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MHC class II-assortative mate choice in European badgers (*Meles meles*)

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Abstract

The major histocompatibility complex (MHC) plays a crucial role in the immune system, and in some species, it is a target by which individuals choose mates to optimize the fitness of their offspring, potentially mediated by olfactory cues. Under the genetic compatibility hypothesis, individuals are predicted to choose mates with compatible MHC alleles, to increase the fitness of their offspring. Studies of MHC-based mate choice in wild mammals are under-represented currently, and few investigate more than one class of MHC genes. We investigated mate choice based on the compatibility of MHC class I and II genes in a wild population of European badgers (*Meles meles*). We also investigated mate choice based on microsatellite-derived pairwise relatedness, to attempt to distinguish MHC-specific effects from genomewide effects. We found MHC-assortative mating, based on MHC class II, but not class I genes. Parent pairs had smaller MHC class II DRB amino acid distances and smaller functional distances than expected from random pairings. When we separated the analyses into within-group and neighbouring-group parent pairs, only neighbouring-group pairs showed MHC-assortative mating, due to similarity at MHC class II loci. Our randomizations showed no evidence of genomewide-based inbreeding, based on 35 microsatellite loci; MHC class II similarity was therefore the apparent target of mate choice. We propose that MHC-assortative mate choice may be a local adaptation to endemic pathogens, and this assortative mate choice may have contributed to the low MHC genetic diversity in this population.

Keywords: Genetic compatibility hypothesis, major histocompatibility complex, MHC-assortative mating, pre- and postcopulatory mate choice, sexual selection

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Introduction

A central question in the study of sexual selection is what drives the choice of a particular mate over other mates. Indirect genetic benefits have been proposed to underpin mate choice, especially when direct benefits have not been detected (Zelano & Edwards 2002). In theory, females [which are often the choosier sex (Tregenza & Wedell 2000)] are predicted to select males based on 'good genes' or genetic compatibility (Trivers 1972; Neff & Pitcher 2005; Kempenaers 2007). Hamilton and Zuk's 'good genes' theory proposed that females select males carrying genes for resistance against the currently prevalent pathogens, to ensure that offspring will carry these good genes (Hamilton & Zuk 1982).

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The good genes hypothesis posits that all breeding individuals in the population will show consistent patterns of mate choice with respect to pathogen prevalence, irrespective of their own genotypes (Neff & Pitcher 2005). Genetic compatibility, by contrast, requires individuals to assess the genotype of potential mates by self-reference to their own genotype (Puurtinen *et al.* 2005).

In vertebrates, some of the assumed polymorphic genes for resistance in the Hamilton–Zuk model are MHC genes (Milinski 2014). The MHC is a diverse gene family that plays a crucial role in the adaptive immune system, as it encodes cell surface glycoproteins that bind and present antigens to T cells and trigger an immune cascade (Swain 1983). These MHC genes are associated with mate choice (Penn & Potts 1999); for example, MHC-based mate choice was first shown in mice, which preferred the odour of potential mates that had the most dissimilar MHC genes to their own (Yamazaki *et al.* 1976). Mating with dissimilar mates will, on average, increase the heterozygosity of offspring, and because pathogen recognition is mediated by the sequence of individual MHC alleles, MHC heterozygotes might have an advantage of being susceptible to fewer pathogens than homozygotes (McClelland *et al.* 2003). The MHC might therefore provide the basis on which individuals discriminate mating partners, to increase the fitness of their offspring (Penn & Potts 1999; Penn *et al.* 2002; Milinski 2006). While the arms race between pathogens and hosts maintains extreme MHC diversity (Jeffrey & Bangham 2000; Piertney & Oliver 2006), MHC-related reproductive mechanisms, such as mating preferences, selective fertilization and abortion, also generate MHC diversity (Ziegler *et al.* 2005; Løvlie *et al.* 2013). MHC genotypes can be detected through olfactory cues (Penn 2002; Leinders-Zufall *et al.* 2004; Milinski *et al.* 2005), or through other traits (e.g. ornamentation; vonSchantz *et al.* 1997; Dunn *et al.* 2012); thus, the MHC is a highly plausible target for mate choice through indirect genetic benefits.

Individuals could select MHC-similar or MHC-dissimilar mates, but availability of mating partners might also play a role, particularly in group-living species. A preference for MHC-similar males has been reported in a handful of species, potentially due to adaptation to local pathogen pressures (e.g. Yamazaki *et al.* 1978; Roberts *et al.* 2005; Bonneaud *et al.* 2006; Bos *et al.* 2009; Bollmer *et al.* 2012). MHC-disassortative rather than MHC-assortative mating is, however, more often detected (Kamiya *et al.* 2014). Choosing mates with dissimilar MHC can maximize MHC diversity (Ejsmond *et al.* 2014) and might consequently enhance disease resistance among offspring by the ability to recognize more pathogenic antigens (Doherty & Zinkernagel 1975; Penn *et al.* 2002). MHC-disassortative mating occurs in a variety of animals (e.g. Landry *et al.* 2001; Gillingham *et al.* 2009; Miller *et al.* 2009; Juola & Dearborn 2012). A recent meta-analysis of 116 effect sizes from 48 studies showed MHC-dissimilar mate choice only in species with multiple MHC loci examined (Kamiya *et al.* 2014). Mating with a dissimilar partner, with highly divergent MHC genes, might be risky, however, because disruption of co-adapted genes may reduce fitness (Kaufman 1999; Hendry *et al.* 2000; Neff 2004). Mate choice can, however, produce offspring that have optimal rather than maximal diversity at the MHC loci (Wegner *et al.* 2003; Milinski 2006). A meta-analysis suggested possible support for the optimizing hypothesis, but pointed out that this should be interpreted with care as it was based on 4 effect sizes from 4 species (Kamiya *et al.* 2014). The direction of MHC-based mate choice may be context dependent. For example, MHC-based mate choice might play a greater role under circumstances where an extra-pair or extra-group mate has been selected, compared to within-pair/group mate choice. If social mate choice is limited, individuals may settle for a social mate with less optimal MHC compatibility, and in these circumstances, they may be more likely to engage in extra-pair/group matings for MHC-compatible mates (Richardson *et al.* 2005; Schwensow *et al.* 2008).

The European badger (*Meles meles*) is well suited for investigating MHC-based mate choice in the wild, given their mating system, life history characteristics and reproductive biology. In high-density populations, *M. meles* is group living (Newman *et al.* 2011), with frequent contacts between neighbouring groups (Macdonald *et al.* 2008), and has a polygynandrous mating system (i.e. they do not have one exclusive social mate; Dugdale *et al.* 2007, 2011) with low fecundity (i.e. 1-2 cubs once a year; Macdonald & Newman 2002; Carpenter *et al.* 2005; Dugdale *et al.* 2007). Extra-group paternity accounts for >40% of offspring (Carpenter *et al.* 2005; Dugdale *et al.* 2007) with no subsequent paternal care (Dugdale *et al.* 2010). Although European badgers can conceive throughout the year, delayed implantation uncouples mating and parturition (Thom *et al.* 2004) and they give birth fairly synchronously around February (Yamaguchi *et al.* 2006). As female badgers are capable of superfoetation and embryo re-absorption (Yamaguchi *et al.* 2006), this extends the opportunity for females to select the most suitable mates, through pre- and postcopulatory mate choice (Andersson & Simmons 2006). Furthermore, we have found no evidence for clear mounting hierarchies in these badgers, that is male mounting frequency is not related to dominance rank; however, male mounting frequency does not correlate with paternity success (Dugdale *et al.* 2011). Promiscuous mounting may therefore promote sperm

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competition and selection for genetic compatibility through postcopulatory mate choice.

