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No evidence for MHC class I-based disassortative mating in a wild population of great tits

I. SEPIL*, R. RADERSMA*, A. W. SANTURE†‡, I. DE CAUWER†§, J. SLATE† & B. C. SHELDON*

*Department of Zoology, Edward Grey Institute, University of Oxford, Oxford, UK
†Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK
‡School of Biological Sciences, The University of Auckland, Auckland, New Zealand
§Laboratoire de Génétique et Evolution des Populations Végétales, UMR CNRS 8198, Université des Sciences et Technologies de Lille-Lille 1, Villeneuve d'Ascq Cedex, France

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Abstract

Genes of the major histocompatibility complex (MHC) are regarded as a potentially important target of mate choice due to the fitness benefits that may be conferred to the offspring. According to the *complementary genes hypothesis*, females mate with MHC dissimilar males to enhance the immunocompetence of their offspring or to avoid inbreeding depression. Here, we investigate whether selection favours a preference for maximally dissimilar or optimally dissimilar MHC class I types, based on MHC genotypes, average amino acid distances and the functional properties of the antigen-binding sites (MHC supertypes); and whether MHC type dissimilarity predicts relatedness between mates in a wild great tit population. In particular, we explore the role that MHC class I plays in female mate choice decisions while controlling for relatedness and spatial population structure, and examine the reproductive fitness consequences of MHC compatibility between mates. We find no evidence for the hypotheses that females select mates on the basis of either maximal or optimal MHC class I dissimilarity. A weak correlation between MHC supertype sharing and relatedness suggests that MHC dissimilarity at functional variants may not provide an effective index of relatedness. Moreover, the reproductive success of pairs did not vary with MHC dissimilarity. Our results provide no support for the suggestion that selection favours, or that mate choice realizes, a preference for complimentary MHC types.

Introduction

Female mate choice is often invoked as a mechanism by which females improve their reproductive success and offspring quality (Mays & Hill, 2004). Genes of the major histocompatibility complex (MHC) have been linked to the evolution of mating preferences due to the potential genetic benefits that may be conferred to offspring through MHC-based mate choice (Brown & Eklund, 1994; Jordan & Bruford, 1998; Penn & Potts, 1999). MHC genes are a central component of vertebrate adaptive immune system, encoding glycoproteins that deliver foreign and self-peptides to the cell surface, to enable self- and non-self-identification by T cells, which then initiate an immune response against pathogens (Klein, 1986; Potts & Wakeland, 1990).

There are two main hypotheses explaining how MHC-based mate choice can act. The *good genes hypothesis* states that female mate choice should be based on male quality regardless of the female's own genotype, so that the female would guarantee resource gain or genetic benefits for the offspring, thus maximizing her own reproductive success (Hamilton & Zuk, 1982). Condition-dependent traits such as sexual secondary characters are predicted to be the cues for male quality,

Correspondence: Irem Sepil, Department of Zoology, Edward Grey Institute, University of Oxford, South Parks Road, Oxford OX1 3PS, UK.
Tel.: +44 1865 281999; fax: +44 1865 310 447; e-mail: irem.sepil@zoo.ox.ac.uk

and females could either prefer more MHC diverse or optimally diverse males, or seek mates carrying specific MHC types.

The alternative, but not exclusive, *complementary genes hypothesis* states that females seek genes that complement their own genes to optimize the genetic diversity and hence fitness of their offspring (Zeh & Zeh, 1996). A preference for MHC dissimilar mates may be an advantage for two reasons: either to maximize offspring MHC heterozygosity, or to avoid inbreeding. Offspring with maximal MHC diversity could cope with more parasites and have enhanced immunocompetence (heterozygote advantage: Doherty & Zinkernagel, 1975) or could be provided with a 'moving target' against rapidly evolving or recently introduced parasites (negative frequency dependence or fluctuating selection: Bodmer, 1972; Slade & McCallum, 1992; Hedrick, 2002). However, maximal MHC diversity may lead to excessive T-cell elimination during negative selection in the thymus (Nowak *et al.*, 1992; Woelfing *et al.*, 2009), and individuals possessing an intermediate number of MHC alleles may be at advantage under varying pathogenic pressures, having higher relative fitness (Madsen & Ujvari, 2006; Kalbe *et al.*, 2009). This would favour an intermediate, optimal, level of MHC dissimilarity between mates (Reusch *et al.*, 2001; Milinski, 2006). On the other hand, a preference for MHC dissimilar mates could also be an adaptation to avoid mating with a kin, MHC genes acting as a marker for assessing relatedness between individuals (Potts & Wakeland, 1990). Because MHC molecules are crucial for distinguishing self and non-self at the molecular and cellular level, it has been suggested that the role of MHC in recognition might have extended to higher biological levels, to enable kin and nonkin differentiation (Potts *et al.*, 1994; but see Sherborne *et al.*, 2007). According to the *inbreeding avoidance hypothesis*, selection should favour a preference for maximal MHC dissimilarity to avoid the deleterious consequences of kin mating (Brown, 1983; Brown & Eklund, 1994). Alternatively, a preference for intermediate dissimilarity could be under selection to optimize between outbreeding and inbreeding depression (Bonneaud *et al.*, 2006). There is increasing support for the suggestion that MHC constitution is olfactorily perceptible in a variety of taxa (Leinders-Zufall *et al.*, 2004; Milinski *et al.*, 2005; Radwan *et al.*, 2008); hence, individual odour profiles are often predicted to be the cues for MHC dissimilarity.

