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Associations between MHC class II variation and phenotypic traits in a free-living sheep population

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Abstract

Pathogen-mediated selection (PMS) is thought to maintain the high level of allelic diversity observed in the major histocompatibility complex (MHC) class II genes. A comprehensive way to demonstrate contemporary selection is to examine associations between MHC variation and individual fitness. As individual fitness is hard to measure, many studies examine associations between MHC variation and phenotypic traits, including direct or indirect measures of adaptive immunity thought to contribute to fitness. Here, we tested associations between MHC class II variation and five phenotypic traits measured in free-living sheep captured in August: weight, strongyle faecal egg count, and plasma IgA, IgE and IgG immunoglobulin titres against the gastrointestinal nematode parasite *Teladorsagia circumcincta*. We found no association between MHC class II variation and weight or strongyle faecal egg count. We did, however, find associations between MHC class II variation and immunoglobulin levels which varied with isotype, age and sex. Our results suggest associations between MHC and phenotypic traits are more likely to be found for traits more closely associated with pathogen defence than integrative traits such as bodyweight and highlight the association between MHC variation and antibodies in wild populations.

KEYWORDS
immune response, major histocompatibility complex, parasite, phenotypic trait, selection, Soay sheep

1 | INTRODUCTION

The immune system provides a variety of mechanisms to protect the host from infection by rapidly evolving and highly variable pathogens. The diversity of immune-related proteins and their associated genes are believed to have evolved in response to such pathogen diversity via the process of coevolution (Eizaguirre et al., 2012b; Pilosof et al., 2014). Among the proteins directly involved in the initiation of adaptive immunity, major histocompatibility complex (MHC) molecules, encoded by MHC class I and class II gene families, are the most variable and have been intensively researched in many species (Bernatchez & Landry, 2003; Edwards & Hedrick, 1998; Piertney & Oliver, 2006; Radwan et al., 2020). MHC genes encode heterodimeric MHC molecules which bind and present self- and pathogen-derived peptides to T cells to invoke and coordinate the adaptive immune response. Classical MHC class I genes

are primarily responsible for presenting peptides derived from intracellular pathogens such as viruses, while classical MHC class II genes typically present peptides derived from extracellular pathogens such as bacteria and parasites (Bernatchez & Landry, 2003). The tight mechanistic link between pathogen infection and MHC molecules leads to the expectation that selection pressure imposed by pathogens, known as pathogen-mediated selection (PMS), is the major force driving high levels of diversity at the MHC (Bernatchez & Landry, 2003; Piertney & Oliver, 2006; Spurgin & Richardson, 2010).

Substantial effort has been made to investigate how PMS can maintain high levels of MHC diversity across a wide variety of vertebrate taxa (reviewed by Bernatchez & Landry, 2003; Piertney & Oliver, 2006; Spurgin & Richardson, 2010). There are several nonmutually-exclusive mechanisms by which PMS may occur. (1) Heterozygote advantage (HA): HA occurs when heterozygotes have greater fitness than either homozygote (Hughes & Nei, 1988; Penn et al., 2002; Takahata & Nei, 1990). (2) Divergent allele advantage (DAA): DAA is an extension of HA. Under DAA, individuals with high levels of functional divergence between MHC alleles have a selective advantage over individuals with lower levels of allelic divergence (Wakeland et al., 1990). (3) Negative frequency-dependent selection (NFDS): NFDS occurs due to rare allele advantage; pathogens are predicted to be under selection to evade the most common MHC alleles, resulting in rare MHC alleles having a selective advantage, and creating a cyclical coevolutionary arms race (Slade & Mccallum, 1992; Takahata & Nei, 1990). (4) Fluctuating selection (FS): Under

FS, directional selection due to variation in pathogen pressure varies in time and space such that it maintains diversity (Hedrick, 2002). Several studies have used experimental methods to examine PMS on MHC genes (Bolnick & Stutz, 2017; Eizaguirre et al., 2012a, 2012b; Phillips et al., 2018). However, experimental studies are rarely capable of replicating the wide array of pathogens and parasites that occur within a wild host and are limited in the conclusions that they can draw about natural processes. Therefore, testing these hypotheses within wild systems is valuable (Piertney & Oliver, 2006; Spurgin & Richardson, 2010).

A direct way to infer contemporary selection on MHC genes in a wild population is to examine associations between MHC heterozygosity or genotypes and fitness. As fitness is difficult to measure in natural populations, we could alternatively examine associations between MHC variation and pathogen load (Spurgin & Richardson, 2010) and other phenotypic traits with potential links with fitness, for example, bodyweight. However, examining phenotypic traits is a less direct approach than examining fitness components and, in the quest to understand selection mechanisms, it is of interest to know how consistent results from these two approaches are. Only a few studies have analysed both MHC-fitness associations and MHC-phenotypic trait associations in the wild (summarized in Table 1). Of these, some studies found consistent results between MHC-fitness associations and MHC-phenotypic trait associations (Kloch et al., 2012; Paterson et al., 1998; Sepil, Lachish et al. 2013a, 2013b), while other studies did not (Dunn et al., 2013). These mixed results could

TABLE 1 Summary of previous studies testing associations between MHC diversity and both Fitness and phenotypic traits

Species	Locus/ region	Genotyping method	Phenotypic traits	Fitness component	Conclusion	Study
Soay sheep (*Ovis aries*)	DRB	MHC-linked microsatellite	Strongyle faecal egg count	Juvenile survival	Two alleles associated with both survival and resistance to intestinal nematodes[a]	Paterson et al. (1998)
Root vole (*Microtus oeconomus*)	DRB	454 NGS	*Babesia* ssp.	Survival	A specific allele associated with both decreased parasite load and increased survival[a]	Kloch et al. (2012)
Yellow throat (*Geothlypis trichas*)	Class II	454 NGS	Malaria	Survival	Allele number associated with increased survival while aspecific allele associated with resistance to malaria[b]	Dunn et al. (2013)
Great tit (*Parus major*)	Class I	454 NGS	Malaria	Survival and lifetime reproductive success	A specific MHC supertype associated with both quantitative resistance to malaria and increased lifetime reproductive success[a]	Sepil, Lachish, Hinks, et al. (2013); Sepil, Lachish, and Sheldon (2013)
Collared flycatcher (*Ficedula albicollis*)	Class II	454 NGS	Malaria	Survival and lifetime reproductive success	No associations between MHC and prevalence of malaria, nor lifetime reproductive success[a]	Radwan et al. (2012)

Note: MHC-fitness association and MHC-trait association were [a]consistent or [b]inconsistent.
Abbreviation: NGS, next-generation sequencing.

be due to some MHC-fitness associations not acting through the phenotypic traits examined. Therefore, it is of interest to determine which types of traits MHC-based selection is most likely to be acting through.