In the European badger, among the four MHC class II genes, the DRB gene is the most variable, with four putatively functional sequences identified (Sin *et al.* 2012c). Seven putatively functional MHC class I sequences have also been identified in the same study population (Sin *et al.* 2012b). In contrast to the highly diverse MHC genes in many other species (e.g. Robinson *et al.* 2003), the low variability of the DRB and class I genes allows the number of loci and haplotypes to be inferred, with at least two DRB loci and two class I loci (Sin *et al.* 2012b,c). This low MHC diversity therefore provides a good system with which to investigate MHC-based mate choice, in the broad sense of both pre- and postcopulatory mechanisms, because there are no complications that arise from analysing high allelic diversity (Richardson *et al.* 2005).

To test the genetic compatibility hypothesis, we examined whether badgers selected mates according to their MHC similarity (class II DRB and class I genes), taking into account the number of shared MHC alleles, the functional differences between alleles, and social-group membership. Given that badgers are group-living in high-density populations, and more related to within-group than to neighbouring-group members (Dugdale *et al.* 2008), MHC similarity is likely to vary with respect to group membership. Additionally, the decision to mate outside of the group may be context dependent, for example in relation to the compatibility of potential within-group mates. We therefore also analysed within-group and neighbouring-group parent pairs separately, in addition to the analyses that combined both within- and neighbouring-group parent pairs. To attempt to distinguish MHC-specific effects from genomewide effects, we then compared MHC similarity with that of potentially neutral microsatellite markers. Mating associated with MHC similarity but not microsatellite similarity would indicate that MHC similarity is the more likely target of mate choice than microsatellite-wide similarity.

Materials and methods

Study site and sample collection

We studied a high-density (44 badgers/km^2; Macdonald & Newman 2002; Macdonald *et al.* 2009) population of badgers in Wytham Woods, a 4-km^2 mixed coniferous–deciduous woodland in Oxfordshire, UK (51°46′26N, 1°19′19W). Trapping events have been typically undertaken three to four times a year since 1987 (for detailed methods, see Macdonald & Newman 2002). Upon first capture, each badger was tattooed with a unique number on their left inguinal region for permanent individual identification. The sex, age class (cub or adult, based on body size) and location (social group) of each badger were recorded. DNA samples were collected: guard hairs were plucked and stored in 80% ethanol, and approximately 3 mL of blood was taken by jugular venipuncture, collected in a vacutainer containing EDTA and stored at −20°C, until DNA was extracted.

Social group ranges were established using bait-marking techniques approximately every 2 years (Macdonald *et al.* 2008). The mean number of badger social groups in this population, 1987–2005, was 19 ± 2 SE (range = 14–26; Dugdale *et al.* 2008). These badgers typically exhibit high group fidelity; only 19% of individuals dispersed permanently to other social groups, although temporary intergroup movements do occur (16%, Macdonald *et al.* 2008). We defined the social group of residence of each individual per year, following the criteria of Macdonald *et al.* (2008): (a) badgers were assigned to the social group in which they were first trapped as a cub (i.e. natal group) unless they satisfied our dispersal rule (c); (b) badgers first caught as adults were assigned to the social group that they were trapped in most frequently unless dispersal events were recorded; and (c) badgers were recorded as dispersing if the 2 most recent captures, as well as at least 1 of 2 captures before that, were made in a different group to their resident social group.

DNA extraction and MHC gene amplification

Genomic DNA was isolated using the GFX Genomic Blood DNA Purification Kit (Amersham Biosciences, Little Chalfont, UK), or from a minimum of 20 hairs with visible follicles using a Chelex protocol (Walsh *et al.* 1991). We used published primers to amplify the regions that encode the antigen-binding domain in class II DRB [Meme-DRBex2, for the exon 2 (the forward primer spanned the boundary of intron 1 and exon 2); Sin *et al.* 2012c] and class I (Meme-MHCIex3, for the exon 3; Sin *et al.* 2012b) genes. Using these primers on 10–30 ng of gDNA, PCR amplification was performed in a 10-μL reaction mix that also contained: 0.5 μM of each primer, 200 μM of each dNTP, 1 × PCR buffer (containing $MgCl_2$; Qiagen, Hilden, Germany) and 1 unit of HotStarTaq (Qiagen). The PCR cycle began with incubation at 94°C for 15 min, followed by 35 incubation cycles at 94°C for 30 s, 59°C for 30 s and 72°C for 60 s, ending with an extension step at 72°C for 10 min.

MHC genotyping

We used reference strand-mediated conformation analysis (RSCA), which can detect genetic variants that differ

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at just a single nucleotide (Argüello *et al.* 1998a,b; Kennedy *et al.* 2005; Lenz *et al.* 2009). Fluorescent-labelled reference strands (FLRs) were generated using alleles from closely related species (American badger *Taxidea taxus*, polecat *Mustela putorius*, stoat *Mustela erminea* and mink *Neovison vison*). FLRs were generated by PCR using cloned alleles as templates and a 5′-FAM-labelled primer. Cloning and sequencing details are described in Sin *et al.* (2012b,c). The same PCR protocol, detailed in the MHC gene amplification section, was used to generate the FLRs, except that the primer proportion was altered to 0.5 μM FAM-labelled primer for Meme-DRBex2R and Meme-MHCIex3F, and 0.1 μM unlabelled primer for Meme-DRBex2F and Meme-MHCIex3R. Nine FLRs were evaluated for each of the class II DRB and the class I genes, to determine a subset that could best resolve all sequences in the control samples (13 and 12 cloned sequences for MHC class II and class I, respectively). A final set of three FLRs derived from the American badger, polecat and stoat were used for the DRB genes (GenBank Accession no.: KM371113–KM371115), and three FLRs derived from the American badger and mink for the class I genes (KM371116–KM371118).

All the resulting FLRs were diluted 1:5 in ddH_2O before use in the hybridization reactions. To form heteroduplexes, 2 μL diluted FLR and 2 μL PCR product (detailed in the MHC gene amplification section) were mixed and incubated in a thermal cycler at 95°C for 10 min, cooled down to 55°C at 1°C/s, hybridized at 55°C for 20 min, cooled to 15°C for 1 min and put on ice for 30 min. The plate was then stored at 4°C. Subsequently, 3 μL of hybridization product was mixed with 7.82 μL water and 0.18 μL Genescan Rox-500 size standard [Applied Biosystems (ABI), Foster City, USA] in a 96-well plate. The samples were then run on an ABI 3100 Genetic Analyzer, using 4% Genescan nondenaturing polymer (ABI), and visualized using matrix Dye set D. The running protocol used an injection voltage of 8 kV, injection time 15 s, run voltage of 15 kV and run temperature of 18°C. The heteroduplex peaks were identified using GENEMAPPER 3.7 (ABI), and their motilities were estimated relative to the ROX size standard. Control alleles from cloned plasmids were included in each run to control for variation between runs. Peaks with the same motility across different FLRs were designated as identical putative alleles. All seven class I alleles and four class II DRB alleles identified were sequenced (Sin *et al.* 2012b,c).

Microsatellite typing and parentage analyses

Individuals were genotyped using 35 microsatellite loci (Annavi *et al.* 2011). The loci were in Hardy–Weinberg and linkage equilibrium (see Dugdale *et al.* 2007; Annavi *et al.* 2014a). Details of candidate parent rules, genotyping procedures and parentage analysis are described elsewhere (Annavi *et al.* 2014a). Briefly, parentage and sibships were assigned with at least 80% confidence (accounting for genotyping error and unsampled individuals: see Annavi *et al.* 2014a) using MasterBayes 2.47 (Hadfield *et al.* 2006) and Colony 2.0 (Wang & Santure 2009), respectively. Candidate fathers included all sexually mature males (i.e. older than 1 year) present in the population in the year before the cub was born (due to delayed implantation; Yamaguchi *et al.* 2006). Candidate mothers included all females aged two or more resident in the cub's natal group in the year the cub was born. We defined a parent pair as a male badger and female badger that were assigned parentage of the same cub with at least 80% confidence.