Major histocompatibility complex-based disassortative mating preferences have been reported across taxa, both in model species (Potts *et al.*, 1991; Ober *et al.*, 1997; Chaix *et al.*, 2008) and in wild populations of nonmodel species (Consuegra & Garcia de Leaniz, 2008; Juola & Dearborn, 2011; – but see Huchard *et al.*, 2010a). Although a number of studies suggested that females target MHC *per se*, by mating with MHC dissimilar (Landry *et al.*, 2001; Miller *et al.*, 2009) or optimally dissimilar males (Forsberg *et al.*, 2007; Eizaguirre *et al.*, 2009), other studies could not differentiate the role of inbreeding avoidance or MHC diversity on mate choice decisions (Freeman-Gallant *et al.*, 2003; Setchell *et al.*, 2010). The method chosen for differentiating between a preference for immunocompetence and inbreeding avoidance is also of great importance, and analyses that incorporate MHC dissimilarity and relatedness in a single model are necessary to clarify whether MHC *per se* is the target of mate choice (see Potts *et al.*, 1994; Thoß *et al.*, 2011). Moreover, to assess female mate choice accurately, studies should consider males that are geographically close to the female to be potential mates (given that she is likely to encounter them). Yet, spatial population structure is only rarely taken into account in tests assessing MHC-based mating decisions (but see Miller *et al.*, 2009). Such factors, if not corrected for, may confound or conceal links between MHC and mate choice. Likewise, few studies to date have investigated the fitness benefits associated with MHC-based disassortative mating, even though documenting such patterns is crucial to understand the relevance of MHC for mate choice in nature (but see Huchard *et al.*, 2010a; Brouwer *et al.*, 2010).

A final issue to consider in MHC studies is that the majority of work to date has investigated the link between MHC genotypes and mating preferences, although allelic differences between MHC alleles are not always functionally relevant, especially when alleles share similar antigen-binding motifs (Schwensow *et al.*, 2008). To assess the functional diversity of MHC alleles accurately, one should consider the predicted properties of their antigen-binding sites (Spurgin & Richardson, 2010). An approach based on clustering alleles with similar effects into supertypes is increasingly being employed in human and nonhuman primate studies, and supertypes are considered as the unit of selection to differentiate the phenotypic effect of the underlying MHC genes (Trachtenberg *et al.*, 2003; Naugler & Liwski, 2008; Huchard *et al.*, 2010b). Therefore, if females seek MHC dissimilar males to maximize MHC genetic diversity and enhance the immunocompetence of the offspring, it is possible that a preference based on functional MHC dissimilarity might be operating. However, in the context of inbreeding avoidance, MHC-based mate choice might depend on the alleles themselves, and not on the functionality of MHC variants. Still, it is unclear whether a preference based on functional MHC dissimilarity between mates could also be a reliable predictor of overall genetic similarity.

In the present work, we investigate the complementary genes hypothesis in a wild population of great tits (*Parus major*). We combine MHC class I allele, amino acid and supertype data with genomewide SNP data to test whether females choose mates based on (i) maximally dissimilar MHC alleles and supertypes, or (ii) optimally dissimilar MHC alleles and supertypes, while

controlling for relatedness between individuals. To account for the males that the female was likely to encounter, we control for spatial population structure in each model. We also test whether MHC allele sharing and supertype sharing are correlated with pairwise relatedness. Finally, to assess the relevance of MHC for female mate choice, we investigate whether the observed mating patterns correlate with reproductive success. We found no evidence of mate choice for MHC dissimilarity and no influence of parental MHC compatibility on pair reproductive success.

Materials and methods

Study population

We investigated MHC-based mate choice in a data set comprising 793 great tits captured over the 2006–2010 breeding seasons as part of a long-term study conducted at Wytham Woods, near Oxford, UK (51°46′N, 1°20′W). The c. 385-ha study site is mixed deciduous woodland, in which 1020 nest boxes are scattered at variable densities. The great tit is a small, short-lived passerine bird species resident in the UK. In this part of their range, great tits have a synchronous, annual breeding season (April–June), are single-brooded and both parents take care of the young until they fledge. Throughout the breeding season, all nest boxes were checked weekly to obtain records of lay date, clutch size, hatching date, brood size and number of fledglings. Breeding birds were captured between day 6 and 14 of the nestling phase, either within the nest box by hand or using traps, or with mist nets in front of the nest entrance. All adults and nestlings were processed and ringed with aluminium bands for individual recognition, and blood samples were collected from each adult under UK Home Office licence (PPL 30/2409). Blood was collected by wing or jugular venipuncture and stored in SET buffer at −80 °C until DNA extraction. Total genomic DNA was extracted using the standard ammonium acetate method and stored in AE buffer (Qiagen, Hilden, Germany).

Genetic analysis

A total of 1536 great tit samples were genotyped at the MHC class I exon 3 using 454 pyrosequencing. The methods for MHC class I characterization and genotyping are described in detail in Sepil *et al.* (2012); brief details are provided here. Ten forward and ten reverse fusion primers with individual multiplex identifier (MID) tags were designed and used in conjunction to amplify a 212- to 221-base pair fragment of exon 3 (the length of exon 3 varied between fragments due to indels). Amplifications were purified using the MinElute 96 UF PCR Purification Kit (Qiagen), and 16 sets of 96 individually tagged amplicons were pooled together in approximately equimolar quantities, to be loaded on the 16 lanes of the Pico Titer Plate gasket. The pools were sent for bidirectional 454 pyrosequencing at the Wellcome Trust Centre for Human Genetics, University of Oxford.