Another possible explanation for variation between MHC-fitness and MHC-phenotype studies may lie in analysis methods. First, MHC–phenotypic trait associations may vary due to heterogeneity in exposure or response to pathogens due to host age and sex or with environmental variables such as population density. For example, in a recent study of black-legged kittiwake (*Rissa tridactyla*), MHC class II diversity was positively associated with growth and tick clearance in female but not in male chicks (Pineaux et al., 2020). Therefore it is important to investigate whether MHC-phenotypic trait associations vary with age, sex and environmental variables. In addition, there is additive genetic variation for most phenotypic traits such that when examining MHC-phenotypic trait associations in data sets including many related individuals, animal models should be used. The animal model framework includes phenotypic information from individuals of varying relatedness to estimate the additive genetic component of the trait. By including the breeding value as a random effect within a mixed effect model, variation in the trait that is due to additive genetic effects located throughout the genome can be controlled for (Wilson et al., 2010). This will reduce the risk of generating false positive associations.

The unmanaged Soay sheep (*Ovis aries*) population on Hirta, St Kilda, UK has been intensively studied for more than three decades (Clutton-Brock & Pemberton, 2004). Since 1985, nearly all individuals living in the Village Bay study area have been followed from birth, through all breeding attempts, to death. As an island isolate, the population has low Ne and low genetic diversity compared with other sheep breeds (Kijas et al., 2012) and is quite inbred (Stoffel et al., 2021) and so relatively low MHC diversity is expected and found (Dicks et al., 2019). The fitness data, combined with a genetically-inferred multigenerational pedigree and phenotypic data for bodyweight, parasite load and plasma antibodies (Berenos et al., 2015; Hayward et al., 2011, 2014; Nussey et al., 2014; Sparks et al., 2018), enable us to investigate the interplay between MHC variation, fitness and phenotypic traits.

Two previous studies investigated MHC variation and fitness in Soay sheep. A study of individuals alive between 1985 and 1994 found negative associations between two alleles at an MHC-linked microsatellite and a key parasite measurement, strongyle faecal egg count (FEC), and these two alleles were also positively associated with juvenile survival (Paterson et al., 1998). In a more recent study, we again found associations between Soay sheep MHC class II variation and fitness measurements (Huang et al., 2020), but this time using greatly superior genetic data and samples.

We were able to identify a total of eight MHC class II haplotypes (named A-H) through sequence-based genotyping of a subset of the population (Dicks et al., 2019) and imputed diplotypes successfully for 5349 sheep sampled from 1985 to 2012 using 13 SNPs (Dicks et al., 2021). We found haplotypes C and D are associated with decreased and increased male total fitness (measured

as the number of offspring that an individual had throughout its life span) respectively. In terms of fitness components, we found MHC divergence (measured as the proportion of the amino acid sequence that differed between the two MHC haplotypes of each individual) was positively associated with juvenile survival. We also found that haplotype C is associated with decreased adult male annual and lifetime breeding success while haplotype F is associated with decreased adult female life span. Consistent with the male total fitness result, the frequency of haplotype D has increased more than expected by drift over the study period (Huang et al., 2020). These results indicate that there is contemporary selection on MHC class II variation in Soay sheep. In the present study, with larger sample sizes and improved genetic resolution of haplotypes compared with the previous study (Paterson et al., 1998), we examine the associations between MHC variation and five phenotypic traits in Soay sheep. These traits were all measured in an annual August sheep catch, and include weight, a fitness-related nonimmune trait, strongyle faecal egg count, FEC, a fitness-related trait with a strong link to the immune system, and three immune traits, *Teladorsagia circumcincta*-specific immunoglobulin isotypes IgA, IgE and IgG (henceforth, "anti-*T. circ* antibodies"). Weight is an important measure of body condition in Soay sheep and high weight is advantageous for both survival and fecundity in Soay sheep (Clutton-Brock & Pemberton, 2004; Coltman, Pilkington, et al. 2001). Gastrointestinal nematodes (GIN) are common in Soay sheep throughout life, with virtually 100% prevalence in lambs, and immunity to GIN develops with age (Craig et al., 2006). GIN are a major selective force on the Soay sheep (Craig et al., 2006; Gulland & Fox, 1992; Hayward et al., 2011) and GIN burden, measured as FEC, is negatively associated with bodyweight (Coltman, Pilkington, et al., 2001) and over-winter survival (Gulland & Fox, 1992; Hayward et al., 2011). Immunoglobulin isotypes IgA, IgE and IgG are involved in the acquired immune response to GIN in sheep (Hayward, 2013; Lee et al., 2011; Stear et al., 1999). GIN-specific IgA acts at mucosal surfaces and is known to reduce worm growth and fecundity (Gutierrez-Gil et al., 2010; Lee et al., 2011; Stear et al., 1999). GIN-specific IgE also acts predominantly at mucosal surfaces and is involved in the degranulation of mast cells, which are white blood cells involved in parasite expulsion (McNeilly et al., 2009; Murphy et al., 2010). IgG is the primary plasma antibody that can interact directly with the parasite. In Soay sheep, anti-*T. circ* IgG is positively associated with increased survival in adult females (Nussey et al., 2014; Sparks et al., 2018; Watson et al., 2016). However, a recent study decomposed the association between IgG and female survival into within-individual and between-individual effects and found the association was driven by within-individual variation late in life linked to senescence rather than by between-individual differences determined by genetics or early-life conditions (Froy et al., 2019). Here, we aimed to determine whether MHC-phenotypic trait associations can indicate contemporary selection on MHC genes in Soay sheep by answering the following questions: (1) which phenotypic traits are associated with MHC

Phenotypic trait	Lambs	Yearlings	Adults/older
Weight	1494 (1986–2012)	841 (1985–2013)	2599/908 (1985–2016)
Strongyle FEC	1183 (1988–2012)	704 (1988–2013)	2205/794 (1988–2016)
IgA	1404 (1990–2012)		3032/1114 (1990–2015)
IgE	1506 (1990–2012)		3034 /1114 (1990–2015)
IgG	1503 (1990–2012)		3018/1112 (1990–2015)

Note: Sample sizes for adult traits are shown as *N* records or *N* records/*N* individuals. Years of measurement are shown in brackets.

class II variation? (2) Does the association between MHC variation and phenotypic traits vary with age, sex or population density? (3) Are there consistent patterns between MHC-phenotypic trait associations and MHC-fitness associations?

