School of Biomedical Sciences

Characterisation of the Ovine Major Histocompatibility Complex class II region

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Declaration of Authenticity

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

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Abstract

The main aim of this project has been to provide insights into the genomic organization of the class II region of the ovine major histocompatibility complex (MHC), a chromosomal region containing genes that control adaptive immune responses in vertebrate species. Assessment of the potential of measuring resting serum IgA levels as a predictor of the resistance of sheep to gastrointestinal parasites was also undertaken.

Loci within the ovine MHC class II region were identified and sequenced either by direct sequencing of known class II genes using ovine specific primers or from sub-clones constructed from bacterial artificial chromosome (BAC) clones containing the class II region. The loci were apportioned into two groups (IIa and IIb) based on analogy with the class II region in the cattle MHC where it is known that a chromosomal inversion has divided and separated the region into two subregions ≈15 cM apart. All sequences obtained have been deposited in GenBank. A relative map of the ovine class IIa and IIb subregions was constructed based on comparative information from the human and cattle MHCs. Additionally, complete nucleotide sequences were obtained for the protein folding chaperone gene PFDN6; phylogenetic analysis confirmed its homology and impressive conservation with orthologues from other vertebrate. Partial sequences were also generated for several other loci including WDR46 and DRA.

Single nucleotide polymorphisms (SNPs) were identified by analysing homologous sequences from a minimum of five individual sheep. In total, 103 SNPs were discovered that were distributed over 20 distinct loci spanning both the putative class IIa and IIb regions. Genotypes were typed for panels of 10 SNPs across each of the ovine class IIa, IIb subregions, plus 10 published SNP loci for the class III region, in a total of 261 sheep. The sheep comprised 68 unrelated parental animals and 193 sheep from nine different family groups for which worm egg count

(WEC) data and estimated breeding values (EBV) were available. Allele frequencies for each SNP locus were estimated and determined to be in Hardy Weinberg proportions. Analysis of SNP heterozygosity in sheep revealed a region within the class IIa subregion where heterozygosity was reduced. This region is similar to a corresponding region within the human MHC manifesting a reduced frequency of SNPs. Analysis of linkage disequilibrium (LD) across the panel of 30 SNP loci showed regions of high and low LD. The region of low heterozygosity within the putative sheep class IIa subregion was characterised by low LD.

The availability of independently confirmed pedigrees permitted identification of 54 Mendelian inherited haplotypes across the class IIa, IIb and III subregions for all 30 loci. Haplotypes for other sheep were inferred using the EM algorithm as implemented in the SNPstats software package. Analysis of the deduced haplotypes showed evidence for the presence of conserved allele groupings or 'blocks' corresponding to each subregion. These 'blocks' seemed to occur more frequently than expected despite some SNP loci being homozygous (especially in the sires). Similar blocks were predicted by haplotype function in SNPstats. This result suggests that conserved subregion haplotypes may be present in the sheep MHC as are known to exist in the human MHC. Further analysis of the sheep pedigrees showed four haplotypic crossovers between the class IIb and class IIa subregions. Four sire derived crossovers in 21 meioses is consistent with a physical separation between these two subregions similar to that observed in cattle. No crossovers were observed between the class IIa and class III subregions.

Total and parasite-specific IgA levels were measured in serum collected post weaning from 171 unchallenged sheep for which WEC data was available. There were no significant correlations between either total or parasite specific IgA serum levels and post natal WEC or WEC at weaning Hence, this parameter is not a useful predictor of worm immunity and cannot replace the more difficult to measure WEC values. Multiple regression analysis of the data did suggest however that IgA synthesis

may have a role in the humoral immune responses of sheep to gastrointestinal parasites.

The discoveries reported in this thesis provide a more detailed description of the ovine MHC class II region, evidence of its division into two separated subregions, and the description of haplotypes based on newly discovered SNPs. Haplotypic associations with breeding values based on WEC were identified, but these did not permit speculation concerning the identity of candidate loci for parasite immunity probably due to the relatively small populations studied. These findings will contribute to association and linkage studies in larger sheep populations and between breeds in the search for genetic determinants of immunity to gastrointestinal worms. It is expected that such determinants in turn will facilitate breeding for more disease-resistant sheep by marker-assisted selection with consequent increases in industry productivity.

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Abbreviations

aa	amino acid
≈	approximately
BAC	Bacterial artificial chromosome
BLV	Bovine leukemia virus
Bp	base pairs (nucleotides)
BSA	bovine serum albumin
cDNA	coding deoxyribonucleic acid
cM	centimorgan
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
dTTP	deoxythymidine triphosphate
dNTP	deoxy nucleotide tri-phosphate
ddNTP	dideoxynucleotide triphosphate
DNA	deoxyribonucleic acid
EBVwec	Estimated breeding values (worm egg count)
EBVwecwean	Estimated breeding values (worm egg count at weaning)
E.coli	*Escherichia coli*
EDTA	ethylenediamine tetraacetic acid (disodium salt)
ELISA	Enzyme linked immunosorbent assay
EM	Expectation maximisation
FEC	faecal egg count
g	Gravitational acceleration
HLA	human lymphocyte antigen
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IPTG	isopropyl-β-D-thiogalactopyranoside
kbp	kilo base pairs (nucleotides)
kDa	kilo Dalton
L1	First stage larvae
L3	Third stage larvae
LB	Luria-Bertolli broth
LBA	Luria-Bertolli agar
Mbp	mega base pairs (nucleotides)

G, mg, μg, ng	gram, milligram, microgram, nanogram
M, mM, μM, pM	molar, millimolar, micromolar, picomolar
Min, hrs	minute, hours
ml, μl	millilitre, microlitre
mm, μm, nm	millimetre, micrometre, nanometre
MCS	multiple cloning site
MHC	major histocompatibility complex
NA	not available
PBS	phosphate buffered saline
PCR	polymerase chain reaction
QTL	Quantitative trait loci
RFLP	restriction fragment length polymorphism
SAP	shrimp alkaline phosphatase
SDS	sodium dodecyl sulphate
SNP	single nucleotide polymorphism
SOC	Super Optimal broth with Catabolite repression
SSC	sodium chloride / sodium citrate buffer
SSCP	single stranded conformational polymorphism
ssDNA	single stranded DNA
TAE	tris acetate EDTA buffer
TE	tris EDTA buffer
TEMED	N, N, N', N'-tetramethylethylenediamine
UV	ultraviolet
V, kV, mV	volts, kilovolts, millivolts
WEC	worm egg count
x-gal	5-bromo-4-chloro-3-idolyl-β-D-galactopyranoside

Chapter 1: General Introduction

1.1 The Immune system

The immune system is an organisation of cells and molecules with specialised roles in defending against infection by foreign antigens, including bacterial, viral and parasitological. There are two fundamentally different types of immune responses towards foreign antigens, innate and adaptive immune responses. Innate responses occur to the same extent however many times the infectious agent is encountered, whereas adaptive (acquired) immunity improves on repeated exposure to a given infection.

1.2 Major Histocompatibility Complex

The importance of the role of the MHC in resisting infections, inflammation, autoimmunity and tissue transplantation has long been discussed. Its role in determining histocompatibility in tissue transplantation was discovered in mice during tissue transplant studies (Gorer *et al*., 1948). The primary function of the MHC is to code for specialised antigen-presenting receptor glycoproteins, known as MHC molecules, which bind processed foreign peptide antigens and present them to T lymphocytes, thereby triggering immune responses (Vyas *et al*., 2008). The MHCs in several species are associated with many diseases with a wide range of etiologies; for example, narcolepsy resulting from a defect in the hypocretin type 2 receptor (Chemelli *et al*., 1999) and hemochromatosis, resulting from a defect in the HFE class I gene (Kuhn, 1999). Many MHC associations seem to have as their basis an interaction with an infectious agent. The mechanism of these associations is not always understood however associations between specific MHC alleles and disease have been reported. For example the presence of the HLA-B*53 allele is associated with reduced risk of death in humans from severe malaria (Hill *et al*., 1992) and the bovine DRB3 and DQB*1804 class II allele are associated with susceptibility to

Dermatophilosis (Maillard *et al*., 2003). In addition, in humans and experimental animal models there have been many reports of MHC associations with diseases with an autoimmune aetiology (Fernando *et al*., 2008).

The human and mouse MHC have been investigated in much more detail than those of other mammals and, among the domesticated species, the ovine MHC is one of the least characterised. The human MHC covers a region of approximately 3.6 megabase-pairs (Horton *et al*., 2004), comprising the class I and class II regions separated by a central region as shown in Figure 1.1. The human MHC contains 224 genes, of which 128 have been predicted to be expressed (The MHC sequencing consortium, 1999; Trowsdale, 2001). It is estimated that about 40% of expressed genes have an immunologically related function (Stewart *et al*., 2004; Flajnik and Kasahara, 2001). The extensive mapping and sequencing of the human MHC provides a valuable reference for intra-species and inter-species comparative genomic studies (Kulski *et al*., 2002). However, it is also likely that interspecies comparisons of the MHC will provide considerable information concerning the internal regulation and functions of the human MHC.

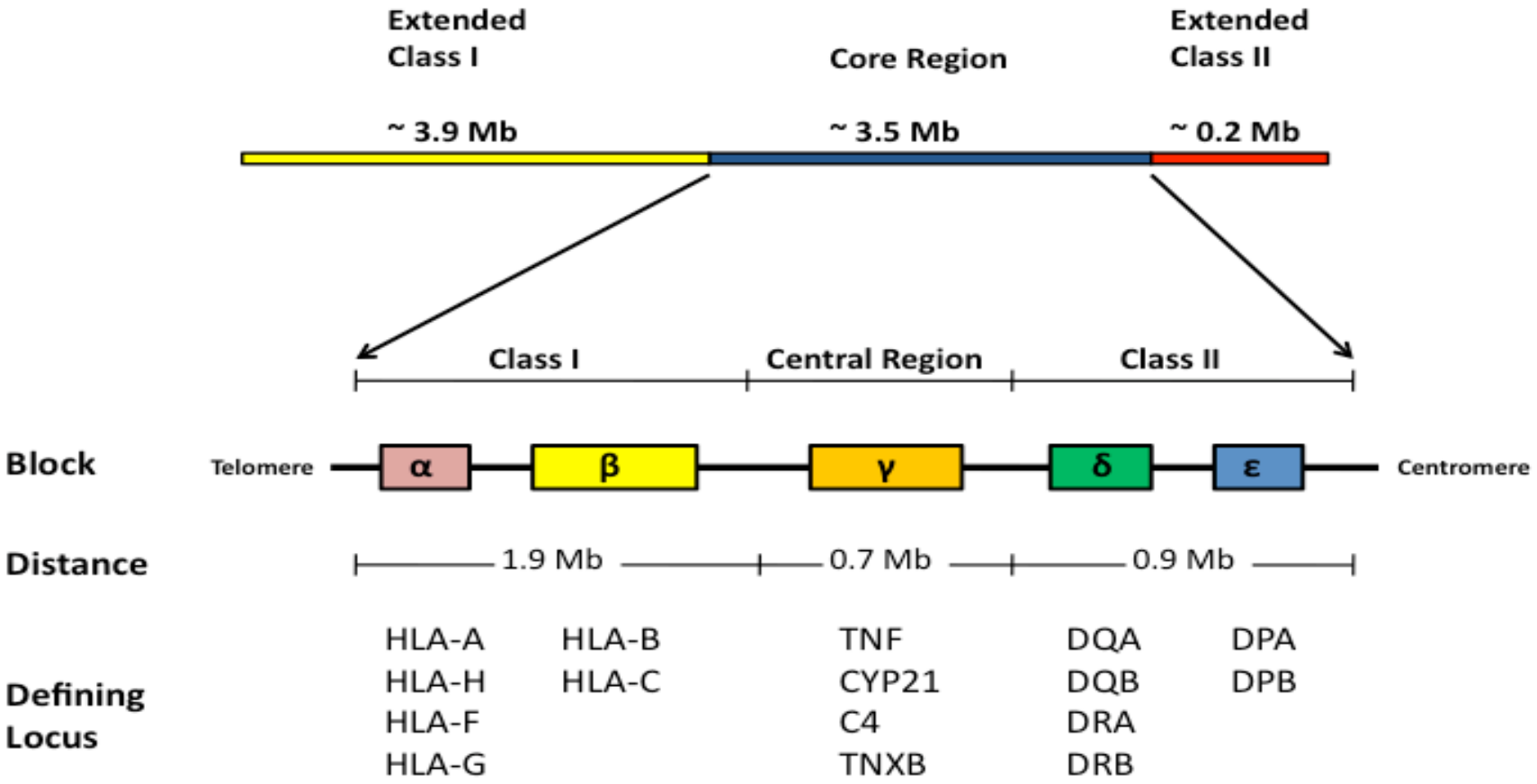


Figure 1.1: Generalised structure of the human MHC showing selective loci to illustrate the internal regions. Greek symbol blocks are characterised by low recombination. HLA-H is also referred to as HFE. Adapted from a figure originally proposed by Dawkins et al. (1983).

1.2.1 MHC class I

The MHC class I genes consists of both classical (MHC class Ia) and non-classical genes (MHC class Ib; Kumanovic *et al*., 2003), and are generally located at the telomeric end of the chromosome in mammals. The MHC class I molecule consists of the alpha- or heavy chain non-covalently linked to a light β2-microglobulin chain (Figure 1.2). The α-chains are encoded by the polymorphic MHC class I loci while the β2-microglobulin light chain is encoded by a non-polymorphic locus outside the MHC region which is involved in the presentation of antigenic peptides to CD8+ cytotoxic T cells (Pamer and Cresswell, 1998; Flutter and Gao, 2004; Raghavan *et al*., 2008). The antigenic peptides are predominantly derived from intracellular proteins and are presented by the class I molecules (Peaper and Cresswell, 2008). The non-classical class I genes are not as well characterised as the classical genes. They have been found to be evolutionarily related to the classical class I genes and appear to have distinct functions related to immune responses and NK cell recognition (Rodgers and Cook, 2005). The presentation of antigenic peptides involves the degradation of the antigenic protein by a multimeric proteolytic complex (Raghavan *et al*., 2008; Vyas *et al*., 2008). The processed antigenic peptides are transported into the endoplasmic reticulum, and are loaded onto MHC class I molecules. This process is regulated by chaperone proteins, including TAP (transporter-associated antigen processing) and tapasin, which are intrinsically involved in the presentation of intracellular antigens (Koch *et al*., 2004; Procko *et al*., 2005). However, despite the close relation of the class I molecules with chaperone proteins, none of the genes that encode the chaperone proteins are located within the MHC class I region. TAP is located in the MHC class II region, while tapasin is in the extended class II region in species including human and mouse.

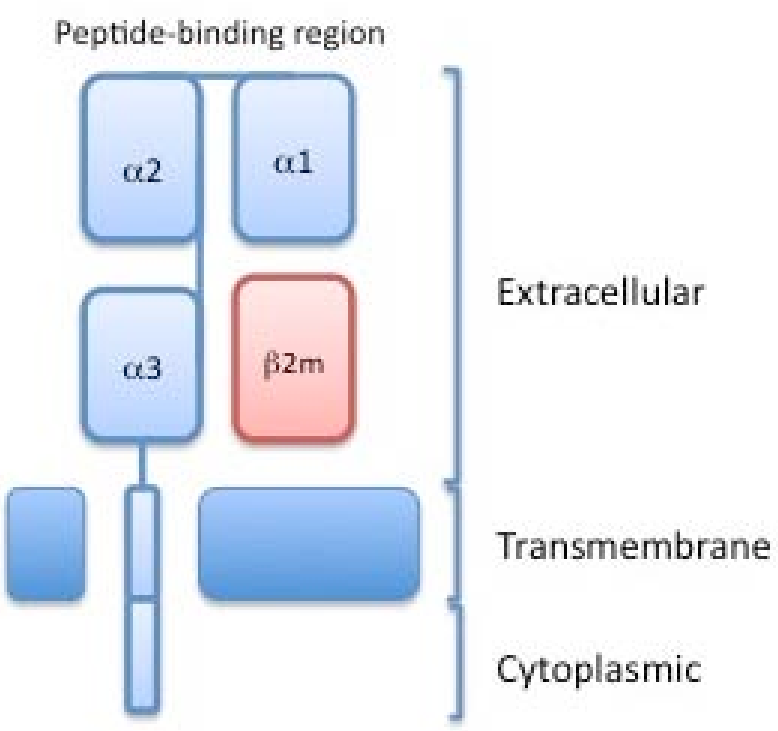


Figure 1.2: Schematic presentation of the structure of MHC class I molecules.

1.2.2 MHC class III

The class III region has the highest gene density of all the MHC regions and is sandwiched between the telomeric class I and centromeric class II gene clusters (Xie *et al*., 2003). In humans, the class III region spans approximately 700 kb and contains more than 60 genes; with the gene density estimated to be one per 9 kbp (Kumanovics *et al*., 2003; Xie *et al*., 2003). The class III genes with a role in immunobiology include those involved in the complement cascade (C4A, C4B, C2 and Bf; Hauptmann and Bahram, 2004), inflammation (MIC; Deakin *et al*., 2006) and extracellular matrix organisation (TNX; Hughes, 1999). Other genes in this region that are not directly associated with immune responses include HSP70, CYP21 and LST1. The genes of the complement cascade encode proteins that are essential components of the innate immune response. The cleaved gene products C2, C4A, and C4B, form a complex with the C3 fragments to generate the classical pathway, the lectin pathway, or the alternative complement pathways respectively (Walport, 2001a; Walport, 2001b). The HSP70 gene is another important gene

within the class III region, as it encodes heat shock protein 70. HSP70 presents intracellular contents of cancer cells to the immune system and therefore plays a significant role in tumour rejection (Castelli *et al*., 2004). In cattle, the HSP70 gene is duplicated, and it has been shown that the loss of one of the duplicated genes in Holstein cattle is responsible for hereditary diaphragmatic myopathy (Sugimoto *et al*., 2003).

1.2.3 MHC class II

The MHC class II genes are member of the immunoglobulin superfamily whose gene products are specialised for the presentation of antigenic peptides (Vyas *et al*., 2008). These antigenic peptides are derived from extracellular proteins and are presented to the receptors on the surface of $CD4^+$ helper T cells (Lennon-Dumenil *et al*., 2002a; Lennon-Dumenil *et al*., 2002b; Paust and Cantor, 2005). In humans, there are the classical DP, DQ and DR genes, together with related genes such as DO and DM which are often referred to as non classical class II genes. (Alfonso and Karlsson, 2000; Beck and Trowsdale, 2000). However, there are other genes within the class II for which functions are still unclear and which may be immunologically important, such as PFDN6. There are also a set of MHC class II genes that have a major role in the presentation of peptides onto class I molecules, namely TAP1, TAP2, TAPBP, PSMB8 and PSMB9 (Trowsdale, 2001). The α-chain and β-chain of the class II molecules are encoded by separate genes within each set of the classical class II genes and, like the class I molecules, are synthesised within the endoplasmic reticulum and associate with the invariant chain (Cresswell, 1996; Bryant and Ploegh, 2004). The α- and β-chain encoding genes are located close to each other within the class II region in mammalian species such as human, mouse and sheep (DQA1, DQB1, DQA2, DQB2), with the exception of DO genes.

MHC class II molecules are expressed primarily on cells that are capable of extracellular antigen uptake, processing and presentation, such as macrophages, dendritic cells and B cells (Castellino *et al*., 1997; Alfonso and Karlsson, 2000; Vyas *et al*., 2008). The expression of class II

molecules can be induced and enhanced by cytokines, especially interferon gamma (IFN-γ; Lennon-Dumenil *et al*., 2002a; Lennon-Dumenil *et al*., 2002b; Tizard, 2004). MHC class II molecules exist as heterodimers but, unlike class I molecules, are composed of α- and β-peptide chains (Figure 1.3)**.** In humans, the class II α- and β- peptide chains are approximately 32 kDa and 29 kDa respectively (Lang *et al*., 2000). The MHC class II molecules consist of two extracellular domains (α1 and α2, β1 and β2 respectively), a connecting peptide, a transmembrane domain and a cytoplasmic domain (Tizard, 2004). One of the main differences between class I and class II molecules is the peptide binding groove, which is closed in class I proteins, but open at both ends in class II proteins, allowing a less restricted range of peptide sizes to be presented (usually 15 to 20 amino acid residues; Chicz *et al*., 1992; Salomon and Flower, 2006). A third polypeptide chain known as the invariant chain (Li or CD74) is associated with intracellular class II molecules (Tizard, 2004). The invariant chain associates with newly synthesised class II molecules to form a stoichiometric complex in the endoplasmic reticulum (Rocha and Neefjes, 2006). It has been predicted that the invariant chains have many functions, including:

- facilitating the transport of the complex through the Golgi complex to the endosome,
- prevention of mis-binding of peptides to class II molecules, and
- facilitating the folding of class II molecules in the ER (Lotteau *et al*., 1990; Cresswell, 1996).

After the degradation of the invariant chain by a series by proteolytic reactions, the resultant peptide occupies the class II binding groove, and is then replaced by the antigenic peptide (Cresswell, 1996; Boss and Jensen, 2003). The resultant MHC class II-antigenic peptide complex is then transported to the cell surface, where it interacts with antigen specific T cells. This triggers the release of cytokines by the helper T cell, triggering the production of antibodies and cell-mediated immune responses (Weir and Stewart, 1993).

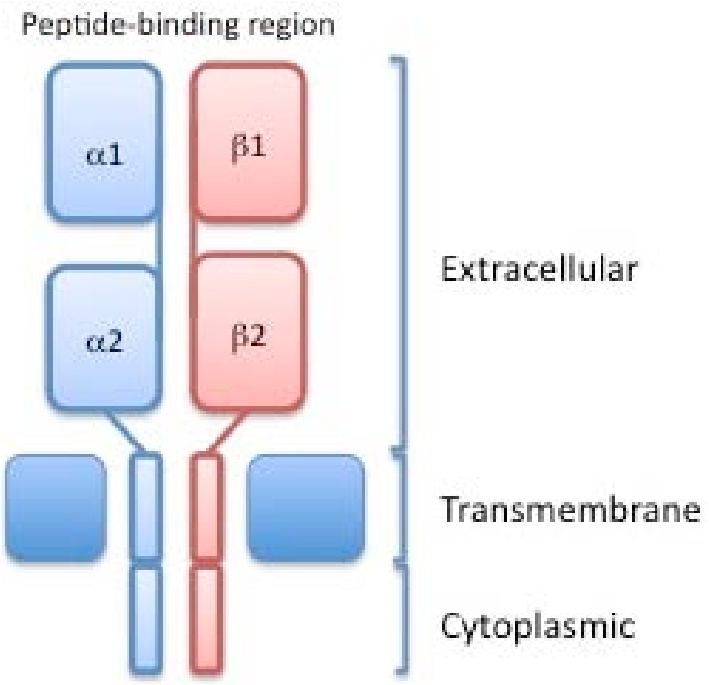


Figure 1.3: Schematic presentation of the structure of MHC class II molecule.

1.3 The Ovine MHC

The ovine MHC was first identified in the late 1970s by serological studies on sheep lymphocyte antigens (Schwaiger *et al*., 1996). MHC molecules were discovered by serological and immunochemical methods to be membrane associated proteins in sheep peripheral blood lymphocytes. The ovine MHC has been located by *in situ* hybridisation to chromosome 20, between bands q15 and q23 (Hediger *et al*., 1991). Unlike the resources devoted to human and mouse MHC characterisation, study of the sheep MHC has been a slow and limited procress. As a result of the lower importance placed on sheep MHC genes, alternative methods were adopted to understand the sheep MHC structure and its involvement in immune responses. Restriction Fragment Length Polymorphism (RFLP) analysis with human MHC probes showed that the ovine MHC was similar in gene organisation to that of humans (Chardon *et al*., 1985; Deverson *et al*., 1991). The investigation revealed banding patterns that corresponded to the human DR, DQ, DO and DZ sub-regions (Scott *et al*., 1987).

The mammalian MHC is generally divided into three regions, the central class III region separating the telomeric class I and centromeric class II regions (Flajnik and Kasahara, 2001; Horton *et al*., 2004). Recent analysis of the regions immediately flanking the MHC region, have shown that the classical class I and class II extend further than expected, and so are referred to as the extended class I and class II regions respectively (Horton *et al*., 2004). The general structure of the MHC, including the three main regions, is conserved across many mammalian species. However, in comparisons among the MHC of different mammals, some regions are more highly conserved than others (Kelley *et al*., 2005).

In general, the MHC class II and class III regions are orthologous with other mammalian species and often manifest regional syntenic conservation. Ruminant animals, seem unique in that the class II region is split into two distinct sub-regions, known as class IIa and IIb, separated by a region of non-MHC related genomic sequence (van Eijk *et al*., 1995; Figure 1.4). The exact size of this region is still not known, but in cattle, it is estimated to be approximately 15 cM (van Eijk *et al*., 1995). Comparative sequence analysis with the human MHC allowed the proximal inversion breakpoint to be estimated as 2 kb away from a DRB-like MHC class II locus (designated DSB) and 2.5 kb away from the 3' end of the glutamate-cysteine ligase catalytic subunit (Childers *et al*., 2006). The splitting of the MHC region has been attributed to the occurrence of chromosomal recombination, a phenomenon that has been shown to occur in the MHC of other species, such as in the class I region of the cat (Beck *et al*., 2005).