The genotyping experiment generated 638 501 reads; however, the final genotypes were based on the 214 357 reads that were retained following a five-step variant validation procedure (Sepil *et al.*, 2012). The variant variation procedure was designed to reliably differentiate real alleles from PCR/sequencing artefacts, to validate MHC variants and to remove individuals that had low read numbers, hence unreliable MHC genotypes. A total of 857 individuals passed our reliability criteria, and the repeatability of the experiment was calculated as 0.94 based on the 12 individuals genotyped twice. In total, 862 MHC class I alleles (GenBank: JQ034624–JQ035485) were detected, of which 39 were nonfunctional alleles bearing stop codons. Of these, 36 alleles had stop codons at the same locations and formed a monophyletic cluster with 68 other alleles that showed a dissimilar pattern of divergence from the rest of the alleles. These alleles were classified as putatively nonfunctional. The remaining 755 alleles were classified as functional. Each individual had between 9 and 32 functional MHC variants; therefore, we estimated that the great tit has at least 16 functional loci. There was a discrepancy in the number of alleles per individual, probably due to the variation in loci number within the species, allele sharing between loci and homozygosity at some loci (Sepil *et al.*, 2012). There was evidence for strong balancing selection among the putatively functional alleles, and the antigen-binding sites were found to be under positive selection according to Bayes empirical analysis (Sepil *et al.*, 2012). Lastly, the positively selected sites were translated into a matrix based on their physico-chemical properties (Doytchinova & Flower, 2005), and the matrix was subjected to a *K*-means clustering algorithm and model selection for identifying genetic clusters (Jombart *et al.*, 2010); the optimal number of supertypes was identified as 17 (Sepil *et al.*, 2012). Each individual had between 6 and 16 MHC supertypes. MHC allele/supertype similarity between females and males was calculated as the MHC allele or MHC supertype sharing value (D). $D = 2F_{AB}/(F_A + F_B)$ where F_A and F_B are the number of alleles/supertypes in individuals A and B, and F_{AB} is the number shared in both (Wetton *et al.*, 1987). We also calculated the average amino acid difference (AAdist) between males and females as a measure of sequence divergence (Landry *et al.*, 2001; Forsberg *et al.*, 2007; Miller *et al.*, 2009). AAdist was calculated for the entire 212- to 221-base pair fragment of exon 3.

In addition to MHC pyrosequencing, a proportion of individuals were also genotyped at 9193 single nucleotide polymorphisms (SNPs) on an Illumina iSelect BeadChip (Illumina, San Diego, CA, USA), according to

the manufacturer's protocol. Following genotyping, the 9193 SNPs were manually quality-checked in Genome Studio v2010.2 (Illumina). After excluding SNPs that did not result in clearly distinctive genotypes, identical by state (IBS) allele sharing was calculated using PLINK (Purcell *et al.*, 2007) across 8221 autosomal SNPs for each pair of the 2644 individuals successfully genotyped on the SNP chip (see van Bers *et al.*, 2012 for further details on the development and genotyping of the SNP chip). IBS values of each individual for which MHC genotypes are available (637 great tits) were extracted. IBS was chosen as a proxy to estimate relatedness because identical by descent-based methods estimating relatedness are typically restricted to a few hundred unlinked loci (e.g. see Wang, 2007). IBS measures were highly correlated ($r = 0.89$) with relatedness calculated from the social pedigree for this population, suggesting that IBS is an appropriate measure to estimate relatedness between breeding pairs (the social pedigree includes cases of paternal mis-assignment due to extra-pair paternity).

MHC and nonrandom mating

Randomization tests

Randomization procedure. We compared the MHC allele sharing, MHC supertype sharing and AAdist measures of 252 mated pairs with the distribution of these parameters under the null hypothesis generated using randomization procedures. We first performed a non-spatial randomization in which MHC-typed breeding males were randomly allocated to MHC-typed breeding females within years (2006: 65 males and 34 females; 2007: 117 males and 47 females; 2008: 193 males and 93 females; 2009: 133 males and 68 females; 2010: 63 males and 10 females). All MHC-typed males were included in the randomizations, whereas females were only included if they were part of an MHC-typed pair; hence, there were more males than females in the analysis.

Preference for maximal dissimilarity. To test whether females preferred males with maximal MHC dissimilarity, we calculated the average allele/supertype sharing and average AAdist for mated pairs within the population and compared it to the average allele/supertype sharing and average AAdist of the randomized mating pairs. We predicted that if females preferred males with maximal MHC dissimilarity, average allele/supertype sharing between mates would be significantly lower and average AAdist between mates would be significantly higher than in random pairs.

Preference for optimal dissimilarity. To test whether females preferred males with optimal MHC dissimilarity, we calculated the variance in allele/supertype sharing and AAdist for mated pairs within the population and compared it with that for randomized mating pairs. Here, we reasoned that if females preferred males with optimal MHC dissimilarity, then the variance in allele/supertype sharing and AAdist would be significantly lower in mated pairs than in randomized mating pairs.

Influence of relatedness. We compared the pairwise relatedness of mated pairs with the distribution of pairwise relatedness under the null hypothesis generated using randomization procedures. The data set was reduced to 203 pairs, because in 49 pairs, either the male or female of the pair had not been included in the genomewide SNP typing panel. We predicted that average relatedness between mates would be significantly lower than in random pairs, if females preferred males with maximal MHC dissimilarity to avoid the deleterious consequences of kin mating. Secondly, we predicted that if females preferred males with intermediate MHC dissimilarity to optimize between outbreeding and inbreeding depression, then the variance in relatedness would be significantly lower in mated pairs than in randomized mating pairs.

Controlling for relatedness. We controlled for background relatedness while investigating nonrandom MHC allele/supertype dissimilarity and AAdist between mates, and calculated standardized average allele/supertype sharing and AAdist and their variance. To do so, we computed the *z*-scores for relatedness, allele/supertype sharing and AAdist of the 203 mated pairs, by subtracting the mean and dividing it by the standard deviation. Then, we subtracted the *z*-scores for relatedness from the *z*-scores for allele/supertype sharing and AAdist. This was done to eliminate the influence of relatedness in MHC-based mate choice decisions. We predicted that if females preferred males with maximal MHC dissimilarity only to enhance the immunocompetence of their offspring, then the standardized average allele/supertype sharing between mates would be significantly lower and the standardized average AAdist between mates would be significantly higher than in random pairs. However, if females preferred males with optimal MHC dissimilarity only to achieve maximal pathogen resistance for their offspring, then the standardized variance in allele/supertype sharing and AAdist would be significantly lower in observed mating pairs than in randomized mating pairs. In all cases, we restricted randomizations within years. In each test, we performed 9999 randomizations, compared these to the value for the mated pairs and calculated *P*-values as two-sided tests.

