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Abstract

Sexual selection has been proposed as a force that could help maintain the diversity of major histocompatibility complex (MHC) genes in vertebrates. Potential selective mechanisms can be divided into pre-copulatory and post-copulatory, and in both cases the evidence for occurrence is mixed, especially in natural populations. In this study, we used a large number of parent-offspring trios that were diplotyped for MHC class II genes in a wild population of Soay sheep (*Ovis aries*) to examine whether there was within-trio post-copulatory selection on MHC class II genes at both the haplotype and diplotype levels. We found there was transmission ratio distortion of one of the eight MHC class II haplotype (E) which was transmitted less than expected by fathers, and transmission ratio distortion of another haplotype (A) which was transmitted more than expected by chance to male offspring. However, in both cases these deviations were not significant after correction for multiple tests. In addition, we did not find any evidence of post-copulatory selection at the diplotype level. These results imply that, given known parents, there is no strong post-copulatory selection on MHC class II genes in this population.

Introduction

The major histocompatibility complex (MHC) is one of the most variable gene families in the vertebrate genome. Classical MHC genes are an essential component of the adaptive immune system and comprise two main classes of genes (class I and II) that are responsible for the recognition and presentation of foreign antigens. Class I-encoded molecules are expressed on all nucleated somatic cells primarily involved in presenting endogenously derived peptides to CD8+ cytotoxic T cells. Class II-encoded molecules are expressed on antigen-presenting cells and are primarily involved in presenting exogenously derived peptides to CD4+ T cells. Pathogen-mediated balancing selection is thought to be the main force maintaining the diversity of MHC genes, but sexual selection is considered to be an important mechanism in some species. MHC genes could be under sexual selection because parents are selected to optimize the immunity of their offspring or because MHC genes are used as a proxy for certain sexually selected traits (1-6).

MHC-dependent sexual selection could occur at both the pre-copulatory and post-copulatory stages, based on different aspects of MHC genes including selection favouring or disfavouring specific alleles, selection favouring more and/or more diverse MHC alleles, and MHC compatibility (similarity/dissimilarity) between partners. A meta-analysis including studies on both pre-copulatory and post-copulatory selection across non-human vertebrates supports female choice for MHC diversity and choice for MHC dissimilarity regardless of which sex chooses (7). Pre-copulatory sexual selection in the form of MHC-dependent mate choice has been reported in a wide range of vertebrate taxa in natural populations including fishes (8, 9), reptiles (10), birds (11, 12), and mammals (13, 14).

MHC-dependent post-copulatory selection may also play an important role in shaping MHC diversity in some species, and this could occur at two stages, either before fertilization, through sperm competition or cryptic female choice, or after fertilization through mother-foetus interactions (15). The "Sperm receptor selection hypothesis" has been proposed to explain selection before fertilization (16). Although the expression of MHC genes in spermatozoa or oocytes is controversial, with both positive and negative evidence, linkage disequilibrium between odorant receptor genes and MHC genes could still contribute to the recognition between spermatozoa and oocytes (15, 17). Thus, in either a polyandrous mating system or within a sire, specific spermatozoa could be selected for fertilization based on their MHC haplotype. After fertilization, females could allocate more energetic resources to genetically "preferred" embryos which could induce MHC-dependent sexual selection (18, 19). Finally, the similarity between maternal and foetal MHC genes could result in selective abortion of embryos. For example in a Hutterite population, significantly increased foetal loss rates were observed among couples with identical MHC haplotypes (20, 21).

MHC-dependent post-copulatory selection has mostly been studied experimentally and different studies have focused on different stages and produced mixed results. At the pre-fertilization stage, some experimental studies of fishes demonstrated cryptic female choice favouring sperm from MHC-similar males (22, 23) while another study of red junglefowl (*Gallus gallus*) found sperm from MHC-dissimilar males were favoured (24). Sperm selection within a sire has also been investigated. Some experimental studies found no evidence of MHC-dependent gamete fusions (25, 26) while other studies have reported haplotype-specific fertilization bias toward gametes with complementary MHC genes (27-29).