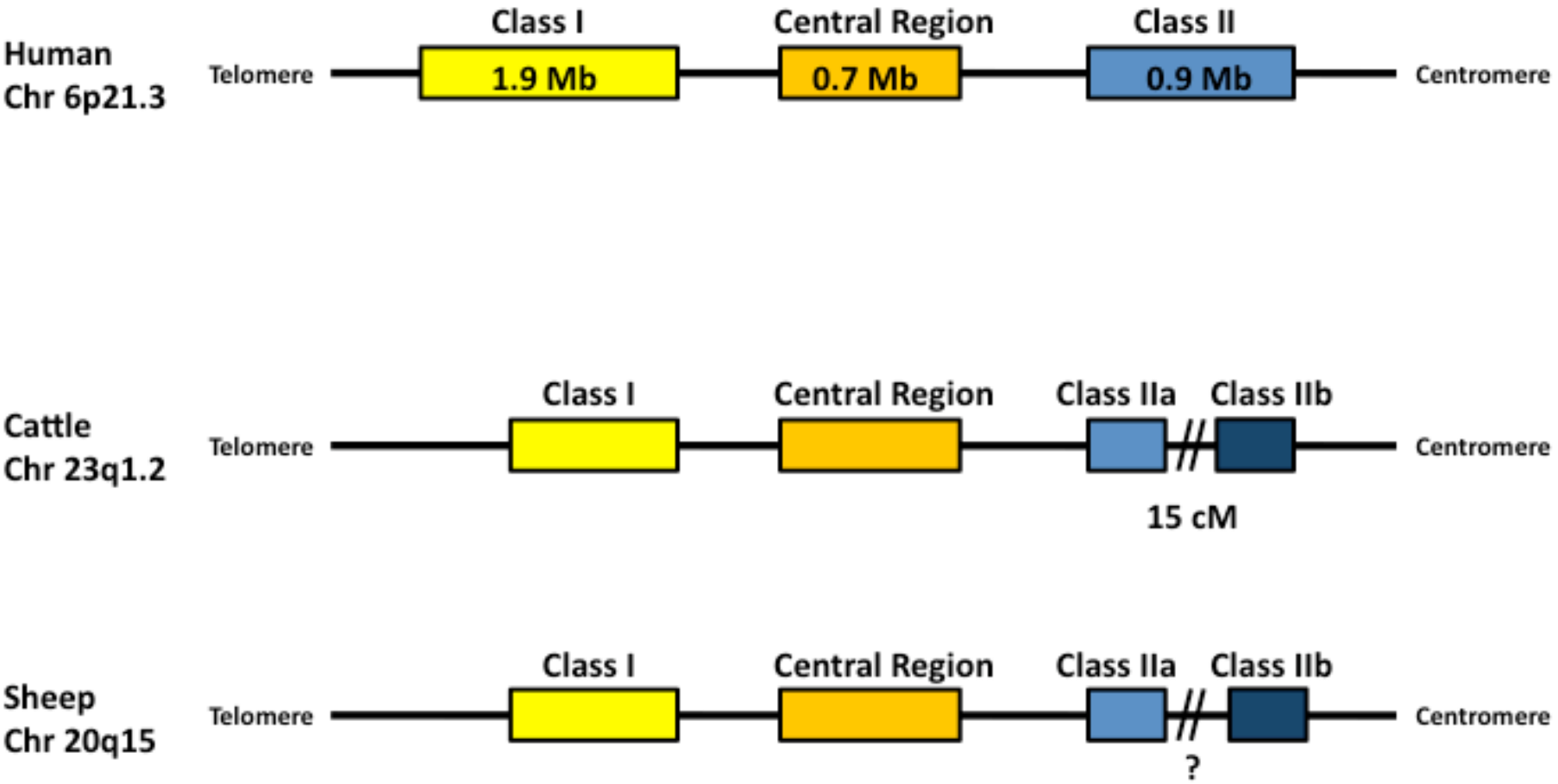


Figure 1.4: Comparison of the structure of the MHC region in human, cattle and sheep (adapted from Qin, 2009).

1.3.1 Ovine MHC class I

The ovine class I region is the least well characterised ovine MHC region, and there have been many debates about the number of classical class I genes present within the ovine MHC. Initial studies revealed two closely linked class I genes in micro-lymphocytotoxicity assays (Hein, 1997). Further studies, in different breeds of sheep, confirmed the existence of two class I genes (Hein, 1997; Jugo *et al*., 2002). Recent studies revealed 12 novel class I transcripts [sequences available on the sheep section of the ImmunoPolymorphism Database (IPD): http:// www.ebi.ac.uk/ipd/mhc/bola/index.html], from which at least four distinct polymorphic class I loci were identified (Miltiadou *et al*., 2005). The predicted existence of four classical class I genes within the ovine MHC is similar to that postulated by Liu *et al*. (2006) in a BAC clone based physical map of the ovine MHC.

The investigation of polymorphisms among ovine MHC class I genes has been limited. Initial RFLP studies in sheep, using a human class I probe, revealed polymorphic bands that co-segregated and correlated with serologically defined lymphocyte antigens (Chardon *et al*., 1985). This confirmed that the sheep leukocyte antigens were encoded by genes within the MHC region and were similar to the human homologs. This was followed by other investigations, including the identification of dinucleotide microsatellites (Groth and Wetherall, 1994), as well as SSCP and SSP-PCR genotyping of exons of MHC class I genes (Miltiadou *et al*., 2005).

1.3.2 Ovine MHC class III

The class III region is poorly characterised in sheep. Initial investigation of the ovine class III region revealed the presence of genes equivalent to those in the human MHC class III, such as C4 and CYP21. The homology was demonstrated using DNA probes based on the human MHC (Schwaiger *et al*., 1996). The relative positions of the loci identified within the sheep class III region were mapped to within an approximately

150kb DNA segment. Further investigation revealed the presence of duplicated C4 and CYP21 loci and their relative orientation, which is unique and quite different from that in human and mouse (Schwaiger *et al*., 1996). More recently, Qin *et al* (2008) and Dalrymple *et al*. (2007) confirmed the relative location and orientation of the genes within the class III region, which was similar to that suggested by Schwaiger *et al*. (1996). Qin *et al*. (2008) also suggested the possibility of a localised inversion resulting in the gene arrangement. However, these observations contradicted those of Wu *et al*. (2008), who constructed a BAC clone-based comparative hybrid map of the sheep chromosomal regions that are homologous to human chromosome 6, and concluded that the gene order and organisation of the ovine CYP21 - C4 region was similar to that of human (Liu *et al*., 2006).

1.3.3 Ovine MHC class II

Genes from the ovine class II region are better characterised than those from the other regions of the MHC. Early studies of the class II region by genomic Southern blot analysis, using human class II gene probes, resulted in a complex pattern of cross-hybridising bands (Chardon *et al*., 1985). This showed the high level of similarity between the human and ovine MHC and showed that sheep contained homologues of DQ and DR genes. Results from subsequent studies confirmed earlier predictions by Chardon *et al*. (1985), identifying DQ and DR, but not DP genes within the ovine MHC (Scott *et al*., 1987). The role of the ovine DQ and DR set of genes in immunological responses against foreign pathogens and parasites, and the effects of mutations within them, has been studied in great detail. This was followed by the identification and analysis of other class II genes, particularly class IIb genes, including DY, DM and DO (Wright *et al*., 1994; Wright *et al*., 1995a).

1.3.3.1 DR-genes

The DR genes (DRA and DRB) are present within the class IIa region of the ovine MHC. Class II molecules encoded by these genes are expressed

in higher concentrations than the DQ molecules on the cell membranes of macrophages and B cells (Outteridge *et al*., 1996).

An early hybridisation study using human HLA-D probes provided evidences for the existence of the DRA gene (Scott *et al*., 1987), which was later isolated and found to be expressed in sheep (Deverson *et al*., 1991). The possibility of the presence of a second DRA gene present in sheep that might have occurred as a result of gene duplication was observed, but it has yet to be confirmed in subsequent studies (Ballingall *et al*., 1992). Complete DNA sequences of the gene have revealed that it encodes for a 253 amino acid polypeptide chain, of which 24 amino acids constitute the signal peptide and the remaining 229 amino acids form the mature polypeptide (Fabb *et al*., 1993).

Unlike the DRA gene, DRB has been studied in greater detail in sheep, especially in association with parasite resistance. Initial RFLP studies on bacteriophage clones of a sheep genomic library revealed two distinct DRB-like genes (Scott *et al*., 1987) which were later confirmed by Dutia *et al*. (1994). Scott *et al*. (1991b) have described the presence of four ovine DRB loci, the functional DRB1 gene and three other pseudogenes, DRB2, DRB3 and DRB4. The basic structure of the ovine DRB1 gene is very similar to other mammalian species (Schwaiger *et al*., 1996). The functional ovine DRB1 gene is an ortholog of the cattle DRB3 gene, as well as the human DRB1 gene.

Exon 2 of DRB1 encodes the β1 domain, which constitutes part of the peptide-binding region (PBR) of the DR molecules (Brown *et al*., 1993), and the high level of variation within exon 2 was thought to have arisen from the need to recognise a diverse range of pathogens that exhibit antagonistic co-evolution with the host to evade immune recognition. Despite the high level of variations within the gene, most of the studies undertaken on this gene have concentrated on exon 2 and the adjoining intron 2 (Ballingall *et al*., 2008). The ovine DRB1-exon 2 has been genotyped by a number of methods including RFLP studies, cloning and sequencing, as well as direct sequencing of PCR products. There are

currently more than 89 ovine DRB1 alleles across different breeds, including Laxta, Karrantzar, Scottish Blackface, Soay, Suffolk, Merino and Romney (Konnai *et al*., 2003; Dukkipati *et al*., 2006). Intron 2 of DRB1 is also of interest, as it contains a short tandem repeat (STR) in the form of $[(GT)(GA)]_n$ approximately 30 bp downstream from the 3' splice site of exon 2 (Schwaiger and Epplen, 1995). The basic structure of this STR is present in almost all the expressed DRB1 loci of all mammalian species, including cattle, red deer and human. It was also reported that the number of $[(GT)(GA)]_n$ repeats in the STR is closely linked to the exon 2 sequence (Ammer *et al*., 1992).

The analysis of DRB1 exon 2 has received great interest because of the association of this region with diseases in sheep, which leads to an effect on production. It was predicted by Schwaiger *et al*. (1995) and Buitkamp *et al*. (1996) that certain alleles of the DRB1 locus have a correlation with low FEC from *Teladorsagia circumcincta* in Scottish Blackface sheep. This association was also shown in the Texel and Suffolk breeds of sheep (Sayers *et al*., 2005). Also, two STR alleles in intron 2 of the DRB1 gene have been linked with the highest observed level of antibody production in response to gastrointestinal nematode parasite infection (Outteridge *et al*., 1996). Apart from associative studies with parasite infection in the gastrointestinal tract of sheep, a correlation was identified between polymorphisms of ovine DRB1 and the development of tumours after experimental infection of sheep with bovine leukemia virus (Nagaoka *et al*., 1999). This suggests that the differences in immune response are affected by differences in MHC class II alleles. Comparisons of the DRB1 locus among different ruminants showed that the levels of polymorphism differed; from monomorphism in musk-ox to extensive DRB diversity in domestic sheep and cattle (Mikko *et al*., 1999). In addition, typing of the ovine DRB1 locus was also used in determining the effects of MHC variation in the desert bighorn sheep against infectious diseases (Gutierrez-Espeleta *et al*., 2001). Phylogenetic tree analysis presented evidence of clustering of DRB sequences within species instead of within allelic lineages across species. Recently, a study by Fernandez-de-Mera

et al. (2009) observed significant correlations between DRB-2 haplotypes and specific parasites in Iberian red deer.

1.3.3.2 DQ-genes

Like DR genes, DQ genes are present within the class IIa region of the ovine MHC. The existence of DQ genes in sheep was first demonstrated by Southern blot analysis using probes that were homologous to the human DQA/B region (Chardon *et al*., 1985). Several studies have since been undertaken to characterise DQA and DQB genes of sheep and their role in immune responses, especially against gastrointestinal nematode parasite infections.

The presence of two DQA genes in each haplotype has been shown by RFLP studies and sequence data from genomic clones (Dukkipati *et al*., 2006). This is consistent with a detailed genomic map of the ovine DQ-region, which revealed two DQ loci. Each DQ locus consists of a DQA and DQB gene arranged in a tail to tail orientation (DQA1-DQB1-DQA2-DQB2; Wright and Ballingall, 1994). This was confirmed by a recent investigation, where a BAC clone containing the DQ region and the DRB1 gene was fully sequenced, and a detailed map of the region was constructed (Hermann-Hoessing *et al*., 2008).

The ovine DQA1 and DQA2 genes were sequenced by Scott *et al*. (1991a) who noted the similarity between ovine DQA1 and DQA2 and the equivalent human genes. Subsequent studies led to the identification of the full length cDNA sequences, which were used in further sequence and amino acid analysis (Fabb *et al*., 1993). The DQA1 and DQA2 genes encode a 255 amino acid chain, of which 23 amino acids compose the signalling peptide and the remainder are retained as the mature polypeptide. Comparison between exon 2 of DQA1 and DQA2 showed similarity of 78% of nucleotide sequence and 71% of coding amino acid sequence, allowing discrimination between the two loci (Snibson *et al*., 1998; Dukkipati *et al*., 2006). Sequence differences between exon 2 of DQA1 and of DQA2 has led to the identification of several distinct alleles in different breeds of sheep (Hickford *et al*., 2004; Zhou and Hickford,

2004). These alleles could then be used for various genetics analyses, such as the evolutionary development of this sub-region in sheep compared to other species, the association between these sheep genes and resistance to gastrointestinal nematode parasites, or even the possible involvement of the DQA genes in vaccine responses by the parasitised host.

Nucleotide sequences of the ovine DQB gene were first reported by Scott *et al*. (1991b), and comparative analysis with human sequences revealed similarities with both human DQB1 and DQB2 genes. Subsequent studies of exon 2 sequences of both ovine DQB1 and DQB2 sequences, derived from cosmid clones, were determined and were shown to have extremely high level of nucleotide sequence similarity (greater than 90%; Wright and Ballingall, 1994). The high level of similarity hindered discrimination between loci, but their close proximity to the ovine DQA1 and DQA2 genes could be used to differentiate between these genes. Several new DQB sequences were also subsequently identified in other studies, including that by van Oorschot *et al*. (1994) from an SSCP sequence analysis of PCR-amplified DQB exon 2 sequences in 13 Merino sheep, as well as by Schwaiger *et al*. (1996), where sixteen PCR-amplified DQB exon 2 sequences were characterised from 18 sheep. However, like the earlier studies, the sequences could not be differentiated between the two loci. More recently, Feichtbauer-Huber *et al*. (2000) adopted a reference-strand-mediated conformation analysis method to identify new sequences of the DQB gene.

The ovine DQA and DQB genes are more closely positioned than in humans, and linkage analysis of ovine DQA1, DQA2, DQB1, DQB2 and DRA loci showed that there is a high level of linkage disequilibrium. In addition, the lack of recombination observed between the DQA and DQB genes, or between DRA and any of the DQ- loci further confirms the genes are physically close (Dukkipati *et al*., 2006). However, haplotype analysis by Hickford *et al*. (2007) identified recombination within the DQA region, between DQA1 and DQA2 genes, which only occurred in the presence of specific DQA1 and DQA2 alleles. It was suggested that this

may be due to recombination occurring in a haplotype-dependent manner, which has been reported in the MHC of other species, including mouse and humans (Thomsen *et al*., 1994; Shiroishi *et al*., 1995). It has been hypothesised that haplotype-specific recombination occurs due to structural constraints on variation in haplotype length and gene organisation (Carrington, 1999).

1.4 Genetics of parasite resistance

Since the late 1980s there has been an increased interest in the importance of MHC genes and their role in disease resistance in many species. This has led to an increased interest in the ovine MHC and it's role in parasitic infestations because this information has a direct benefit to the sheep industry by virtue of enhanced productivity and reduced production costs. There is abundant evidence, both anecdotal and scientific, that individual sheep vary in their resistance to gastrointestinal parasites, and probably all parasites, and that this variation is genetically determined. Evidence supporting this statement is described below.

1.4.1 The link between MHC and parasite resistance

It is well established that ingestion of parasite antigens into the host's gastrointestinal system triggers innate immune responses, as well as both humoral and cell mediated adaptive responses, with recruitment of T cells along the gastrointestinal mucosa (Acheson and Luccioli, 2004; Maizels and Yazdanbakhsh, 2003). In the small intestine, soluble antigens (metabolic or excretory-secretory components) are absorbed by specialised microfold cells in the follicle-associated epithelium overlying the Peyer's patches, either through phagocytosis or pinocytosis (Janeway *et al*., 2001). The antigens are transported from the intestinal lumen to the subepithelial dome, where antigen presenting cells, especially dendritic cells, interact with T cells. In contrast, the mechanisms of antigen priming in the abomasum are unclear. Ruminants do, however,

respond to foreign antigens in the abomasum, for example cattle infected by *Ostertagia ostertagi* (Claerebout and Vercruysse, 2000) and sheep infected with *Teladorsagia circumcincta* or *Haemonchus contortus*.

Disease association studies have mapped many disease predisposing loci to the human MHC. However, despite the significance of these association studies and the information they have generated, the specific disease-related loci remain unidentified for many diseases. The difficulty in deriving genetic information in outbred populations illustrates the importance of any information generated from such immune response studies. The high degree of variation within the MHC region and its association with diseases in humans has increased the interest in similar association studies in a variety of mammalian species, in particular farm animals. In addition, research aimed at delineating the role of the MHC in regulating adaptive immune responses in mammals, as well as the involvement in a variety of immunologically related mechanisms, further supports the significance of these association studies. One of the earlier disease association studies in farm animals demonstrated the strong association between the MHC and Marek's disease in chickens (Briles *et al*., 1977; Haeri *et al*., 2005) but more importantly to this study, the chicken MHC was recently found to be involved in the response to helminth infection (Schou *et al*., 2007). Many studies have also implicated variation within the MHC as a determinant of host resistance and/or sensitivity to gastrointestinal parasite infection in several species, including mice, water voles and long-tailed giant rats (Behnke *et al*., 2006; Tollenaere *et al*., 2008; Lenz *et al*., 2009).

There is currently a vast amount of literature implicating genes within the MHC in determining resistance or susceptibility to a wide variety of pathogens. This is relevant in understanding the role and effects of the MHC in domesticated animals such as sheep and cattle. In Australia, earlier work demonstrated the effects of genetic variation on the magnitude of the immune response by ruminants. This work was extended to examine the genetics of resistance and susceptibility of the host to worm infestation. DNA probes for cDNA sequences derived from

human MHC class II loci (DQA, DQB and DRB), cross-reacted with sheep orthologues and led to the identification of statistically significant associations between class II alleles and FEC in both *Trichostrongylus colubriformis* susceptible and resistant sheep (Outteridge *et al*., 1988). Subsequently, RFLP analysis was performed, using human DQB and DRB probes, to investigate the association of the MHC with parasite resistance (Blattman *et al*. 1993). This study did not find any significant association between the RFLP genotypes and resistance to *H. contortus*. However, in another RFLP study, using a human-leukocyte antigen DRB probe, a significant association between the presence of a 19 kb DRB fragment and susceptibility to gastrointestinal nematodes was established in ewes, but not in rams (Wetherall *et al*., 1991). The lack of consistency in findings by RFLP studies was suggested to be due to the lack of discriminatory capability with significantly larger number of alleles.

More recently, DRB1 alleles, characterised by length polymorphisms of a microsatellite in intron 2, were found to be correlated with FEC across different breeds of sheep (Schwaiger *et al*., 1995; Outteridge *et al*., 1996). A significant MHC effect was observed when substitution of the common allele with a resistant allele resulted in a reduction in FEC. The role of MHC in the control of nematode parasite resistance in sheep was also demonstrated by analysis of quantitative trait loci (QTL). Davies *et al*. (2006) observed evidence of loci associated with severity of gastrointestinal nematode parasite infection in Scottish Blackface sheep on chromosomes 2, 3, 14 and 20. Analysis of the entire chromosome 20 showed that variations within the MHC region had a statistically significant correlation with gastrointestinal nematode parasite infection, supporting involvement of the MHC in the severity of parasite infection. In addition, there were also quantitative genetic analysis in sheep and cattle that have clearly shown that resistance to nematode infection is under genetic control (Bisset *et al*., 1992; Stear *et al*., 1990; Stear *et al*., 1994; Bishop *et al*., 2004). It was identified that the heritability of a single FEC measurement varies between populations but is usually between 0.2 and 0.4 in animals that have been previously exposed to infection (Stear *et al*., 1997).

Apart from the association between MHC and FEC, loci within the MHC region have also been shown to be associated with other productivity traits including body weight, wool fibre strength and fat-marbling of muscle. Bot *et al*. (2004) have shown that specific alleles at two microsatellite loci, close to the regions encoding complement factor B and MHC class II DRB1, showed significant correlation with increased fleece weight. Associations between productivity traits and MHC loci have also been demonstrated in cattle. Schook and Lamont (1996) reported a correlation between certain MHC genetic markers and growth rate, milk yield, milk fat, milk protein and fertility in cattle.

In a comparative study using bovine cDNA microarray, an increased expression of a range of genes was observed in an analysis of duodenum tissue from an outbred population of resistant and susceptible lambs which had been subjected to two natural challenges with a range of gastointestinal parasites (Diez-Tascón *et al*., 2005). Upregulated genes included *DQB1*, *DRA* and *DQA1* from the MHC class II region (Diez-Tascón *et al*., 2005). This observation highlights key differences between resistant and susceptible animals in the early immune response to gastrointestinal nematodes. In another study, using transcriptional profiling of duodenum tissue samples from resistant and susceptible sheep (Keane *et al*., 2007), upregulation of several MHC class II genes (*DQA1*, *DQB1* and *DRA*) was observed in resistant animals. Subsequent RT-PCR analysis of *DQA1* showed that it had an average 8.4 fold increase in expression in resistant animals relative to susceptible animals. (Keane *et al*., 2007). However, these observations seemingly contradict earlier findings by the same group, where no observed increase in expression of either MHC class II genes or any association with antigen presentation or processing was found (Keane *et al*., 2006). Interestingly, a significant increase in expression of a MHC class I gene (*HLA-A* orthologue) in resistant animals was also observed, indicating possible cross talk between the different Th type responses.

The involvement of the MHC region with resistance to gastrointestinal nematodes has also been demonstrated in mice. The first analysis of QTL

the mouse genome, for resistance to gastrointestinal nematode parasites was by Iraqi *et al*. (2003). Six QTLs, identified on chromosomes 1, 2, 8, 13, 17 and 19, were found to be associated with resistance to the intestinal worm, *Heligsomoides polygyrus*. Interestingly, the MHC region in mice is located on chromosome 17. This observation of a QTL in the mouse MHC region was confirmed by Behnke *et al.* (2006). Eight immunological traits (FEC at weeks 2, 4 and 6; mucosal mast cell protease 1; granuloma score; IgG1 against L5; IgG1, and IgE to L4) were used and identified QTLs on chromosomes 1 and 17 that were associated with resistance to the *H. polygyrus* infection. More specifically, the MHC genes, most notably the class II molecules and TNF located on chromosome 17 correlated with gastrointestinal parasite infection. A recent elegant study by Rodrigues *et al*. (2008) using class I or class II loci knockout mice challenged with the gastrointestinal parasite *Strongyloides venezuelensis* has confirmed the relative importance of the class II region loci for resistance to this parasite.

1.4.2 Selective influences on ovine MHC loci

The MHC region has evolved as a main coordinator of specificity in both adaptive and innate immunity. This may explain some of the characteristics of the MHC region, such as high gene density and diversity, relatively low frequency of recombination and high levels of variation. The mechanisms involved in the variability of the MHC region have been proposed to be either balancing selection (parasite or disease mediated) or sexual reproduction selection (Piertney and Oliver, 2006).

The influences of MHC on mating patterns are often not random and reflect disassortative mating. Therefore, MHC-mediated mate-choice mechanisms are likely to exist, and to contribute to polymorphism in MHC genes (Apanius *et al*., 1997; Penn and Potts, 1998). This form of MHC-mediated sexual reproduction selection has been demonstrated in several species including salmon (Landry *et al*., 2001) and humans (Chaix *et al*., 2008). Parasite mediated balancing selection constitutes

two theories that demonstrate the link between the MHC and parasite resistance, and the resulting level of MHC diversity:

- negative frequency-dependent selection or
- symmetrical overdominance selection (heterozygote advantage).

The negative frequency-dependent selection proposes that new or rare alleles may incur a selective advantage as fewer pathogens have been exposed and adapted to it. Selective advantage as a result of symmetrical overdominance occurs because high heterozygosity allows the recognition of a wider variety of antigens originating from multiple pathogens.

Several recent studies have shown evidence of balancing selection in the sheep MHC and that disease association studies in sheep, therefore supports the evolutionary theory. Gutierrez-Espeleta *et al*. (2001) investigated genetic variation at the MHC class II loci and resistance to diseases of Desert Bighorn sheep. It was discovered that low MHC variation was not the cause of high susceptibility to infectious disease, and the eventual decrease in the number of the population, since there is extensive genetic variation at the DRB locus. However, higher levels of heterozygosity in the DRB amino acid domain involved in antigen presentation relative to other domains that is not involved in antigen binding was observed. These findings imply that diversity in this gene is functionally significant and is maintained by balancing selection (Gutierrez -Espeleta *et al*., 2001). Charbonnel and Pemberton (2005) have also demonstrated the presence of balancing selection in free living Soay sheep in St Kilda (Scotland) by analysing both MHC and neutral loci. These sheep were subjected to infection with the nematode *T. circumcincta* over an eight year period. For the later part of the experiment, lower levels of temporal genetic differentiation were observed at the MHC loci than at neutral loci. This suggests balancing selection activity at the MHC loci acting through spatial and temporal heterogeneity in selection pressure (Charbonnel and Pemberton, 2005). These observations confirmed earlier work by Paterson (1998) who

revealed evidence of balancing selection but not of negative frequency-dependent selection. Overdominance and negative frequency-dependent selection have both been suggested as modes of balancing selection in sheep populations. In striped mice, MHC DRB heterozygosity influenced their susceptibility to parasites, with particular alleles more frequently present than expected in both high and low parasitised individuals, suggesting that both overdominance and negative frequency-dependent selection may operate simultaneously (Froeschke and Sommer, 2005).

1.5 Gastrointestinal parasitism in sheep

Sheep production constitutes an important part of livestock farming in many parts of the world. In many areas this is the only form of livestock, and therefore is the sole source of income for both owners and land-users. In Australia, an estimated gross value of 4.5 billion Australian dollars, from wool production and sheep and lamb slaughtering, made up approximately 5% ($ 2 billion) of the gross value of Australian agricultural production in 2006/2007 (7503.0, Australian Bureau of Statistics). However, economic costs on production hinder the full exploitation of the industry's potential revenue generation. Parasitism, in particular gastrointestinal parasitism, is considered to be one of the most serious limitations affecting small ruminant production worldwide.

Economic losses resulting from parasite infections are both direct and indirect. Direct effects include sheep mortality and decreased productivity, and the indirect effects of increased cost of prevention, cost of treatment with biochemical products, increased labour burden and enforced changes of pasture management also contribute to the costs of infection (Dominik, 2005). As a result of the diverse costs incurred from parasite infection, it is difficult to establish an accurate figure for the total cost through even the most elaborate surveys and estimation procedures. Many diseases or disorders may result in continuous interactions with nutritional and environmental stresses, management methods, concurrent diseases, genetic predispositions and many other

factors (Stear *et al*., 2006). However, periodic reports from government agencies on sheep industries have released estimates on the scale of hundreds of millions of dollars per year, including all phases of production in Australia (McLeod, 1995; Marshall *et al*., 2009).

1.5.1 Gastrointestinal parasites

The three most economically important gastrointestinal parasites that infect small ruminants across the world are *H. contortus*, *T. circumcincta* and *T. colubriformis* (Urquhart *et al*., 1987; Zajac, 2006). All three parasites belong to the superfamily Trichostrongyloidea, of the order Strongylida, and the class Nematoda.