2 | MATERIALS AND METHODS

2.1 | MHC data

The genetic data used in this study was obtained through two previous studies (Dicks et al., 2019, 2021). 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 Sanger sequencing, including 94 sheep genotyped on the Ovine Infinium HD chip. A total of eight MHC haplotypes were identified (named A to H) and confirmed in an additional 94 Soays which were also genotyped on the Ovine Infinium HD chip (Dicks et al., 2019). The 188 sheep run on the HD chip were selected from the pedigree to provide broad genetic representation of the whole study population, that is, we genotyped individuals with low relatedness. A panel of 13 SNPs located in the region of MHC class IIa haplotypes, including 11 SNPs from the Ovine Infinium HD chip and two other SNPs located within the *DQA1* gene, were selected using the 188 Soay sheep and genotyped in 5951 Soay sheep using Kompetitive allele-specific PCR (KASP) to impute the MHC class II haplotypes (Dicks et al., 2021). After quality control, which included pedigree checking, the diplotypes of 5349 individuals sampled between 1985 and 2012 were identified (Dicks et al., 2021). For each individual successfully diplotyped, the functional divergence between an individual's two haplotypes (MHC divergence) was calculated by hand as the proportion of the amino acid sequence that differed between the two MHC haplotypes (p-distance) (Henikoff, 1996; Huang et al., 2020).

2.2 | Phenotypic traits

During an annual August catch when we tried to catch as many individuals as possible, all sheep were weighed to the nearest 0.1 kg.

Faecal samples were collected from as many individuals as possible at capture, and counts of nematode worm eggs were performed using a modified McMaster technique (MAFF 1986). This protocol enumerates Strongyle-type eggs per gram of wet weight faeces but does not differentiate between several morphologically indistinguishable species. Of the species contributing to FEC on St Kilda, *Trichostrongylus axei, Trichostrongylus vitrinus* and *Teladorsagia circumcincta* eggs are the most abundant, but eggs from *Chabertia ovina, Bunostomum trigonocephalus* and *Strongyloides papillosus* may also be present (Craig et al., 2006). This measure of FEC is correlated with adult worm count within the abomasum in Soay sheep on St. Kilda and on Lundy (Grenfell et al., 1995).

IgA, IgE and IgG activity against antigens of the third larval stage of *T. circ* were measured using ELISA from blood samples obtained from captured sheep. Full laboratory details and quality control measures were described in the previous study (Sparks et al., 2018). Of 3189 samples assayed, 13 samples failed quality control for IgA, eight samples for IgE and 27 samples for IgG. Due to the lack of standard solutions, all results were measured as optical density (OD) ratio values. The OD ratio of each sample was calculated as (sample OD−blank OD)/(positive control OD−blank OD). If the blank OD was greater than the sample OD, the OD ratio was set to zero to prevent negative OD ratios. The mean OD ratio was then calculated for each duplicated sample, and these values were used in all subsequent analyses.

For all the phenotypic traits used in this study, we only included individuals which were successfully genotyped for MHC class II diplotype, and we excluded individuals which had received an experimental treatment prior to sampling, for example, an anthelminthic bolus. The number of records that we used for each analysis is shown in Table 2.

2.3 | Statistical analysis

All analyses were conducted in the R package MCMCglmm (Hadfield, 2010). We used generalised linear mixed models called animal models (AMs) to study potential associations between MHC variation and phenotypic traits. An AM was built for each phenotypic trait by fitting an additive genetic effect with a covariance structure proportional

to the pedigree relatedness matrix. Each null model included fixed and random effects relevant to the studied phenotypic trait and age-group based on the findings of previous studies (Berenos et al., 2016; Hayward et al., 2011; Sparks et al., 2018) which are shown in Table 3. Genome-wide inbreeding, F_{grm} (Fhat3 from (Yang et al., 2011)) based on 38K genome wide SNPs (Berenos et al., 2016), was included in all models as a fixed effect to ensure any MHC heterozygosity or divergence associations did not simply reflect inbreeding depression. First, for each null model, we added MHC effects as MHC heterozygosity (0 for homozygous diplotype, 1 for heterozygous diplotype) and each MHC haplotype as dosage (0, 1 or 2) to test whether there are nonadditive dominance effects (Hu et al., 2015; Lenz et al., 2015). In order to test any sex-dependent association between MHC variation and phenotypic traits, we also fitted MHC by sex interactions including heterozygosity by sex and haplotype by sex interactions for each model. In all models, haplotype H was treated as a reference haplotype so any differences between individual haplotypes were relative to haplotype H. In another sets of models, we also tested the association between MHC divergence and phenotypic traits by adding MHC divergence and a divergence by sex interaction into each null model. Finally, we fitted population density and MHC (heterozygosity and specific haplotypes) by population density interactions in addition to the original models including both MHC heterozygosity and individual haplotypes to test whether the association between MHC variation and phenotypic traits varied with population density.

We used a conservative statistical framework to determine the significance of MHC effects on phenotypic traits in Soay sheep.

For models including MHC heterozygosity and haplotypes, the significance of MHC heterozygosity and MHC heterozygosity by sex interactions can be directly determined by whether the 95% credibility interval overlapped with zero. Similarly, the significance of MHC heterozygosity by population density interactions in additional models can also be directly determined by whether the 95% credibility interval overlapped with zero. The significance of specific haplotypes was first determined using Wald tests for models with or without all MHC haplotypes fitted in the same model. When the Wald test was significant ($p < .05$) we examined the significance of specific MHC haplotypes by conducting an additional analysis comparing the estimated effect of each haplotype against the mean of the effect estimates of all the other haplotypes (see Supporting Information 1 for detailed methods). Similarly, Wald tests for each model with and without haplotype by sex interactions in the same model were used to determine the significance of haplotype by sex interactions. If the Wald test was significant, we conducted an additional analysis comparing the estimated effect of each haplotype by sex interaction with the mean of the effect estimates of all the other haplotype by sex interactions (see Supporting Information 1 for detailed method). Finally, the Wald tests were also used to determine the significance of haplotype by population density interactions. For models including only MHC divergence, the significance of MHC divergence and MHC divergence by sex interactions can be directly determined by whether the 95% credibility interval overlapped with zero. Finally, if any MHC effect by sex interaction was significant, we ran sex-specific

TABLE 3 Fixed and random effects fitted in null models of each of the phenotypic traits

Effects	Weight			Strongyle FEC			Immunoglobulins	
	Lambs	Yearlings	Adults	Lambs	Yearlings	Adults	Lambs	Older
Fixed effects								
Sex	×	×	×	×	×	×	×	×
Litter size	×			×			×	
Age	× (days)		× (years)	× (days)		× (years)	× (days)	× (years)
Age^2			×			×		×
F_{grm} [a]	×	×	×	×	×	×	×	×
Maternal age	×			×			×	
Maternal age^2	×			×			×	
Random effects								
ID			×			×		×
Birth year	×		×	×		×	×	×
Capture year		×	×		×	×		×
Pedigree	×	×	×	×	×	×	×	×
Maternal ID	×			×	×	×	×	
ELISA plate ID							×	×
ELISA plate run date							×	×

Note: A "x" indicates that the effect was fitted.