As badgers may be present in the population but not caught in the year of mating (Dugdale *et al.* 2007), we included individuals as candidate parents for: (a) 2 years beyond their last capture, unless death was confirmed; and (b) the period between their consecutive captures. We analysed mate choice in the 6 years in which the most parent pairs were assigned. See Table S1 (Supporting information) for numbers of assigned parent pairs and candidate fathers in the different analyses. Assigned parent pairs that were resident in the same social group in the year in which the cubs were conceived were categorized as within-group pairs, whereas parents that were from neighbouring social groups were categorized as neighbouring-group pairs.

Data analysis

MHC similarity parameters. To examine whether assigned parent pairs had more (or less) similar MHC genes than expected under random mating, we examined MHC compatibility using both the extent of allele sharing and the magnitude of the functional differences between genotypes. The allele sharing value indicates the number of class I or II alleles shared between assigned parent pairs. To incorporate the functional similarity between class I and II alleles in the analysis, we also calculated the amino acid distance (proportion of amino acid sites that differed) from pairwise combinations of alleles in assigned parent pairs (Landry *et al.* 2001). We used the average amino acid distance (Landry *et al.* 2001; Forsberg *et al.* 2007; Miller *et al.* 2009) because individuals might carry different numbers of alleles, and hence, the number of pairwise comparisons could be different between parent pairs. We calculated the amino acid distance for both the entire exon sequences [exon 2 for DRB (285 bp) and exon

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2 + 3 for class I (546 bp); Sin *et al.* 2012b,c] and just the antigen-binding sites (ABS) in these exons (Sin *et al.* 2012b,c). The ABS is the basis of antigen recognition, so only variation in this region may be functionally important. We further analysed functional differences between alleles using the physiochemical properties of amino acids (Schwensow *et al.* 2007; Agbali *et al.* 2010). Each amino acid of the ABS was described using five *z*-descriptors: z_1 (hydorphobicity), z_2 (steric bulk), z_3 (polarity), and z_4 and z_5 (electronic effects) (Sandberg *et al.* 1998); these act as quantitative measures of functional differences that are important for variation in antigen-binding ability. A matrix (Doytchinova & Flower 2005) was constructed for the DRB and class I loci, respectively, and the Euclidean distance between alleles was calculated (Agbali *et al.* 2010). Although the MHC class I and class II genes are in linkage disequilibrium (Sin *et al.* 2014), they have different structure, function and expression patterns (Hughes & Yeager 1998) and could influence mate choice differently (Strandh *et al.* 2012); therefore, we analysed them separately.

Randomization tests. Randomization tests (Landry *et al.* 2001) enabled us to compare the mean allele sharing value, amino acid distance and functional distance for assigned parent pairs with the frequency distribution of mean values generated from 1000 simulations of the same number of randomly selected parent pairs. Specifically, we disassociated all parent pairs assigned in the parentage analyses and selected parent pairs at random (detailed in the next paragraph) with replacement. We then calculated mean values 1000 times to derive a distribution under random mating. We calculated *P*-values as the proportion of the total number of iterations greater or smaller than the observed mean (Fisher 1935). We applied a 2-tailed test with $\alpha = 0.05$; any values that fell outside of the 97.5–2.5% confidence interval (CI) were significant. We ran the randomizations separately for MHC class I and class II data sets. All statistical analyses were performed in R 2.15.0 (R Core Development Team 2011).

Forty-three per cent of the paternities were assigned to extra-group males (6 year data set: 98/230 assignments), of which 91% were neighbouring-group males (89/98). We therefore restricted our analyses of extra-group mating to assigned parent pairs from neighbouring-groups only. Likewise, during randomizations, random extra-group parent pairs were only selected from neighbouring-groups. We first compared the MHC similarity parameters of assigned parent pairs [by analysing within- and neighbouring-group parent pairs together, which produced 96% (221/230) of the cubs] with randomly paired assigned mothers and candidate fathers (irrespective of whether the candidate fathers were assigned paternity). This randomization included all within- and neighbouring-group candidate fathers and all candidate mothers that were assigned maternity. This randomization was weighted based on the probability of candidate mothers pairing with a within-group or neighbouring-group mate, according to the proportion of within- versus neighbouring-group paternity in a particular data set for each year (Table S1 in Supporting information). This was required to simulate random mating given that there is invariably a much greater availability of neighbouring males than within-group males; the rate of neighbouring-group mating would be inflated erroneously if the simulations were conducted without weighting.

In addition, badgers are more related to within-group than to neighbouring-group members (Dugdale *et al.* 2008) and within-group relatedness of assigned mothers and candidate fathers has a negative quadratic effect on the rate of extra-group paternity (Annavi *et al.* 2014b). We therefore examined the MHC similarity of assigned parent pairs and of randomly paired fathers and mothers in another two sets of randomizations according to social-group membership. First, we compared the MHC similarity parameters of assigned within-group parent pairs with within-group parents that were paired randomly. Our randomizations included all within-group candidate fathers (i.e. including males that were not assigned paternity, in addition to the assigned fathers). Second, we compared the MHC similarity of assigned neighbouring-group parents with random neighbouring-group pairs, where a random mate was selected for each assigned mother from all of their neighbouring candidate fathers.

These randomizations enabled us to compare the MHC similarity of each parent pair to that of the majority of males that a particular female could have mated with, according to social-group membership. Specifically, when females choose a (i) within- or neighbouring-group; (ii) within-group or (iii) neighbouring-group mate, we investigated whether they selected this mate based on MHC compatibility, given the (i) within- and neighbouring-group; (ii) within-group and (iii) neighbouring-group mates they could have chosen, respectively.

To test whether any observed MHC-based mating was a consequence of selection on wider genetic diversity, we also calculated the genetic similarity of assigned parent pairs based on 35 microsatellite loci (Annavi *et al.* 2011) using a pairwise relatedness measure *R* (Queller & Goodnight 1989). The mean pairwise relatedness of assigned parent pairs was compared with 1000 simulations of randomly selected parent pairs.

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We ran the randomizations of relatedness separately for MHC class I and class II data sets, using the same numbers of parent pairs as for the randomizations of MHC similarity parameters.

Combining probabilities. We assessed the overall significance of detecting larger or smaller mean values of the five genetic similarity measures (allele sharing, amino acid distance of the entire exon sequences, amino acid distance of the ABS, functional distance and relatedness), across years, using Fisher's method of combining probabilities for independent tests (Sokal & Rohlf 1994), by combining either left- or right-sided *P*-values ($\alpha = 0.025$). The parent pairs, analysed over 6 years, can be considered different reproductive events, because females can prevent males from mounting them (59/257 = 23% of mounting events were failed mountings; Dugdale *et al.* 2011), so they are not coerced into reproducing with the same male(s) each year (the behavioural mating system is polygynandrous, Dugdale *et al.* 2011). We analysed 116 females that were assigned maternity; 16 of these females were assigned the same mate in >1 year. We therefore excluded these repeated occurrences of parent pairs by including these parent pairs in only 1 year, which we selected at random, to meet the assumption of combining *P*-values from independent tests. Results are considered significant after false discovery rate control (Benjamini *et al.* 2001) for multiple tests (n = 10, $\alpha = 0.025$, adjusted *P*-value = 0.0025–0.025) using the same data.

Correlations between variables. We tested for associations between different parameters [i.e. allele sharing, relatedness, pairing type (within- or neighbouring-group) and year] and amino acid distance of assigned within-group and neighbouring-group parent pairs, to strengthen our hypothesis that MHC amino acid distance was the actual target of selection. We ran general linear mixed models, with four fixed main effects: two continuous (allele sharing and relatedness) and two categorical (pairing type and year). We modelled first-order interactions between pairing type, and allele sharing, relatedness and year. We did not include amino acid distance of the ABS, as it was highly correlated with amino acid distance of all sites [correlation coefficient = 1.0 (class II) and 0.97 (class I)]. We controlled for females that were assigned maternity in more than 1 year, and social groups with more than one assigned mother, by including individual identity and social group residency of assigned mothers as random effects. Model selection was based on Akaike's information criterion corrected for sample size (AICc, Akaike 1973). Models that better fit the data produce lower AICc values. Multimodel inference (Burnham & Anderson 2002) was performed for models with ΔAICc <7 (Burnham *et al.* 2011). We report model averaged parameter estimates and their 95% confidence intervals (Anderson 2008).