1.5.1.1 Epidemiology

Depending on the geo-climatic factors in which parasites exist, different species will survive better or populate their hosts more efficiently than others. *H. contortus* infection of sheep occurs in the tropical and sub-tropical areas, or in regions with heavy summer-dominant rainfall. This is because it thrives in warm wet climates, but less well in cold conditions (O'Connor *et al*., 2006). The optimum temperature for larval development is about 20°C. Unlike *H. contortus*, nematode parasites *T. circumcincta* and *T. colubriformis* prefer temperate climates with cool winters and uniform rainfall (O'Connor *et al*., 2006; Zajac, 2006). As a result of such characteristics, the parasites are able to survive desiccation better than *H. contortus* and are able to develop at lower temperatures over the winter periods. All three parasites are very successful parasites of sheep and goats in their respective regions.

1.5.1.2 Description

In many parts of the world *H. contortus* is an economically significant nematode species. The adult worms reside in the abomasum of small ruminant gastrointestinal systems. The female *H. contortus* is one of the largest strongylid nematodes that infects ruminants (3 cm in length) and is highly prolific, producing approximately a thousand eggs each day. The eggs are deposited in the faecal mass in the pastures. The eggs then hatch and the larvae pass through three stages before infecting the

grazing animals. The resulting disease is a direct consequence of the adult worms feeding on host blood, leading to parasitic gastroenteritis and haemonchosis in animals that are immuno-compromised, or highly infected. The chronic loss of blood, often compounded by poor nutrition, leads to weight loss, weakness and decreased productivity.

T. circumcincta is the second most important trichostrongylid nematode that contributes to parasitic gastroenteritis. Like *H. contortus*, it resides in the abomasum, but it does not primarily feed on the blood of the host, nor are the larvae as prolific. *T. circumcincta* are smaller than *H.contortus*: approximately 1cm long. The L4 larvae develop in the abomasum, leading to changes within the gastric glands and the formation of nodules on the mucosal surface. These changes lead to an increase in pH and failure to convert pepsinogen to pepsin, thus reducing digestive efficiency. Heavily infected animals develop diarrhoea, anaemia and hypoproteinemia, and die in severe cases. The prepatent period of *T. circumcincta* is similar to that of *H. contortus* and, like *H. contortus*, the life span of the adult parasite is a few months.

The third common genus of trichostrongylid nematode, Trichostrongylus, causes many different diseases in heavily infected animals. There are several species of Trichostrongylus that infect small ruminants, including *T. axei* and *T. colubriformis*. Unlike the two parasites describe above, *T. colubriformis* is not found in the abomasum but in the small intestine. These small parasites (often less than 1cm) are very difficult to detect and are only seen after removing the intestinal content. Also, unlike the other two parasites, it can survive over the winter periods in the host. The development of the larvae in the small intestine of the animal causes considerable haemorrhage and loss of plasma into the lumen of the gut, which leads to weight loss. *T. colubrifomis* infection also causes severe diarrhoea.

1.5.1.3 Life Cycle

The three trichosytrongylid nematodes, *T. circumcincta*, *T. colubriformis* and *H. contortus* have very similar life cycles. The adult female parasite in the abomasum or small intestine, depending on the species, produces eggs that are excreted in the faeces. This releases the eggs onto the pasture on which the sheep grazes. Hatching of the eggs and development of the first stage larvae (L1) occurs within the faecal mass, which provides some protection from environmental factors. First stage larvae feed on the bacteria in the faecal mass, undergoing two moults to reach the infective third stage (L3). The L3 larvae migrate from the faecal material onto the surrounding vegetation. Infective larvae are ingested by the grazing animal and exsheath in the rumen. From there, they pass into the abomasum or small intestine and develop through a fourth larval-stage into the adult worm.

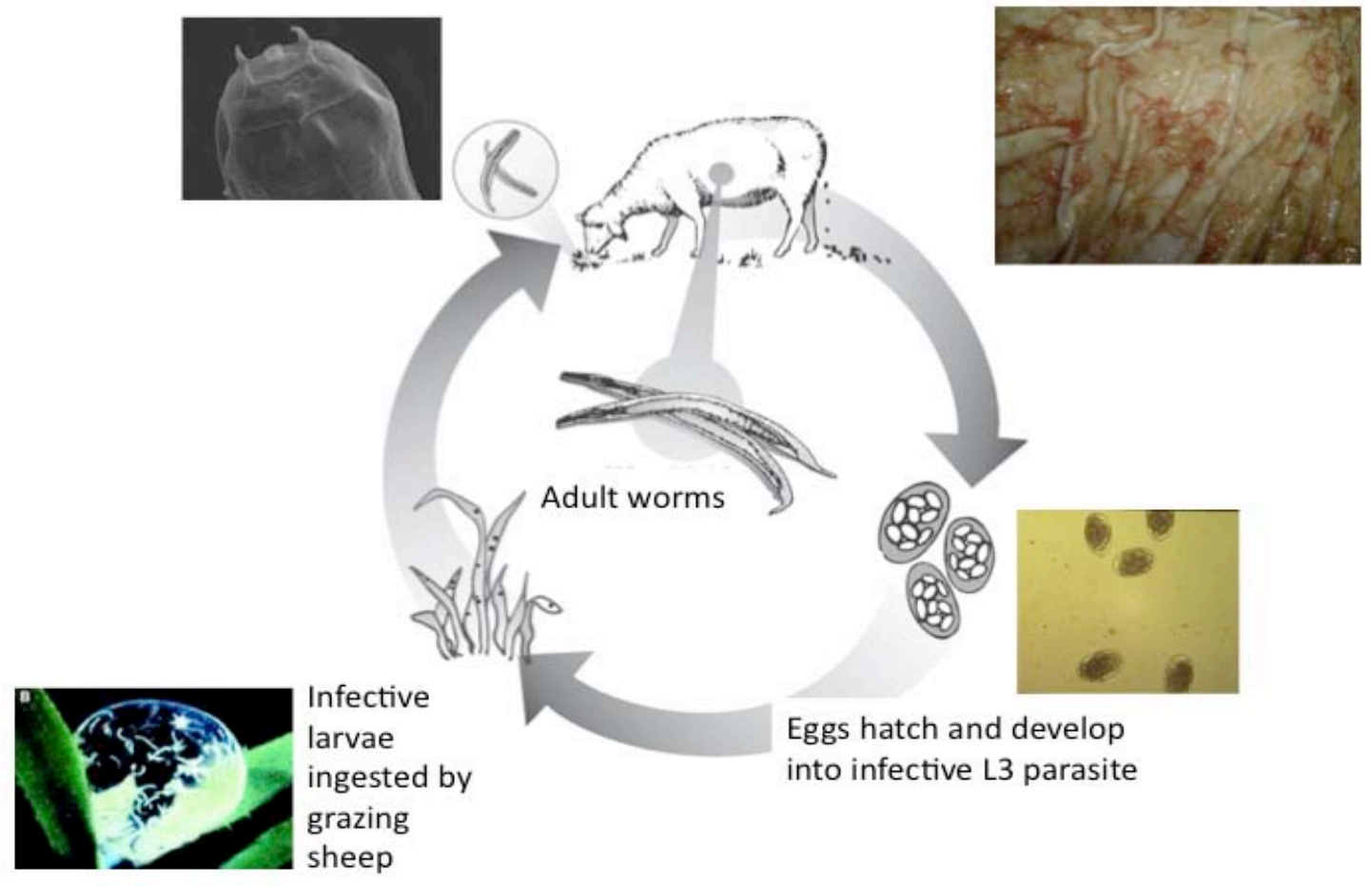


Figure 1.5: Diagram of a generalised life cycle of gastrointestinal parasites in sheep.

Sources: Virginia Cooperative Extension (http://pubs.ext.vt.edu/410/410-027/410-027.html); Ray M. Kaplan – Science Watch (http://sciencewatch.com/dr/erf/2008/08aprerf/08aprerfKap1/); Emerging Concepts in Small Ruminant Parasite Control (http://smallfarms.oregonstate.edu/sfn/spring08parasitecontrol); Hair Sheep Workshop (http://www.sheepandgoat.com/HairSheepWorkshop/parasitism.html

1.5.2 Current strategies for parasite control

For decades, the control of gastrointestinal parasites in small ruminants has been made possible through the use of chemicals and pasture management, and more recently the development of alternative strategies such as vaccines, selective breeding and biological control (Barger, 1996; Woolaston and Baker, 1996).

1.5.2.1 Management

Pasture management can be applied to reduce the exposure of livestock to parasites. Over-wintered larvae die during spring, allowing pasture rotation, grazing animal rotation and crop rotation to be used as a form of parasite control. However, the efficiency of these methods has fluctuated over time (Colvin *et al*., 2008). The management of grazing pastures is often impractical due to the costs of labour and land usage. In addition, uncontrollable factors such as temperature, drought and the types of parasite species present cannot be predicted. Rotation of animals grazing on a pasture would also be problematic because of the parasites' ability to cross-infect between species (Morley and Donald, 1980), as well as the hardiness of the infective L3 larvae, which can reside on the pastures for long periods of time (Wanyangu *et al*., 1997).

1.5.2.2 Anthelmintic Drugs

In addition to pasture management, the application of anthelmintic drugs has been used in a variety of ways to control parasite infection of livestock (Waller, 2003). Initially, anthelmintics offered a simple, cheap, cost effective method of controlling nematodes then. In sheep, one of the sources of infection for sheep is the peri-parturient rise in faecal egg output in ewes during the last trimester of pregnancy and early lactation (Beasley *et al*., 2009).

However, recent developments have led producers to reassess their reliance on chemicals for parasite control, with the emergence of anthelmintic resistant parasites (Bisset and Morris, 1996; Kaplan, 2004). The evolution of resistance in parasites is thought to be due to the unregulated use of anthelmintics. Initially, resistance developed slowly

because of the lower efficiency of benzimidazoles. But with the introduction of more efficient thiabendazole-based anthelmintics, selection pressure for resistance has increased dramatically (Kaplan, 2004). Further studies investigating the prevalence of resistance among breeds have found more parasites resistant to the newer drugs. By the 1990s, anthelmintic resistance was a major threat to small ruminant production in many parts of the world (Waller, 1999; Waller and Thamsborg, 2004).

1.5.2.3 Vaccines

Vaccines have proven to be a useful means of protection against a range of microorganisms. This success and the need for alternative and efficient strategies for the prevention of parasitic infection have led to a large volume of research towards the development of anthelminthic vaccines (reviewed by Newton and Munn, 1999). However, vaccines against nematode parasites have had very limited commercial success.

Several types of vaccine are currently being studied and used. This includes ones based on antigens that are accessible to the hosts' immune system during natural infection. The advantage of such vaccines is that the continuous natural exposure to antigens acts as a booster to the primary vaccine (Knox, 2000; Meeusen and Piedrafita, 2003). Another approach to vaccination in sheep is attenuated vaccination. Early attempts at this were made using attenuated whole parasites, which showed promise and marketing opportunities (Waller and Thamsborg, 2004). However, this approach failed against the more crucial gastrointestinal nematode parasites. Currently, commercially available irradiated larval vaccines are used against bovine and ovine lungworm infection (Bain, 1999). The limited success of attenuated and natural infection based vaccines has led to a shift in attention, to a molecular approach of identifying protective antigens from parasites. The third method that is being studied is the search for hidden antigens that are not accessible to the hosts' afferent immune system, such as intestinal cells of parasites (Knox, 2000).

1.5.2.4 Biological Control

Biological control is a novel alternative method for control of parasitic nematodes. Currently, research on biological control of nematodes in livestock is almost exclusively associated with nematode-destroying microfungi. The microfungal spores are able to survive and pass through the extreme conditions of the gastrointestinal tract and then develop within the faecal matter. The development of hyphae traps the infective larvae before they migrate onto the pasture (Larsen, 1999). An example of such a microfungus is *Duddingtonia flagrans*, which has the capacity to reduce the number of infective larvae on the pasture and reduce the intensity and severity of infection (Waller, 2004; Waller *et al*., 2004). The fungal spores are administered as a food additive and need to be continuously fed, to create the constant shedding of the spores in the faeces (Waller and Thamsborg, 2004). Field evaluation of this form of biological control in a variety of geo-climatic regions has been carried out with promising results (Chandrawathani *et al*., 2003; Waller, 2003).

1.6 Aims and relevance of this study

The work described in this thesis has sought to further characterize the class II region of the sheep MHC in Merino sheep with known resistance to gastrointestinal parasites. The main aims were to:

- Indentify known loci within the MHC class IIa and IIb subregions
- Discover SNPs within the IIa and IIb regions and determine SNP genotypes in the sheep populations available for study
- Identify sheep haplotypes by deduction from sheep pedigrees and by inference using the EM algorithm
- Assess the predictive role of resting serum IgA levels in post weaning sheep for parasite resistance and/or susceptibility
- Look for associations between specific haplotypes and estimated breeding values based on WEC and resting serum IgA levels

Chapter 2

General Materials and Methods

This Chapter highlights the general laboratory resources and methods used throughout the project. Specific laboratory methods and reagents specific to individual experiments are described in their respective chapters.

2.1 Sources of sheep DNA

Sheep from two panels of animals that have been maintained as part of the Rylington Merino Project (Western Australia) were used as donors of blood for extraction of individual DNA. The gastrointestinal parasite resistance status of these animals, as measured by worm egg count (WEC), was known. In addition, estimated breeding values WEC (EBVwec) and estimated breeding values WEC at weaning (EBVwecwean) information are also available. The two groups of animals are:

i. ***Cohort 1***. This cohort of animals consisted of 68 unrelated parental animals (sire and dams). Sheep blood was collected into Vacuette 6 ml K_3EDTA tubes and was kept at -20°C until the DNA was extracted. Semen samples of the sires from this group were obtained from the Department of Agriculture and Food of Western Australia (DAFWA), and were stored at -20°C prior to DNA extraction.

ii. ***Cohort 2***. This cohort of animals consisted of 193 animals that comprised 10 family groups. Blood from these animals was collected into 6ml K_3EDTA tubes and was kept at -20°C until DNA was extracted. Offspring of each family group were bred from selected sire and dams.

2.2 Source of sheep serum

Sheep from a panel of animals maintained as part of the Rylington Merino Project (Western Australia) were used as donors of blood for extraction of serum samples. The blood was collected into Vacuette 8ml Z Serum Sep Clot Activator tubes and was left to coagulate at room temperature. The coagulated blood was centrifuged at 2600 g for 20 minutes to separate the coagulated blood cells from the serum. The serum was transferred to a sterile 5ml tube and stored at -20°C until further use in the IgA antibody ELISA assays.

2.3 DNA purification

DNA was extracted from either blood, semen or from cultures of bacteria. The quality and quantity of the extracted DNA was determined by agarose gel electrophoresis (1.0% w/v), as well as by spectroscopy using a Thermo Scientific Nanodrop 1000. All DNA was stored at -20°C.

2.3.1 Sheep DNA

2.3.1.1 DNA purification from blood

Genomic DNA was extracted from whole sheep blood using the Qiagen DNeasy Blood and Tissue kit as per the manufacturer's protocol with slight modifications. Whole blood (500 µl) was mixed with 1 ml highly purified water and centrifuged at 16000g for 15 minutes. The supernatant was removed and the pellet washed with 1 ml of water and centrifuged at 16000g for 15 minutes. A pellet, composed mainly of white blood cell nuclei, was then used for extraction of genomic DNA according to manufacturer's instructions.

2.3.1.2 DNA purification from semen

A proteinase K/SDS based method was adopted to extract DNA from sheep semen. Sheep semen (200 µl) was mixed with 1 ml of Tris-EDTA buffer (TE; 10 mM Tris, 1 mM EDTA pH 8.0) and centrifuged at 16000g for 10 minutes. The pellet was resuspended in Lysis buffer (White Blood Cell lysis buffer: 10 mM Tris, 10 mM EDTA, 50 mM NaCl, 0.2 % SDS, 1.6

% v/v 2-Mercapto-ethanol, 160 µg of proteinase K) and incubated at 50°C until the pellet dissolved. The sample was then extracted using the Qiagen DNeasy Blood and Tissue kit according to the manufacturer's instructions.

2.3.2 Plasmid and cosmid DNA purification

A QIA prep® Spin Miniprep Kit was used for the extraction of plasmid and cosmid DNA from ELECTROMAX™ DH5α-E™ *Escherichia coli*.

A single bacterial colony containing either plasmid or cosmid was inoculated into 10 ml of Luria Bertani (LB) broth culture media containing ampicillin (100 µg/ml for plasmid and 50 µg/ml for cosmid). The culture was incubated at 37°C for 18 hours with constant agitation and then the bacteria were harvested by centrifugation at 2600g for 15 minutes. The plasmid or cosmid DNA was extracted as per the manufacturer's instructions except that cosmid was eluted in 50 µl of Tris-HCl (pH 8.0) at 60°C, and both plasmid and cosmid DNA were incubated at room temperature with Tris-HCl (pH 8.0) for 1 minute prior to centrifugation to elute the DNA.

2.3.3 Bacterial Artificial chromosome (BAC) DNA purification

The ovine CHORI-243 BAC library filters for radioactive screening were provided by PACBAC Resources (Dr Noelle Cockett – personal communication). BAC clones identified from screening of the filters were obtained from Australian Genome Research Facility (AGRF). A single BAC clone was inoculated in 5 ml of LB broth culture media containing chloramphenicol (12.5 µg/ml). The culture was incubated at 37°C for 8 hours under constant agitation. Five 1 ml aliquots of the culture were sub-cultured into five identical pre-warmed media (100 ml each) and incubated at 37°C for another 18 hours with constant agitation. The bacterial culture was harvested and BAC DNA was extracted using the QIAGEN Large-construct Kit according to the manufacturer's standard protocol, with the exception of an additional elution of BAC DNA with 100 µl of TE buffer.

2.3.4 DNA amplicons from polymerase chain reaction (PCR)

PCR products were purified using the QIAquick® PCR Purification Kit. The manufacturer's standard protocol was used with the following modifications.

Five independent 10 µl PCR reactions were pooled and adjusted to a pH of 6.5 prior to loading into the PCR cleanup column. Elution buffer (30 µl) was used to elute amplified DNA from the column.

2.3.5 Purification of DNA products

Ethanol was used to precipitate DNA following laboratory modification such as restriction enzyme (RE) digestion or ligation. Ethanol (2.5X volumes of 100% ethanol) and 3 M sodium acetate (pH 5.2; 1/10th volume) were added to the DNA solution that was incubated at -20°C overnight or at -80°C for 4 hours. A DNA pellet was obtained by centrifugation at 16000 g for 30 minutes (at 4°C). The supernatant was removed and the pellet was washed with 70% ethanol and centrifuged at 16000g for 15 minutes (at 4°C). The supernatant was removed, and the pellet was air-dried for 15 minutes, and then resuspended in TE buffer.

2.4 Sub-cloning of DNA fragments into a Plasmid Vector

2.4.1 pGEM® - 4z vector

The sub-cloning of cosmid and BAC DNA was performed using the pGEM®-4z vector (Promega). This vector was selected for its high copy-number, presence of two universal primer binding sites (M13 Forward and M13 Reverse), bacterial colony colour screening for recombinants, and an extensive restriction enzyme repertoire in the multiple cloning site.

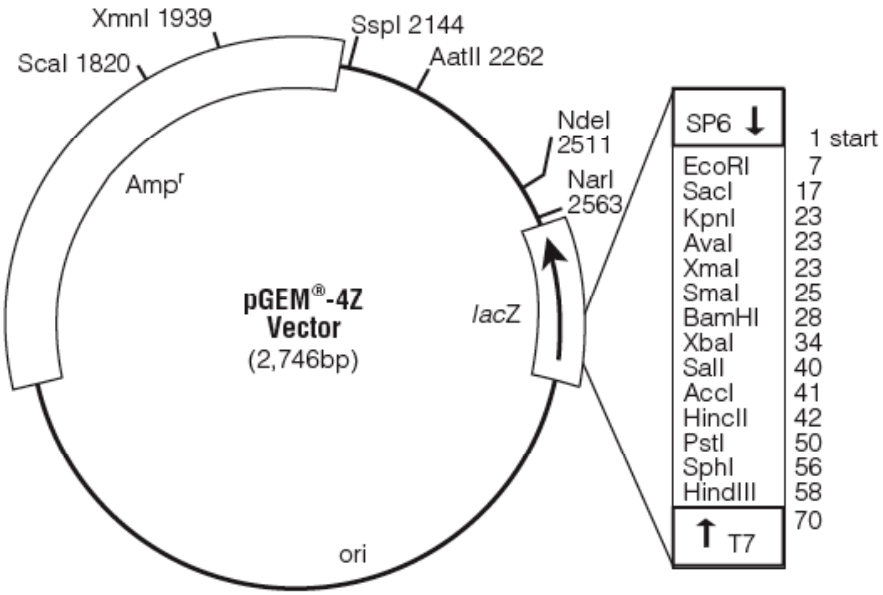


Figure 2.1: A map of pGEM® -4z showing the multiple cloning site, a selection of restriction enzyme active sites, the Ampicillin resistance gene and other genes essential for blue/white colony screening and DNA replication.

2.4.2 Restriction enzyme digestion of DNA

All restriction enzymes and their buffers were purchased from Promega Life Science. A standard restriction enzyme digestion reaction included 500 to 1000 ng of DNA, 20 units of restriction enzyme and restriction buffer in a 20 µl reaction (final concentration of buffer in Table 2.1), incubated at 37°C for 2-3 hours. The restriction enzyme digestion reaction was terminated by heat-denaturing the enzyme at 65°C for 15 minutes. Purified cosmid, plasmid vector pGEM® -4z and BAC DNA were each digested with restriction enzymes BamH1, EcoR1, HindIII and Pst1 in separate reactions.

Vectors for the sub-cloning process were digested and treated differently. The vector pGEM® -4z DNA was digested in a final volume of 50 µl consisting of 5µg of purified vector pGEM® -4z, 30 units of restriction enzyme, 5 µg of acetylated bovine serum albumin, restriction buffer (final concentration of buffer in Table 2.1) and made up to volume with ultra high-pure water (hpH_2O). The digested vector was then purified (Section 2.2.5), de-phosphorylated using shrimp alkaline phosphatase

(SAP) in a 50 µl reaction containing 40 µl of purified DNA digest, 5 units of SAP (Promega) and reaction buffer (Table 2.1). The reaction was incubated at 37°C for 30 minutes and terminated by heat-denaturation at 65°C for 15 minutes. Digested and de-phosphorylated vector DNA was stored at -20°C.

Table 2.1 Restriction enzymes and buffer at working concentrations

Enzyme	Components in buffer
BamH1; HindIII (Buffer E)	6 mM Tris-HCl, 6 mM $MgCl_2$, 100 mM NaCl pH 7.5
EcoR1; Pst1 (Buffer H)	90 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl pH 7.5
Shrimp Alkaline Phosphatase (10X Reaction Buffer)	0.5 M Tris-HCl (pH 9.0), 100 mM $MgCl_2$
T4 DNA Ligase (10X Reaction Buffer)	300 mM Tris-HCl (pH 7.8 at 25 °C), 100 mM $MgCl_2$, 100 mM DTT, 10 mM ATP

2.4.3 DNA ligation

Ligation of restriction enzyme digested DNA with dephosphorylated vector DNA was performed in 10 µl reactions comprising 150 ng of de-phosphorylated pGEM® -4z DNA, 50 ng of insert DNA, ligation buffer (30 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP pH 7.8) and 3 units of T4 DNA ligase (Promega), which were incubated at 4°C overnight. The ligated products were purified as described in Section 2.2.5 and were resuspended in 5 µl of hpH_2O.

2.4.4 Transformation

An aliquot of the purified ligation mix (1-2 µl; Section 2.4.3) was mixed with 20 µl of ELECTROMAX™ DH5α-E™ *Escherichia coli* cells and transferred into an ice-cold electroporation cuvette (1mm). Electroporation was performed using the BioRad GenePulse II at the settings; 1.8 kilovolts, 25 µF capacitance, and 200 to 500 resistance range (ohms). Transformations were considered successful if a time constant between 4.0 to 4.6 milliseconds was achieved. The transformation mix was then immediately mixed with 800 µl of Super Optimal broth with Catabolite repression (SOC; Invitrogen) media and incubated at 37°C for 45 minutes with gentle agitation. An aliquot of the culture (100 µl) was spread onto agar plates [isopropyl β-D-1 thiogalactopyranoside (IPTG; 50 µg/ml), 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-gal; 50 µg/ml), ampicillin (100 µg/ml) Luria Bertani broth with Agar (LBA)] and were incubated at 37°C for 18 hours.

2.4.5 Selection of Recombinant clones

Transformants containing an insert DNA were identified via two methods:

i. Plasmid DNA extraction, followed by gel electrophoresis. Selected white colonies were grown in 6 ml of LB broth culture media containing ampicillin (100µg/ml) in preparation for plasmid DNA extraction as per Section 2.2.2. Positive recombinant plasmid DNA were identified by size comparison to a DNA molecule marker together with unmodified pGEM® -4z DNA after electrophoresis in a 1.5% w/v TAE agarose gel.

ii. The second method is by PCR amplification of insert DNA in the plasmid using M13 forward and M13 reverse primers, followed by gel electrophoresis. PCR was carried out for each selected white colony in a 10 µl reaction including 1 µl of diluted bacterial culture (1 µl of bacterial culture suspended in 9 µl of sterile hpH_2O). Standard PCR reaction mix and PCR cycling conditions were applied (Section 2.5). Positive amplicons were identified by size comparison to a DNA marker as well as with amplicons from unmodified pGEM® -4z DNA on a 1.5% w/v TAE agarose gel.

2.5 DNA amplification by polymerase chain reaction (PCR)

One of the main components of the experimental work involved amplification of regions of DNA within the sheep genome as a template for DNA sequencing. Sheep genomic DNA was amplified using specifically designed oligonucleotide primers. These primers were designed using information generated from sequences of BAC sub-clones, as well as from sequence alignments between human genomic DNA and cattle genomic DNA sequences of gene loci within the MHC region. The composition of a standard PCR reaction are (0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.2 mg/ml, 0.1 unit/µl *Taq* DNA polymerase, 1X PCR buffer (Invitrogen) and 10pmol/µl each primer) and the PCR cycling conditions were as follows; 95 °C for 8 minutes, 30X (95 °C for 30 seconds, for 30 seconds, 72 °C for 2 minutes) 72 °C for 10 minutes, and held at 14 °C. Minor modifications in regards to $MgCl_2$ concentrations, primer annealing temperatures and number of PCR amplification cycles were applied according to individual PCR reactions.

2.6 DNA probe labelling and hybridisation

2.6.1 Labelling overlapping oligonucleotides (Overgo probes)

Overlapping oligonucleotide primers (Overgo primers) were designed based on information derived from two different sources:

i. Sheep specific sequences derived from previously cloned PCR amplicons (Dr David Groth - personal communication).

ii. Conserved coding sequences obtained by alignment of mouse, human and cattle mRNA with genomic sequences of genes within the sheep MHC region.