[a]Genomic inbreeding coefficient (Yang et al., 2011).

models to determine whether there was a significant association between the MHC effect and phenotypic trait within either females or males.

Since the phenotypic traits have different distributions, we used different transformations for each trait. For weight, we used the raw data as the distributions were normally distributed in all age groups. However, as mean weight is known to vary with age, separate models were run for the different age classes (lambs, yearlings and adults) (Figure S2.1) (Wilson et al., 2007). Strongyle FEC was not normally distributed so we carried out log transformation as Log(FEC + 50) by adding half the minimum detection limit (100 eggs/g), and we again ran separate models for lambs, yearlings and adults as means were different (Figure S2.2). Distributions of immunological traits were largely normal except for lamb IgE for which we could not find an appropriate transformation. Although MCMCglmm takes a Bayesian approach which mitigates the effect of non-Gaussian response variables (Hadfield, 2010), the lamb IgE results presented below (which are actually null results) should be treated with caution. A pronounced increase in antibody levels occurs between lambs and yearlings (Sparks et al., 2018) but there was no difference in the distributions between yearlings and adults (Figure S2.3). Thus, we fitted separate models for lambs and older sheep (including yearlings and adults). All models were run for 60,000 iterations with a sampling interval of 30 after 5000 iterations (Hadfield, 2010; R Core Team, 2013).

3 | RESULTS

3.1 | Weight

There was no significant association between MHC heterozygosity or divergence and weight in any age group (Figure 1, Supporting Information 3 and 4). There was also no significant heterozygosity by sex interaction and no heterozygosity by population density interaction (Supporting Information 7). However, there was an MHC divergence by sex interaction on lamb weight in which the effect of MHC divergence on male lamb weight was significantly more positive than that on female lamb weight (Supporting Information 3 and 4). However, MHC divergence was not significantly associated with lamb weight in sex-specific models (Table S5.1).

The Wald tests for haplotype differences were not significant in any age group, so no significant association was identified between specific MHC haplotypes and weight (Table 4, Figure 2). The Wald test indicated that there was no significant haplotype by sex interaction and no haplotype by population density interaction for weight (Supporting Information 6 and 7).

3.2 | Strongyle FEC

There was no association between MHC heterozygosity or divergence and strongyle FEC in any age group. Also, there was no significant MHC heterozygosity by sex interaction and no heterozygosity

by population density interaction or MHC divergence by sex interaction (Figure 1, Supporting Information 3, 4 and 7) The Wald test for haplotype differences was not significant in any age group, so no significant association was identified between specific MHC haplotypes and strongyle FEC (Table 4, Figure 3). The Wald test indicated that there was no significant haplotype by sex interaction and no haplotype by population density interaction for strongyle FEC (Supporting Information 6 and 7).

3.3 | Immunoglobulins

3.3.1 | Anti-*T. circumcincta* IgA

We found that both MHC heterozygosity and MHC divergence were positively associated with IgA levels in lambs but not in older age groups (Figure 1, Supporting Information 3 and 4). There was neither a MHC heterozygosity by sex interaction nor a MHC divergence by sex interaction (Supporting Information 3 and 4). There was a significant MHC heterozygosity by population density interaction on lamb IgA such that the effect of MHC heterozygosity on IgA became stronger at higher density' (Supporting Information 7).

When testing for haplotype differences, Wald tests were significant in both lambs and older sheep (Table 4). In lambs, haplotype C was associated with higher IgA while haplotype G was associated with lower IgA (Figure 4a, Supporting Information 8). In older sheep, haplotype A was associated with lower IgA (Figure 4b, Supporting Information 5). In addition, the Wald test for haplotype by sex interactions was significant for lambs but not significant for older sheep. The effect of haplotype E on IgA in male lambs was significantly more positive than that on female lambs (Supporting Information 6). When testing in sex-specific models, haplotype E was positively associated with lamb IgA level in males but not in females (Table S5.3). Finally, the Wald tests for haplotype by population density interaction were not significant in both lambs and older sheep (Supporting Information 7).

3.3.2 | Anti-*T. circumcincta* IgE

There was no association between MHC heterozygosity or divergence and IgE levels, nor any heterozygosity by sex, heterozygosity by population density, or divergence by sex interactions, in any age group (Figure 1, Supporting Information 3, 4 and 7).