Results

MHC class II DRB genotypes were determined for 366 individuals (201 assigned parent pairs and their cubs) and MHC class I genotypes for 356 individuals (186 assigned parent pairs and their cubs). Candidate males that were not assigned parentage were also genotyped (250 and 263 individuals for DRB and class I genes, respectively) for the randomization analyses. We identified six class II DRB and 13 class I genotypes, from combinations of four class II DRB and seven class I putatively functional sequences (for sequence information, see Sin *et al.* 2012b,c). We inferred three DRB and five class I haplotypes (Fig. 1) from parentage assignments, based on the putatively functional sequences and assumption of Mendelian inheritance. These MHC genotyping results fit the parentage data, assuming Mendelian inheritance, suggesting we did not miss any MHC alleles stochastically. However, we could not rule out the possibility that we might not have amplified all MHC loci.

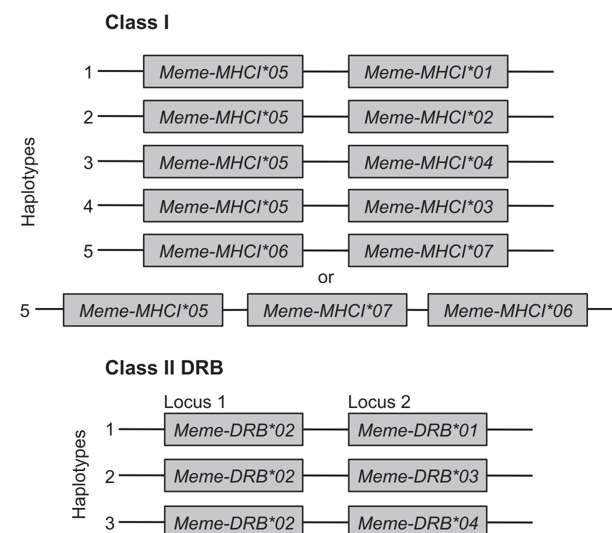

Fig. 1 The three MHC class II DRB haplotypes and five MHC class I haplotypes in the study population of European badgers (*Meles meles*), based on putatively functional sequences. It is uncertain whether the MHC class I haplotype 5 comprises *Meme-MHCI*05* or not. This is because *Meme-MHCI*05* was present in all examined individuals and the frequency of haplotype 5 was low in the population (0.9% in 1117 individuals; Sin *et al.* 2014), with no homozygote identified.

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(a) Combined data set (within- and neighbouring-group parents)

Assigned within-group and neighbouring-group parent pairs had smaller amino acid distances (both at all amino acid sites and the ABS only) and functional distance than random parent pairs, at the MHC class II DRB gene but not at the class I gene, over all years by combining probabilities from 6 years (Table 1). Year-to-year variation in these patterns occurred; for example, in four of 6 years, the assigned within-group and neighbouring-group parent pairs had smaller DRB amino acid distances, compared to random parent pairs, but it did not differ in the other 2 years (Fig. 2; Fig. S1 in Supporting information). MHC allele sharing did not differ between assigned and random parent pairs over all 6 years (Table 1; Fig. S1 in Supporting information). Assigned within-group and neighbouring-group parent pairs were less related than random within-group and neighbouring-group parent pairs overall (Table 1; Fig. S1).

(b) Within-group parents

Over all 6 years, assigned within-group parent pairs shared similar numbers of class I and class II alleles, similar amino acid distances and similar functional distances as randomized within-group pairs (Table 1; Figs S2, S3 in Supporting information). Although there was year-to-year variation (Fig. S2 in Supporting information) overall the results showed that assigned within-group pairs were less related than random within-group pairs (Table 1).

(c) Neighbouring-group parents

Assigned neighbouring-group parent pairs had smaller amino acid distances and functional distance at MHC class II genes than random neighbouring-group pairs, but they had similar amino acid distances at MHC class I genes, similar numbers of class I and II alleles, and similar relatedness to random neighbouring-group pairs (Table 1; Figs S2, S3 in Supporting information). Year-to-year variation was detected (Fig. S2 in Supporting information).

Correlations between variables

Greater MHC class I amino acid distances correlated with fewer shared alleles and lower relatedness of assigned within-group and neighbouring-group parent pairs (Fig. 3a). The MHC class I amino acid distances of assigned within-group and neighbouring-group parent pairs was similar in all years, except in 2010 when it was greater than in 1993 (Fig. 3a).

No significant association was found between the amino acid distances of the MHC class II DRB gene (all sites) and allele sharing (Fig. 3b). MHC class II DRB amino acid distances increased with relatedness for assigned within-group parent pairs, but the distances did not vary with relatedness for assigned neighbouring-group parent pairs (Fig. S4 in Supporting information). Amino acid distance in 2008 and 2010 was lower than in 1993 for within-group parent pairs compared to neighbouring-group parent pairs (Fig. 3b).

Discussion

We demonstrate MHC-assortative mate choice in badgers, based on the amino acid distance and functional distance of class II genes of within-group and neighbouring-group parent pairs. In addition, neighbouring-group parent pairs (but not within-group parent pairs) had MHC class II DRB alleles that were smaller in

Table 1 MHC-based and relatedness-based mate-choice results across 6 years (1993, 2004, 2005, 2008, 2009 and 2010), calculated using Fisher's method of combining probabilities (Sokal & Rohlf 1994)

Parent pairs	MHC gene	Allele sharing, χ^2_{12}	Smaller a.a. distance (all), χ^2_{12}	Smaller a.a. distance (ABS), χ^2_{12}	Smaller functional distance, χ^2_{12}	Lower *R*, χ^2_{12}
Combined	Class I	18.62 (higher)	25.42	24.55	16.43	**29.78***
	Class II	12.87 (higher)	**40.90*****	**40.43*****	**40.64*****	**28.36***
Within-group	Class I	20.54 (lower)	21.12	20.97	26.86	**44.81*****
	Class II	13.04 (lower)	21.90	21.35	21.38	**42.12*****
Neighbouring-group	Class I	16.27 (higher)	20.70	20.79	11.49	10.72
	Class II	12.23 (higher)	**32.32****	**39.00*****	**36.48*****	9.86

P-values are combined for a higher (or lower) number of shared alleles, smaller amino acid (a.a.) distance (all sites and ABS only), smaller functional distance and lower Queller and Goodnight's index of pairwise relatedness (*R*) of assigned parent pairs compared to randomly selected parent pairs. Significant results, after false discovery rate control (number of tests = 10, α = 0.025, adjusted *P*-value = 0.0025–0.025), are shown in bold (*$P < 0.005$, **$P < 0.002$, ***$P < 0.001$). See supporting information materials for yearly results.