Even fewer studies have investigated post-copulatory selection in semi-natural or natural populations, and again the results are equivocal. First, some studies used behavioural observations combined with molecular parentage data to examine post-copulatory selection caused by cryptic female mate choice or sperm competition between different males. For example, fathers were reported to have more MHC supertypes (MHC variants with similar physicochemical properties) different from those of the mother than randomly assigned males in a population of grey mouse lemur (*Microcebus murinus*), although such a deviation was not observed in behavioural data (30). Other studies have used molecular parentage data to study within-trio post-copulatory selection. For example in a semi-natural rhesus macaque (*Macaca mulatta*) colony, although there was no evidence for post-copulatory selection against MHC-homozygous individuals, the distribution of paternally and maternally inherited MHC haplotypes tended to differ from expected (31). A similar pattern was observed in a lesser kestrel population (*Falco naumanni*) at the allele level: an MHC supertype including two common alleles showed significant transmission ratio distortion when inherited from males but not from females (32). However, in a semi-natural population of mandrills (*Mandrillus sphinx*), no evidence of post-copulatory selection on MHC genes was found (33). As experimental studies cannot reflect the complexity of the natural environment, more studies in natural populations are needed to understand the generality of patterns of post-copulatory selection on MHC genes.

Here, we used an unmanaged population of Soay sheep (*Ovis aries*) living on the island of Hirta, St Kilda, to study post-copulatory selection on MHC genes. Since 1985, a large number of sheep have been individually followed from birth to death and a multigenerational pedigree covering nearly all studied individuals has been constructed using genome-wide SNP genotypes. A previous study using MHC-linked microsatellite markers of several hundred individuals born between 1985 and 1994 found all loci were in Hardy-Weinberg proportions and strong evidence of balancing selection (34). Recently, eight functional MHC class II haplotypes were identified in this population using sequence-based genotyping (35). Using 13 selected SNPs in the MHC class II region, we successfully characterized MHC class II diplotypes, variants of all possible combinations of the MHC haplotypes that exist in the population, in 5349 sheep and found that the data are in Hardy-Weinberg equilibrium (36, 37). Combining the MHC class II genotyping with the pedigree information, we identified a large number of trios with offspring and both parents successfully diplotyped for MHC genes. Using these trios, we tested within-trio post-copulatory selection on MHC class II haplotypes in Soay sheep by answering several questions using different parental groups classified by their MHC class II diplotypes: 1) Is there selection against homozygote offspring? 2) Is there selection against offspring which have an identical diplotype to their mother? 3) Is there selection favouring offspring with more divergent MHC class II haplotypes? 4) Is any specific MHC class II haplotype favoured? 5) Is there transmission ratio distortion of specific MHC class II haplotypes from fathers or mothers or to male or female offspring?

Methods

Study population and parentage data

The Soay sheep population used in this study has lived on the island of Soay, in the St. Kilda archipelago for many centuries. In 1932, 107 Soays were introduced to the larger neighbouring island of Hirta and have been living there unmanaged since. From 1985, a longitudinal individual-based study has been conducted on the sheep resident in the Village Bay area of Hirta to investigate ecological and evolutionary questions (38). 90% of lambs, born in April or May of each year, are ear-tagged and tissue sampled for DNA extraction soon after birth. Any missed lambs or immigrant adults are captured, tagged and sampled in an August catch up or in the rut in November. As far as possible all sheep alive since 1989 have been genotyped on the Illumina Ovine 50K SNP array. Parentage is inferred for each individual using a subset of 315 SNPs in low linkage disequilibrium derived from the SNP array using the pedigree reconstruction software Sequoia (39, 40). In cases where no SNP genotypes were available, a small number of parentage inferences were made using field observations (for mothers) or a previous microsatellite genotyping approach (41).