When testing for haplotype differences, Wald tests were only significant in older sheep (Table 4). Haplotypes C and D were associated with higher IgE while haplotypes G and H were associated with lower IgE (Figure 4b, Supporting Information 8). The Wald tests for haplotype by sex interactions were not significant for either lamb or older sheep, which indicated there were no significant haplotype by sex interactions for IgE (Supporting Information 6). Also, The Wald tests for haplotype by population density interaction were not significant in either lambs or older sheep (Supporting Information 7).
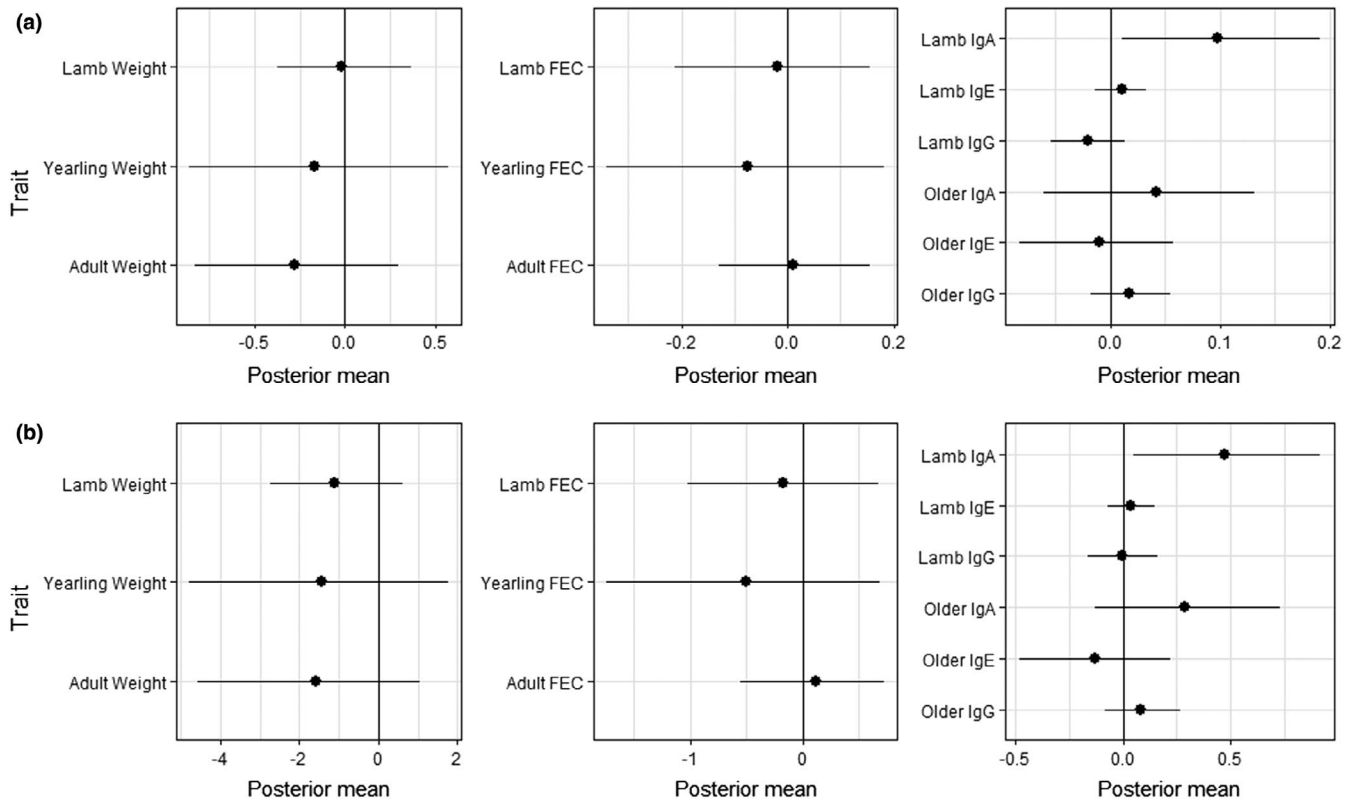
FIGURE 1 Associations between (a) MHC heterozygosity and (b) MHC divergence and phenotypic traits in Soay sheep. Results are presented as posterior means of the sampled iterations with the 95% credible intervals. Significance can be assumed if the 95% credible intervals do not overlap with zero

TABLE 4 Summary of results (*p*-values) for Wald tests for associations between MHC haplotypes and trait values (df = 7)

Phenotypic trait	Age class		
	Lambs	Yearlings	Adults/older
Weight	0.32	0.23	0.93
Strongyle FEC	0.38	0.22	0.19
Anti-Tc IgA	**0.0038**	—	**0.043**
Anti-Tc IgE	0.26	—	**0.00019**
Anti-Tc IgG	0.088	—	**0.0036**

Note: Bold values indicate significant associations (*p* < .05). "—" indicates category was not tested.

3.3.3 | Anti-*T. circumcincta* IgG

There was no association between MHC heterozygosity or divergence and IgG in either lambs or older sheep (Figure 1, Supporting Information 3 and 4). We found no significant heterozygosity by sex interaction while the effect of divergence on IgG in older sheep was significantly more negative in males than that in females (Supporting Information 3 and 4). However, MHC heterozygosity was not significantly associated with IgG in either older females or males in sex-specific models (Table S5.2). Finally, the Wald tests for haplotype by

population density interaction were not significant in either lambs or older sheep (Supporting Information 7).

When testing for haplotype differences, the Wald test was significant only in older sheep (Table 4). We found haplotype A was associated with lower IgG (Figure 4b, Supporting Information 8). However, the Wald test for haplotype by sex interactions was not significant in either lambs or older sheep, which indicated there were no significant haplotype by sex interactions for IgG (Supporting Information 6). Finally, the Wald tests for haplotype by population density interaction were not significant in either lambs or older sheep (Supporting Information 7).