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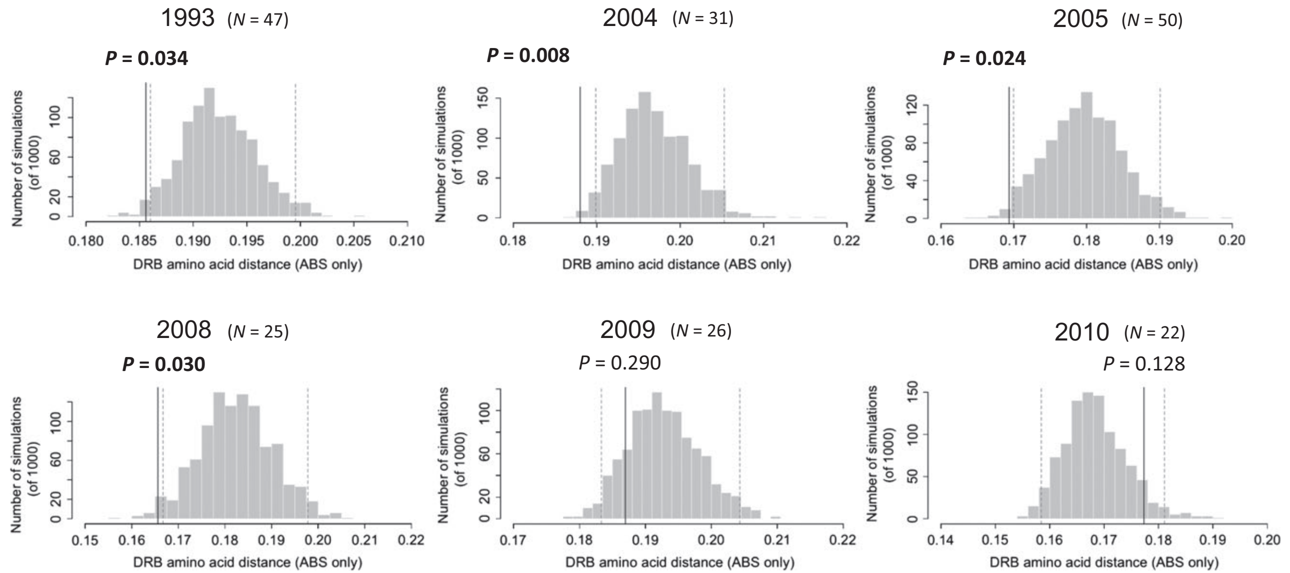

Fig. 2 Mean MHC class II DRB amino acid distances (ABS only) of assigned within-group and neighbouring-group parent pairs (solid line), compared to random within-group and neighbouring-group parent pairs. The frequency distributions (grey bars) are mean values generated from 1000 simulations of random pairings between all assigned mothers and all candidate fathers from both within the group and neighbouring groups. The two-tailed 95% confidence intervals (dashed lines) indicate cut-offs for significant departures from random mating. Randomizations were performed separately for each year: 1993, 2004, 2005, 2008, 2009 and 2010. *N* indicates the number of assigned parent pairs. Significant p-values are shown in bold.

amino acid distance and functional distance than expected from random neighbouring-group parents. In contrast to mate choice for similar MHC genes, parent pairs (both combined data set and within-group) exhibited disassortative mating at microsatellite loci. The different direction of this relationship with the microsatellite versus MHC class II similarity indicated that the smaller MHC amino acid distance of assigned parent pairs versus random pairs was not due to genomewide inbreeding. In combination, these MHC and microsatellite results suggest that MHC functional similarity is the actual target of female choice. Our results indicate that mate choice for MHC similarity might benefit offspring by having less divergent MHC alleles and maintaining co-adapted gene complexes (Kaufman 1999; Hendry *et al.* 2000; Neff 2004). Because cubs could only be trapped after they have emerged from below the ground when around 12 weeks old (Macdonald & Newman 2002), selective MHC-based mortality before emergence could not be ruled out, although this would not explain the within-/neighbouring-group differences we observed.

We found evidence that badgers selected neighbouring-group mates with reference to MHC class II gene similarity, but this was not observed in within-group pairs. Indeed, the structuring of the badger population into social groups might affect MHC-based mate choice (Huchard *et al.* 2010), because within-group relatedness was high in this population (Dugdale *et al.* 2008). Inbreeding avoidance was indicated by analyses based on randomization of within-group and neighbouring-group candidate mate partners, and the significance was much greater when only within-group parents were considered. Potentially, inbreeding avoidance occurred only when candidate mates were more highly related; which was the case in within-group compared to neighbouring-group pairs (Table 1; Fig. S2 in Supporting information; Dugdale *et al.* 2008). In addition, high relatedness between within-group assigned mothers and candidate fathers was correlated with a higher rate of extra-group paternity (Annavi *et al.* 2014b). Therefore, the apparent lack of MHC-assortative mate choice in within-group pairs could result from the high level of within-group relatedness and the potential inbreeding avoidance, which could override MHC-assortative mating. Alternatively, the lack of within-group MHC-based mate choice could be an artefact of data selection, if badgers that had fewer MHC-compatible within-group mates only mated outside of their group. However, when all parent pairs were considered in one analysis, MHC-assortative mate choice was detected.

The MHC-assortative mate choice that we demonstrated has been reported by only a few other studies (Yamazaki *et al.* 1978; Jordan & Bruford 1998; Roberts *et al.* 2005; Sommer 2005; Bonneaud *et al.* 2006; Bos *et al.*

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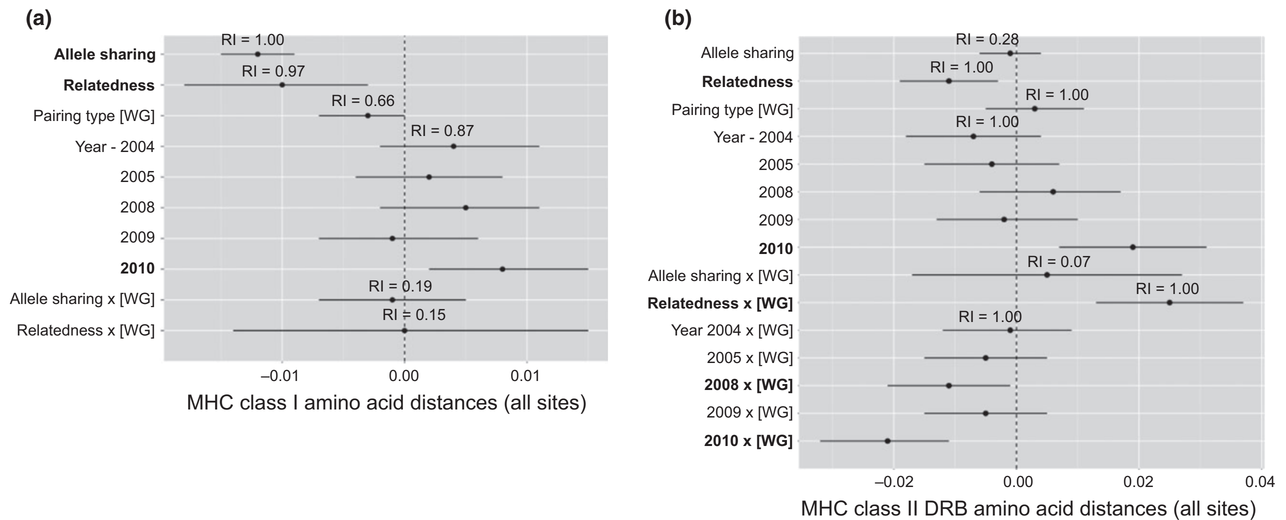

Fig. 3 The effect of allele sharing, Queller and Goodnight's index of pairwise relatedness, pairing type (within-group [WG] or neighbouring-group pair), year and parameter interactions on the amino acid distances (all amino acid sites) of (a) MHC class I and (b) MHC class II DRB genes of assigned within-group and neighbouring-group parent pairs. Parameter estimates are presented with their 95% confidence intervals (CI) and relative importance after model averaging (models with ΔAICc < 7). Estimates where the 95% confidence intervals do not overlap zero have their *y*-axis label in bold. Relative importance (RI) is the sum of Akaike weights for all models (ΔAICc < 7) including the parameter. Neighbouring-group pairs and 1993 were the reference categories for pairing type and year, respectively. For MHC class I data set, the interaction between pairing type and year was not included in multimodel inference after model selection for models with ΔAICc < 7.