Accounting for spatial structure

Great tit natal (Greenwood *et al.*, 1979) and breeding dispersal distances (Harvey *et al.*, 1979) are considerably smaller than the study area; the mean natal dispersal

distance for males is 674 m, and the mean natal dispersal distance for females is 911 m (Garant *et al.*, 2005). Therefore, there is likely to be variation in the probability that two individuals would encounter and form a breeding pair, and there is a possibility that this would be related to their degree of genetic similarity. Recent work has already revealed fine-scale spatial genetic structure in our study population (Garroway *et al.*, 2013). Thus, we performed spatially restricted randomizations as a means to generate null models that incorporated such spatial effects. We used GIS-derived measures of tit nest box coordinates to estimate the geographical locations of the breeding great tits (Wilkin *et al.*, 2007). Then, we used a floating grid permutation technique to account for space. A grid of squares of a given size was projected over a map of the research area. For each iteration, this grid was randomly moved and rotated. Breeding male identities were shuffled within each square, while breeding female identities and the locations of individuals were kept constant. Over a large number of iterations, the probability that two individuals were selected as a pair decreased near-linearly with distance.

We performed 9999 randomizations at 12 different spatial scales; hence, the area that the breeding female could choose a mate from varied in each analysis. The spatial scales (length of the edges of the squares) that we considered were 1, 50, 100, 200, 400, 600, 800, 1000, 1500, 2000, 5000 and 10 000 m, and the average number of mates that was randomized per female at these distances were 1, 1.08, 1.42, 2.72, 6.46, 10.79, 15.37, 20.00, 31.26, 43.30, 91.22 and 114.12. A distance of 1 m was included to specify the value of the observed pair. Again, we restricted randomizations within years, included the values for the observed mating pairs and calculated *P*-values as two-sided tests. Both the spatial and the nonspatial randomizations were performed in R (R Development Core Team, 2012), using the package 'fgpt' (available at CRAN).

MHC and reproductive success

We examined associations between MHC allele/supertype sharing values and reproductive success in the 252 pairs of great tits (each involving different pairs of individuals) that bred between the years 2006 and 2010. Twenty-four females bred twice with different males, 31 males bred twice with different females, and three females bred three times (each involving different males). We used generalized linear mixed effects models to investigate the effect of pair MHC dissimilarity on great tit reproductive success, measured at four consecutive stages of the breeding attempt. The specific reproductive measures we considered were the following: (i) clutch size, modelled as a Poisson response with a log link; (ii) brood size, modelled as a Poisson response; (iii) the number of young fledged (fledging success), a Poisson response, adjusted for overdispersion; and (iv) recruitment success, a binary variable indicating whether any of the young recruited to the population as an adult, modelled as a binomial response with a logit link. The reproductive measures were assessed in relation to the MHC allele or MHC supertype sharing value of the pair and local breeding density (measured as nest box densities at the nine Wytham woodland sections); the latter is known to significantly influence several measures of reproductive performance in the great tit (Bouwhuis *et al.*, 2009, 2010). Year was included as a random effect in each model to control for temporal environmental heterogeneity. In addition, we fitted clutch size as a covariate when modelling brood size, brood size as a covariate when modelling fledging success, and fledging success as a covariate when modelling recruitment success; hence, each analysis addressed the additive effect of MHC dissimilarity at that reproductive stage, controlling for any influence from preceding stages. We also repeated the same analyses without fitting the previous stage as a covariate to consider the total effect of parental MHC dissimilarity at the reproductive stage. Lastly, we fitted relatedness as a covariate and repeated each analysis. Inclusion of relatedness reduced the data set to 203 pairs. Models were performed using the R package 'lme4' (Bates *et al.*, 2012).

We used Akaike's information criterion (AIC) to determine the combination of variables that best explained the data with minimal parameters. Model selection was performed by backward stepwise elimination, and the fit of each new model was assessed by comparison of AIC values. Terms were eliminated from the model when their removal resulted in an improved fit (i.e. a < 2 reduction in AIC, Burnham & Anderson, 2002) and were retained if their removal resulted in an increase in AIC > 2. Where the removal of a term resulted in a model with an approximately equal fit (i.e. a change in AIC of < 2), the model with fewer terms was considered the most parsimonious model (Burnham & Anderson, 2002). To confirm the validity of the minimum model, removed variables were added individually to assess any potential improvement in the model fit.

Results

MHC and nonrandom mating

Major histocompatibility complex allele sharing values varied between 0.100 and 0.757, MHC supertype sharing values varied between 0.444 and 1.000, and SNP-based IBS relatedness values varied between 0.745 and 0.800 among mated pairs (Fig. S1). We found a weak but significant relationship between MHC allele sharing and SNP-based estimates of relatedness among mated pairs ($R^2 = 0.047$, $P = 0.002$) and a marginally nonsignificant relationship between MHC supertype

sharing and SNP-based estimates of relatedness (R^2 = 0.018, P = 0.053), implying that MHC allele sharing and supertype sharing are poor predictors of kinship (Fig. 1).