The Soay sheep has a promiscuous mating system. Both females and males mate multiply and often with different partners within a year. Females usually have single lambs, less commonly twins, and very rarely triplets (38), with twins and triplets accounting for approximately 20% of new-born lambs (Supplementary table 1). Twins and triplets are always non-identical (J.

Pemberton & S. Johnston, pers. obs.) and usually have different fathers (Supplementary table 1). Since each offspring therefore represents a separate fertilisation, we treated each offspring as an independent data point.

MHC data

The ovine class II region comprises two distinct subregions, class IIa and IIb, which both contain a number of loci, with pathogen resistance mainly reported to be associated with class IIa loci (42). The MHC data used in this study were obtained from a previous study (35, 36). First, seven expressed loci (*DRB1*, *DQA1*, *DQA2*, *DQA2-like*, *DQB1*, *DQB2* and *DQB2-like*) within the MHC class IIa region were characterised in 118 Soay sheep using genotyping-by-sequencing. As a consequence, a total of eight MHC class II haplotypes were identified and named A to H, and confirmed in an additional 94 Soays selected from the pedigree to maximise genetic diversity (35). Second, a panel of 13 SNPs, mostly located in the flanking regions of the MHC class IIa haplotypes, including 11 SNPs from the Ovine Infinium HD chip and two other SNPs located within *DQA1* gene, were selected for imputation of the eight haplotypes and genotyped in 5951 Soay sheep using Kompetitive Allele-specific PCR (KASP). After imputation and quality control, we rejected 276 individuals on 3 plates with high genotyping error rate, 297 individuals with missing SNP genotypes, 26 individuals with novel MHC haplotypes potentially caused by genotyping errors and 3 individuals with diplotypes which were inconsistent with their parents. Finally, the diplotypes of 5349 individuals that lived in the study area between 1985 and 2012 were identified (36, 37). The frequency of each haplotype is shown in Supplementary Figure 1.

Analytical methods

In this study, we only used offspring-mother-father trios in which all three members were diplotyped (N=2459 trios). We omitted all trios in which the diplotyped offspring died as a foetus when its mother died. We characterized seven parental groups based on the parental diplotype combination (Table 1). Groups 1 and 2 are of no further interest because all offspring will have the same diplotypes. For all other groups, Monto-Carlo simulations were conducted by randomly choosing one haplotype from each true parent in a pair to create a simulated offspring. Each trio was simulated for 10,000 iterations using a custom script in R v.3.5.2. The observed sample size of offspring for each group is shown in Table 1.

Table 1. Classification and sample size of parental groups. The letters in parental diplotypes are here used as examples to describe the seven possible combinations of parental diplotypes. Since there are eight haplotypes in the population named A to H, in reality there are multiple different diplotype combinations in each group, e.g. group 1 includes AA-AA, BB-BB, CC-CC etc.

Group	Parental diplotype combination	Expected ratio of heterozygotes: homozygotes in offspring	Number of trios
1	MM-MM	all homozygote	18
2	MM-NN	all heterozygote	74
3	MM-NO	all heterozygote	432

4	MN-OP	all heterozygote	894
5	MM-MN	1:1	164
6	MN-MN	1:1	78
7	MN-MO	3:1	799
total			2459

After simulation, we conducted five kinds of analyses to test for post-copulatory selection on MHC variation in Soay sheep, comparing the observed value with the simulated distribution. For all the analyses, significance was determined by comparing the observed value with the 2.5% and 97.5% tails of the distribution of the values of the 10,000 iteration simulations.

(1) At the diplotype level we examined whether there was a deficit or excess of MHC homozygotes in groups 5-7 using the ratio of heterozygote : homozygote. We did this separately for each group as the expected ratio of heterozygote : homozygote is different across the three groups (Table 1).

(2) We investigated whether there was a deficit or excess of offspring with MHC class II diplotypes identical with a parent using groups 5 and 7. We compared the number of offspring which had identical diplotypes with the mother and those which had identical diplotypes with their father separately. This test was not performed on group 6, since both parents have identical diplotypes.