4 | DISCUSSION

In this study, we tested for associations between MHC class II variation (heterozygosity, divergence and specific haplotypes) and five phenotypic traits in a large sample of Soay sheep using a modelling approach that accounted for genome-wide additive genetic and inbreeding effects. While we found no associations between MHC variation and weight or strongyle faecal egg count, we found a number of associations with anti-*T. circ.* antibody levels. Specifically, we found associations between MHC heterozygosity or divergence and IgA levels in lambs and a number of associations between specific MHC haplotypes and antibodies that vary with isotype, age and sex.
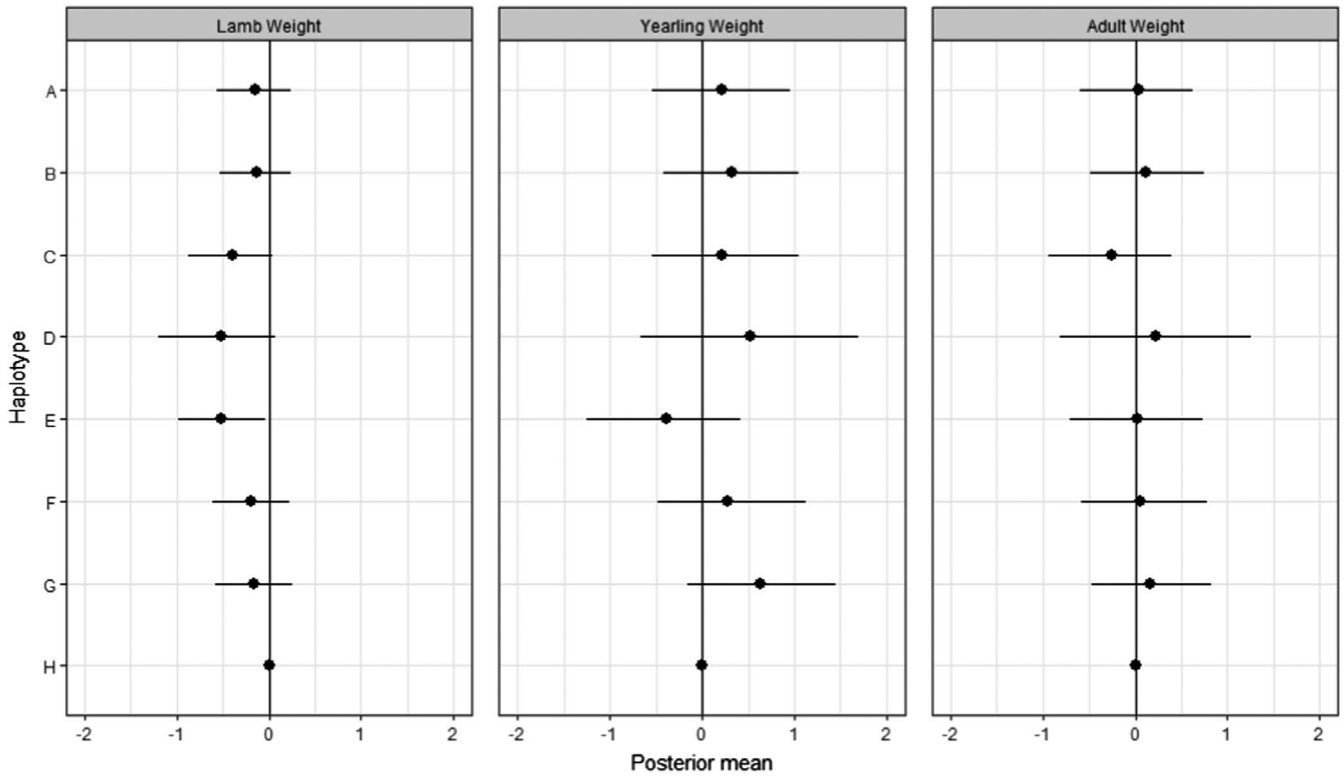
FIGURE 2 Associations between MHC haplotypes and weight in Soay sheep. Posterior means and 95% credible intervals for each haplotype are plotted relative to haplotype H from the original model outputs. None of the Wald tests for these models were significant
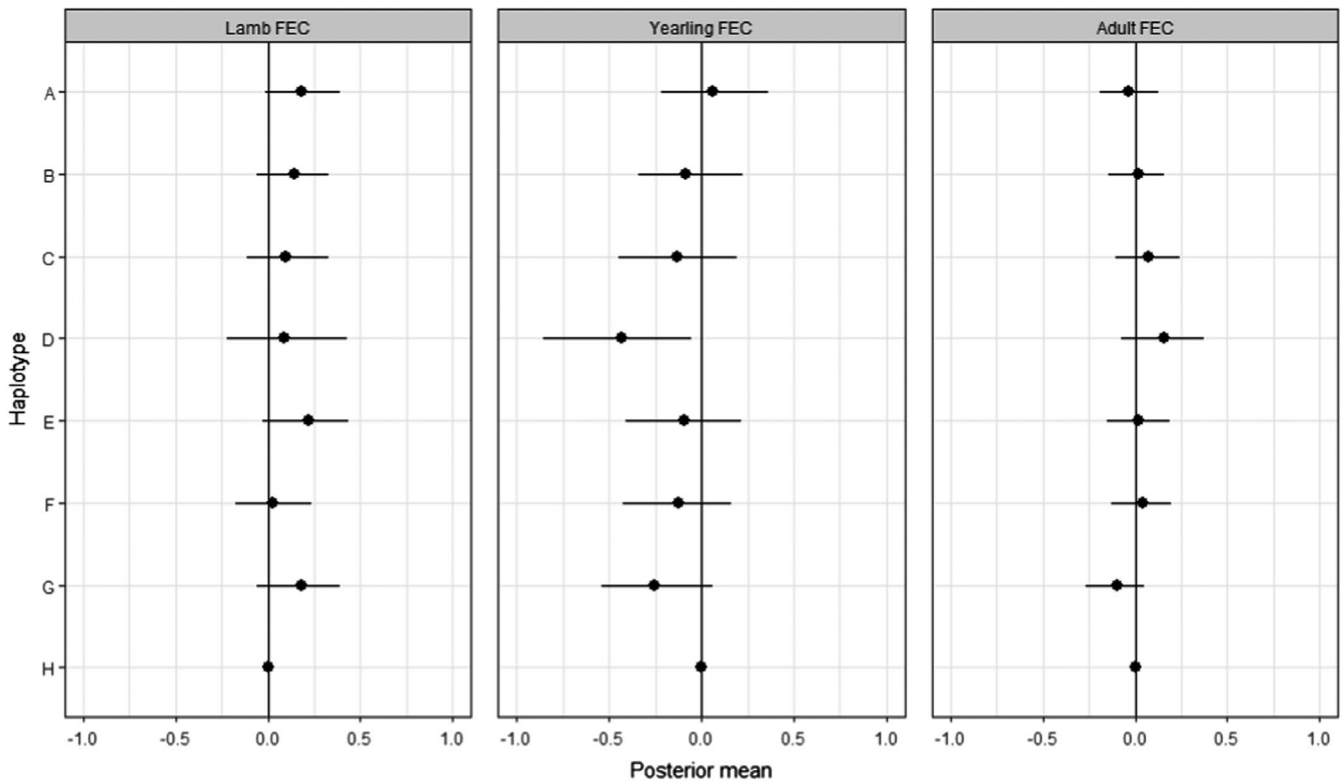