Results of the randomization tests yielded little support for an association between female mate choice and pair MHC allele or supertype dissimilarity. There was no difference in mean MHC allele sharing (Fig. 2a, nonspatial: P = 0.646) or variance in MHC allele sharing (Fig. 2b, nonspatial: P = 0.227) between mated and randomly assigned pairs. The same pattern was observed for mean MHC supertype sharing (Fig. 3a, nonspatial: P = 0.726) and variance in MHC supertype sharing (Fig. 3b, nonspatial: P = 0.554). Likewise, there was no difference in mean AAdist (Fig. 4a, nonspatial: P = 0.736) or variance in AAdist (Fig. 4b, nonspatial: P = 0.993); mean relatedness (Fig. 5a, nonspatial: P = 0.145) or variance in relatedness (Fig. 5b, nonspatial: P = 0.338) between mated and randomly assigned pairs. However, randomly assigned mates tended to be less similar to the observed mates in terms of SNP-based IBS relatedness as the spatial scale increased, suggesting a weak influence of

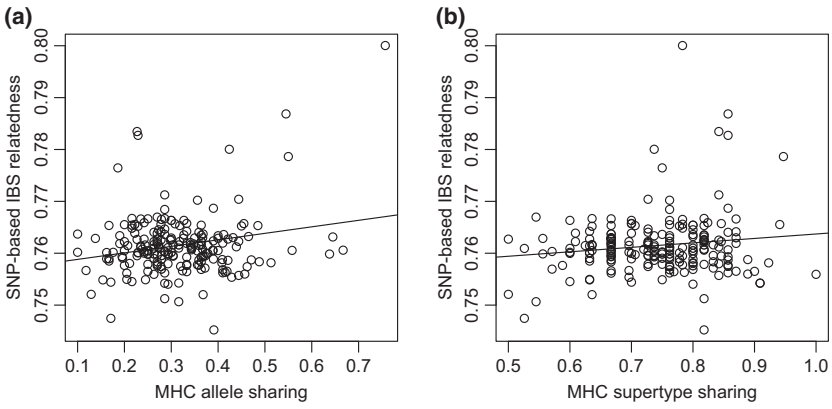

Fig. 1 Relationship between SNP-based identical by state (IBS) relatedness and (a) MHC allele sharing, (b) MHC supertype sharing for 203 mated pairs.

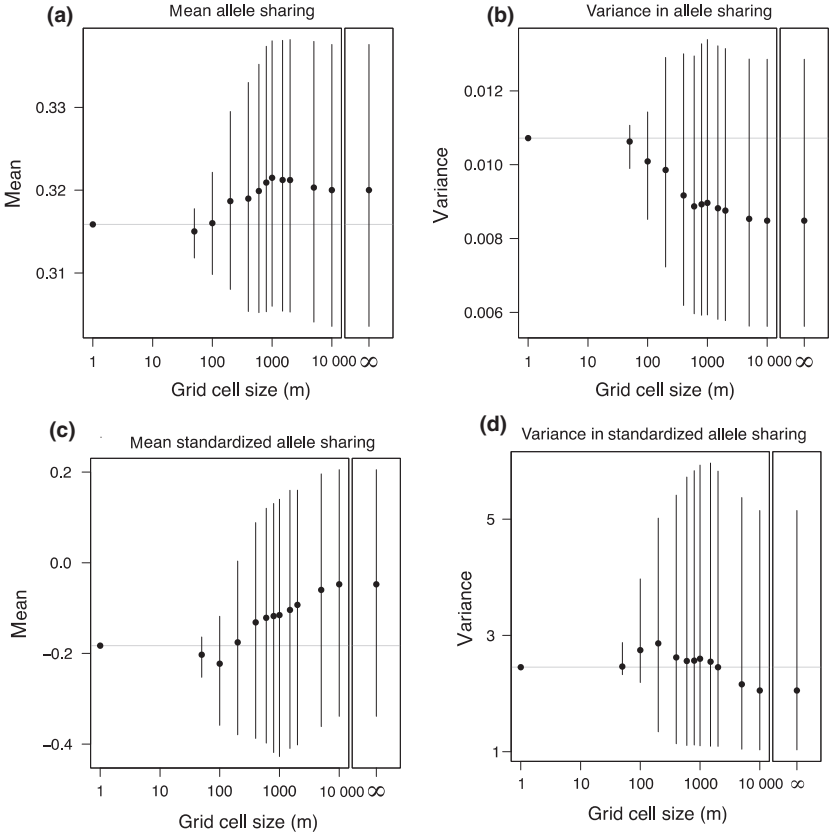

Fig. 2 Measures of MHC allele sharing in observed great tit pairs compared with the distribution of values generated from 9999 simulations at a range of spatial scales (1–10 000 m), including the entire population scale (∞, the nonspatial randomization). (a) Mean allele sharing, (b) variance in allele sharing, (c) mean standardized allele sharing and (d) variance in standardized allele sharing. Horizontal grey lines indicate the observed values. Bars indicate the 95% confidence interval in the simulated data sets.