Overgo primers were labelled using the method adopted by Gustafson et al. (2003). A 10 µl reaction was prepared for each pair of Overgo primers, comprising of 1 µM each of forward and reverse primer, 25 µM dTTP and 25 µM dGTP (Invitrogen), 1 µl each of 3000 Ci/mmol ^{32}P dATP and ^{32}P dCTP (Perkin-Elmer), 2 units Klenow fragment DNA polymerase (Invitrogen), REact® 2 buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM

NaCl pH 8.0; Invitrogen) and 2 µl of hpH_2O. The reaction was incubated at 37°C for 30 minutes, followed by the addition of 25 µM non-radioactive dATP and dCTP, and incubated for a further 15 minutes at 37°C. Following incubation, reactions for all probes were pooled and purified to eliminate unincoporated nucleotides using a Sephadex G-15 gravity column with PBS (Phosphate Buffered Saline; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4) as running buffer. Radioactively labelled DNA probes were denatured at 96°C for 10 minutes immediately prior to addition to the hybridisation buffer for overnight hybridisation with the target DNA.

2.6.2 Radioactive hybridisation

2.6.2.1 Hybridisation of the sheep BAC library

Dry ovine CHORI-243 BAC library filters (PACBAC Resources) were wet with hpH_2O, arranged on a sheet of nylon muslin and rolled inside a 500 ml hybridisation bottle. The filters were then incubated at 53 °C in a hybridisation oven (Hybaid) with 20 ml of hybridisation buffer [5X SSPE (750 mM NaCl, 45 mM $NaH_2PO_4.H_2O$, 5 mM EDTA), 7 % SDS, 1 % BSA, 2.5 mM EDTA] added into the bottle. This pre-hybridisation step was carried out for 1 hour. After pre-hybridisation, the buffer was removed and replaced with 15 ml of fresh hybridisation buffer containing the denatured radioactively-labelled DNA probes. The filters were then incubated at 53°C overnight with constant rotation.

2.6.3 Post hybridisation washing and film development

After overnight hybridisation, the BAC filters were washed three times with Washing Buffer [1X Saline-sodium citrate (SSC) buffer, 0.1 % SDS] for 20 minutes at 53°C. The washed filters were then rinsed twice in 1X SSC buffer. The washed filters were sealed in plastic and exposed to X-ray film [Amersham Hyperfilm™ MP (8” X 10”); GE Healthcare] using an intensifying screen cassette at -80°C for 3 days. The film was developed in developer (Phenisol High contrast Film Developer; ILFORD) for 5 minutes, rinsed briefly in water and then fixed in fixer (Rapid Fixer; ILFORD) with gentle agitation until the background turned clear. The film was then rinsed and dried at 37°C. After autoradiography, the BAC filters

were treated with Transfer buffer for 10 minutes, followed by neutralisation buffer (TAE). The moist BAC filters were sealed in plastic and stored at -20°C for future use.

2.7 DNA sequencing

The sequencing of cloned DNA fragments generated by PCR amplification, recombinant plasmid DNA fragments generated by sub-cloning of sheep BAC DNA and PCR products using sheep-specific primers was performed using the dideoxy-termination sequencing method.

2.7.1 ABI 3730 automated sequencing

Purified plasmid or cosmid DNA (Section 2.2.2) and PCR product (Section 2.2.4) were used as templates for sequencing in a dideoxy termination reaction system (Big Dye Terminator; Applied Biosystems) and a 3730 DNA Analyser performed at the WA State Agricultural Biotechnology Centre (Murdoch University, WA). Each 10 µl sequencing reaction consisted of 2 µl of ABI BigDye® terminator mix, primers (3.2 pmoles for plasmids and PCR products, 6 pmoles for cosmids), 1X Sequencing buffer, DNA template (50 ng for PCR products, 350 ng for plasmids, 1000 ng for cosmids) and was made up to volume with hpH_2O. The sequencing reaction was as follows: 96°C for 2 minutes, 30X (96°C for 10 seconds, for 10 seconds, 60°C for 4 minutes).

After the sequencing reaction, the DNA product was purified by a modified ethanol precipitation method as recommended by Applied Biosystems. The DNA was added to 2.5 X volume 100 % ethanol, 1/10th volume 3 M sodium acetate (pH 5.2) and 1/10th volume 125 mM ethylenediaminetetraacetic (EDTA). The sample was incubated at room temperature for 20 minutes, and then centrifuged at 16000g for 30 minutes. The supernatant was removed and the DNA pellet was washed with 125 µl of 70 % ethanol, and centrifuged at 16000g for 15 minutes. The supernatant was removed, and the DNA pellet was air-dried for 20

minutes in the dark at room temperature and stored at -20°C until analysis.

2.7.2 DNA sequence analysis

Sequence information obtained from the ABI 3730 sequencer was checked and screened for any ambiguous results. Ambiguous sequences were re-sequenced or sequenced from another direction using another primer to confirm the sequence information. Contiguous sequences were determined using bioinformatics software packages, Vector NTI (Invitrogen) and ClustalX (Higgin *et al*., 2007). Identification of sequences was achieved by searching the GenBank database [blastn (somewhat similar sequences)].

2.8 Detection of genetic polymorphisms

Single nucleotide polymorphisms (SNP) were identified by comparison of locus specific sequences within the MHC class II from several unrelated sheep. Oligonucleotide primers were designed to amplify the region or gene of interest, to generate genomic PCR products which were then purified and sequenced directly. Sequences were generated from at least five different unrelated Australian Merino sheep. Insertions or deletions were also identified by comparison of the sequences, and confirmed when the heterozygous state of the mutation was observed in same sequences.

Chapter 3
Identification and characterisation of polymorphic loci (SNP) within the ovine MHC class IIa and IIb region

This Chapter describes the identification of genetic markers spanning approximately 900 kbp of the ovine MHC class II region. The loci were identified through sequencing and comparative analysis, and this allowed the generation of a predicted physical map spanning the ovine class IIa and IIb gene regions. This map was obtained by identifying overlapping clones from a sheep bacterial artificial chromosome (BAC) library, which were subsequently sub-cloned and sequenced. In addition, some ovine genomic sequences were generated using ovine-specific PCR primers. Subsequently, these loci were used in the discovery of single nucleotide polymorphisms (SNP). In addition, the PFDN6 and WDR46 genes were sequenced and further characterised.

3.1 Introduction

The high level of polymorphism and the observed association with a diverse range of diseases in humans has made the MHC a region of significant interest in agricultural research. However, in comparison to many other mammalian species, the ovine MHC is poorly characterised. Early studies of the ovine MHC region showed that the genetic structure was very similar to that of the human MHC, with the central class III, separating the class I and II regions. More recently, Liu *et al.*, (2006) identified and end sequencing of BAC clones from a Chinese Merino sheep, further supporting this observation. This allowed a reconstruction of the region and provided a broad description of the ovine MHC, confirming the orthologous nature of ovine MHC relative to the human MHC. The presence of a chromosomal inversion within the class II region was originally identified in cattle (van Eijk *et al.*, 1995), which was subsequently confirmed and characterised (Childers *et al*., 2006). However, the length of the region separating class IIa and IIb region has yet to be confirmed. More recently, the ovine MHC class III region was independently characterised and fine-mapped by Qin *et al.* (2008) and Qin (2009).

Despite early identification attempts of ovine MHC molecules using serological studies, the structural characterisation of the ovine MHC has been relatively slow. Several dinucleotide microsatellite genetic markers were identified and mapped to the ovine MHC; OMHCI (Groth and Wetherall, 1994), located within the class I region and, OLADRB and OLADRBps loci present in the class II region (Blattman and Beh, 1992; Schwaiger *et al.*, 1996) and one close to the Factor B gene in the class III region (Groth and Wetherall, 1995). Several of these microsatellite markers have been used in QTL studies investigating associations between the MHC and resistance to gastrointestinal parasites. However, the number of genetic markers within the ovine MHC is limited and therefore studies using them in QTL analysis lacked sufficient power for identification of potential disease predisposing candidate genes. For example, Beh *et al.* (2002) applied only one microsatellite from the ovine MHC region in their QTL analysis. Furthermore, there are also inherent limitations with the use of microsatellites for association studies as alleles at the loci are derived from both convergent and divergent evolutionary pathways (Chistiakov *et al.*, 2006).

In order to have a better understanding of the ovine MHC class II region and its contributions to immunologically related functions, ideally genetic markers spread evenly across the ovine MHC class IIa and IIb are required. This would facilitate the identification of MHC haplotypes for both disease association studies (parasitic, microbial or viral), as well as providing insights into the evolution of subregions within the ovine MHC.

3.2 Materials and Methods

3.2.1 Strategy for identification of ovine MHC class IIa and IIb region BAC clones

The identification of BAC clones containing DNA derived from the ovine MHC class IIa and IIb region was initially performed through screening a sheep CHORI-243 BAC library. Several Overgo oligonucleotides (Gustafson *et al*., 2003) were designed from conserved DNA sequences identified between human and mouse MHC regions through the alignment of human and mouse MHC genomic sequences, as well as from existing

BAC end sequences. In addition, BAC clones were also identified using the ovine virtual map developed using BAC end sequence that was made publicly available subsequent to the start of this project (Dalrymple *et al.*, 2007). The BAC clones identified through these processes were obtained from the Australian Genome Research Facility (AGRF). These clones were cultured in LB (chloramphenicol; 12.5 µg/ml) media and the BAC DNA subsequently extracted, purified, sub-cloned and sequenced (Section 2.3.3, 2.4 and 2.7).

Target regions of the ovine MHC class IIa and IIb regions were also identified and characterised by designing PCR DNA primers which could be used to amplify regions of the ovine MHC class II. These primers were designed using sequence information derived from the cattle MHC class IIb region, as well as GenBank deposited DNA sequence information from a BAC clone obtained from a different BAC library (Rambioullet; Herrmann-Hoesing *et al.*, 2008). This Rambouillet BAC clone covers a significant proportion of the ovine MHC class IIa region.

SNP discovery was achieved by using DNA primers to amplify targeted regions of the ovine MHC, which were subsequently sequenced. SNPs were identified through sequence alignments of these sequences, which were derived from at least five unrelated animals.

3.2.2 Labelling of DNA and oligonucleotide probes and screening

3.2.2.1 Labelling of overlapping oligonucleotide probes

Overgo primers were labelled using the method described by Gustafson *et al.* (2003). Overgo primers used in this project are listed in Table 3.1. A 10 µl reaction was prepared for each pair of Overgo primers, comprising of 1 µM each of forward and reverse primer (Geneworks), 25 µM of dTTP and 25 µM of dGTP (Invitrogen), 1 µl each of 3000 Ci/mmol $a^{32}P$ dATP and $a^{32}P$ dCTP (Perkin-Elmer), 2 units of Klenow fragment of *E. coli* DNA polymerase I, 1 µl of REact® 2 buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl pH 8.0; Invitrogen) and 2 µl of hpH_2O. The reaction was incubated at 37°C for 30 minutes, followed by the addition of non-radioactive dATP (25 µM) and dCTP (25 µM), and incubated for a further

15 minutes at 37°C. Following incubation, reactions for all probes were pooled and unincorporated nucleotides were removed using a Sephadex G-15 (Amersham) gravity column with PBS buffer.

Short PCR products≈(100 bp) were also labelled with r adioactive nucleotides to be used as probes. The PCR products were generated by amplification of ovine genomic DNA using ovine specific primers (Table 3.1) and then purified. The purified PCR products were denatured and PCR primers were added into the hybridisation mixture. Radioactive dinucleotides were incorporated by primer extension with Klenow fragment of *E. coli* DNA polymerase I extending from the PCR primer complementarily bound to the single-stranded PCR product.

Table 3.1: Overgo primers used to identify CH243-BAC clones

Gene/Region	**Forward Primer (5'-3')**	**Reverse Primers (5'-3')**
RING3	TAGAGCCTTCATCACCACCTTGTG	CCAGTTGCAATATCTGCACAAGGT
TAPBP-1	TGCAGAGAGGCTTACAGAGCCATC	CCTGAGACATCACTCAGATGGCTC
TAPBP-2	ATGAAGCCTCCTCTTCCTCCTTTC	GGAGGAAAGTGAAAGTGAAAGGAG
DQA	ACCTGACTCACCTGACCACATTGG	GTTTACGCCATACGTGCCAATGTG
DYB	TACGTGGAGACGCTGTGTAGACAC	CCGTCTCCTTGTAGTTGTGTCTAC
DYA	GAATTTGATGGAGACGAGCTCTTC	TCCTCAGGTCCACGTAGAAGAGCT
DRB	AGTCGCTGTCGAAGCGCACGTACT	TTCTATAATGGAGAAGAGTACGTG
DSB	AGGCTCCTGGACAGTAATTCTGCC	ATTCTGCCAGTGATACTGATGGTG
B3GALT4	TTCCAGGACTCCTACCGCAACCTC	TGAGGGTCTTTAGGGTGAGGTTGC
BING4-1	GAAGTCTCGGATCTCTAAGAAGCC	TTTCTTCGGGACCTGAGGCTTCTT
BING4-2	CTGCTGCTGACATTGTCAACTTCC	ACAGACCCCACTGTAAGGAAGTTG
A23-1	TAACTACTGAGCCCAGTGCTGCCA	GCACTGGGCTCAGTAGTGGCAGCA
A23-2	AGTACAGACGTCAGTGACTGATGC	GCCTCCCGGTACTGGTGCATCAGT
K16	AGTCCTAGGGGACTTGACCGAGGC	CCATTCATTGTTGTGTGCCTCGGT
OvarDYB*	CACGGGCTCTCCCGCAGAGAATT	CCCACAAGGCTCTGTGTGAAGGCAT
OvarDYA*	TGACCACGTGGGCACTTA	ACCACTGGTGGCAGGTGT
OvarDRB*	CGCTGCACAGTGAAACTC	CTCTCTCTGCAGCACATTTCCT

* PCR primers

3.2.2.2 BAC library screening

Six high-density filters, each containing 18,000 distinct BAC clones, from the ovine CHORI-243 BAC library (PACBAC Resources) were hybridised with ^{32}P labelled Overgo primers to detect the presence of specific loci known to be within the class II region of the human MHC. The denatured, radioactively labelled probes were add to 15 ml of buffered solution (5X SSPE, 7 % w/v SDS, 1 % w/v BSA, 2.5 mM EDTA), and hybridised at 53°C overnight, followed by three washes at 53°C for 20mins in 1X SSC, 1 % w/v SDS. The hybridised filters were sealed in plastic and exposed to x-ray film (X-Omat AR - Kodak) with an intensifying screen at -80°C for 2 days. The film was developed according to manufacturer's protocols.

3.2.3 Sub-cloning and sequencing of BAC clones

BAC DNA was extracted from overnight cultures using QIAGEN Large construct kit (Section 2.3.3). The purified DNA was digested with the restriction enzymes EcoR1, BamH1, Pst1 and Hind III in separate reactions and sub-cloned into the appropriate site within the pGEM-4z vector (Promega) multiple cloning site using the manufacturer's protocols (Section 2.4.2, 2.4.3 and 2.4.4). Recombinant plasmid colonies were identified by PCR using M13 Forward (M13-F: CCCAGTCACGACGTTGTAAA) and M13 Reverse (M13-R: AGCGGATAACAATTTCACAC) primers and PCR product size determined using a TAE agarose gel (1% w/v) and appropriate size markers.

Sequences were generated from recombinant plasmid clones using dideoxy termination sequencing (Big Dye Terminator; Applied Biosystems), a 3730 DNA Analyser and M13 primers performed at the WA State Agricultural Biotechnology Centre (Murdoch University, WA). Contiguous sequences were generated using Vector NTI contig express (Invitrogen). Analysis of the genomic DNA sequences was performed using BLAST software and GenBank databases, while intron/exon organization and cDNA sequence of genes were determined using ClustalX logarithmic analysis (http://www.clustal.org/).

3.2.4 Amplification and sequencing using ovine specific oligos

Ovine-specific oligonucleotide primers were designed using Primer 3 (http://frodo.wi.mit.edu/primer3/). These were used to amplify targets from the ovine MHC class IIa and IIb regions. The PCR primer sequences used in this project are summarized in Table 3.2. The amplification of DNA from sheep genomic DNA is described in Section 2.5. PCR amplified DNA fragments were sequenced using dideoxy termination sequencing (Applied Biosystems) and a 3730 DNA Analyser at the WA State Agricultural Biotechnology Centre (Murdoch University, WA). Analysis of the genomic DNA sequences was performed using BLAST software and GenBank databases, while intron/exon organization and cDNA sequence of genes were determined using ClustalX logarithmic analysis (http://www.clustal.org/).

3.2.5 SNP detection

Ovine specific oligonucloetide primers (Table 3.2) were based on the DNA sequences of ovine MHC class IIa and IIb regions. The primers were designed so that fragments of between 500 bp and 1300 bp were amplified. Individual PCR products were generated from DNA derived from at least five unrelated Australian Merino sheep. SNPs were identified from alignment of the sequences derived from the different animals, with each SNP polymorphism defined when observed both in at least two heterozygotic individuals and at least one of each homozygote states.

Table 3.2: Locus specific oligonucleotide primers used to identify SNPs within the MHC class IIa and IIb region.

Gene/ Region	**Forward Primer (5'-3')**	**Reverse Primer (5'-3')**	**MHC Region**
DQB2-DQA2	ACTTGTATGCCTTCTCCTGA	ACATCAGCAACATGATCAAA	IIa
DQA2-DQB1	TATATTCCTCCCTGCCCTTA	ACATCCTCTTGCTTCTCTCC	IIa
DQA2-DQB1	TGTATGCCCAAACCTAAGAC	ACCCAAAATTAAACCCAATC	IIa
DQA1-DRB1	TAAATCGCTATCATGTGGTG	TTCATCTAACTGTGCTGTGC	IIa
DRB1-DRA	CCATGTAAACTCATTCATTCAA	CCTCAGAGAAGACAACCATC	IIa
DRB1-DRA	ATCCTGCAGCTTTTAATTTG	TTCAACACTTCTTGGAGAGG	IIa
DRB1-DRA	ACTCAGGTTTTTCACTCAGG	AGGAAGGAATCTAAAGGTGAG	IIa
DRA	ACCAATACAAGAAGGAGCAG	TCCTGGCTTTATATTTCCTG	IIa
BTNL2	AAATCCTCTGTGTTCAGTCC	TTAGGGATAGGACGGGATAG	IIa
BTNL2-	CCAACCTCGTCTTATCTGAG	GGACAGCTTTCTTGAACAAC	IIa
DOB-TAP2	AGGAAGATTTGAAAAACAGG	TGCTTCATAGAAGCTTTACC	IIb
PSMB8-	GCCAGATGTGTGATCTGTCG	CAGTAATGGTGGCGGTGAC	IIb
PSMB9	AACTATCTGGTGAGGGAGGTG	TGCAAAAGGGAAACAGACTC	IIb
H2B-DMB	TTATCCCTTCGTGTCTCACC	CTCCATTCTGATAACCAGAG	IIb
DMA-BRD2	AGTCTCCCCCTATATGCTTG	GTTTTGTACCTTCAGTAACC	IIb
BRD2-DOA	AACTCATGCATTGAGGAATC	GGATAAAAATGCACAAGTCC	IIb
RG1	CTGACACCCTTCCCCCATC	TGAGCTCTGAGTGCAGTGACC	IIb
WDR46	AGATGGAGAAACCGAGATGG	GAATCTTTGCCGTGTCCTC	IIb
PFDN6	ATCTTGCCTTAAATCCTTCC	TGTTTAGAGACATCTCATGTGC	IIb
PFDN6	CCCCTTCTCTGTAGTCTTCC	ATTCATACCGCTTACTGAGG	IIb
PFDN6	GCTCAAGTTTCTGACCAATC	ATTACCATGTTCCATTCTGG	IIb
PFDN6	TTGACAGTGAAACAATTTGG	AAACCAGACACTACCTGAGC	IIb
RGL2	GAATGGAACATGGTAATCCTC	TGTGACACTGGCAGTCTTG	IIb
TAPBP	ACCTGCCTCCCACGAGCTTT	AAACTGAGGCCCGAGGTCAC	IIb

NB: TAPBP gene was fully sequenced by Siva (2007) in a joint collaborative project

3.3 Results

3.3.1 Identification of BAC clones containing the ovine class IIa and IIb region

The ovine CHORI-243 BAC library contains 18,000 x 6 distinct sheep BAC clones, with an average insert size of 182 Kbp. The initial screening of the ovine BAC library using radioactive-labelled locus specific Overgo probes for the putative orthologous loci chosen to cover the length of the human and cattle MHC class II region identified 30 positive clones. Out of the 30 BAC clones identified, only 3 clones were subsequently positively confirmed to contain identifiable class IIb gene sequences (CH243-373A23, CH243-375K16 and CH243-486M14).

The publishing of the ovine virtual map (Dalrymple *et al*., 2007) facilitated the identification of additional BAC clones covering the class IIa and IIb regions. The ovine virtual map was constructed using BAC-end sequences of all BAC clones from the CHORI 243 library to construct a putative BAC coverage map of the whole ovine genome (Dalrymple *et al.*, 2007; http://www.livestockgenomics.csiro.au/sheep/vsheep.php).

The six BAC clones selected and used for further characterisation are listed in Table 3.3. Sub-cloning and partial sequencing of these six BAC clones, plus BAC end sequences available in GenBank permitted the assembly of the putative map as shown in Figures 3.1 and 3.2

Table 3.3: GenBank accession numbers for the six BAC clones characterised for sub-cloning

Clone ID	GenBank Accession Number
CH243-373A23	DU227088/DU212887
CH243-375K16	DU183676/DU174233
CH243-486M14	DU188592/DU175764
CH243-404H8	DU191483/DU189962
CH243-228N17	DU458722/DU453502
CH243-404O10	DU201508/DU194517

In addition to identification of locus specific ovine sequences from BAC subclones, sequence information that was available in GenBank [cattle MHC class IIb (Childers *et al*., 2006); BAC sequence containing a large portion of the ovine MHC class IIa (Herrmann-Hoesing *et al*., 2008)] further facilitated the identification of target regions, which could be amplified by PCR to aid SNP discovery. All locus specific ovine sequences identified in Merino sheep from this study were lodged with GenBank and a summary of the allocated accession is shown in Table 3.4. Further information regarding these loci is also available in Appendix (3A.1).

Table 3.4: Deposited DNA sequences of genes/region within the class IIa and IIb region resulting from this project.

Locus (Gene/Region)	**GenBank Accession Number**
DOB-TAP2	FJ999659
PSMB8-TAP2	GQ131517
PSMB9	GQ131516
H2B-DMB	GQ131511
DMA-BRD2	FJ999656
BRD2-DOA	FJ999658
RING1	GQ131518
PFDN6	GQ867665
WDR46	GU056180
RGL2	GQ131514
DQB2-DQA2	GQ131508
DQA2-DQB1 (1)	GQ131509
DQA2-DQB1 (2)	GQ144417
DQA1-DRB1	GQ131512
DRB1-DRA (1)	GQ131510
DRB1-DRA (2)	FJ999657
DRB1-DRA (3)	GU056181
DRA	GQ131513
BTNL2	GQ131519
BTNL2-c6orf10	GQ131515

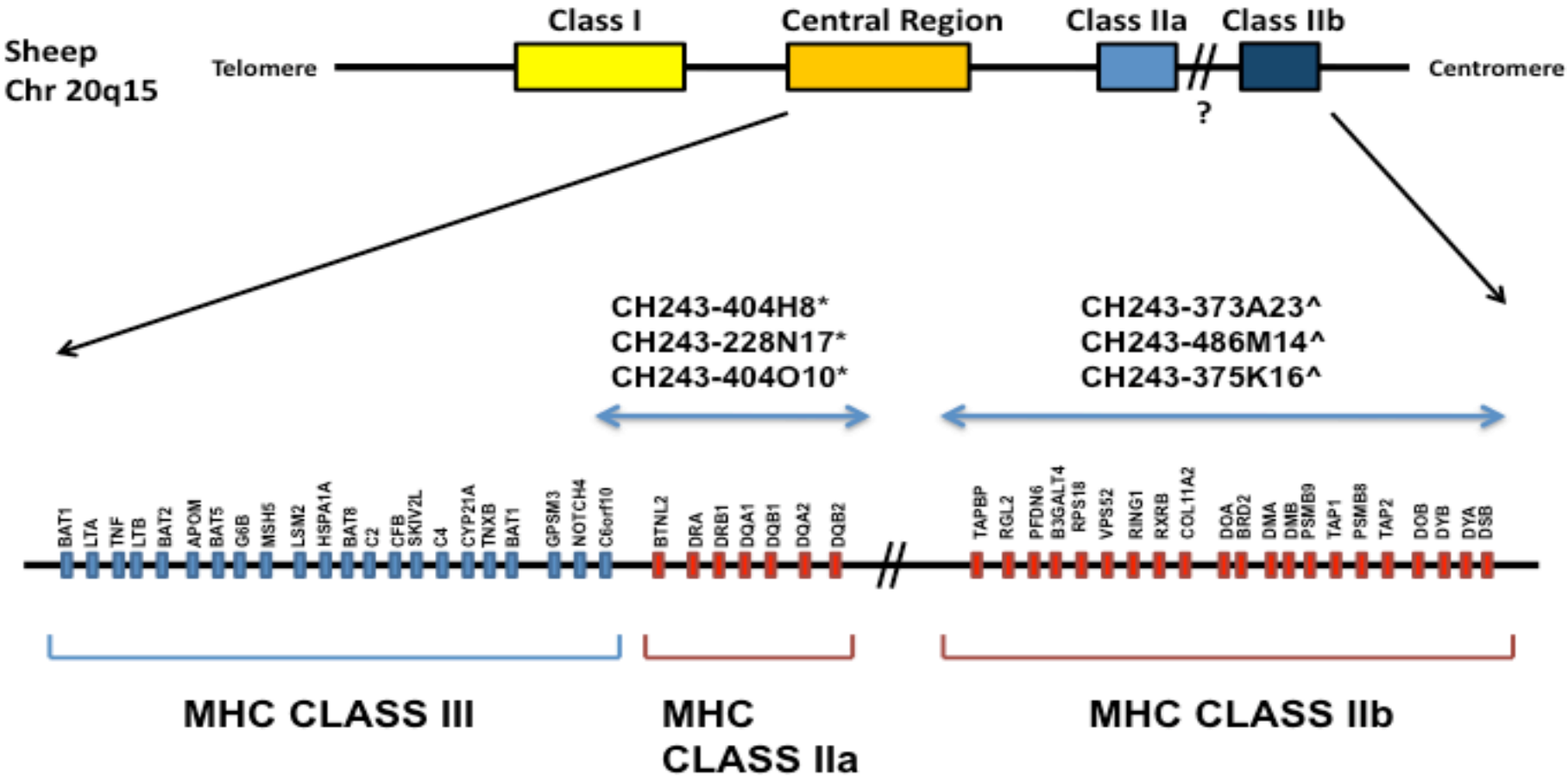


*Figure 3.1: Predicted physical map showing the relative positions of loci within the ovine MHC class IIa, IIb and III regions based on human and cattle orthologues. The identified CHORI ovine BAC clones are represented in the map showing the regions that they cover. * represent the BAC clones identified from the ovine virtual map; ^ represent the BAC clones identified by radioactive hybridization.*