(3) We investigated whether offspring had more or less divergent MHC class II diplotypes than expected in groups 3-7. The pairwise divergence of each pair of MHC class II haplotypes was measured by the proportion of the amino acid sequence that differed (p-distance; Supplementary Table 4) (36). We compared the mean divergence of MHC class II diplotypes across all the offspring in simulated data with that in the real data.

(4) At the haplotype level, we investigated whether specific haplotypes were over- or under-represented in comparison with the parental generation across all the simulated groups (3-7).

(5) We focused on whether there is transmission ratio distortion of MHC class II haplotypes in Soay sheep from either fathers or mothers. For each haplotype, we assessed the frequency with which it was inherited from a father and a mother separately. We did this in all simulated groups except for group 6 in which it was not possible to tell which parent a haplotype came from.

(6) Finally, we investigated whether the frequency of a haplotype received by an offspring was over- or under-represented in male or female offspring, using all simulated groups (3-7).

Results

Here we present the results of the six tests described above in turn.

(1) The expected ratio of heterozygote: homozygote for each group is shown in Table 1. In all tested groups (5, 6, 7) the ratio of heterozygote: homozygote diplotypes was in line with random expectation (Supplementary Figure 2, Supplementary Table 5).

(2) The expected number of offspring with diplotypes identical to a parent was 82 in group 5 (half of 164 trios) and 200 in group 7 (a quarter of 799 trios). The number of offspring with identical diplotypes to their mother or father was in line with random expectation (Supplementary Figure 3, Supplementary Table 6).

(3) We found that the divergence of MHC class II diplotype in offspring was in line with random expectation (Supplementary Figure 4, Supplementary Table 7).

(4) We found that no specific haplotype was either over- or under-represented across all offspring (Supplementary Figure 5, Supplementary Table 8).

(5) We found evidence for transmission ratio distortion of haplotype E. Observed paternally inherited haplotype E was under-represented compared with simulated data (Figure 1A), but maternally inherited haplotype E was neither over- nor under-represented (Supplementary Figure 6, Supplementary Table 9). The nominal P-value for paternal haplotype E distortion is p=0.0065, but after Bonferroni correction for 16 tests (8 haplotypes x 2 sexes, critical p after correction: 0.0015625) it was not significant and is hereafter referred to as marginally significant.

(6) We found that haplotype A was over-represented in male offspring (Figure 1B) but not in female offspring (Supplementary Figure 6, Supplementary Table 9). The nominal P-value for male haplotype A distortion is p=0.007, but after Bonferroni correction for 16 tests (as above) it was not significant and is hereafter referred to as marginally significant.

As shown in Supplementary Table 1, some twins are full sibs. Twins are always dizygotic, so represent separate fertilisation events (J. Pemberton & S. Johnston, pers. obs.). In addition, a small number full sibs are born in different years, also from separate fertilisation events. Nevertheless, to eliminate the possibility of non-independence of parental pairs affecting our results, we repeated the whole analysis after retaining only the first instance of a parental pair in the data set. The results were consistent with those reported above (Supplementary Table 10).

Discussion

In this study, we investigated post-copulatory selection on MHC class II haplotypes in a wild population of Soay sheep using a large number of informative parent-offspring trios. We found no evidence of selection against homozygous offspring, no deficit or excess of identical diplotypes between offspring and either parent, and no selection favouring offspring with more divergent MHC class II diplotypes. Thus, we did not find any evidence of post-copulatory selection at the diplotype level. At the haplotype level, we did not find any haplotype was either over- or under-represented across all offspring. However, we found that haplotype E was underrepresented when inherited from fathers and haplotype A was overrepresented in male offspring, although neither result survived Bonferroni correction.