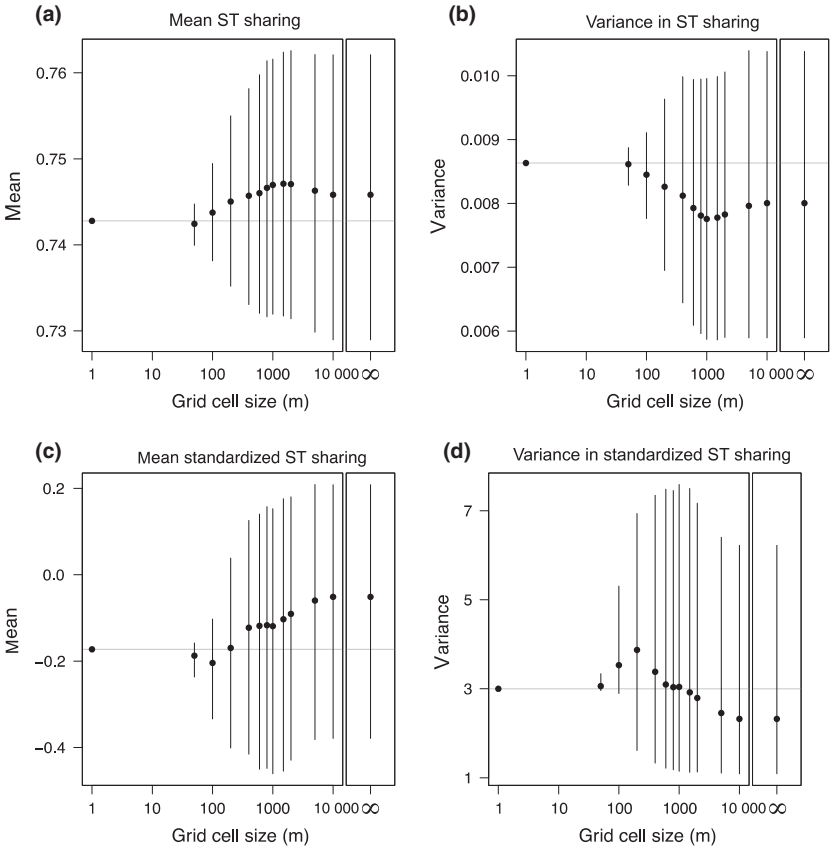

Fig. 3 Measures of MHC supertype (ST) sharing in observed great tit pairs compared with the distribution of values generated from 9999 simulations at a range of spatial scales (1–10 000 m), including the entire population scale (∞, the nonspatial randomization). (a) Mean supertype sharing, (b) variance in supertype sharing, (c) mean standardized supertype sharing and (d) variance in standardized supertype sharing. Horizontal grey lines indicate the observed values. Bars indicate the 95% confidence interval.

genetic population structure on the similarity of observed mating partners (Fig. 5a). A similar pattern to the unstandardized MHC sharing values was observed when MHC allele sharing and supertype sharing and AAdist were assessed while controlling for relatedness (Fig. 2c, nonspatial: $P = 0.313$; Fig. 3c, nonspatial: $P = 0.384$; Fig. 4c, nonspatial: $P = 0.226$). Likewise, the variance in standardized MHC allele sharing and supertype sharing and AAdist was not significantly different than would be expected under random mating (Fig. 2d, nonspatial: $P = 0.507$; Fig. 3d, nonspatial: $P = 0.455$; Fig. 4d, nonspatial: $P = 0.522$). Hence, there is, from these analyses, little evidence to suggest that females choose mates on the basis of maximal or optimal MHC dissimilarity.

MHC and reproductive success

Results of the mixed effect models revealed no evidence that the reproductive performance of pairs varied according to MHC dissimilarity. There was no effect of MHC allele sharing (Table S1) or MHC supertype sharing (Table S2) on clutch size, hatching success, fledging success or recruitment success. Reproductive performance did not vary as a function of nest box density in these models, but did vary significantly with the effect of the former stage (Tables S1 and S2). Adding relatedness as a covariate to the models did not change the results of any analysis. Likewise, removing the former reproductive stages from the models did not qualitatively change the results of any analysis (results not shown).

Discussion

Major histocompatibility complex genes are regarded as an important target of mate choice due to the genetic benefits that can be obtained by the offspring; however, it is still unclear whether selection favours a preference for genetic compatibility to enhance immunocompetence or avoid inbreeding, especially in wild populations. Here, we investigated the role that MHC class I allelic diversity and functional diversity (MHC supertypes) play in female mate choice decisions while controlling for relatedness and spatial population structure, and also examined the reproductive fitness consequences of MHC-dependent mating patterns in a wild great tit population. Our results offer little support for the hypotheses that females select males based on maximal or optimal MHC class I dissimilarity. Similarly, we did not detect any reproductive advantage for mating with MHC dissimilar males. Moreover, both MHC allelic similarity and supertype similarity were weak predictors

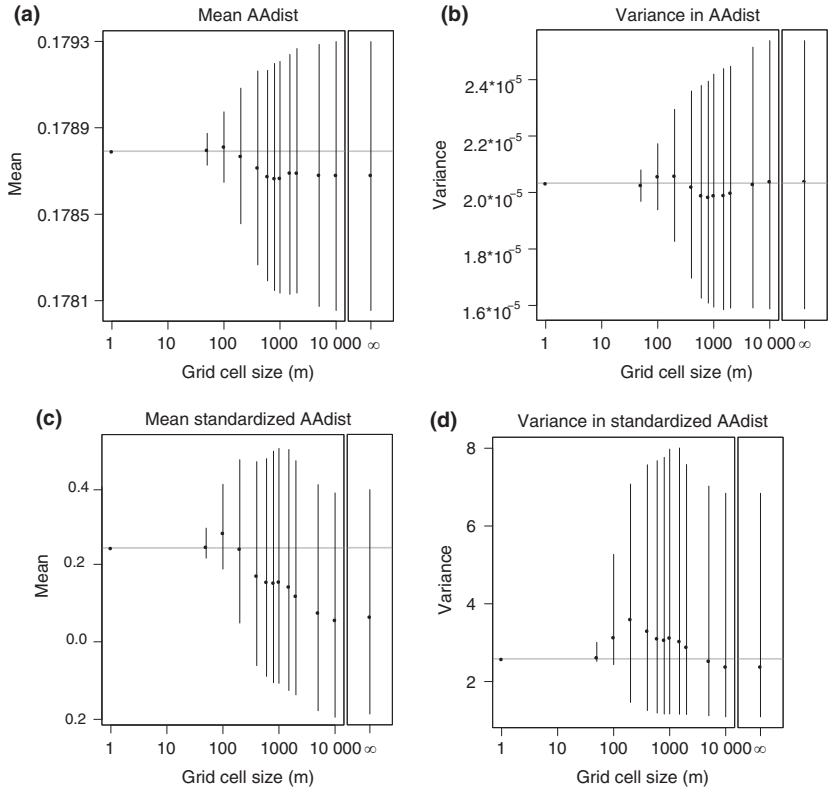

Fig. 4 Measures of MHC amino acid differences (AAdist) in observed great tit pairs compared with the distribution of values generated from 9999 simulations at a range of spatial scales (1–10 000 m), including the entire population scale (∞, the nonspatial randomization). (a) Mean amino acid differences, (b) variance in amino acid differences, (c) mean standardized amino acid differences and (d) variance in standardized amino acid differences. Horizontal grey lines indicate the observed values. Bars indicate the 95% confidence interval.