FIGURE 3 Associations between MHC haplotypes and strongyle FEC in Soay sheep. Posterior means and 95% credible intervals for each haplotype are plotted relative to haplotype H from the original model outputs. None of the Wald tests for these models were significant
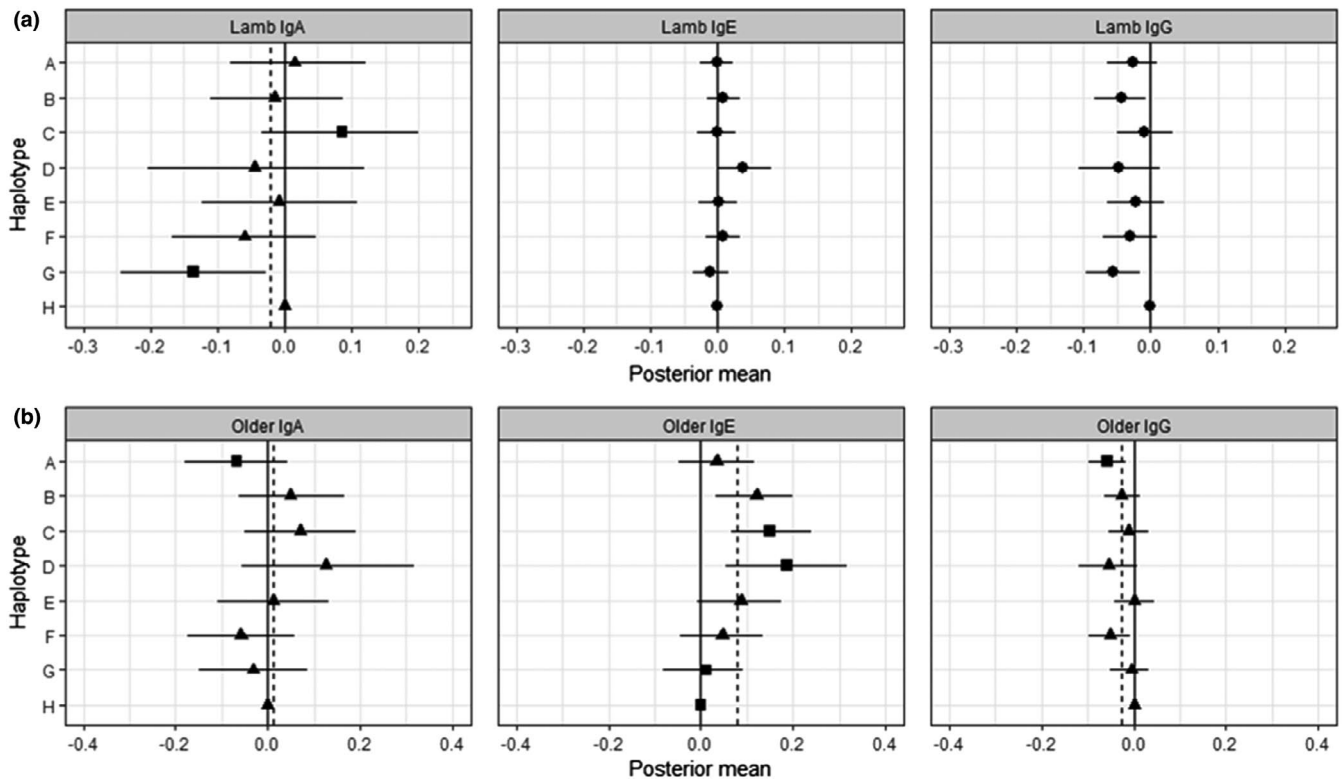
FIGURE 4 Associations between MHC haplotypes and anti-*T. circ* antibodies in (a) lambs and (b) older Soay sheep. Posterior means and 95% credible intervals for each haplotype are plotted relative to haplotype H from the original model outputs, with the solid vertical lines representing the haplotype H values. Circles show that Wald tests of the models were not significant, and other shapes show that Wald tests of the models were significant. When Wald tests were significant, the dashed vertical line shows the average posterior mean of all eight haplotypes, and haplotypes that differed significantly from this mean are shown as squares, while ones that did not are shown as triangles

However, we did not find that associations between MHC variation and phenotypic trait varied with population density except in the case of lamb IgA.

Several previous studies have found evidence that MHC heterozygosity or specific MHC alleles are associated with weight or body size in other species (e.g. Lenz et al., 2009; Lukasch et al., 2017). Although fitness is associated with body size in Soay sheep (Coltman, Pilkington, et al., 2001), we did not find any MHC associations with weight in our study. Weight is a polygenic trait with modest heritability (Supporting Information 10) (Berenos et al., 2015). As a nonimmunological trait, there is also no direct connection between the function of MHC genes and weight. Thus, it may not be surprising that we have not found any associations between MHC variation and weight.

MHC class II molecules are involved in the presentation of peptides from GIN for recognition by the immune system (Janeway et al., 1996). Variation in such responses to GIN infection within a population has been implicated in driving balancing selection which maintains MHC variation (Froeschke & Sommer, 2005; Kloch et al., 2010; Lenz et al., 2009; Madsen & Ujvari, 2006). Association between MHC class II variation and FEC has been reported in a number of sheep breeds (reviewed in [Valilou et al., 2015]) and previous analyses have demonstrated heritable variation in FEC as well as selection for parasite resistance in Soay sheep (Beraldi et al., 2007;

Coltman, Pilkington, et al., 2001; Coltman, Wilson, et al., 2001; Hayward et al., 2011). However, in this study, we did not find any association between MHC variation and strongyle FEC.

Several features of the FEC data may contribute to the lack of association in Soay sheep. The measure of FEC used here is a crude measure of parasite burden, both in terms of the way it was measured and what the measure actually represents in terms of parasite species. Because of the dilution factor used in the modified McMasters method (MAFF, 1986) for estimating FEC, the data are in multiples of 100, which combined with overdispersion, makes modelling FEC challenging. Also, individuals with 0 eggs per gram may have no strongyle worms or may have a very low burden. There may also be a very complicated relationship between FEC and the community composition of worm burden. For example in naturally infected Scottish Blackface sheep, high FEC was associated with a wider range of strongyle species (Stear et al., 1996). Therefore, increased FEC may be confounded with increased species diversity. If there is variation among MHC haplotypes in their ability to present peptides from different worm species, we may not expect to detect a direct association between MHC haplotype and strongyle FEC.

Our results differ from an earlier study of MHC-FEC association in the same population that used the DRB1-linked microsatellite OLADRB as a marker of MHC class II haplotypes. In that study, a positive association between FEC and OLADRB allele 257 in lambs,

a positive association between FEC and OLADRB allele 267 in yearlings and a negative association between FEC and OLADRB allele 263 in adults were identified (Paterson et al., 1998). However, we did not recover such associations in the current study. The previous study used 370 individuals, whereas in this study, we used FEC measures from 1183 lambs, 704 yearlings and 2205 FEC measures from 794 adults (Table 2). Also, the MHC genotyping method used in the current study captures MHC class II composition accurately, while some OLADRB alleles correspond to multiple MHC class II haplotypes (Dicks et al., 2021). The advances in sample size and genotyping method are likely to contribute to this disparity in results.