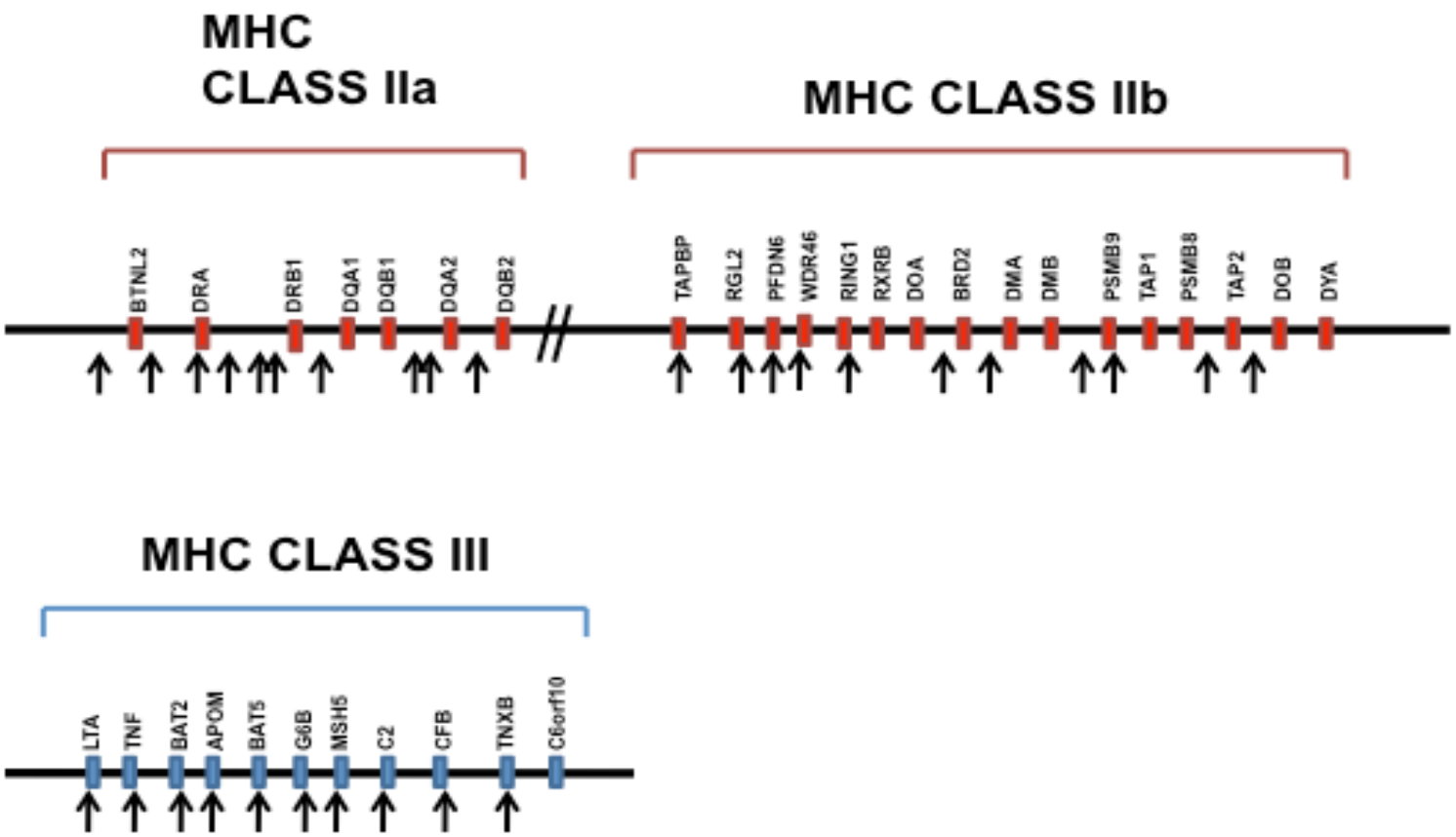


Figure 3.2: Location of locus specific ovine sequences spread across the ovine MHC class IIb, IIa, and III region identified in this study. Arrows show regions for which panels of SNPs were identified. The SNPs located in the ovine MHC class IIa and IIb region were identified during this project while the SNPs located in the ovine MHC class III region were identified by Qin (personal communications).

3.3.2 Detection of SNPs

The loci shown in Figure 3.2 and Table 3.4 were selected to provide a relatively even coverage of the ovine class IIa and IIb regions. These loci were amplified from genomic DNA obtained from at least five individual sheep using primers listed in Table 3.2, and the resulting amplicons sequenced. As an example, a typical electrophoretogram showing the presence of a heterozygous SNP within the PFDN6 locus is shown in Figure 3.3. From the 20 target sequences spanning the ovine class IIa and IIb regions, a total of 96 SNPs were identified. Complete sequencing of the PFDN6 gene also identified an additional 7 SNPs. The SNPs discovered in this project are summarised in Tables 3.5 and 3.6. A panel of SNPs was selected and used to genotype a group of sheep. The information generated was subsequently used to identify ovine class II MHC haplotypes (further described in Chapters 5 and 6).

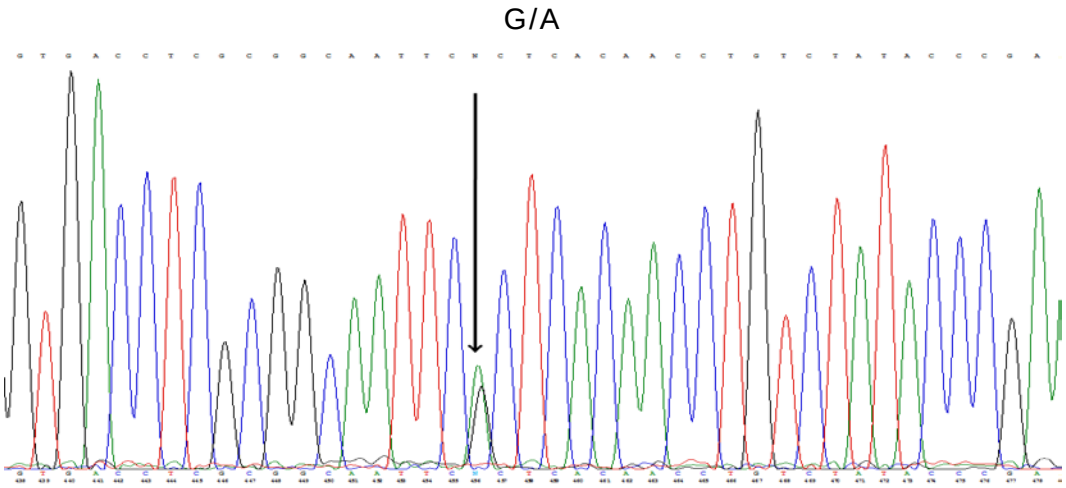


Figure 3.3: Identification of a SNP in the PFDN6 gene after sequencing. A double peak, annotated as 'N' with half signal strength indicates a heterozygote G/A SNP site.

Table 3.5: SNPs identified in the ovine MHC class IIa and IIb region; location and allelic variation of SNPs present at each locus.

Locus Name	GenBank ID	SNP	Position	Sequence
DB-TP2s1	FJ999659	G/A	163	AGAAACGTAA(G/A)ATTTAGGTGG
DB-TP2s2	FJ999659	G/A	201	TCACTATAAT(G/A)TCAGGAAGAA
DB-TP2s3	FJ999659	C/T	253	AAAATTCAAG(C/T)CGTCTAGAAA
DB-TP2s4	FJ999659	C/A	284	TGAAGCTGAT(C/A)AAGTTGATAT
DB-TP2s5	FJ999659	C/T	423	TTGGCCACAA(C/T)AAATATATGT
DB-TP2s6	FJ999659	C/T	486	TTTTTTCTTT(C/T)TCACTTCCCT
DB-TP2s7	FJ999659	C/T	534	TAAGGTGAGA(C/T)TGTAATGGTA
PB8-TP2s1	GQ131517	G/T	307	GAGCCTTCCA(G/T)CGGTCCTGGA
PB8-TP2s2	GQ131517	C/T	322	CCTGGAGGAA(C/T)GAGAGTGGGA
PB9s1	GQ131516	C/T	43	TCAAAATGGA(C/T)AAACAACAAG
PB9s2	GQ131516	G/A	123	GAATATGAAA(G/A)AAAGTATATA
PB9s3	GQ131516	G/T	202	GATACTTAAA(G/T)TTTTTTTAAT
PB9s4	GQ131516	C/T	237	CTGATGACTT(C/T)GCACTGCTTG
HB-DMBs1	GQ131511	G/A	107	ATGTGGGCAA(G/A)GGAAGCTCTT
HB-DMBs2	GQ131511	C/T	150	CCATCCAAGG(C/T)GCCTTAAGCA
HB-DMBs3	GQ131511	G/T	151	CATCCAAGGC(G/T)CCTTAAGCAG
HB-DMBs4	GQ131511	G/A	184	AGCCAAGAGA(G/A)AATCAGAAGC
HB-DMBs5	GQ131511	A/T	196	ATCAGAAGCC(A/T)TCTCCCTTCC
HB-DMBs6	GQ131511	C/T	202	AGCCTTCTCC(C/T)TTCCTCTACT
HB-DMBs7	GQ131511	G/A	289	AAATTTAAGG(G/A)AAAAAAAACC
HB-DMBs8	GQ131511	A/T	423	TGAAAGTAAA(A/T)ATATTAACTT
DMA-BD2s1	FJ999656	G/A	91	TACTATCACT(G/A)CAAGTAACAA
BD2-DAs1	FJ999658	G/A	22	TCACAAACAC(G/A)CACACTTTAT
BD2-DAs2	FJ999658	G/A	81	AAGAATACTT(G/A)ACTACCTGTG
BD2-DAs3	FJ999658	C/A	133	TATTTATTGC(C/A)ACTGTGTGTG
BD2-DAs4	FJ999658	C/T	216	AACTGTCTTG(C/T)TACATAACCA
BD2-DAs5	FJ999658	C/T	285	ATTGATTATC(C/T)GGTCTATTTC
BD2-DAs6	FJ999658	G/A	453	TTGCCTTATA(G/A)AATATCTCAT
BD2-DAs7	FJ999658	A/T	527	ATCCAGTGTA(A/T)TTTCTGTAAA
RG1s1	GQ131518	G/A	44	CGCAGGTGAC(G/A)GGGGCTCTCC
RG1s2	GQ131518	C/T	99	ATCCGCAGAC(C/T)CCCTTCTCAC
RG1s3	GQ131518	C/T	146	TTCCACCCAG(C/T)TTCTCACTCC
RL2s1	GQ131514	C/G	260	GACTAGCTGC(C/G)GGGGGCGGGG
RL2s2	GQ131514	G/A	680	TGCCAGGGCT(G/A)TTCTGAGGAT
WDR46s1	GU056180	G/A	357	TCTCCAAGAA(G/A)CCCCGAGAAC
TAPBPs1	EU814901	G/T	1278	ACGTGGCGGG(G/T)CTCACCTCCC
A2As1	GQ131508	C/G	19	TGTTCTCTCC(C/G)TGGCTTACAG
A2As2	GQ131508	C/T	24	TCTCCGTGGC(C/T)TACAGGGACT
A2As3	GQ131508	C/G	44	TGGCTTTGGA(C/G)TGGCTTTCTG

Table 3.5: cont.

A2As4	GQ131508	G/T	64	GTGCTGCTTT(G/T)TAGTTGTCTG
A2As5	GQ131508	G/A	100	GCTGCTGTCA(G/A)GAGTGCACAC
A2As6	GQ131508	A/T	107	TCAGGAGTGC(A/T)CACAGGGAGC
A3As1	GQ131509	C/T	19	TCATTCTGTA(C/T)ATTAACTCCT
A3As2	GQ131509	C/T	66	GCGATGGCAA(C/T)CATAGTCAAA
A3As3	GQ131509	G/A	100	TTACTGTGTC(G/A)TTTTGCAGAA
A3As4	GQ131509	G/A	157	GAGAACCCAA(G/A)TTTATAGGAA
A3As5	GQ131509	C/A	217	GGAGTCACTC(C/A)GTCTTGGTTT
A3As6	GQ131509	C/G	266	TCTTAGAGGA(C/G)AGTGTCCTCC
A3As7	GQ131509	C/A	285	CCAGTCAACA(C/A)TGTTCAATCA
A3As8	GQ131509	C/T	301	AATCATTCTA(C/T)TGGGTAGATA
A3As9	GQ131509	C/T	486	AACCCATATG(C/T)GAAATACATA
A3As10	GQ131509	G/A	487	ACCCATATGC(G/A)AAATACATAC
A3Cs1	GQ144417	G/A	47	AAGCTTTCCA(G/A)TGGCCAAATT
A3Cs2	GQ144417	C/A	90	AATGGTCAAA(C/A)TTTTGAGCTA
A3Cs3	GQ144417	C/T	104	TGAGCTAGGA(C/T)TTTCACTTTA
A3Cs4	GQ144417	G/A	134	AGAGTAATTA(G/A)TAACAGACAT
A3Cs5	GQ144417	C/T	206	TTTTCAAATA(C/T)TGGACAACAA
A3Cs6	GQ144417	G/A	225	AAATCCTATC(G/A)GATTGTGATT
A3Cs7	GQ144417	C/T	237	ATTGTGATTC(C/T)TGAAAAAGGG
A3Cs8	GQ144417	G/A	323	CACACAGGAG(G/A)AAGTCTCAAG
A5Bs1	GQ131512	G/A	228	ACTGCTATGT(G/A)TTTTGCTTGG
A6As1	GQ131510	C/G	94	ATATTTTAAC(C/G)AGGTATTTTT
A6As2	GQ131510	G/A	97	TTTAACGAG(G/A)TATTTTTGAA
A6As3	GQ131510	G/A	198	CTTGTCAAAA(G/A)TATATGATGA
A6As4	GQ131510	G/A	350	AGTTGGATAT(G/A)TTCCGAGAAA
A6As5	GQ131510	G/A	485	TCTGAAACTC(G/A)TTTCAGAGTA
A6As6	GQ131510	C/T	549	TATTTTGTTT(C/T)CAACCATAGT
A6Bs1	FJ999657	G/A	49	CTCACCAGGA(G/A)TATTCTTAAA
A6Bs2	FJ999657	C/T	133	ACAATACTTA(C/T)GATGCTTTCT
A6Bs3	FJ999657	C/T	507	CCAGTAATGT(C/T)TAAAACAGCG
A6Bs4	FJ999657	G/T	515	GTCTAAAACA(G/T)CGCATTTTTG
A6Bs5	FJ999657	G/A	551	TACAACACAT(G/A)GAACTTAAGT
A6Cs1	GU056181	G/A	54	TCTGGAGTGG(G/A)TGAAGTTGAT
A6Cs2	GU056181	G/T	79	AAATTCTGTT(G/T)TGTGTAAATA
A6Cs3	GU056181	G/A	81	ATTCTGTTGT(G/A)TGTAAATAAA
A6Cs4	GU056181	C/T	96	AATAAATACT(C/T)CTCTATTCTG
A6Cs5	GU056181	G/A	118	TCCCCACTCC(G/A)TTGCCATTTC
DRAs1	GQ131513	G/A	175	CGTAGTTACT[G/A]TAGCGTTTAA
BL2s1	GQ131519	C/T	64	TCTTGGCCTC(C/T)GCTGCCTGAA
BL2s2	GQ131519	G/A	121	CACCTCTGGA(G/A)GCCTCTCCTT

Table 3.5: cont.

BL2s3	GQ131519	C/T	223	ATCTATACTT(C/T)CCCAGTTTTC
BL2-C10s1	GQ131515	C/G	32	TTCACCCAGA(C/G)AGACACGAAC
BL2-C10s2	GQ131515	G/A	72	AAGTAAAAAC(G/A)AGGACAGAAG
BL2-C10s3	GQ131515	G/A	154	GTTTGTAGAA(G/A)CTCCAGAGAA
BL2-C10s4	GQ131515	G/T	162	AAGCTCCAGA(G/T)AAGAATTTTT
BL2-C10s5	GQ131515	G/T	182	TGGGTTTTGG(G/T)ACACGAGGAA
BL2-C10s6	GQ131515	C/T	186	TTTTGGGACA(C/T)GAGGAAGGAA
BL2-C10s7	GQ131515	A/T	205	AAAAGTCATA(A/T)TTCCCAACTC
BL2-C10s8	GQ131515	G/A	220	CAACTCCCAG(G/A)AAAACTCTCC
BL2-C10s9	GQ131515	G/A	277	CCTGGACTGG(G/A)AAGCTGGGAA
BL2-C10s10	GQ131515	C/T	333	TCTATTCTTT(C/T)ACTGGCATCA
BL2-C10s11	GQ131515	C/T	363	GGACCCTTTT(C/T)CCTTATTTTA
BL2-C10s12	GQ131515	C/T	449	GATTCTTCTC(C/T)AAAGACCTCT
BL2-C10s13	GQ131515	A/T	535	GAATGTCAGC(A/T)CCTCCGCTGC
BL2-C10s14	GQ131515	G/A	541	CAGCTCCTCC(G/A)CTGCCTCTGC
BL2-C10s15	GQ131515	C/T	562	TCAGGCTTCT(C/T)ACAGGTATG

Table 3.6: SNPs identified in the ovine PFDN6 locus from the ovine class IIb region (GenBank accession number: GQ867665)

SNP	Position*	SNP	Type	Sequence
PFDN6s1	52	A/T	5′ UTR	TGAGAGCGTG(A/T)GTAGGGGAGC
PFDN6s2	149	G/C	5′ UTR	GCAGTGGGTG(G/C)CCCGAGGGGC
PFDN6s3	216	C/A	5′ UTR	CGGGTACCTT(C/A)CAGAGCGTGA
PFDN6s4	416	C/T	Intron 1	CTGTCCCACC(C/T)CTGGCCCCAG
PFDN6s5	470	G/C	Intron 1	CATTCCCTGT(G/C)CCGCCTGCTC
PFDN6s6	595	G/A	Exon2 (synonymous)	AATCCATGTC(G/A)GGGAGGCAGA
PFDN6s7	689	G/A	Intron 2	ACCTGTCTTG(G/A)AAGTGATTCT

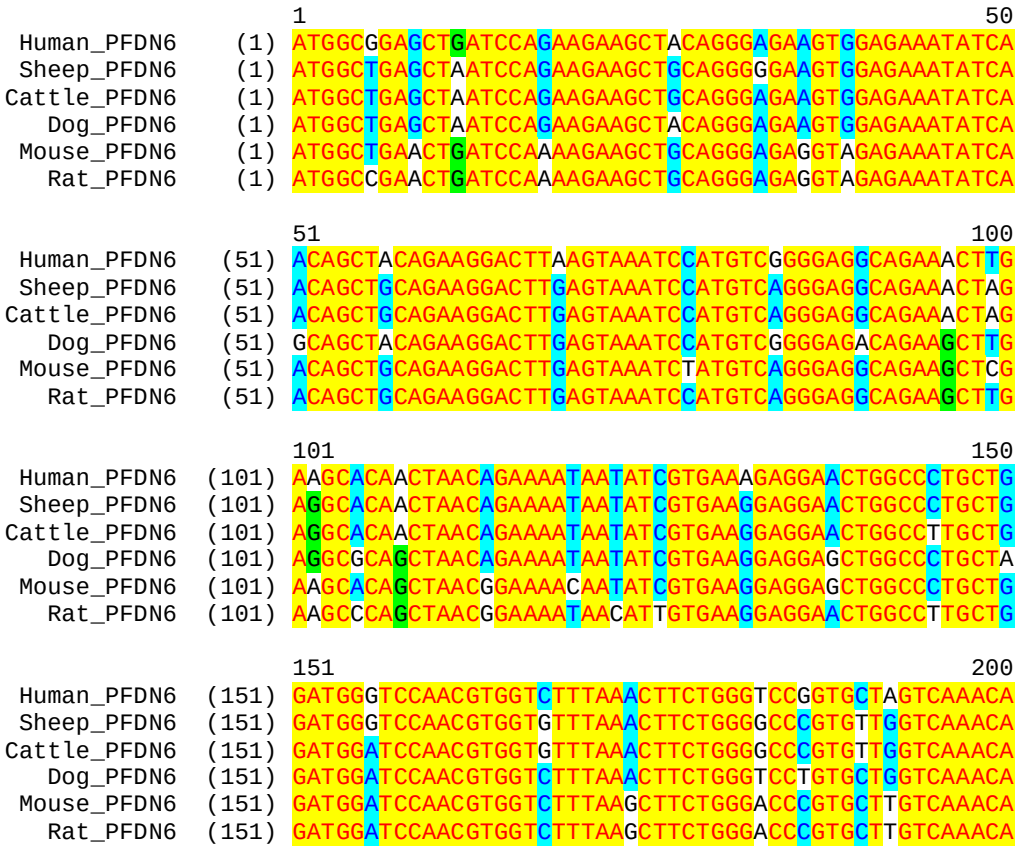
‘Position*’ refers to the base number in the genomic sequence.
PFDN6s1, s2 and s3 are located in the predicted 5’UTR region.

3.3.3 Sequence analysis of ovine PFDN6 gene

The complete genomic sequence of the PFDN6 gene was obtained by alignment allowing the construction of contiguous DNA sequence. The sequences were derived from BAC sub-clones known to contain PFDN6. Furthermore, ovine specific primers were used for sequence-walking to fill in any remaining sequence gaps. Sequence-walking was performed

using a cosmid clone (cosmid 4.103; unpublished data) known to contain PFDN6 which had previously been identified within our research laboratory. SNPs were identified using primers derived from this sequence and are also shown in Table 3.2.

A multiple sequence alignment of the coding region (CDS) of the PFDN6 gene, using previously described orthologous sequences for the human, cattle, mouse, rat and dog was performed using Clustal X (Figure 3.4). The ovine PFDN6 coding region contains 390 nucleotides and encodes a peptide of 129 amino acids. The peptide sequence is shown in Figure 3.5 aligned with the amino acid sequence of its mammalian orthologues. The ovine PFDN6 peptide shows 100% identity to cattle PFDN6 peptide (NP_001068889); both peptides also contain a glutamine (polar; positively charged) at position 99 relative to that of the human PFDN6 protein which contains an arginine (polar; neutral charge).



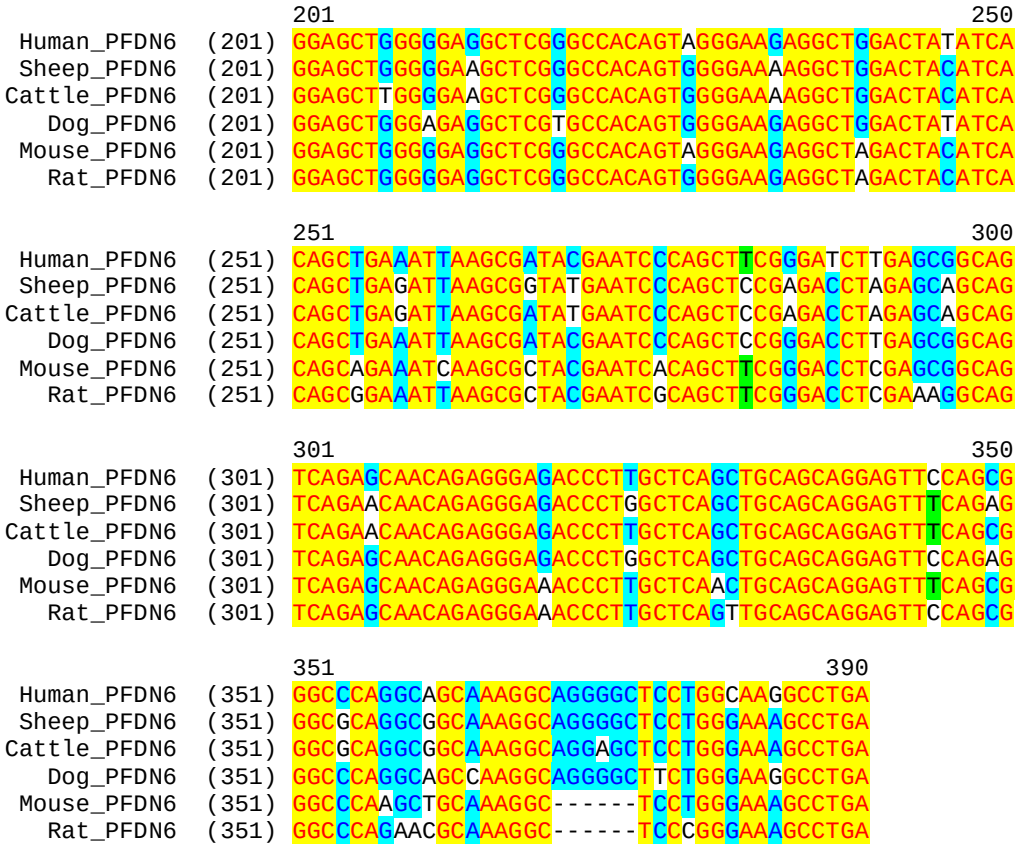


Figure 3.4: Multiple sequence alignment of cds sequences of ovine PFDN6 with their human, cattle, dog, mouse and rat orthologues

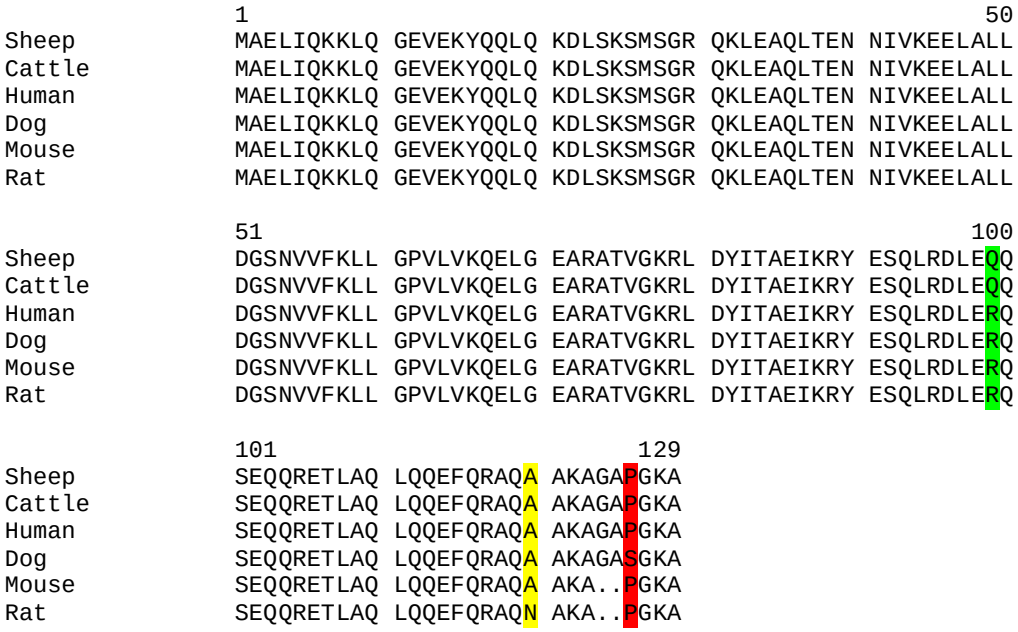


Figure 3.5: Multiple sequence alignment of the peptide sequence of ovine PFDN6 with its human (NP_055075), cattle (NP_001068889), mouse (Q03958), rat (NP_997671) and dog (NP_001041554) orthologues.