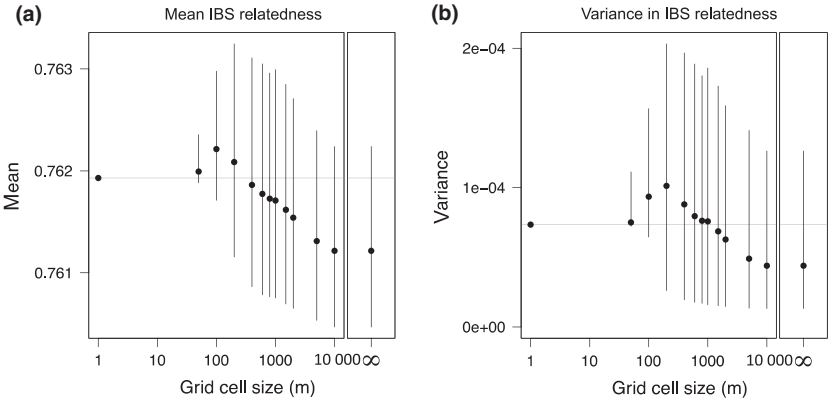

Fig. 5 Measures of SNP-based identical by state (IBS) relatedness in observed great tit pairs compared with the distribution of values generated from 9999 simulations at a range of spatial scales (1–10 000 m), including the entire population scale (∞, the nonspatial randomization). (a) Mean identical by state relatedness and (b) variance in identical by state relatedness. Horizontal grey lines indicate the observed values. Bars indicate the 95% confidence interval.

of relatedness, implying that diversity at MHC class I would be unlikely to act as a cue for kinship. Overall, we found no support for the hypothesis of MHC compatibility.

The results of the randomization tests varied slightly depending on the spatial scale chosen for encountering males. Because the breeding and foraging distances of animals are often expected to be smaller than study areas, it is important to control for spatial population structure, in order to provide a more direct assessment of female mate choice and to control for any bias that may arise as a result of spatial genetic structuring (Miller *et al.*, 2009). For instance, our analyses revealed a nonsignificant trend towards mating with more related individuals than expected when all mated males were compared with randomized pairs; however, the tendency disappeared when we restricted our comparisons to the males that were within few hundred metres. In the Wytham Woods great tits, this result is expected, because limited natal dispersal causes related individuals to occur closer together than nonrelated individuals while breeding (e.g. Szulkin & Sheldon, 2008; Szulkin *et al.*, 2009). Therefore, our results highlight the importance of considering fine-scale

genetic structure on small geographic distances (e.g. Garroway *et al.*, 2013), a phenomenon that has perhaps not been given enough consideration in other studies investigating mating patterns (though see Szulkin *et al.*, 2009).

Although a preference for maximally or optimally MHC dissimilar males has been documented in other avian species (Bonneaud *et al.*, 2006; Juola & Dearborn, 2011; Strandh *et al.*, 2012), we found no evidence to suggest that mate choice is based on genetic compatibility in this population of great tits, despite a slight tendency to mate with more MHC dissimilar males. Moreover, there was no indication of a reproductive advantage linked with MHC compatibility between mates, implying that MHC diversity of the progeny may have little effect on their survival to adulthood. In agreement with this finding, recent analyses have shown no fitness advantage for individuals possessing maximal or optimal functional diversity at the MHC in terms of adult survival, individual reproductive performance and lifetime reproductive success (Sepil *et al.*, 2013b). Hence, the absence of an association between MHC compatibility, female mate choice and reproductive success in the present study is perhaps not unexpected. Likewise, selection for MHC-based disassortative mating might be weak in the Wytham Woods great tit due to the outbred nature of the population. Although the fitness consequences of inbreeding depression are substantial, close inbreeding (i.e. mating with close kin where inbreeding coefficient is equal to or higher than 0.125) occurs rarely, in < 1.5% of all breeding events analysed over 41 years (Szulkin *et al.*, 2007). Moreover, no evidence was found to suggest birds avoid mating with related partners (Szulkin *et al.*, 2009); hence, our findings might reflect the lack of inbreeding avoidance. Finally, our analysis revealed a weak correlation between MHC supertype sharing and SNP-based IBS relatedness; therefore, it is plausible to suggest that functional diversity at MHC is unlikely to be a cue for assessing relatedness. The mechanisms by which MHC acts as a cue for kinship are not fully understood, but are likely to involve detection of differences in the MHC molecules produced by expressed genes (Howard, 1977). In that case, comparison of the functional properties of these molecules (as done here) is more relevant than a comparison of the sequence differences among alleles. However, it is also possible that the MHC alleles themselves might act as a cue for odour detection, influencing kin and nonkin differentiation (Singh *et al.*, 1987). In support of this argument, we found a weak but significant correlation between MHC allele sharing and SNP-based IBS relatedness. Yet, there was no evidence for MHC-dependent mate choice based on alleles to avoid mating with a kin.

