heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S5.5 The association of *Eimeria* infection intensity in badgers with (a) presence or absence of alleles and (b) MHC genes heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S5.6 The association of *Salmonella* infection prevalence (presence or absence) in badgers with (a) presence or absence of alleles and (b) MHC genes heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S5.7 The association of *Salmonella* infection intensity in badgers with (a) presence or absence of alleles and (b) MHC genes heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S5.8 The association of *Yersinia* infection prevalence (presence or absence) in badgers with (a) presence or absence of alleles and (b) MHC genes heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S5.9 The association *Yersinia* infection intensity in badgers with (a) presence or absence of alleles and (b) MHC genes heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S5.10 The association of *Campylobacter* infection prevalence (presence or absence) in badgers with (a) presence or absence of alleles and (b) MHC genes heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S5.11 The association of *Campylobacter* infection intensity in badgers with (a) presence or absence of alleles and (b) MHC genes heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S5.12 The association of fleas infection prevalence (presence or absence) in badgers with (a) presence or absence of alleles and (b) MHC genes heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S5.13 The association of fleas infection intensity in badgers with (a) presence or absence of alleles and (b) MHC genes heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence

intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S5.14 The association of lice infection prevalence (presence or absence) in badgers with (a) presence or absence of alleles and (b) MHC genes heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S5.15 The association of lice infection intensity in badgers with (a) presence or absence of alleles and (b) MHC genes heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S5.16 The association of co-infection status in badgers with (a) presence or absence of alleles and (b) MHC genes heterozygosity: Parameter estimates are provided with their unconditional standard errors (SE), 95% confidence intervals and relative importance, after model averaging (models with ΔAICc < 7).

Table S6.1 Kendall rank correlation coefficient (τ) between MHC heterozygosity and MHC class II−class I haplotype presence, in instances where effect was found for MHC heterozygosity and specific haplotype presence.