Table 3.7: Identity plot between the cds sequences of ovine PFDN6 with their human, cattle, dog, mouse and rat orthologues

	Cattle	Sheep	Dog	Human	Mouse	Rat
Cattle	100	98	90	91	90	89
Sheep		100	91	91	89	88
Dog			100	94	89	88
Human				100	90	90
Mouse					100	94
Rat						100

3.3.4 Sequence analysis of ovine WDR46 gene

The genomic sequence obtained from the sub-clone library was used to design ovine specific primers to PCR amplify and subsequently sequence genomic DNA to identify SNPs within this gene. The genomic sequence obtained covers the first four exons of the WDR46 gene as determined by alignment with its human orthologue. A multiple sequence alignment of the partial genomic sequence of the WDR46 gene with corresponding sequences for cattle and human orthologues was generated and is shown in Figure 3.6. It was observed that there was 95% nucleotide sequence similarity between cattle and sheep. Furthermore, several large indels were identified in the multiple sequence alignment between the ungulate and human versions, the largest being 18 bases in length. The level of identity between the sheep, cattle and human shown in Table 3.8, highlights the level of similarity between the three species. A complete sequence of the gene was not obtained due to resource and time constraints since the gene is relatively largely (≈ 10kb in human) with a relatively large intron (≈ 5kb in human), and consisting of a pseudogene within it (BING5).

```
                    1                                                  50
Homo sapiens      (1) -GAAAAGACAGTCCCATAAGGCAGGGAATGGGG--GTCCAGATTAGGGCT
  Bos taurus      (1) AGAAATGAGAGCCCCATAAAGCAGGGGTTGGGGAGGCCCACATCAGGGTC
  Ovis aries      (1) ---------AGCCCCATAAATCAGGGATTGGGGAGGCCCACATCAGGGTC
   Consensus      (1)  GAAA GA AGCCCCATAAAGCAGGGATTGGGGAGGCCCACATCAGGGTC
                   51                                                 100
Homo sapiens     (48) CACTCACCCAGGTTCTTCAGCAAGCAGCAGCTCAGAACGAGCAGCTTTGA
  Bos taurus     (51) CACTCACCCAGGTTCTTCAGTAAGCAGAAGCTCAGATCGAGCAGCTCTGA
  Ovis aries     (42) CACTCACCCAGGTTCTTCAGCAAGCAGAAGCTCAGATCGAGCAGCTCTGA
   Consensus     (51) CACTCACCCAGGTTCTTCAGCAAGCAGAAGCTCAGATCGAGCAGCTCTGA
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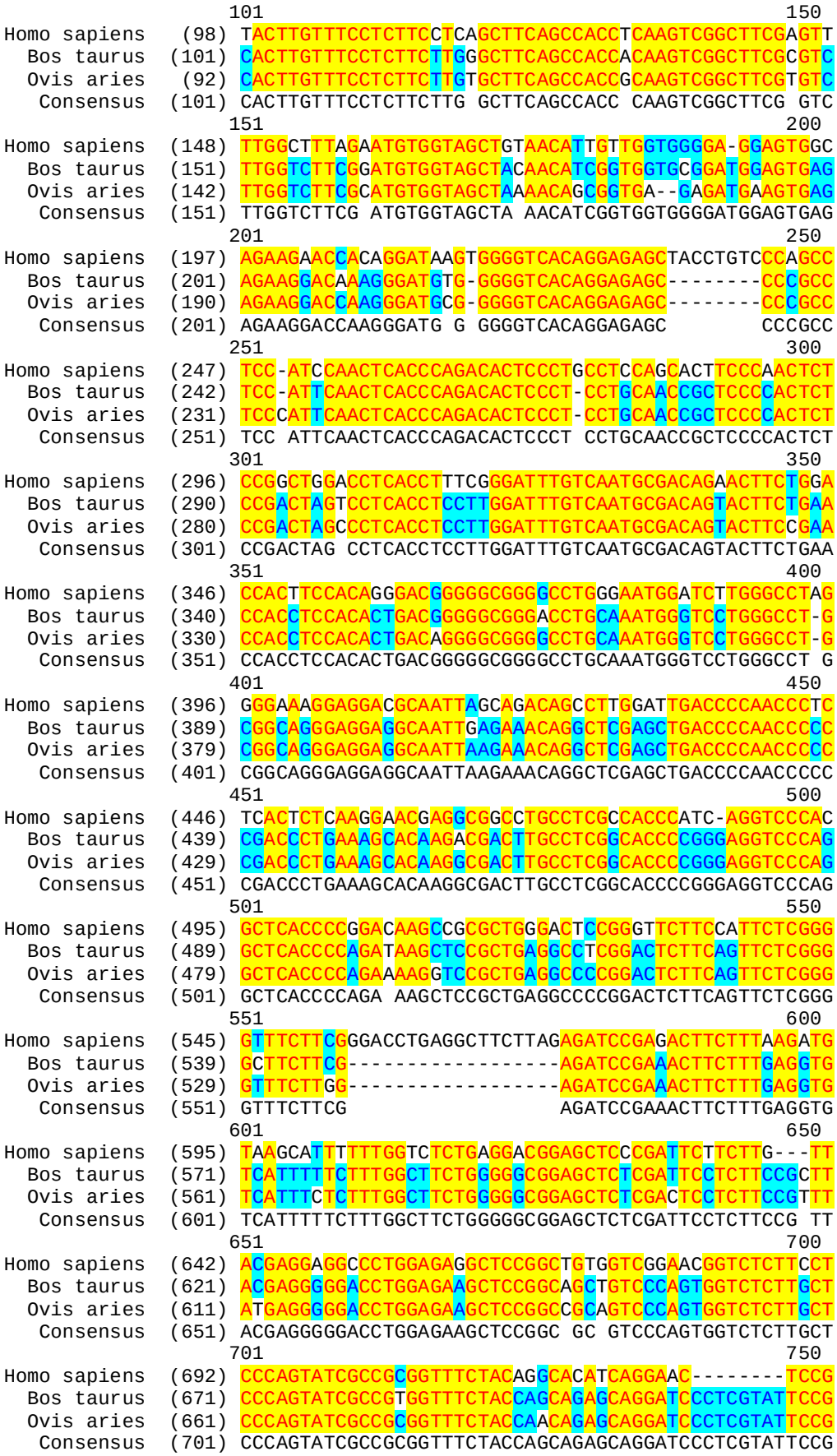

101 150
Homo sapiens (98) TACTTGTTTCCTCTTCCTCAGCTTCAGCCACCTCAAGTCGGCTTCGAGTT
Bos taurus (101) CACTTGTTTCCTCTTCTTGGGCTTCAGCCACCACAAGTCGGCTTCGCGTC
Ovis aries (92) CACTTGTTTCCTCTTCTTGTGCTTCAGCCACCGCAAGTCGGCTTCGTGTC
Consensus (101) CACTTGTTTCCTCTTCTTG GCTTCAGCCACC CAAGTCGGCTTCG GTC
151 200
Homo sapiens (148) TTGGCTTTAGAATGTGGTAGCTGTAACATTGTTGGTGGGGA-GGAGTGGC
Bos taurus (151) TTGGTCTTCGGATGTGGTAGCTACAACATCGGTGGTGCGGATGGAGTGAG
Ovis aries (142) TTGGTCTTCGCATGTGGTAGCTAAAACAGCGGTGA--GAGATGAAGTGAG
Consensus (151) TTGGTCTTCG ATGTGGTAGCTA AACATCGGTGGTGGGGATGGAGTGAG
201 250
Homo sapiens (197) AGAAGAACCACAGGATAAGTGGGGTCACAGGAGAGCTACCTGTCCCAGCC
Bos taurus (201) AGAAGGACAAAGGGATGTG-GGGGTCACAGGAGAGC--------CCCGCC
Ovis aries (190) AGAAGGACCAAGGGATGCG-GGGGTCACAGGAGAGC--------CCCGCC
Consensus (201) AGAAGGACCAAGGGATG G GGGGTCACAGGAGAGC CCCGCC
251 300
Homo sapiens (247) TCC-ATCCAACTCACCCAGACACTCCCTGCCTCCAGCACTTCCCAACTCT
Bos taurus (242) TCC-ATTCAACTCACCCAGACACTCCCT-CCTGCAACCGCTCCCCACTCT
Ovis aries (231) TCCCATTCAACTCACCCAGACACTCCCT-CCTGCAACCGCTCCCCACTCT
Consensus (251) TCC ATTCAACTCACCCAGACACTCCCT CCTGCAACCGCTCCCCACTCT
301 350
Homo sapiens (296) CCGGCTGGACCTCACCTTTCGGGATTTGTCAATGCGACAGAACTTCTGGA
Bos taurus (290) CCGACTAGTCCTCACCTCCTTGGATTTGTCAATGCGACAGTACTTCTGAA
Ovis aries (280) CCGACTAGCCCTCACCTCCTTGGATTTGTCAATGCGACAGTACTTCCGAA
Consensus (301) CCGACTAG CCTCACCTCCTTGGATTTGTCAATGCGACAGTACTTCTGAA
351 400
Homo sapiens (346) CCACTTCCACAGGGACGGGGGCGGGGCCTGGGAATGGATCTTGGGCCTAG
Bos taurus (340) CCACCTCCACACTGACGGGGGCGGGACCTGCAAATGGGTCCTGGGCCT-G
Ovis aries (330) CCACCTCCACACTGACAGGGGCGGGGCCTGCAATGGGTCCTGGGCCT-G
Consensus (351) CCACCTCCACACTGACGGGGGCGGGGCCTGCAAATGGGTCCTGGGCCT G
401 450
Homo sapiens (396) GGGAAAGGAGGACGCAATTAGCAGACAGCCTTGGATTGACCCCAACCCTC
Bos taurus (389) CGGCAGGGAGGAGGCAATTGAGAAACAGGCTCGAGCTGACCCCAACCCCC
Ovis aries (379) CGGCAGGGAGGAGGCAATTAAGAAACAGGCTCGAGCTGACCCCAACCCCC
Consensus (401) CGGCAGGGAGGAGGCAATTAAGAAACAGGCTCGAGCTGACCCCAACCCCC
451 500
Homo sapiens (446) TCACTCTCAAGGAACGAGGCGGCCTGCCTCGCCACCCATC-AGGTCCCAC
Bos taurus (439) CGACCCTGAAAGCACAAGACGACTTGCCTCGGCACCCCGGGAGGTCCCAG
Ovis aries (429) CGACCCTGAAAGCACAAGGCGACTTGCCTCGGCACCCCGGGAGGTCCCAG
Consensus (451) CGACCCTGAAAGCACAAGGCGACTTGCCTCGGCACCCCGGGAGGTCCCAG
501 550
Homo sapiens (495) GCTCACCCCGGACAAGCCGCGCTGGGACTCCGGGTCTTTCCATTCTCGGG
Bos taurus (489) GCTCACCCCAGATAAGCTCCGCTGAGGCCTCGGACTCTTCAGTTCTCGGG
Ovis aries (479) GCTCACCCCAGAAAAGGTCCGCTGAGGCCCCGGACTCTTCAGTTCTCGGG
Consensus (501) GCTCACCCCAGA AAGCTCCGCTGAGGCCCCGGACTCTTCAGTTCTCGGG
551 600
Homo sapiens (545) GTTTCTTCGGGACCTGAGGCTTCTTAGAGATCCGAGACTTCTTTAAGATG
Bos taurus (539) GCTTCTTCG----------AGATCCGAAACTTCTTTGAGGTG
Ovis aries (529) GTTTCTTGG----------AGATCCGAAACTTCTTTGAGGTG
Consensus (551) GTTTCTTCG AGATCCGAAACTTCTTTGAGGTG
601 650
Homo sapiens (595) TAAGCATTTTTTGGTCTCTGAGGACGGAGCTCCGATTCTTCTTG---TT
Bos taurus (571) TCATTTTTCTTTGGCTTCTGGGGGCGGAGCTCTCGATTCCTCTTCCGCTT
Ovis aries (561) TCATTTCTCTTTGGCTTCTGGGGGCGGAGCTCTCGACTCCTCTTCCGTTT
Consensus (601) TCATTTTTCTTTGGCTTCTGGGGGCGGAGCTCTCGATTCCTCTTCCG TT
651 700
Homo sapiens (642) ACGAGGAGGCCCTGGAGAGGCTCCGGCTGTGGTCGGAACGGTCTCTTCCT
Bos taurus (621) ACGAGGGGGACCTGGAGAAGCTCCGGCAGCTGTCCCAGTGGTCTCTTGCT
Ovis aries (611) ATGAGGGGGACCTGGAGAAGCTCCGGCCGCAGTCCCAGTGGTCTCTTGCT
Consensus (651) ACGAGGGGGACCTGGAGAAGCTCCGGC GC GTCCCAGTGGTCTCTTGCT
701 750
Homo sapiens (692) CCCAGTATCGCCGCGGTTTCTACAGGCACATCAGGAAC--------TCCG
Bos taurus (671) CCCAGTATCGCCGTGGTTTCTACCAGCAGAGCAGGATCCCTCGTATTCCG
Ovis aries (661) CCCAGTATCGCCGCGGTTTCTACCAACAGAGCAGGATCCCTCGTATTCCG
Consensus (701) CCCAGTATCGCCGCGGTTTCTACCAGCAGAGCAGGATCCCTCGTATTCCG

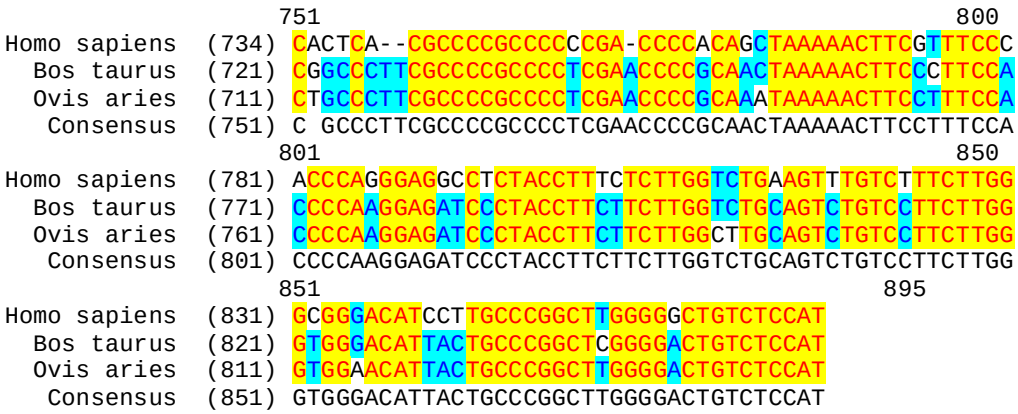


Figure 3.6: Multiple sequence alignment of genomic DNA sequences of ovine WDR46 with their human and cattle orthologues

Table 3.8: Identity plot between the genomic sequences of ovine WDR46 with their human and cattle orthologues

	Bos taurus	Homo sapien	Ovis aries
Bos taurus	100	80	95
Homo sapien		100	80
Ovis aries			100

3.4 Discussion

Two main approaches were used in this work, which allowed the construction of a predicted physical map of the ovine MHC class IIa and IIb regions. This broad map based upon analysis of the ovine BAC clone library was supplemented by the characterisation of additional BAC clones identified from the ovine virtual map. Finally, 103 SNPs were identified representing 21 loci spanning across the class IIa and IIb regions. From the 103 SNPs identified in this study, 20 SNPs spanning most of the class IIa and IIb region were combined with 10 SNPs from a previous PhD study (Qin 2009 - PhD) to give a panel of 30 SNPs covering three main regions of the ovine MHC.

The six BAC clones identified and used in this study comprised DNA representing the majority of the ovine class II region. However, the

actual coverage of the BAC clones across this region was not able to be determined due to the lack of gene position information in the ovine MHC and possible differences between ovine and other mammalian MHCs. In addition, the boundary between the class II/III regions remains to be confirmed through the characterisation of a BAC clone which has been shown to contain both class II region genes (BTNL2 and DRA) and a central region gene (Notch4) (clone no. CH243-375L21).

The SNPs reported in Table 3.5 and 3.6 cover most of the length of the ovine class IIa and IIb region, with SNPs identified in 21 intragenic and intergenic regions. It was hypothesised that the gene order in sheep would be similar to the corresponding syntenic grouping in the human MHC class II region. The main difference would be the occurrence of a chromosomal inversion resulting in a region of approximately 15 – 30 cM separating the class II region into class IIa and IIb as described by van Eijk *et al.* (1993) in cattle. This inversion may be a feature of the class II region in ungulates. The gene order of the DQ region has been predicted by Liu *et al* (2006), and subsequently confirmed by Herrmann-Hoesing *et al.* (2008). A similar physical map of this region was reported in the Sheep Predicted Map ver. 1.4. A radiation hybrid map constructed by Wu *et al* (2008) placed the DQ-DR region within the MHC class III region between TNF and BAT3 thereby casting some uncertainty on the relative positions of these loci. This observation by Wu *et al*. (2008) is inconsistent with those by Liu *et al*. (2006), Herrmann-Hoesing *et al*. (2008) and the sheep virtual map (Dalrymple *et al*., 2007). In the ovine MHC class IIb region, as a result of the chromosomal inversion, the extended class II region (DAXX, TAPBP, PFDN6) is now closer to the class IIa region. This was demonstrated previously in cattle by Band *et al,.* (1998) using a radiation hybrid map of BTA23. Current knowledge of this region in sheep is unfortunately still very unclear.

A total of 103 SNP were discovered in this work. It was estimated that there is one SNP for every every 180 bp in the class IIb and 1 for every 100 bp in the class IIa. These estimated SNP frequencies are

comparable, although slightly higher in the IIa region with frequencies to that reported previously for the whole genome (Kijas et al, 2009). These SNPs will provide a panel of genetic markers that can be used for elucidating class IIa and IIb haplotypes. The use of this panel will be described in Chapter 5, for the recognition of haplotypic combinations and in Chapter 6, their use in association studies.

A complete genomic sequence of the ovine PFDN6 gene was reported and it encodes a protein of 129 aa and was found to contain seven SNPs in Merino sheep, three of which are located in the predicted 5' UTR region. One SNP (PFDN6s6) was located in exon 2 and the mutation was synonymous. Human PFDN6 has been proposed to be similar to heat-shock protein (HSP) 70 genes in that the orthologs are present in multiple species that predate the emergence of the MHC, have a function in protein folding pathways and can be regulated by cellular activation (Ostrov *et al*., 2007). PFDN6 encodes a β sub-unit (sub-unit 6) of prefoldin, which binds unfolded proteins (mostly actin and tubulin) and delivers them to c-chaperonin in protein folding and trafficking pathways (Vainberg *et al*., 1998; Ostrov *et al*., 2007). This process facilitates the folding of other proteins into their correct active conformational structure. PFDN6 orthologs (cDNA and amino acid) were analysed and aligned to identify evolutionary conserved residues. As shown in Figures 3.4, 3.5 and Table 3.7, the PFDN6 cDNA and amino acid sequences are highly conserved. The level of similarity in cDNA ranged from as low as 88% between sheep and rat to as high as 98% between sheep and cattle. Despite the difference in cDNA sequences between species, the PFDN6 proteins are highly homologous. The ovine PFDN6 protein sequence is 100% identical to cattle, and 99% between sheep and human. Homologous genes have been described in a diverse set of mammalian species including human, cattle, rat, mouse and dog. The high level of similarity between PFDN6 orthologues highlights the importance of the gene product and its conservation in protein folding pathways. PFDN6 is one of the few genes in the class II region whose immunologically related function is still unknown. However, the high level of conservation of this gene sequence and its product between species

further emphasises the potential importance of this gene. In addition, the aberrant expression of PFDN6 in malignancy suggests the potential utility of the gene as a cancer biomarker (Ostrov *et al*., 2007).

A partial sequence of the WDR46 gene was also reported. The sequence obtained consists of the first four exons of the gene. This gene is located next to the 5' end of the PFDN6 gene, through genomic sequence alignment with the human orthologue. Sequence alignment of ovine WDR46 with the cattle and human orthologue showed high levels of similarity between these different species, especially between cattle and sheep (95% similarity; Figure 3.6; Table 3.8). Interestingly, comparison of the mRNA sequences of cattle, human, mouse and rat showed significant differences (data not shown). Unlike that observed in PFDN6, the mRNA sequences of WDR46 are not conserved across species. The function of the WDR46 protein is still unknown, but has been shown to be a potential antigen for immunotherapy in patients with melanoma (Rosenborg *et al*., 2002).

The main results from this chapter are the identification and super-positioning on a physical map of the ovine MHC of a panel of SNP loci. Sequences characterising these SNPs have been deposited in the international GenBank database where they will be accessible by others pursuing similar genetic association studies in sheep. Furthermore, the SNPs identified in these regions will facilitate identification of block-like structures, haplotypes, as well as disease and productivity associations. The physical map presented herein has been obtained by using clones from the freely available ovine CHORI-243 BAC library that have been identified screening with radioactively labelled locus specific probes together with interrogation of the ovine virtual map. The ovine MHC class II region is very similar to that of the human and cattle MHCs. However, the sheep and cattle MHCs manifest a chromosomal inversion partitioning the class II region into two distinct subregions (IIa and IIb) separated by a non MHC intervening sequence. The break point for this inversion in sheep is as yet unidentified, however in cattle it is known to be close to *TAP2* (Childers *et al*., 2006).

Chapter 4

Serum Immunoglobulin A levels and parasite resistance in sheep

This Chapter describes experimental work to determine whether serum immunoglobulin A levels are associated with gastrointestinal nematode parasite resistance in sheep post weaning and not under artificial challenge. Total and parasite specific IgA levels were determined in a panel of 171 sheep with known WECs and by inference their susceptibility/resistance to gastrointestinal parasites.

4.1 Introduction

4.1.1 Immunoglobulin A (IgA)

Immunoglobulin A (IgA) is the major class of antibody present in mucosal secretions of all mammals and represents a key first line of defence against enteral infections. IgA is also found in significant concentrations in the serum of all mammals. Due to its relatively short half-life, the daily energy requirement for production of IgA, at least in higher mammals, exceeds that of all other antibody classes combined. This emphasises, from an evolutionary standpoint, the importance and benefits provided by IgA in terms of humoral immune responses.

Human IgA has been extensively characterised. It consists of both monomeric and polymeric forms (predominantly dimers) and the latter being stabilized with a J chain peptide. The IgA alpha chain constant regions are encoded by the Cα genes (Woof and Kerr, 2004). Two allotypic forms and two isotypes are present in normal human serum. A secretory form of IgA is present in saliva and along mucosal surfaces. This consists of an IgA dimer covalently bound to another peptide called secretory component, that increases resistance to proteolytic degradation. The IgA molecules of other species, particularly ruminants, are less well characterised although it has been reported that only a single isotype of IgA is present in cows and horses (Wagner *et al*., 1997;

Brown *et al*., 1997). More recently, Zhou *et al.* (2005) have reported the presence of three isotypes in sheep, however the significance of this finding is yet to be assessed. It is clear however that sheep serum contains IgA and that a secretory form of IgA is present in mucosal fluids (Stear *et al*., 1995; Meeusen *et al*., 2004).

4.1.2 Immunology and gastrointestinal parasites infection

Many studies have shown that mammalian humoral immune responses are generated following infection with gastrointestinal parasites (reviewed by: Behnke *et al*., 2003; Acheson and Luccioli, 2004). Because of its importance in mucosal immunity, many of these studies have focussed on IgA antibodies as well as the more usual IgG isotypes.