2009; Yeates *et al.* 2009; Bollmer *et al.* 2012; Gasparini *et al.* 2015). MHC-assortative mate choice is consistent with mechanisms evolved to limit out-breeding depression, such that MHC-assortative mating (operating through behavioural choice and/or intrinsic physiological mechanisms) would prevent the production of offspring with highly MHC divergent genes, which are hypothesized to be less likely to survive. This survival disadvantage potentially operates through highly divergent MHC genes associated with a smaller T-cell repertoire (Lawlor *et al.* 1990; Nowak *et al.* 1992), increased risk of autoimmune diseases and disruption of local adaptations or co-adapted gene complexes (Kaufman 1999; Hendry *et al.* 2000; Neff 2004). Selection for local adaptation, particularly to endemic pathogenic challenges, could drive mate choice towards genetically similar individuals that carry specific MHC genotypes (Dionne *et al.* 2007). British badger populations have a reduced genetic diversity compared to other populations in mainland Europe due to a genetic bottleneck (Frantz *et al.* 2014), and this low genetic diversity may be further exacerbated by MHC class II-assortative mate choice. New MHC genetic variants, arising from mutation, recombination and immigration events, would be selected against through MHC-assortative mate choice if they were very divergent from those alleles already present in the population. Consequently, the frequency of common MHC alleles might be maintained or increased, and new rare alleles should not increase in frequency if sexual selection on this genetic variation was greater than natural selection, such as pathogen-mediated selection.

Studies of MHC-based mate choice can give different patterns depending on the MHC gene examined (Huchard *et al.* 2013). The divergent patterns of MHC class I and class II genes in contemporary sockeye salmon populations suggest different MHC genes might be responding to different selective pressures (McClelland *et al.* 2013). MHC-disassortative mating was reported based on MHC class II B but not class I genes in blue petrel (Strandh *et al.* 2012). The MHC-based mating observed in our study appears to be mediated by the MHC class II gene only, but not by the MHC class I gene. MHC class II molecules principally bind exogenous antigens, such as those derived from extracellular pathogens such as most bacteria, while MHC class I molecules present intracellular antigens (Hughes & Yeager 1998). Bacteria are known to contribute to the actual odour of the host, through metabolization of the scent gland secretion (Singh *et al.* 1990), offering a potential link between mate choice and MHC genes (Brown 1995). MHC-based odour differences have been proposed to be produced directly by MHC-specific microbial communities or by secondary metabolites generated by those bacteria (Penn & Potts 1998). Badgers exchange information about individual specific

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parameters through subcaudal gland scent (Buesching *et al.* 2002). If the microbiota found in the subcaudal gland secretion (Sin *et al.* 2012a) is affected by MHC genotypes, then the secondary metabolites generated could encode information on the host's MHC profile.

There were considerable annual fluctuations in the occurrence of MHC-based mate choice, either relating to real differences in interannual mate-choice criteria, or as an artefact of varying statistical power. We only analysed the 6 years in which the most cubs were produced, which is related to environmental conditions such as food availability (Macdonald *et al.* 2009); thus, we were unable to identify robustly which annual conditions favour MHC-based mating. Nevertheless, the implication is that MHC-based mating might have an environment-dependent basis if it only happens in specific years related to cub production, given that the 2 years in which the mate choice was not seen were in 2 of the 3 years with the fewest cubs. Consequently, other mechanisms, such as reproductive suppression, which in contrast to other species (e.g. Clutton-Brock *et al.* 2010) operate more strongly in years with high food availability in the study population (Woodroffe & Macdonald 1995), might also affect MHC-based mate choice. A further possibility is that MHC-based mate choice might be affected by ecological constraints such as interannual variation in disease risk (Jaatinen *et al.* 2012), and the specific conditions required for MHC-based mate choice might vary over time.

In conclusion, our evidence of MHC class II genes assortative mate choice in badgers contrasts with other research reporting MHC-dissimilar mating patterns. Importantly, the identification of MHC-based patterns depends on the MHC genes examined, mating group compositions and interannual criteria. This highlights the importance of examining both MHC class I and II genes, over a broad time series, within the context of the species' mating system, to inform the evolution of mate choice mechanisms.

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References

Agbali M, Reichard M, Bryjová A, Bryja J, Smith C (2010) Mate choice for nonadditive genetic benefits correlate with MHC dissimilarity in the rose bitterling (*Rhodeus ocellatus*). *Evolution*, **64**, 1683–1696.

Akaike H (1973) Information theory and an extension of the maximum likelihood principle. In: *Second International Symposium on Information Theory* (eds Petrov BN, Csaki F), pp. 267–281. Akademiai Kiado, Budapest.

Anderson DR (2008) *Model-Based Inference in the Life Sciences: A Primer on Evidence*, 2nd edn. Springer, New York.

Andersson MB, Simmons LW (2006) Sexual selection and mate choice. *Trends in Ecology & Evolution*, **21**, 296–302.

Annavi G, Dawson DA, Horsburgh GJ *et al.* (2011) Characterisation of twenty-one European badger (*Meles meles*) microsatellite loci facilitates the discrimination of second-order relatives. *Conservation Genetics Resources*, **3**, 515–518.

Annavi G, Newman C, Buesching CD *et al.* (2014a) Heterozygosity–fitness correlations in a wild mammal population: accounting for parental and environmental effects. *Ecology and Evolution*, **4**, 2594–2609.

Annavi G, Newman C, DugdaleHL HL *et al.* (2014b) Neighbouring-group composition and within-group relatedness drive extra-group paternity rate in the European badger (*Meles meles*). *Journal of Evolutionary Biology*, **27**, 2191–2203.

Argüello J, Little A, Pay AL *et al.* (1998a) Mutation detection and typing of polymorphic loci through double-strand conformation analysis. *Nature Genetics*, **18**, 192–194.

Argüello JR, Little AM, Bohan E *et al.* (1998b) High resolution HLA class I typing by reference strand mediated conformation analysis (RSCA). *Tissue Antigens*, **52**, 57–66.

Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I (2001) Controlling the false discovery rate in behavior genetics research. *Behavioural Brain Research*, **125**, 279–284.

Bollmer JL, Dunn PO, Freeman-Gallant CR, Whittingham LA (2012) Social and extra-pair mating in relation to major histocompatibility complex variation in common yellowthroats. *Proceedings of the Royal Society B*, **279**, 4778–4785.

Bonneaud C, Chastel O, Federici P, Westerdahl H, Sorci G (2006) Complex Mhc-based mate choice in a wild passerine. *Proceedings of the Royal Society B*, **273**, 1111–1116.

Bos DH, Williams RN, Gopurenko D, Bulut Z, Dewoody JA (2009) Condition-dependent mate choice and a reproductive disadvantage for MHC-divergent male tiger salamanders. *Molecular Ecology*, **18**, 3307–3315.

Brown RE (1995) What is the role of the immune system in determining individually distinct body odours? *International Journal of Immunopharmacology*, **17**, 655–661.

Buesching CD, Waterhouse JS, Macdonald DW (2002) Gas-chromatographic analyses of the subcaudal gland secretion of the European badger (*Meles meles*) Part I: chemical differences related to individual parameters. *Journal of Chemical Ecology*, **28**, 41–56.

Burnham KP, Anderson DR (2002) *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, 2nd edn. Springer, New York.

Burnham KP, Anderson DR, Huyvaert KP (2011) AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behavioral Ecology and Sociobiology* **65**, 23–35.

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Carpenter PJ, Pope LC, Greig C *et al.* (2005) Mating system of the Eurasian badger, *Meles meles*, in a high density population. *Molecular Ecology*, **14**, 273–284.

Clutton-Brock TH, Hodge SJ, Flower TP, Spong GF, Young AJ (2010) Adaptive suppression of subordinate reproduction in cooperative mammals. *American Naturalist*, **176**, 664–673.

Dionne M, Miller KM, Dodson JJ, Caron F, Bernatchez L (2007) Clinal variation in MHC diversity with temperature: evidence for the role of host-pathogen interaction on local adaptation in Atlantic salmon. *Evolution*, **61**, 2154–2164.

Doherty P, Zinkernagel R (1975) Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex. *Nature*, **256**, 50–52.

Doytchinova IA, Flower DR (2005) In silico identification of supertypes for class II MHCs. *The Journal of Immunology*, **174**, 7085–7095.

Dugdale HL, Ellwood SA, Macdonald DW (2010) Alloparental behaviour and long-term costs of mothers tolerating other group-members in a plurally breeding mammal. *Animal Behaviour*, **80**, 721–735.