Our results provide little evidence for within-trio post-copulatory selection on MHC class II haplotypes. Although some experimental studies have reported post-copulatory selection on MHC genes both before fertilization (22, 24) and after fertilization (29), evidence in semi-natural or natural populations is weak. Only one study of lesser kestrel showed significant transmission ratio distortion of an MHC supertype inherited from fathers (32). In our study, we also identified transmission ratio distortion of particular MHC class II haplotypes both in the parental generation and filial generation. However, we could not rule out the possibility that these results are false-positives due to multiple testing. In addition, we did not identify any signature of post-copulatory selection at the diplotype level. Our results are consistent with the lack of deviation from Hardy-Weinberg equilibrium in the wider Soay sheep MHC class II dataset (36).

Our study contrasts somewhat with the two previous studies that found significant or suggestive evidence of post-copulatory selection on MHC genes in semi-natural or natural populations (31-33). The difference is potentially due to differences in MHC diversity, sample size, analytical method and species. First, MHC class II diversity in Soay sheep, with only eight haplotypes, is much lower than in the other two studies. Moreover, the eight haplotypes are at relatively even frequencies, as demonstrated by significant deviation from expected in the Ewens-Watterson test at different life history stages and within the standing population each year (36), which maximises analytical power. Using microsatellite genotyping, a total of 176 MHC haplotypes were identified in Rhesus macaques. As a result, the number of informative trios that had a 1:1 expected ratio of homozygous and heterozygous offspring was too low to use for further analysis, which reduced the study to only the parental category which had an expected 3:1 heterozygote:homozygote ratio. In the study of lesser kestrel, as the allele number of each individual was very high, MHC supertypes were used as the MHC marker for the study of transmission ratio distortion, which may not reflect actual selection at either the allele or haplotype level. Second, our sample size was larger than previous studies: in the Rhesus macaque there were 154 informative trios and in the lesser kestrel there were 228 meiotic events from 44 families. With several hundred trios in each test, our study had more statistical power. Third, our results were produced by comparing the results of Monto-Carlo simulation and real data while the study of Rhesus macaques and lesser kestrel used Bayesian t-test and Kolmogorov–Smirnov test respectively. Monto-Carlo simulation does not require specific statistical assumptions and thus could avoid the use of models with potentially inappropriate assumptions (43). These things said, there remains the possibility that there are species differences in post-copulatory selection on MHC genes, which could explain variation between studies.

Our study focused on within-trio post-copulatory selection on MHC genes using parentage data. However, in polyandrous mating systems there may also be MHC-dependent mating, sperm competition and cryptic female choice favouring sperm from particular males among mated partners (44, 45) which were not examined in the present study. To test these ideas, a large number of mating observations and associated parentage data should be available simultaneously to differentiate MHC-dependent pre-copulatory selection and post-copulatory selection. To date, this hypothesis has only been tested in a population of mouse

lemurs (30). Consort data has been collected in Soay sheep over many years and there is some evidence for assortative mating in the population (46). Thus, further studies could combine the consort data, molecular parentage data and actual MHC diplotypes together to investigate whether there is MHC-dependent selection via sperm competition in this polyandrous mating system.

In conclusion, we have identified a large number of informative trios using MHC genotyping and parentage data to study within-trio post-copulatory selection on MHC class II genes in a wild population of Soay sheep. With the advantage of limited MHC diversity and large sample size, this is the first study to investigate post-copulatory selection thoroughly at both the diplotype and haplotype levels in a free-living population. We found evidence of transmission ratio distortion of specific MHC class II haplotypes inherited from fathers and inherited by male offspring, but we could not rule out the possibility of false positive results in these tests. These results imply little evidence of MHC-dependent post-copulatory selection in the study population. Our study also highlights the value of large-scale genetic parentage inference and Monte Carlo simulation for investigating post-copulatory selection on the MHC in free-living population.

Ethics

Ethical approval for the research on Soay sheep has been granted by the appropriate UK Home Office licences.

Data accessibility

Data used in this paper are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.m63xsj40t (47)

Author's contributions

W.H and J.M.P designed the study. W.H analyzed the data and wrote the manuscript with editorial input from J.M.P.

Competing interests

We declare we have no competing interests.

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