In several host-parasite systems, parasite-specific IgA has been associated with resistance (Kanobana et al., 2002; Inaba et al., 2003; Wilkes et al., 2007; Yanming et al., 2007; Matsuzawa et al., 2008). However, careful design and interpretation is needed because IgA responses to nematode infection are correlated with IgE production, together with infiltration of eosinophils, mast cells and their subsequent degranulation (Stear et al., 1995). The correlations presumably arise because Th2 cells release a variety of cytokines that recruit the relevant cells. Therefore it is possible that IgA synthesis may be an epiphenomenon associated with an IgE mediated protective response. IgA is not complement fixing but has been implicated in anti-inflammatory mechanisms (Lamm, 1997). However, this would seem to be a simplistic view of the potential role of IgA in gastrointestinal infections. In mouse models, the humoral immune response has been reported to exert a direct effector role against the gastrointestinal nematode parasite. Immunity against murine *Trichuris muris* could be achieved through monoclonal IgA antibody infusion resulting in the expulsion of the parasites from the gastrointestinal tract (Roach et al., 1991). The immune mechanism was thought to be mediated by antibody

binding directly to parasite excretion/secretion antigens (Roach et al., 1991).

Smith *et al.* (1985) were the first to show an association between an increased IgA response and reduced worm length. Since then, subsequent experiments have established and confirmed a correlation between parasite-specific IgA synthesis and reduced worm length (Gill *et al*., 1993). Although the mechanism(s) by which IgA may contribute to worm stunting are not known, Stear *et al*. (1995) observed variations in pathophysiological factors such as concentration of mast cells, globular leucocytes, eosinophilia, IgA-positive plasma cells and parasite-specific IgA in the abomasal mucosa in experimentally infected Scottish Blackface sheep matched for age, sex, breed, farm of origin and parasite exposure history. Statistical analysis showed that there was a significant inverse correlation between IgA and worm length. The inverse correlation between an increase in IgA activity and decrease in worm length was also reported in Santa Ines, Suffolk and Ile de France lambs infected by *H. contortus*, Scottish Blackface lambs infected by both *T. circumcincta* and *H. contortus* in separate experiments, as well as Churra lambs infected with *T. circumcincta* (Strain and Stear, 2001; Amarante *et al*., 2005; Martinez-Valladeres *et al*., 2005). It is significant that naturally infected Scottish Blackface lambs infected by *T. circumcinta* also showed a significant negative correlation between IgA concentration and worm length thereby enhancing the biological relevance of this phenomenon (Strain *et al*., 2002; Stear *et al*., 2004).

There are several methods by which IgA could influence nematode growth. Nematodes release a variety of proteases that partially pre-digest proteins and may also break down antibodies and other mediators of host resistance. Antibodies against these enzymes could inhibit enzyme activity and reduce feeding rates (Chandler, 1932; Chandler, 1935a; Chandler, 1935b; Chandler, 1936a; Chandler, 1936b). Alternatively, ingested antibodies could bind to parasite molecules in the digestive tract and reduce digestive efficiency. This appears to be a mechanism underlying the success of vaccination against H-Gal Gp from

Haemonchus contortus (Knox et al., 2003). In mice infected with *H. polygyrus*, passive transfer of purified polyclonal IgG into naïve JH -/- mice, limited parasite fecundity without complement C3 or Fc gamma chain receptor involvement, thus suggesting direct interaction of the antibody with essential parasite proteins (McCoy et al., 2008). A third possibility is that IgA interacts with eosinophils to control nematode growth and fecundity.

An observation by Stear *et al*. (1995) found that sheep with more mast cells had more globule leucocytes, eosinophils, IgA plasma cells and more larval antigen specific IgA antibody. One-way regression analysis established the correlation between increased abomasal concentrations of eosinophils, globule leucocytes and parasite specific IgA with shorter worm length. However, when the data is analysed using multiple regression, only the effect of IgA was significant. This suggested that the apparent link with globule leucocytes and eosinophil numbers was due to the strong association of these with IgA activity (Stear et al., 1995). Henderson and Stear (2006) measured the level of IgA and eosinophil numbers in Scottish Blackface lambs over a period of 60 days post challenge and observed that both variables had similar response kinetics. IgA and eosinophil activity peaked at 8-10 days after infection and declined subsequently. Stear *et al*. (1995) measured eosinophil numbers at the end of the experiment during necropsy of the animals while Henderson and Stear (2006) measured mucosal eosinophilia over a 60 day period. These two variables accounted for much of the variation in worm length: 38% for IgA activity and 40% for eosinophil numbers. Interestingly, when both variables were analysed together in correlation studies the combination accounted for 53% of worm length variation. Therefore, it appears that IgA and eosinophilia have a combined or synergistic effect on worm length (2006). Eosinophils have receptors for IgA (van Egmond et al., 2001; Woof and Kerr, 2006). Therefore IgA could help target eosinophils to nematodes. Interestingly, mice eosinophils lack receptors for IgA (van Egmond et al., 2001) and this

could explain the relative ineffectiveness of eosinophils in some murine models (De Andres et al., 1997; Behm and Ovington, 2000).

4.1.3 Outline of this study

While is clear that IgA plays an important role in host responses to gastrointestinal parasite infection, most studies have focussed on determining parasite specific IgA levels following experimental challenge with the parasites. This chapter, investigates whether either total or parasite-specific IgA concentrations in sheep serum is a useful predictor of susceptibility to infection with nematodes, predominantly *T. circumcincta*. It will then be ascertained whether serum IgA levels are correlated with WECs.

4.2 Materials and Methods

4.2.1 Source of sheep serum

Sheep from a panel of animals maintained as part of the Rylington Merino Project (Western Australia) were used as donors of blood for extraction of serum samples. Serum IgA levels were determined in sheep approximately 6 months post weaning and maintained on pasture with a low parasite burden. The WEC of these sheep, and by inference their susceptibility to parasites, had been measured shortly after weaning. Preparation of the serum samples are as described in Chapter 2.2. The test population in this study is a subset of the whole test population described in Chapter 2.1. A total of 171 sheep (33 animals making up the control group and the remaining animals from 9 different family groups). This sub-division of the animals is used in the analysis of this study.

4.2.2 Preparation of Parasite Homogenate

4.2.2.1 Parasite protein homogenate

Infective parasite larvae (stage L3; *T. circumcincta*) were generously provided by Novartis (personal communications). An aliquot of 50 ml of L3 gastrointestinal parasite (≈ 50,000 -100,000 worms) suspended in water was centrifuged at 3000 g for 15 minutes at 4°C. The supernatant was carefully removed to prevent loss of parasites, and the pellet was resuspended in 2 ml of PBS buffer with protease inhibitor (1 tablet dissolved in 10 ml PBS buffer). The parasite suspension was transferred into a 2.5 ml screw-cap Cryo tube with twenty sterilised 2mm stainless steel ball bearings. The parasite and ball bearing suspension was centrifuged at 13000 rpm for 3 minutes. The supernatant was removed and the pellet was resuspended with PBS buffer (with dissolved protease inhibitor). This process was repeated twice. The parasites were then disrupted using the ball bearings in the Fastprep FP120 machine (Thermo). The process for parasite disruption using the Fastprep FP120 machine is as follows: 9 sets of 3X Pulse: 5.5 Time: 30 seconds, with the parasite suspension chilled in ice in between each set to prevent increase in temperature of the parasite protein homogenate. The disrupted parasite protein homogenate with suspended stainless steel ball bearings was centrifuged at 16000 g for 5 mins. The clear supernatant was transferred into a clean tube and stored at -80°C until later use.

4.2.2.2 Measurement of protein homogenate concentration

The measurement of protein concentrations was performed using the Lowry protein assay method. An aliquot of 2.5 ml of Working Tartrate solution [45 ml Alkaline Tartrate solution (188 mM Na_2CO_3, 1.8 mM $KNaC_4H_4O6._4H_2O$, 0.1 M NaOH) and 5 ml of 4 mM Copper Sulphate solution] was mixed with 50 µl of the sample. The mixture was left to stand at room temperature for 10 minutes, followed by the addition of 250 µl of diluted Phenol reagent of Folin and Ciocalteau. The solution was mixed immediately and left to stand at room temperature for 30 minutes.

The optical density of the sample was measured using a spectrophotometer at 750 nm.

4.2.3 Enzyme-linked immunosorbent assay

4.2.3.1 Total IgA

The wells of a flat-bottomed microtitre plate (Nunc) were coated with 50 µl of rabbit polyclonal anti-sheep IgA (Novus Biologicals) at a dilution of 1:100 in carbonate buffer (15 mM Na_2CO_3 and 35 mM $NaHCO_3$; pH 9.6) and incubated overnight at 4°C (minimum of 16 hours). The plate was washed three times with Tris-Tween 20 [0.1% v/v Tween 20 in Tris buffer (10 mM Tris, 145.5 mM NaCl and 0.001 % w/v Gelatine); pH 7.4], followed by incubation for 1 hour at 37 °C with 100 µl of Blocking buffer (4 % BSA in Tris buffer). The wells were then washed three times in Tris-Tween 20. An aliquot of 50 µl of sheep serum samples diluted 1:100 in Blocking buffer was added and incubated for 30 minutes at 37°C. After another three washes with Tris-Tween 20, 50 µl of isotype specific rabbit polyclonal anti-sheep IgA conjugated to alkaline phosphatase (Novus Biologicals) diluted at 1:60 in Blocking buffer was added and incubated for 30 minutes at 37°C. After the final three washes in Tris-Tween 20, 50 µl of 4-nitrophenyl (Sigma) dissolved in diethanolamine buffer (0.1% w/v diethanolamine and 1 mM $MgSO_4$: pH 10; final substrate concentration of 1 mg/ml) was added and incubated for a further 15 minutes at room temperature. The reaction was then read on a microplate reader (Thermo Labsystems – Multiskan Ascent) at 405 nm. Total IgA reactivity was determined by measuring the mean of three optical density replicates for each animal. The mean of the replicates was corrected by subtracting the optical density value of a negative control.

Since a mass standard for sheep IgA protein was not available commercially, and preparation of such a standard was not feasible, total sheep IgA concentrations were reported as an index representing the IgA level as a proportion of the range between a high level serum sample and a low level serum sample. The Optical Density Index (ODI), as used by Strain *et al*. (2002), for each sheep serum sample was determined by

measuring the difference between the mean of three replicates from a pool of low-total IgA samples and the adjusted sample mean, divided by the mean of three replicates from a pool of high-total IgA samples subtracted from the sample of low-total IgA samples. The ODI is given by the formula:

$$ODI = \frac{\text{Mean sample} - \text{Mean low count}}{\text{Mean high count} - \text{Mean low count}}$$

The three replicates for the high- and low- total IgA samples were made by combining equal amounts of serum from five lambs that gave strong or weak IgA responses respectively. The value of total IgA activity was therefore expressed as a proportion of a reference range reflecting the difference between high and low serum IgA concentrations in the sheep under study.

4.2.3.2 Parasite-specific IgA

The wells of a flat-bottomed microtitre plate (Nunc) were coated with 100 µl of parasite protein homogenate (5 µg/ml) in carbonate buffer and incubated overnight at 4 °C (minimum of 18 hours). The plate was washed five times with Tris-Tween 20, followed by incubation for 2 hours at 37°C with 200 µl of Blocking buffer. The wells were then washed five times in Tris-Tween 20. An aliquot of heat-treated sheep serum samples (neat) was added and incubated for 1 hour at 37°C. After another five washes with Tris-Tween 20, 100 µl of rabbit polyclonal anti-sheep IgA conjugated to alkaline phosphatase (Novus Biologicals) diluted in Blocking buffer (1:50 dilution) and incubated at 37°C for 30 minutes. After five washes with Tris-Tween 20, 100 µl of 4-nitrophenyl (Sigma) dissolved in diethanolamine buffer (pH 10; final substrate concentration of 1 mg/ml) were added and incubated for a further 30 minutes at room temperature. The reaction was then read on a microplate reader (Thermo Labsystems – Multiskan Ascent) at 405 nm. Total IgA activity was determined by estimating the mean of three replicates from each animal. The mean of the replicates were corrected by subtracting with the negative control.

Parasite-specific IgA concentrations were reported as an index representing the IgA level as a proportion of the range between a high level serum sample and a low level serum sample. The Optical Density Index (ODI) for each sheep serum sample was determined as described in Section 4.2.3.1.

4.2.4 Statistical analysis

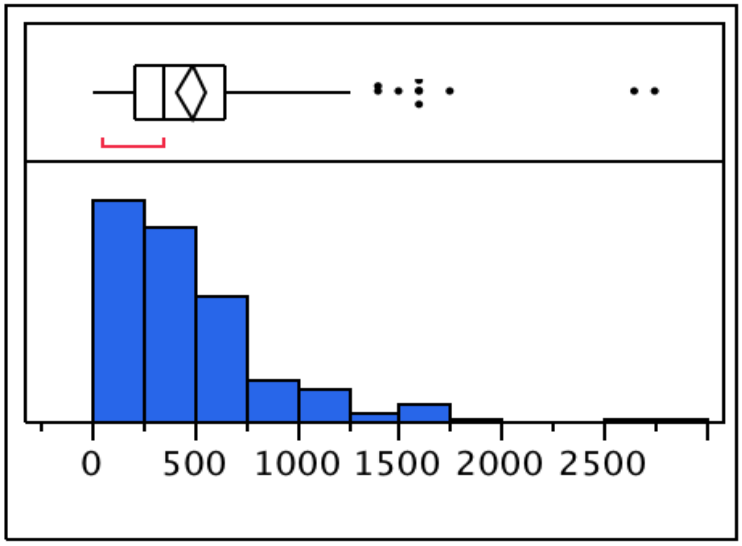
Statistical analyses were performed using the JMP® 7 statistical package (SAS Institute Inc. 2008). In general, associations between two variables were examined using the following correlation coefficients; standard Pearson and the non-parametric Spearman correlation coefficient. A general linear model was used to describe the relationship between Family groups and parasite-specific IgA. The model was examined and residual plotted. The WEC distribution was found to be heteroskedastic, and therefore subsequent analyses were done on log-transformed data (log WEC).

4.3 Results

4.3.1 Analysis of Worm Egg Count (WEC) in Rylington Merino population

The distribution of WECs in 171 sheep from the Rylington Merino project used in this experiment is shown in Figure 4.1. These animals comprised 9 different family groups each with one sire and a control group generated from multiple sires (33 animals). Animals from each family group have varying parasite resistant/susceptibility levels based on WEC and pedigree information. The distribution was positively skewed with a mean of 481.7 and a median of 350.

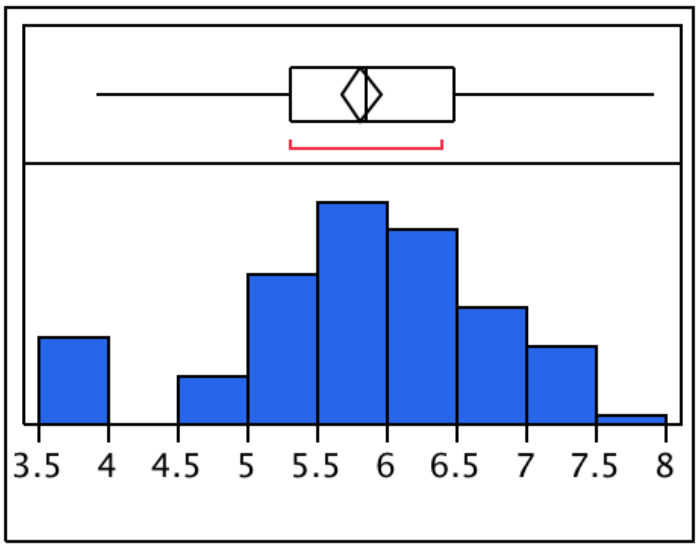
A



Mean	481.71
Median	350.00
Std Dev	452.16
Std Err Mean	34.89

Figure 4.1 continued on next page.
Figure 4.1 cont.

B



Mean	5.81
Median	5.86
Std Dev	0.95
Std Err Mean	0.07

Figure 4.1: (A) WEC and (B) Log WEC distribution of naturally infected animals that were selected for resistance to parasite (JMP® 7). The logarithmetic distribution clearly is a better approximation to a normal distribution.

The mean WEC for Family 1 is significantly different from the control group ($F_{9,156}$ = 3.73, p = 0.0003) (Figure 4.2). The WEC for Family 1 is significantly greater than for the control group plus the other Family groups (p = 0.0228).

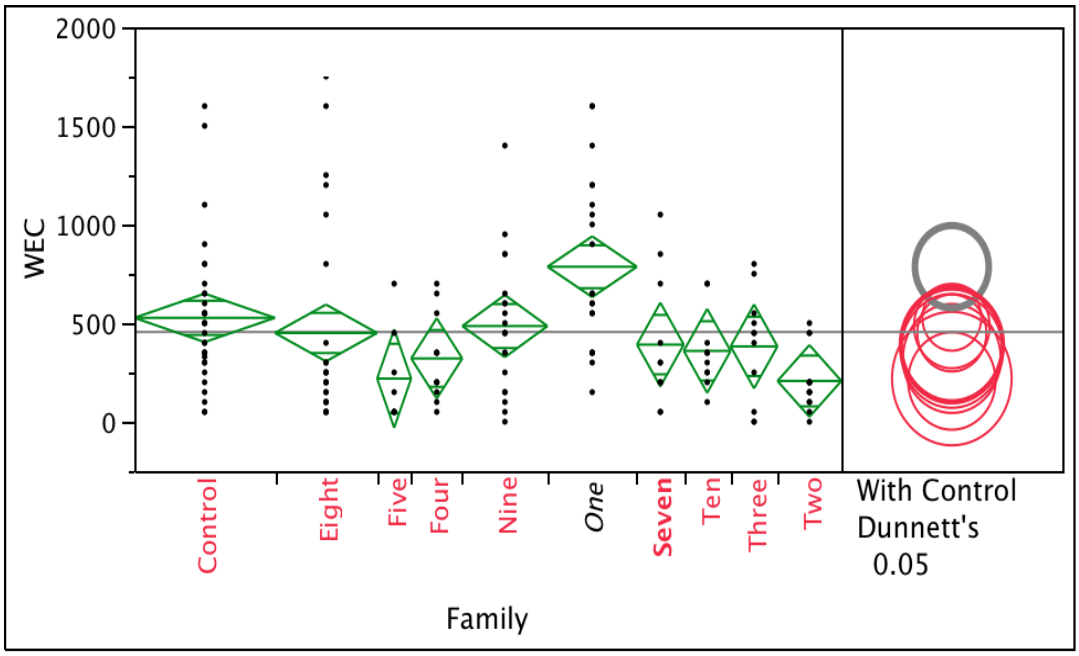


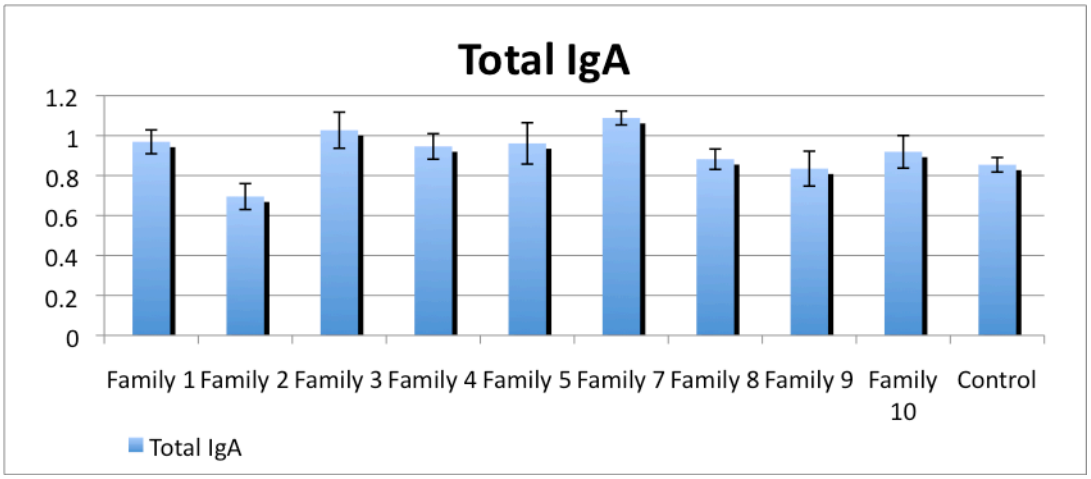
Figure 4.2: Means and standard deviations of WECs for individual sheep families (green boxes). The circles show the results of a Dunnert analysis which visually displays a differences between the mean WECs of the families groups and the control group. Family one differs at the 5% probability level. (JMP® 7)[1].

4.3.2 Correlation between IgA levels and WECs

The mean and standard error for total IgA and parasite-specific IgA was constructed (Figure 4.3). The mean total IgA level for each family group was relatively constant ranging from 0.69 to 1.09 ODI. In contrast, parasite-specific IgA levels were significantly more variable between the family groups, ranging from 0.27 to 1.18 although it should be noted that due to the nature of the assay used, the ODI for total IgA is not directly comparable with the ODI for parasite-specific IgA. The standard error for total IgA and parasite-specific IgA for each family group reflects the wide variation of WEC for each animal in each family group, as shown in Figures 4.1 and 4.2.

[1] The Dunnetts test, ANOVA results and leverage tests are presented graphically using the JMP® output wherever it was applicable within the results.

A



B

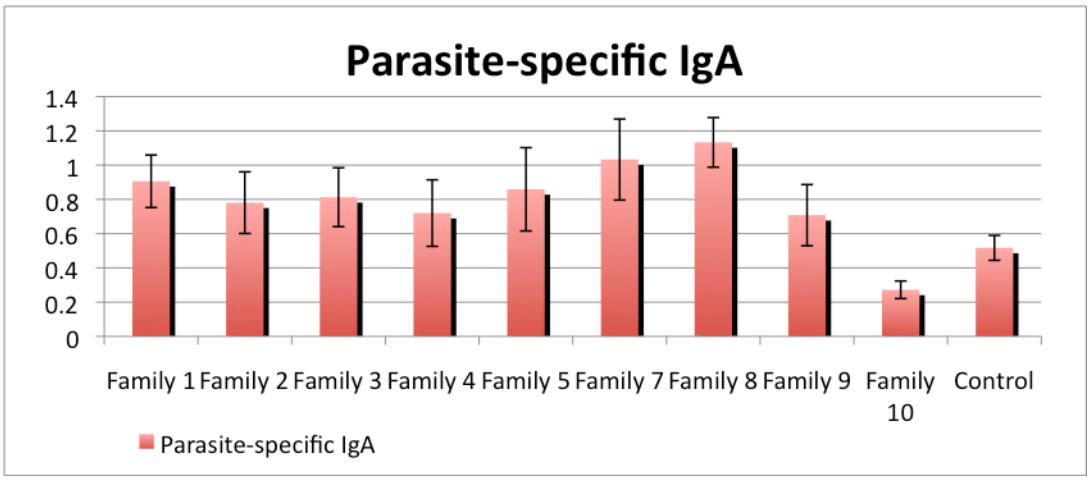
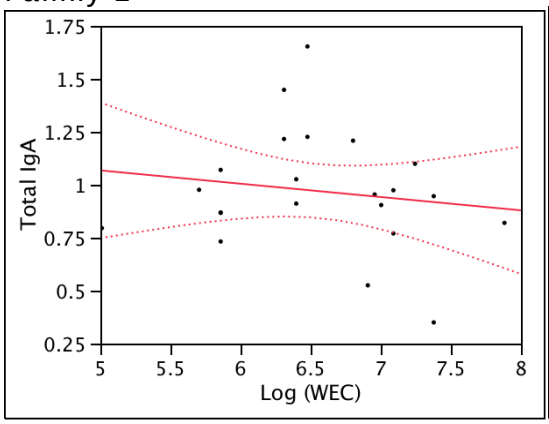


Figure 4.3: Mean and Standard Error of ODIs for (A) Total IgA and (B) Parasite-specific IgA for the 9 family groups and control group of sheep.

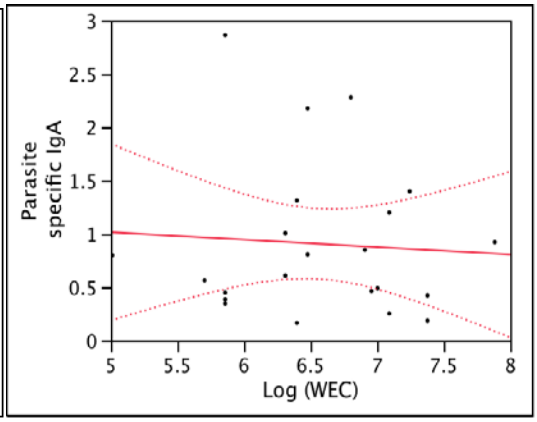
NB: The ODIs for total and parasite specific IgA are relative values and cannot be directly compared.

In all 9 families and the control family, a negative correlation was observed between the ODIs for both total IgA activity and parasite-specific IgA with the log transformed WEC except for Family 2. Scatter plots for all family groups, and the control group are shown in Figure 4.4, for total IgA and parasite-specific IgA. Figure 4.5 displays the scatter plots for total IgA and parasite-specific IgA for the whole test population. Two extreme outlier data points, one in Family 7 and the other in the Control family, were excluded from the correlation and modelling analysis on the advice of a statistician.