Previous studies have demonstrated associations between MHC variation and antibody response in wild populations (summarized in [Gaigher et al., 2019]). Some studies found significant associations (Bonneaud et al., 2005; Charbonnel et al., 2010; Cutrera et al., 2011; Gaigher et al., 2019) while others did not (Cutrera et al., 2014; Ekblom et al., 2013). A recent study suggested that the disparity of findings in previous studies examining associations between MHC variation and immunocompetence is probably caused by insufficient sample size and recommended a minimum sample size of 200 individuals to achieve sufficient power for testing associations of small effect size between MHC variation and immunocompetence (Gaigher et al., 2019). From this perspective, our sample size was large enough to have sufficient statistical power to test associations between MHC variation and immune measures (Table 2). In addition, previous studies investigating the association between MHC variation and antibody response have mainly used nonspecific challenges, such as sheep red blood cell antigens (SRBC) and hemagglutinin, to elicit an antibody response (Bonneaud et al., 2005; Cutrera et al., 2011, 2014; Gaigher et al., 2019). An exception is a study of Great snipe (*Gallinago media*) that used diphtheria and tetanus toxoid as antigens (Ekblom et al., 2013). Antibody against such challenges may be informative on the host's general immunocompetence, but may not reflect the host's immunocompetence in response to the actual pathogens imposing selection in the host's natural environment. By using the antigen of *Teladorsagia circumcincta*, our study was able to test associations between MHC variation and a relevant pathogen-specific antibody response. Although we did not find a significant association between MHC variation and FEC, we still expected to find significant associations between MHC variation and anti *T. circ* antibodies because of the functional link between MHC genes and adaptive immune response (Janeway et al., 1996). Indeed, we found several associations between MHC variation and anti-*T. circ* antibodies in Soay sheep, in contrast to our results for weight and FEC. Such results suggest MHC class II variation could contribute the inter-individual heterogeneity of immune response to GIN in Soay sheep.

The associations between MHC class II variation and anti-*T. circ* antibodies varied between different age classes and among different isotypes. First, we only found an association between MHC heterozygosity or divergence and IgA level in lambs. This result is consistent with our study of MHC-fitness associations which identified a positive association between MHC divergence and juvenile survival (Huang et al., 2020). However, when including MHC heterozygosity,

individual MHC haplotypes and MHC divergence in the same model, the model showed neither MHC heterozygosity nor MHC divergence was significant (Supporting Information 9). Thus, we could not conclude the positive effect of MHC divergence on lamb IgA was independent of MHC heterozygosity and vice versa. Second, although we found associations between specific MHC haplotypes and the IgA titre in both lambs and older sheep, IgE and IgG titres were only associated with MHC haplotypes in older sheep. This is consistent with a previous study in domestic sheep which found that associations with specific MHC haplotypes were only present for lamb IgA but not for lamb IgE (Ali et al., 2019). Since the acquired immune response develops over the first year of life (Stear et al., 1999), the change in adaptive immune response may result in significant associations with specific MHC haplotypes in IgE and IgG level of older sheep.

The MHC-antibody associations are also partially consistent with the genetic architecture of anti-*T. circ* antibodies described in a recent genome-wide association study (GWAS) of Soay sheep. Lamb IgA and older sheep IgE levels were both associated with the MHC class II region on chromosome 20 (Sparks et al., 2019) and we recovered those associations in this study. However, the associations with IgA and IgG level in older sheep were not detected in the previous GWAS study. An explanation for this difference probably lies in the different approaches. Even with high density SNPs, a GWAS tests each SNP independently, and each SNP has only two alleles. Furthermore, the Illumina ovine 50K SNP array which was used for the GWAS has very sparse coverage of the MHC and as we have found it requires ~13 SNPs to define the eight haplotypes in this region (Dicks et al., 2021). Therefore, it is not surprising that a GWAS missed some MHC-antibody associations in this hypervariable region.

In order to test whether there are sex-dependent associations between MHC variation and phenotypic traits, we fitted MHC by sex interactions throughout our statistical models. We found the effect of MHC divergence on lamb weight and older IgG level were significantly different between males and females. However, MHC divergence was associated with neither lamb weight nor older IgG level when testing in sex-specific models. Regarding specific MHC haplotypes, we found that only the association between haplotype E and lamb IgA was significantly different between males and females. When testing in sex-specific models, haplotype E was positively associated with lamb IgA level in males but not in females. These results suggest sex-dependent effects of haplotype E on lamb IgA level in Soay sheep which could result from the differences in ranging behaviour and life history between males and females (Clutton-Brock & Pemberton, 2004). We also tested whether associations between MHC variation and phenotypic traits varied with population density by including MHC by population density interactions in the models. However, we only found one interaction, between MHC heterozygosity and population density for lamb IgA. No interactions between MHC haplotypes and population density affecting phenotypic traits were identified. For this one trait in one age class there is thus tentative support for fluctuating selection which could be investigated further in future.

In the present study, we have investigated associations between MHC variation and multiple phenotypic traits. Most of the traits have been found to be associated with fitness or fitness components in previous studies (Coltman, Pilkington, et al., 2001; Hayward et al., 2011; Sparks et al., 2018). Thus, we hypothesised that we would find links between MHC-phenotypic trait associations and MHC-fitness associations. In this study, we only found associations between MHC class II variation and antibody titres. Neither weight nor FEC was associated with MHC class II variation. When comparing MHC-antibody associations with the associations between MHC variation and fitness components identified in a parallel study (Huang et al., 2020), we found MHC heterozygosity and divergence were both positively associated with both lamb IgA level and juvenile survival. In terms of specific MHC haplotypes, haplotype C was positively associated with older sheep IgE level but negatively associated with adult male annual and lifetime breeding success. However, haplotype F was associated with decreased adult female life span but not associated with antibodies (Huang et al., 2020). Although lamb IgA level was not significantly associated with juvenile survival, there is a consistent pattern between lamb IgA level and FEC (Sparks et al., 2018). A raised level of IgA is negatively associated with lamb FEC and lamb FEC is negatively associated with annual fitness (Hayward et al., 2011; Sparks et al., 2018). Thus, it is likely that lambs with divergent MHC diplotypes have a survival advantage through raised anti-*T. circ* IgA level. However, such a consistent pattern is not observed in adults as older IgE level was not associated with adult fitness component (Sparks et al., 2018). Therefore, it is not clear whether selection on MHC variation could act through anti-*T. circ* antibody response.

Overall, we can conclude three points from our study. First, we only identified associations between MHC variation and immune traits, suggesting that associations are more likely to be found as one moves from highly integrative traits such as bodyweight to immune traits which are arguably closer to actual gene expression. Second, associations between antibody traits and MHC variation varied with isotype, age and sex. Associations with MHC heterozygosity or divergence were only found in lambs while associations with individual haplotypes were found in both lambs and adults. Third, we found few MHC-phenotypic trait associations that were consistent with MHC-fitness associations, except for an association between MHC divergence and lamb IgA level. Overall, our results suggest that examining the association between MHC variation and pathogen-specific immune response is useful in the study of association between MHC variation and phenotypic traits in wild populations.

AUTHOR CONTRIBUTIONS

Wei Huang and Josephine M. Pemberton designed the study. Kara L. Dicks conducted the MHC genotyping. Alexandra M. Sparks and Kathryn Watt generated the antibody data. Jill G. Pilkington collected the data from the field. Wei Huang analysed the data and wrote the manuscript. All the authors contributed to the final version of the manuscript.

DATA AVAILABILITY STATEMENT

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