A further possible explanation for the absence of a preference for MHC dissimilar males is that tits might be unable to discriminate the MHC profiles of conspecifics on the basis of odour. Experimental work on mammal and fish species has revealed compelling evidence that females use olfactory cues to evaluate the MHC genotype of their prospective mating partners (Reusch *et al.*, 2001; Milinski *et al.*, 2005; Radwan *et al.*, 2008); however, little is known about whether birds (especially passerines) could use MHC-related cues to choose compatible mates. Although birds have long been thought to be anosmic, a growing number of studies on different avian orders indicate that birds are able to use olfactory information in a variety of contexts (Balthazart & Taziaux, 2009). For instance, Strandh *et al.* (2012) have documented MHC class II-based disassortative mating in blue petrels (*Halobaena caerulea*), a bird with highly developed olfaction. Experimental studies investigating the link between MHC-based mate choice and individual odour profiles are necessary to clarify whether birds can assess the MHC content of potential partners using olfactory cues.

Major histocompatibility complex-based mating preferences may also target specific alleles, supertypes or male MHC diversity, regardless of females' own MHC, as predicted by the *good genes hypothesis*. Elsewhere, we have shown that the possession of specific MHC supertypes was strongly associated with avian malaria (*Plasmodium* spp.) resistance, adult survival and lifetime reproductive success (Sepil *et al.*, 2013a,b). Moreover, a recent meta-analysis of MHC-based mating preferences in nonhuman vertebrates revealed that the effect of MHC dissimilarity appears smaller than that of diversity in mate choice regardless of taxa (Kamiya *et al.*, 2014). Overall, the authors detected small effect sizes for diversity- and dissimilarity-based mate choice and concluded that a sample size of 243 choosing individuals' is required to detect nonrandom MHC dissimilarity with multiple loci (assuming statistical power of 80% and alpha-level of 5%). In this study, the sample sizes are sufficient (793 individual great tits and 252 choosing females'), to conclude that great tits do not prefer males that are dissimilar at MHC class I as their social mate. However, in the present study, we were unable to test preferences for certain alleles, supertypes and diversity, because all the males in our data set were part of a breeding pair and we did not screen nonmated males in the population. Sampling males that failed to form a breeding pair and examining the probability of pair formation as a function of male MHC diversity and possession of specific alleles/supertypes would be ideal for testing the good genes hypothesis and is an area that awaits further exploration.

In this study, we only investigated the role that MHC class I dissimilarity plays in mate choice decisions; hence, we cannot discount the possibility that a linkage between MHC class II loci and mate choice may exist. Strandh *et al.* (2012) investigated MHC-based disassortative mating for both class I and class IIB loci and revealed nonrandom MHC class II dissimilarity between

blue petrel mating pairs. However, there was no such pattern for MHC class I, and the authors concluded that this could be due to the different targets of MHC class I (intracellular parasites) and MHC class II (extracellular parasites). Infections with intracellular parasites (*Plasmodium* spp., *Haemoproteus* spp., *Leucocytozoon* spp. and avian pox) have previously been recorded in the Wytham Wood tit population, and both *Plasmodium* and avian pox infections are associated with reduced survival and reproductive output (Lachish *et al.*, 2011, 2012; Sepil *et al.*, 2013b). However, little is known about the extracellular parasites infecting the tits and an exhaustive parasite screening effort would be required to identify these.

Finally, it should also be emphasized that despite their social monogamy, great tit extra-pair copulations are frequent in the population, and 13% of nestlings are reported to be extra-pair young (Patrick *et al.*, 2012). Extra-pair copulations occur regularly in passerine bird species that form social mating pairs (Birkhead & Møller, 1992; Griffith *et al.*, 2002) and can act to enhance the genetic quality of offspring, when females are restricted in their choice of a social mate (reviewed in Jennions & Petrie, 2000). Therefore, the absence of MHC-based social mate choice in the great tit might be due to the occurrence of extra-pair copulations, if females engage in extra-pair mating to correct for any genetic disadvantage gained from paring up with a suboptimal male. For instance, Gohli *et al.* (2013) have recently shown a positive relationship between MHC class IIB functional diversity and population promiscuity in a comparative analysis of passerine birds and suggested that female promiscuity is a driving force maintaining MHC genetic diversity through balancing selection (but see Spurgin, 2013). Evidence for MHC-based extra-pair copulations has been provided in several wild bird studies (Freeman-Gallant *et al.*, 2003; Richardson *et al.*, 2005; Promerová *et al.*, 2011; but see Bollmer *et al.*, 2012). Particularly, Richardson *et al.* (2005) showed that females were more likely to obtain extra-pair paternity when their social mate had low MHC diversity, but failed to detect any association between MHC and choice of social mate in the Seychelles warbler (*Acrocephalus sechellensis*). Moreover, in a follow-up study, Brouwer *et al.* (2010) revealed a positive association between MHC diversity and juvenile survival and argued that this pattern was indicative of the hidden genetic benefits gained through extra-pair copulations. In this study, we could not examine MHC-dependent extra-pair mating preferences and their fitness benefits; hence, the possibility of hidden genetic benefits for offspring could not be tested.

To our knowledge, this study is among the first to test MHC-based nonrandom mating patterns while controlling for relatedness and population genetic structure and to investigate the genetic benefits associated with MHC-based disassortative mating in a wild population. Our results indicate that great tits do not prefer males that are dissimilar at MHC class I as their social mate, and there is no fitness benefit associated with mate choice. Therefore, we found no evidence supporting the suggestion that selection favours preference for complimentary MHC types.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Frequency distributions of (a) MHC allele sharing, (b) MHC supertype sharing, and (c) SNP-based

identical by state relatedness among observed mated pairs.

Table S1 Results of model selection based on AIC for mixed effect models examining the influence of MHC allele sharing on four stages of reproductive performance.

Table S2 Results of model selection based on AIC for mixed effect models examining the influence of MHC supertype sharing on four stages of reproductive performance.

Data deposited at Dryad: doi: 10.5061/dryad.gd5qb

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