Dugdale HL, Griffiths A, Macdonald DW (2011) Polygynandrous and repeated mounting behaviour in European badgers, *Meles meles*. *Animal Behaviour*, **82**, 1287–1297.

Dugdale HL, Macdonald DW, Pope LC, Burke T (2007) Polygynandry, extra-group paternity and multiple-paternity litters in European badger (*Meles meles*) social groups. *Molecular Ecology*, **16**, 5294–5306.

Dugdale HL, Macdonald DW, Pope LC, Johnson PJ, Burke T (2008) Reproductive skew and relatedness in social groups of European badgers, *Meles meles*. *Molecular Ecology*, **17**, 1815–1827.

Dunn PO, Bollmer JL, Freeeman-Gallant CR, Whittingham LA (2012) MHC variation is related to a sexually selected ornament, survival, and parasite resistance in common yellowthroats. *Evolution*, **67**, 679–687.

Ejsmond MJ, Radwan J, Wilson AB (2014) Sexual selection and the evolutionary dynamics of the major histocompatibility complex. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20141662.

Fisher RA (1935) *The Design of Experiment*. Hafner Press, New York.

Forsberg LA, Dannewitz J, Petersson E, Grahn M (2007) Influence of genetic dissimilarity in the reproductive success and mate choice of brown trout—females fishing for optimal MHC dissimilarity. *Journal of Evolutionary Biology*, **20**, 1859–1869.

Frantz AC, McDevitt AD, Pope LC *et al*. (2014) Revisiting the phylogeography and demography of European badgers (*Meles meles*) based on broad sampling, multiple markers and simulations. *Heredity*, **113**, 443–453.

Gasparini C, Congiu L, Pilastro A (2015) MHC-similarity and sexual selection: different doesn't always mean attractive. *Molecular Ecology*, doi:10.1111/mec.13222.

Gillingham MAF, Richardson DS, Løvlie H *et al.* (2009) Cryptic preference for MHC-dissimilar females in male red junglefowl, *Gallus gallus*. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 1083–1092.

Hadfield JD, Richardson DS, Burke T (2006) Towards unbiased parentage assignment: combining genetic, behavioural and spatial data in a Bayesian framework. *Molecular Ecology*, **15**, 3715–3730.

Hamilton WD, Zuk M (1982) Heritable true fitness and bright birds: a role for parasites? *Science*, **218**, 384–387.

Hendry AP, Burg JK, Bentzen P, Volk EC, Quinn TP (2000) Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. *Science*, **290**, 516–518.

Huchard E, Baniel A, Schliehe-Diecks S, Kappeler PM (2013) MHC-disassortative mate choice and inbreeding avoidance in a solitary primate. *Molecular Ecology*, **22**, 4071–4086.

Huchard E, Knapp L, Wang J, Raymond M, Cowlishaw G (2010) MHC, mate choice and heterozygote advantage in a wild social primate. *Molecular Ecology*, **19**, 2545–2561.

Hughes AL, Yeager M (1998) Natural selection at major histocompatibility complex loci of vertebrates. *Annual Review of Genetics*, **32**, 415–434.

Jaatinen K, Noreikiene K, Merila J, Öst M (2012) Kin association during brood care in a facultatively social bird: active discrimination or by-product of partner choice and demography? *Molecular Ecology*, **21**, 3341–3351.

Jeffrey KJ, Bangham RM (2000) Do infectious diseases drive MHC diversity? *Microbes and Infection*, **2**, 1335–1341.

Jordan WC, Bruford MW (1998) New perspectives on mate choice and the MHC. *Heredity*, **81**, 239–245.

Juola FA, Dearborn DC (2012) Sequence-based evidence for major histocompatibility complex-disassortative mating in a colonial seabird. *Proceedings of the Royal Society of London. Series B*, **279**, 153–162.

Kamiya T, O'Dwyer K, Westerdahl H, Senior A, Nakagawa S (2014) A quantitative review of MHC-based mating preference: the role of diversity and dissimilarity. *Molecular Ecology*, **23**, 5151–5163.

Kaufman J (1999) Co-evolving genes in MHC haplotypes: the 'rule' for nonmammalian vertebrates? *Immunogenetics*, **50**, 228–236.

Kempenaers B (2007) Mate choice and genetic quality: a review of the heterozygosity theory. *Advances in the Study of Behavior*, **37**, 189–278.

Kennedy LJ, Quarmby S, Fretwell N *et al*. (2005) High-resolution characterization of the canine DLA-DRB1 locus using Reference Strand-mediated Conformational Analysis. *Journal of Heredity*, **96**, 836–842.

Landry C, Garant D, Duchesne P, Bernatchez L (2001) Good genes as heterozygosity: the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). *Proceedings of the Royal Society of London. Series B*, **268**, 1279–1285.

Lawlor DA, Zemmour J, Ennis PD, Parham P (1990) Evolution of class-I MHC genes and proteins: from natural selection to thymic selection. *Annual Review of Immunology*, **8**, 23–63.

Leinders-Zufall T, Brennan P, Widmayer P *et al.* (2004) MHC class I peptides as chemosensory signals in the vomeronasal organ. *Science*, **306**, 1033–1037.

Lenz TL, Eizaguirre C, Becker S, Reusch TBH (2009) RSCA genotyping of MHC for high-throughput evolutionary studies in the model organism three-spined stickleback *Gasterosteus aculeatus*. *BMC Evolutionary Biology*, **9**, 57.

Løvlie H, Gillingham MAF, Worley K, Pizzari T, Richardson DS (2013) Cryptic female choice favours sperm from major histocompatibility complex-dissimilar males. *Proceedings of the Royal Society B*, **280**, 20131296.

Macdonald DW, Newman C (2002) Population dynamics of badgers (*Meles meles*) in Oxfordshire, UK: numbers, density

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and cohort life histories, and a possible role of climate change in population growth. *Journal of Zoology*, **256**, 121–138.

Macdonald DW, Newman C, Buesching CD, Johnson PJ (2008) Male-biased movement in a high-density population of the Eurasian badger (*Meles meles*). *Journal of Mammalogy*, **89**, 1077–1086.

Macdonald DW, Newman C, Nouvellet PM, Buesching CD (2009) An analysis of Eurasian badger (*Meles meles*) population dynamics: implications for regulatory mechanisms. *Journal of Mammalogy*, **90**, 1392–1403.

McClelland EE, Penn DJ, Potts WK (2003) Major histocompatibility complex heterozygote superiority during coinfection. *Infection and Immunity*, **71**, 2079–2086.

McClelland EK, Ming TJ, Tabata A *et al.* (2013) Patterns of selection and allele diversity of class I and class II major histocompatibility loci across the species range of sockeye salmon (*Oncorhynchus nerka*). *Molecular Ecology*, **22**, 4783–4800.

Milinski M (2006) The major histocompatibility complex, sexual selection, and mate choice. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 159–186.

Milinski M (2014) The past and the future of behavioral ecology. *Behavioral Ecology*, **25**, 680–684.

Milinski M, Griffiths S, Wegner KM *et al.* (2005) Mate choice decisions of stickleback females predictably modified by MHC peptide ligands. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 4414–4418.

Miller HC, Moore JA, Nelson NJ, Daugherty CH (2009) Influence of major histocompatibility complex genotype on mating success in a free-ranging reptile population. *Proceedings of the Royal Society of London. Series B*, **276**, 1695–1704.

Neff BD (2004) Stabilizing selection on genomic divergence in a wild fish population. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 2381–2385.

Neff BD, Pitcher TE (2005) Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Molecular Ecology*, **14**, 19–38.

Newman C, Zhou Y-B, Buesching CD, Kaneko Y, Macdonald DW (2011) Contrasting sociality in two widespread, generalist, mustelid genera, *Meles* and *Martes*. *Mammal Study*, **36**, 169–188.

Nowak MA, Tarczy-Hornoch K, Austyn JM (1992) The optimal number of major histocompatibility complex molecules in an individual. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 10896–10899.

Penn D, Potts W (1998) How do major histocompatibility complex genes influence odor and mating preferences? *Advances in Immunology*, **69**, 411–436.

Penn DJ (2002) The scent of genetic compatibility: sexual selection and the major histocompatibility complex. *Ethology*, **108**, 1–21.

Penn DJ, Damjanovich K, Potts WK (2002) MHC heterozygosity confers a selective advantage against multiple-strain infections. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 11260–11264.

Penn DJ, Potts WK (1999) The evolution of mating preferences and major histocompatibility complex genes. *American Naturalist*, **153**, 145–164.

Piertney SB, Oliver MK (2006) The evolutionary ecology of the major histocompatibility complex. *Heredity*, **96**, 7–21.

Puurtinen M, Ketola T, Katiaho JS (2005) Genetic compatibility and sexual selection. *Trends in Ecology and Evolution*, **20**, 157–158.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic-markers. *Evolution*, **43**, 258–275.

R Development Core Team (2011) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0.

Richardson DS, Komdeur J, Burke T, vonSchantz T (2005) MHC-based patterns of social and extra-pair mate choice in the Seychelles warbler. *Proceedings of the Royal Society B*, **272**, 759–767.

Roberts SC, Little AC, Gosling LM *et al.* (2005) MHC-assortative facial preferences in humans. *Biology Letters*, **1**, 400–403.

Robinson J, Waller MJ, Parham P *et al.* (2003) IMGT/HLA and IMGT/MHC: sequence databases for the study of the major histocompatibility complex. *Nucleic Acids Research*, **31**, 311–314.

Sandberg M, Eriksson L, Jonsson J, Sjöström M, Wold S (1998) New chemical descriptors relevant for the design of biologically active peptides. A multivariate characterization of 87 amino acids. *Journal of Medicinal Chemistry*, **41**, 2481–2491.

Schwensow N, Fietz J, Dausmann K, Sommer S (2008) MHC-associated mating strategies and the importance of overall genetic diversity in an obligate pair-living primate. *Evolutionary Ecology*, **22**, 617–636.

Schwensow N, Fietz J, Dausmann KH, Sommer S (2007) Neutral versus adaptive genetic variation in parasite resistance: importance of major histocompatibility complex supertypes in a free-ranging primate. *Heredity*, **99**, 265–277.

Sin YW, Annavi G, Dugdale HL *et al.* (2014) Pathogen burden, co-infection and major histocompatibility complex variability in the European badger (*Meles meles*). *Molecular Ecology*, **23**, 5072–5088.

Sin YW, Buesching CD, Burke T, Macdonald DW (2012a) Molecular characterization of the microbial communities in the subcaudal gland secretion of the European badger (*Meles meles*). *FEMS Microbiology Ecology*, **81**, 648–659.

Sin YW, Dugdale HL, Newman C, Macdonald DW, Burke T (2012b) Evolution of MHC class I genes in the European badger (*Meles meles*). *Ecology and Evolution*, **3**, 285.

Sin YW, Dugdale HL, Newman C, Macdonald DW, Burke T (2012c) MHC class II genes in the European badger (*Meles meles*): characterization, patterns of variation, and transcription analysis. *Immunogenetics*, **64**, 313–327.

Singh PB, Herbert J, Roser B *et al.* (1990) Rearing rats in a germ-free environment eliminates their odors of individuality. *Journal of Chemical Ecology*, **16**, 1667–1682.

Sokal RR, Rohlf FJ (1994) *Biometry: Principles and Practice of Statistics in Biological Research*, 3rd edn. W.H. Freeman, New York.

Sommer S (2005) Major histocompatibility complex and mate choice in a monogamous rodent. *Behavioral Ecology and Sociobiology*, **58**, 181–189.

Strandh M, Westerdahl H, Pontarp M *et al.* (2012) Major histocompatibility complex class II compatibility, but not class I, predicts mate choice in a bird with highly developed olfac-

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tion. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 4457–4463.

Swain SL (1983) T cell subsets and the recognition of MHC class. *Immunological Reviews*, **74**, 129–142.

Thom MD, Johnson DDP, Macdonald DW (2004) The evolution and maintenance of delayed implantation in the Mustelidae (Mammalia: Carnivora). *Evolution*, **58**, 175–183.

Tregenza T, Wedell N (2000) Genetic compatibility, mate choice and patterns of parentage: invited review. *Molecular Ecology*, **9**, 1013–1027.

Trivers RL (1972) *Parental Investment and Sexual Selection*. Aldine, Chicago.

vonSchantz T, Wittzell H, Goransson G, Grahn M (1997) Mate choice, male condition-dependent ornamentation and MHC in the pheasant. *Hereditas*, **127**, 133–140.

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques*, **10**, 506–513.

Wang J, Santure AW (2009) Parentage and sibship inference from multilocus genotype data under polygamy. *Genetics*, **181**, 1579–1594.

Wegner KM, Kalbe M, Kurtz J, Reusch TBH, Milinski M (2003) Parasite selection for immunogenetic optimality. *Science*, **301**, 1343.

Woodroffe R, Macdonald DW (1995) Female/Female Competition in European Badgers *Meles meles*: effects on Breeding Success. *Journal of Animal Ecology*, **64**, 12–20.

Yamaguchi N, Dugdale HL, Macdonald DW (2006) Female receptivity, embryonic diapause, and superfetation in the European badger (*Meles meles*): implications for the reproductive tactics of males and females. *Quarterly Review of Biology*, **81**, 33–48.

Yamazaki K, Boyse EA, Mike V *et al*. (1976) Control of mating preference in mice by genes in the major histocompatibility complex. *The Journal of Experimental Medicine*, **144**, 1324–1335.

Yamazaki K, Yamaguchi M, Andrews P, Peake B, Boyse EA (1978) Mating preferences of F2 segregants of crosses between MHC-congenic mouse strains. *Immunogenetics*, **6**, 253–259.

Yeates SE, Einum S, Fleming IA *et al*. (2009) Atlantic salmon eggs favour sperm in competition that have similar major histocompatibility alleles. *Proceedings of the Royal Society B*, **276**, 559–566.

Zelano B, Edwards SV (2002) An MHC component to kin recognition and mate choice in birds: predictions, progress, and prospects. *American Naturalist*, **160**, S225–S237.

Ziegler A, Kentenich H, Uchanska-Ziegler B (2005) Female choice and the MHC. *Trends in Immunology*, **26**, 496–502.

Y.W.S., H.L.D., C.N. and D.W.M. planned the study. Y.W.S., H.L.D., G.A., C.N. and C.D.B. 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 G.A. and H.L.D. genotyped the microsatellite loci. Y.W.S. analysed the data and created the first draft of the manuscript, to which all the authors then contributed.

Data accessibility

MHC and microsatellite genotyping data, parentage and social group information, and lists of candidate male mating partners are available via Dryad (doi:10.5061/dryad.d8080).

Supporting information

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

Table S1 The number of assigned within-group and neighboring-group parent pairs, total number of candidate fathers and mean number of candidate father per female in different years and MHC datasets.

Fig. S1 Mean MHC class I and class II DRB allele sharing, mean amino acid distance (all amino acid sites and ABS only) and mean Queller & Goodnight's index of pairwise relatedness (Q&G's *r*) of assigned within-group and neighboring-group parent pairs (solid line), compared to random within-group and neighboring-group parent pairs.

Fig. S2 Mean MHC class I (a–f) and class II DRB (g–l) allele sharing, mean amino acid distance (all amino acid sites and ABS only) and mean Queller & Goodnight's index of pairwise relatedness (Q&G's *r*) of assigned parent pairs (solid line).

Fig. S3 Mean MHC class I and class II DRB functional distance of combined (both within-group and neighboring group), within-group (WG), and neighboring-group (NG) assigned parent pairs (solid line), compared to random combined, within-group, and neighboring-group parent pairs, respectively

Fig. S4 Plots of MHC class II DRB amino acid distances (all sites) against Queller & Goodnight's index of pairwise relatedness, for assigned within-group and neighbouring-group parent pairs.

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