Family 1

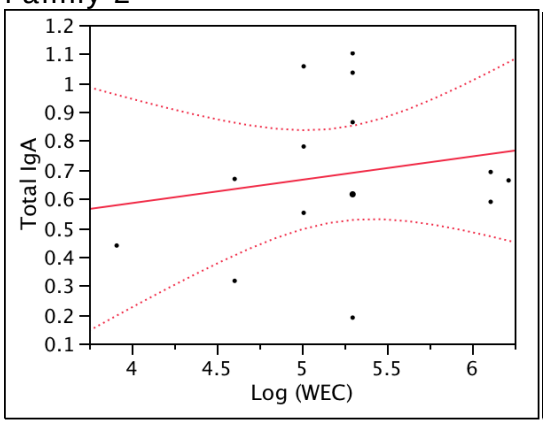


R^2 : 0.005

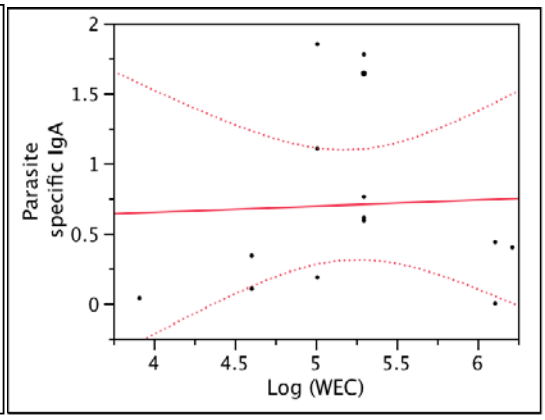


R^2 : 0.03

Family 2

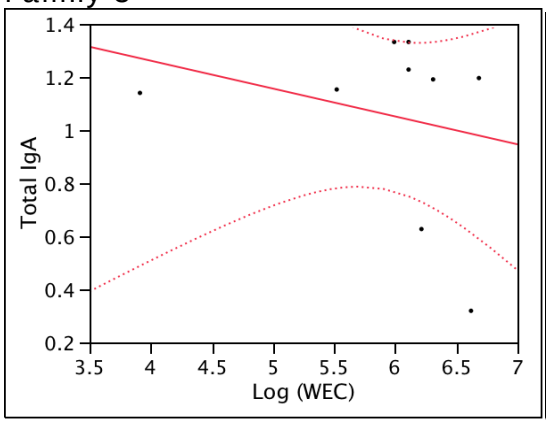


R^2 : 0.04

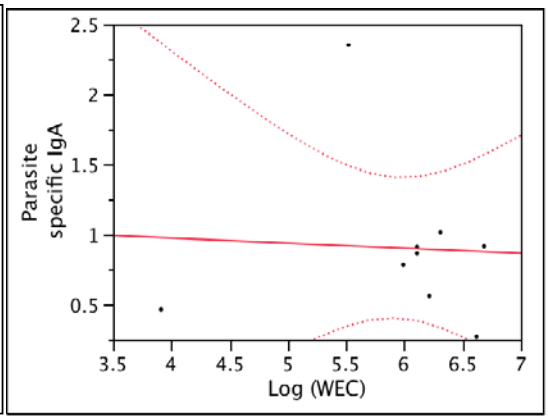


R^2 : 0.002

Family 3



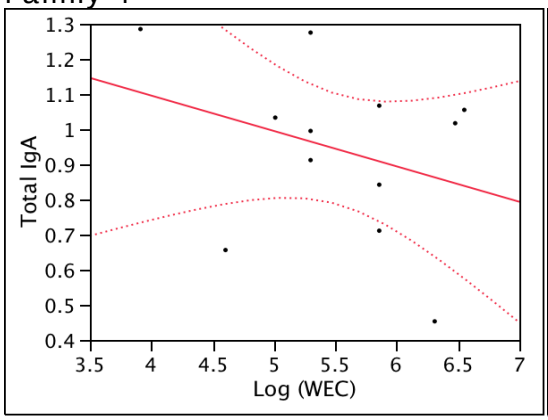
R^2 : 0.06



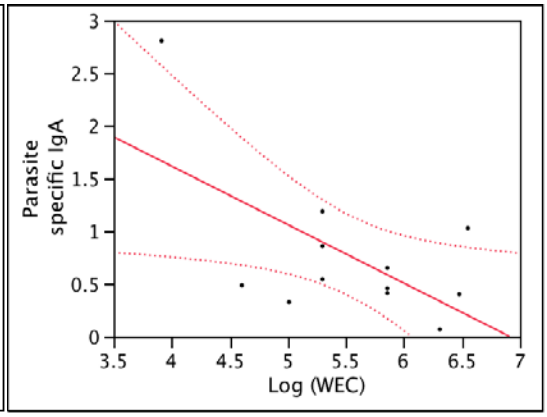
R^2 : 0.002

Figure 4.4 cont.

Family 4

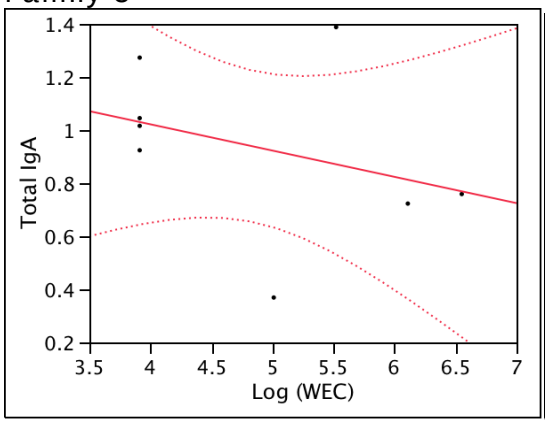


R^2 : 0.10

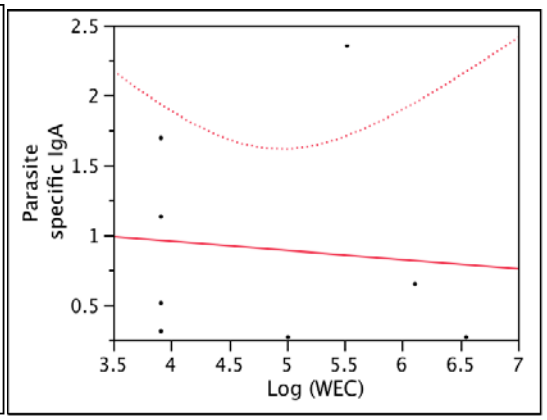


R^2 : 0.37

Family 5

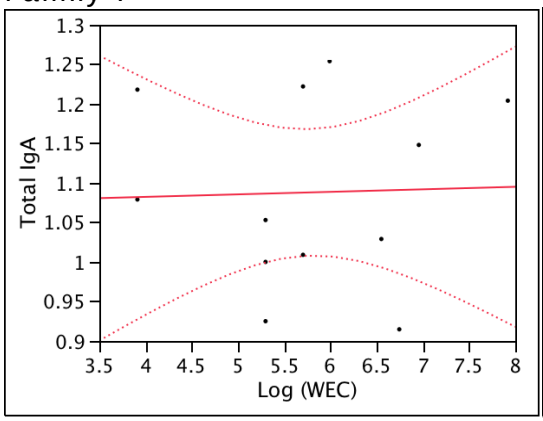


R^2 : 0.11

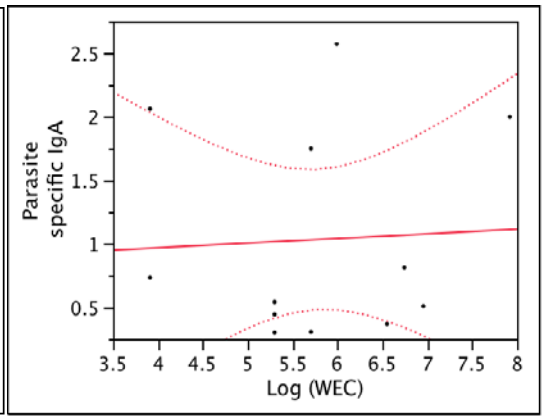


R^2 : 0.009

Family 7



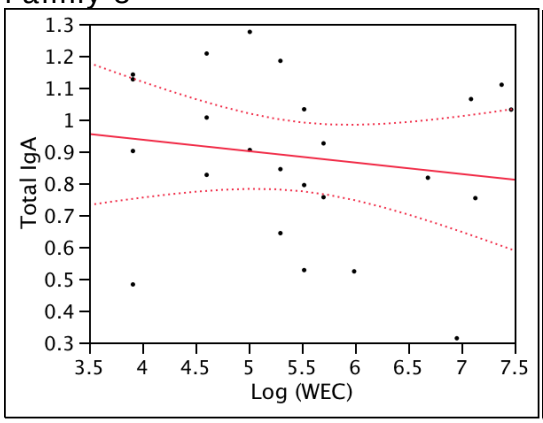
R^2 : 0.03



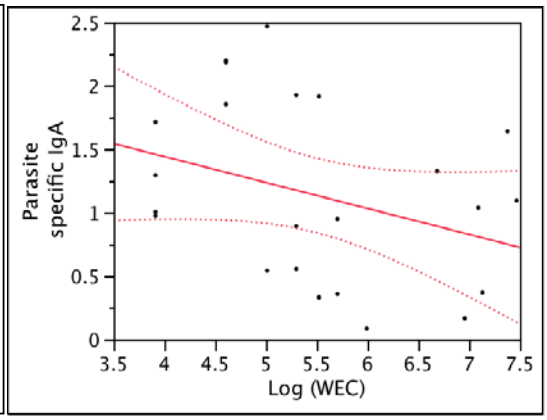
R^2 : 0.04

Figure 4.4 cont.

Family 8

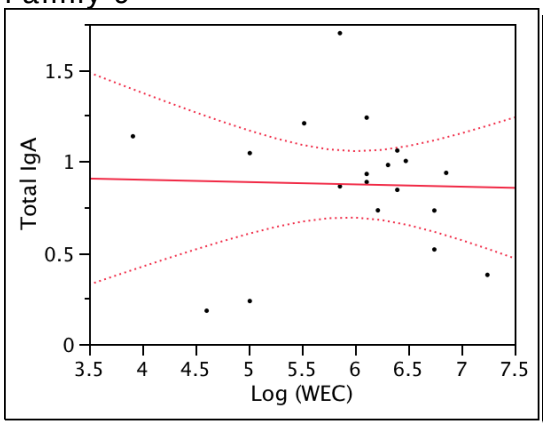


R^2 : 0.03

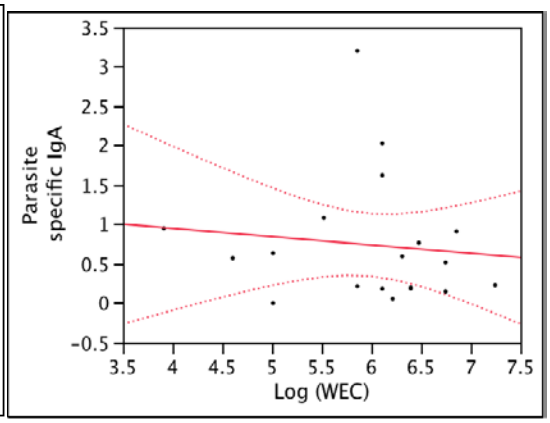


R^2 : 0.10

Family 9

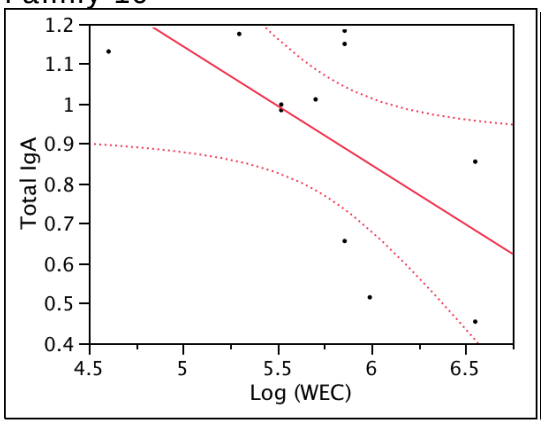


R^2 : 0.0008

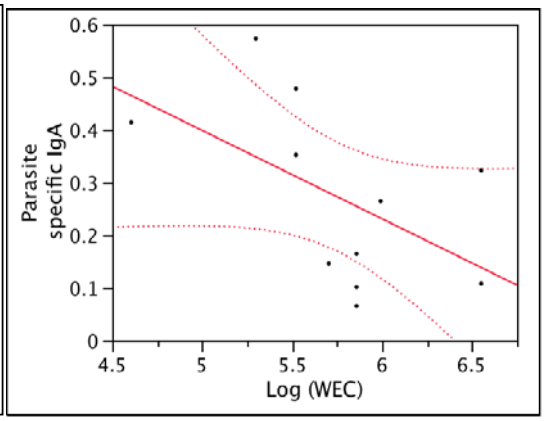


R^2 : 0.01

Family 10



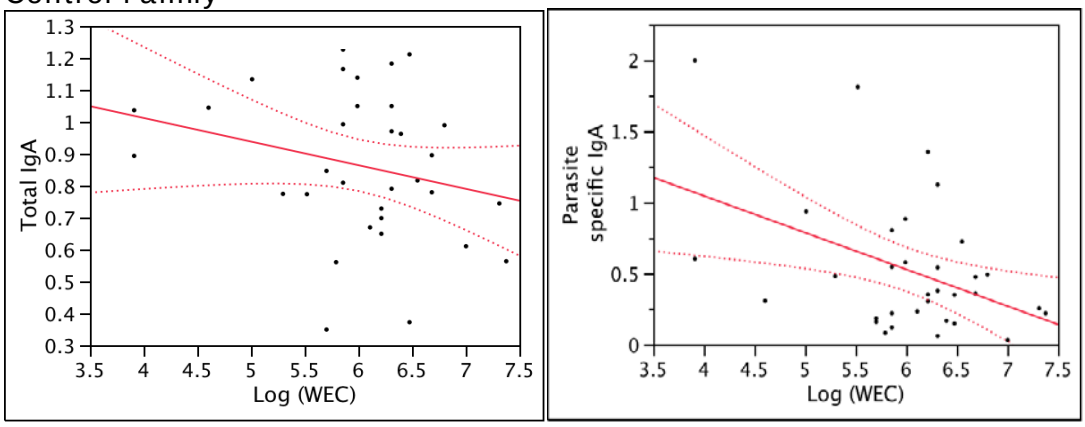
R^2 : 0.38



R^2 : 0.30

Figure 4.4 cont.

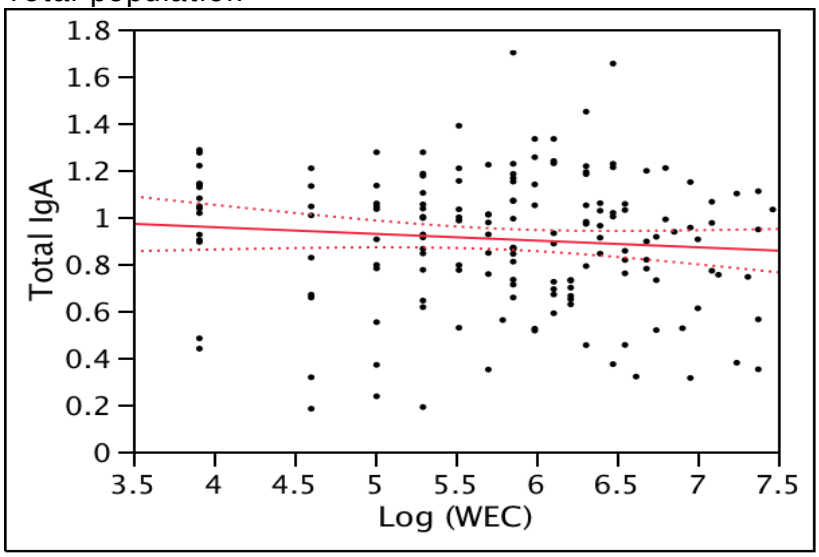
Control Family



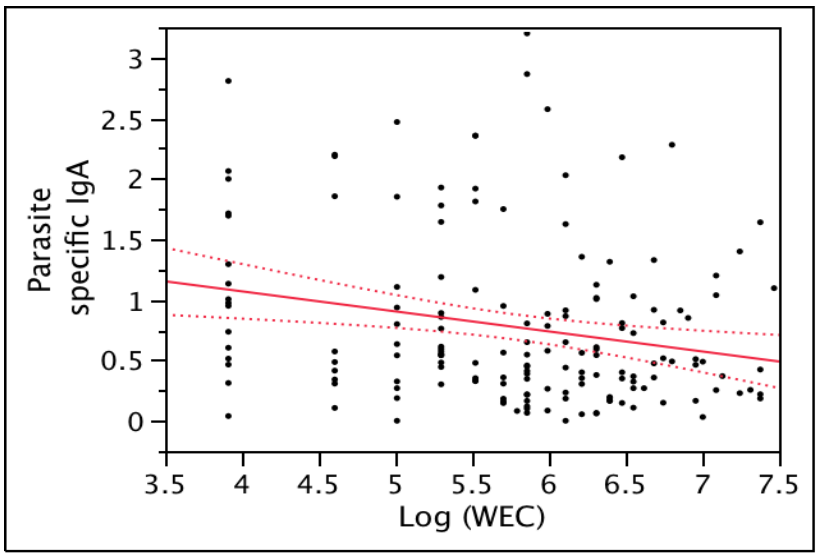
R^2 : 0.07 R^2 : 0.19

Figure 4.4: Scatter plots of Total IgA and Parasite-specific IgA vs log (WEC) for 9 sheep families and the control group. The red lines are linear least squares trend lines and the dotted red lines are the 95% confidence limits.

Total population



R^2 : 0.008



R^2 : 0.05

Figure 4.5: Scatter plots of Total IgA and Parasite-specific IgA vs log WEC for the whole population (all 9 sheep families and the control group). The red lines are linear least squares trend lines and the dotted red lines are the 95% confidence limits.

The correlation coefficients between total IgA and parasite-specific IgA with log WEC are summarised in Tables 4.1 and 4.2. Two correlation studies were performed, pairwise correlation and a non-parametric Spearman's correlation test. A negative correlation was observed for all families except Family 2 in both the pairwise correlation test and for the non-parametric Spearman's test. However, a positive correlation was observed for parasite-specific IgA in Family 3.

Table 4.1: Pairwise correlation of Total IgA and Parasite-specific IgA with log WEC for each family group

Family	Total IgA	Parasite-specific IgA
1	-0.115	-0.076
2	0.189	0.043
3	-0.253	-0.051
4	-0.323	-0.611*
5	-0.336	-0.095
7	-0.186	-0.211
8	-0.161	-0.324
9	-0.029	-0.111
10	-0.613*	-0.541
Control	-0.255	-0.434*
Whole population	-0.094	-0.224*

* p-value < 0.05

Table 4.2: Non-parametric Spearman's correlation test for Total IgA and Parasite-specific IgA with WEC for each family group

Family	Total IgA	Parasite-specific IgA
1	-0.072	-0.065
2	0.055	0.124
3	-0.167	0.374
4	-0.184	-0.305
5	-0.406	-0.242
7	-0.055	-0.046
8	-0.230	-0.371
9	-0.048	-0.059
10	-0.581	-0.618*
Control	-0.188	-0.273
Whole population	-0.094	-0.168*

* p-value < 0.05

4.3.3 General Linear modelling

Worm egg count was significantly influenced by the sheep Family groups and parasite-specific IgA ($F_{10,151}$= 4.9, p<0.001). Family groups had variable WECs and were ordered lowest to highest; Family 5, 2, 4, 10, 8, 7, Control, 9, 3 and 1 (p<0.0001). However, variability in WEC attributed to the influence of parasite-specific IgA is less important than the family (p=0.0038; Figure 4.6). However, this Family group - parasite-specific IgA relationship is not a strong association (r^2 = 0.25). Parasite-specific IgA and Family groups do not influence each other (no interaction effect), and therefore it was concluded that the two variables, parasite-specific IgA and family groups, were independent of each other.

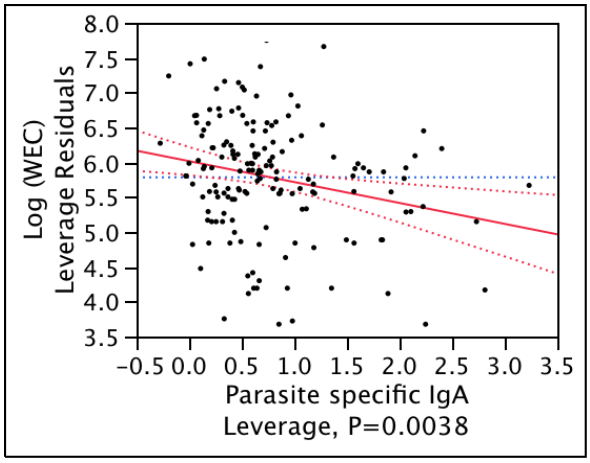


Figure 4.6: Parasite-specific IgA vs Log WEC Leverage plot

4.4 Discussion

It was observed that the distribution of WECs in the sheep used in this study were positively skewed and clearly not normally distributed (Figure 4.1). A natural logarithmic transformation (base e) resulted in a much less skewed distribution and these transformed values were then used in all analyses. The results described in this chapter have shown in the control group and all but one of the family groups that total and parasite-specific serum IgA levels in sheep, not presently under challenge from parasites, correlate negatively with WECs. This result is consistent with

the observations of Stear *et al*. (1995) that IgA affects gastrointestinal worm growth and that this is one factor (of possibly many factors) that determine the susceptibility of sheep to gastrointestinal worm infections. These observations were not only in Scottish Blackface sheep, but were also observed in other breeds, such as Churra, Suffolk and Santa Ines, as well in both natural and experimental infections (Amarante *et al*., 2005; Strain *et al*., 2002). Therefore, the negative correlations between IgA (total and parasite-specific) and WEC are biologically significant. Unfortunately, the strength of the correlation coefficients observed did not permit a reliable prediction of WEC for individual sheep and hence cannot be used in place of WEC as a predictor of susceptibility (or resistance) to gastrointestinal parasites. This is because only several families showed a statistically significant correlation between IgA (total and parasite-specific) and WEC (Table 4.1 and 4.2). Thus the aims proposed at the start of this Chapter has not been substantiated.

One of the reasons for the lack of significant correlations between IgA levels and WEC could be due to the latter measure not being a good indicator of parasite resistance. Most of the current studies on IgA and parasite resistance have been based on worm length measurements, but these were not available in this project. Another possibility may be that IgA is only one of several factors contributing to worm resistance and that its influence therefore is masked. Stear *et al.* (1995), Amarante *et al.* (2005) and Martinez-Valladares *et al.* (2005) found that only 38% of variation in worm length could be attributed to parasite-specific IgA (Stear *et al*., 1995; Amarante *et al*., 2005; Martinez-Valladares *et al*., 2005). In addition, despite the many studies supporting a role for IgA in inhibiting worm growth, there are some studies with contradicting results. For example, Halliday *et al*. (2007) have shown that the peak of IgA activity post challenge did not synchronize with parasite stunting. Hertzberg *et al*. (1999) have also questioned the involvement of IgA in protection against incoming larvae. In contrast in a more recent study, Henderson and Stear (2006) observed a peak in IgA activity at a similar time post-infection (8-10 days) and a significant negative correlation between parasite-specific IgA and worm length. Interestingly, in this

later study Henderson and Stear (2006) also observed an eosinophilia in parallel with IgA activity, suggesting a potential allergic response against the parasites.

Analysis of WEC within family groups has been shown to be highly variable (Figures 4.1 and 4.2). The high level of variability of WEC between animals within each family group will reflect also the genetic contribution from the dam since all offspring in each family group are bred from a single designated sire. Therefore, differences in parasite resistance in the offspring will also be contributed partially by the dam. In addition, high level of variability in WEC between animals within a family group may mask the effects of inter-group variability. This therefore results in the observed biologically significant trends but not to a sufficient extent that a statistically significant correlation between the IgA and WEC is seen.

Based on the linear model, WEC in sheep is influenced by parasite-specific IgA and the Family groups. However, total IgA was not predictive of WEC. This is consistent with the observation that the average ODI for all families for parasite specific IgA was higher relative to the control unselected population. This was not observed for the total IgA measurements. The relationship with family indicates that susceptibility to infection, as measured by WEC, varies with genetic relatedness as would be expected from previous work (see Chapter 1). In addition, WEC has been used as an indicator of parasite resistance in sheep breeding programmes where animals with low WEC were bred together to produce offspring with low WEC. The heritability of WEC has been determined to be 0.2-0.4 (Stear *et al*., 1997). The relationship with parasite specific IgA, but not with total IgA, in serum from resting (not under parasitic stress) sheep is consistent with a role for this immunoglobulin in adaptive immunity against gastrointestinal parasites. Of interest, an informal analysis by Dr Johan Greeff of the IgA data generated from this study using BLUP analysis showed IgA levels were not useful predictors of WEC. The BLUP analysis determines whether the inclusion of total IgA or parasite-specific IgA allows for a better prediction of animals with lower

susceptibility or higher resistance to parasite infection. This result contrasts with a recent study where it was proposed that measurement of parasite-specific IgA in animals under artificial challenge would enhance selection of breeding animals (Beraldi *et al*., 2008).

Currently, all studies that have reported a significant negative correlation between parasite-specific IgA and worm length have used mucosal or serum IgA samples post-challenge. It is possible that IgA levels measured close to weaning time after parasite challenge may be better predictors of parasite resistance however this procedure is more laborious and does not offer any practical or economic advantage over measuring WEC as a predictor of susceptibility or resistance to infection.

The absence of a suitable mass standard for the ELISA method to allow for quantitative measurements of IgA levels necessitated using an optical density index that provides only a relative measure of IgA levels. Thus the total IgA levels measured in this study were not directly comparable with the parasite specific IgA levels, which precluded estimates of the proportion of parasite specific IgA in sheep serum which may be a more meaningful parameter. Future studies of IgA levels in sheep will require mass standardization of IgA concentrations in order for work by different laboratories to be directly comparable.

The work reported in this Chapter supports previous studies implicating IgA in parasite immunity. Further, it is known that in humans a locus (or loci) within the MHC is associated with humoral IgA deficiency (Vorechovsky *et al*., 2000; Martinez *et al*., 2003). Thus the possibility of an MHC linked locus affecting IgA synthesis in sheep that contributes to susceptibility to gastrointestinal parasites remains an hypothesis worthy of further investigation.

Chapter 5
Genotyping of polymorphic loci within the ovine MHC class IIa, IIb and central region

This Chapter describes the genotyping of sheep from the Rylington Merino Project, sub-divided into two sub-populations; a cohort of relatively unrelated sires and dams (Cohort 1) and a cohort of offspring (Cohort 2) some of which shared a common sire. Allele frequencies for the unrelated animals in these two cohorts were determined together with heterozygosities for each SNP locus. All SNP loci manifested Hardy Weinberg equilibrium for Cohort 1. Pairwise linkage disequilibria were estimated across all 30 loci and revealed regions of high and low linkage disequilibrium. The class IIa subregion contained a segment exhibiting low heterozygosity and low LD.

5.1 Introduction

One of the early discovered characteristics of the human MHC region is the presence of extensive linkage disequilibrium (LD) across the region, as evidenced by the inheritance of alleles at adjacent polymorphic loci without recombination between generations.

Recombination is the physical exchange of genetic material that occurs between homologous chromosomes where there is crossing over (chiasmata), breakage and recombination of chromosome segments or blocks (Collins, 2009). As a result of this block like substructure, there are many combinations of alleles within each haplotype that are rarely separated over long periods of evolutionary history (Jeffreys *et al*., 2003; Arnheim *et al*., 2007). Strong linkage between loci within MHCs may be functionally advantageous due to enhanced coordination of gene expression and facilitated sequence exchange among sequence-related gene duplicates. For example, the clustering of the closely linked TAP1, TAP2, LMP2 and LMP7 genes within the human class II region, whose gene products mediate the loading of peptide antigens onto class I molecules (Herberg *et al.*, 1998). The expression of TAP1 and LMP2 are

coordinately controlled by a shared bi-directional promoter (Wright *et al.*, 1995b).

As mentioned previously, recombination "hotspots" are considered to be regions within chromosomes in which recombination occurs more frequently (Arnheim *et al*., 2007) and which delineate relatively conserved regions of chromosomes often referred to a "blocks". The nature and distribution of hotspots is still poorly understood and is of much contemporary interest. An interesting instance of hot spot characterization was published by Jeffreys *et al*., (2001) who described a 216kbp region in the class II region of the human MHC, previously known from familial crossovers. This DNA segment showed extended regions of strong LD interrupted by a relatively short subregion with low LD that corresponded precisely with regions of meiotic crossover. This study was extended by McVean *et al.* (2004), who described similar results for a human population. Jeffreys *et al.* (2003) described the presence of sites of recombination initiation within human recombination hotspots and suggested that such sites may also be involved in initiating gene conversion.

A recent study found that the patterns of recombination are poorly conserved between human and chimpanzee despite the similarity of the two genomes (Wall *et al.*, 2003; Ptak *et al.*, 2004). As a result, there have been much speculation about the nature of these regions. For example, Coop and Myers (2007) have suggested that recombination hotspots are transient features of the genome, which may be disrupted by mechanisms involving biased gene conversion. Hey (2004) has suggested that individual hotspots arise as an adjunct of linkage disequilibrium between two loci under selection pressure Kauppi *et al*., (2003) used SNP genotyping to show the presence of corresponding hotspots in three ethnic populations across a large segment of the human class II region. Recombination hotspots in the ovine MHC region have not been clearly identified to date with the exception of an apparent hotspot in the class I region (Bot *et al.*, 2004), and possibly in the class III region (Qin, 2009).

5.2 Material and Methods

5.2.1 Animals genotyped

DNA samples used in this study were isolated from sheep comprising Cohorts 1 and 2 (Chapter 2.1). Cohort 1 consisted of 68 unrelated sires and dams from a flock of 261 sheep. Fifteen of the sires and dams from Cohort 1 comprised a parental animals three generation family. Cohort 2 consisted of the remaining 193 offspring from a collection of sheep families some of which shared sires (see Table 5.1).

Table 5.1: Average WEC of the test populations of 261 sheep used in determining the allele frequencies for each loci. In this Table all sheep (cohort 1 and cohort 2) are grouped according to family groups, the control group and the three generation half sibling sheep family.

Sheep Family	Number of animals	Average Weaning Egg Count (WEC)
Family 1	29	~871
Family 2	22	~206
Family 3	20	~369
Family 4	23	~340
Family 5	14	~218
Family 7	17	~587
Family 8	28	~450
Family 9	29	~502
Family 10	19	~359
Control Family	45	~616
Three Generation Family	15	N/A

5.2.2 Single Nucleotide Polymorphism (SNP) genotyping

All genotyping was performed by KBioscience (*http://www.kbioscience.co.uk*). SNPs were genotyped using the KASPar chemistry, which is a competitive allele specific PCR SNP genotyping system using FRET (VIC/FAM) based homogenous format (*http://www.kbioscience.co.uk/genotyping/genotyping-chemistry.html*). Blind duplicates and plate-identifying blank wells were used as quality control tests. The associated loci names and SNPs genotyped are summarized Table 5.2.

Table 5.2: Summary of the SNP loci genotyped.

Locus	Gene/Region	Sequence
MHC class IIb		
DB-TP2 (s7)	DOB-TAP2	GTGTGTAAGGTGRGA[C/T]TGTAATGGTACTAGA
PB8-TP2 (s2)	PSMB8-TAP2	KCGGTCCTGGAGGAA[C/T]GAGAGTGGGAGCCCA
PB9 (s3)	PSMB9	TCAACGATACTTAAA[G/T]TTTTTTTAATTGGCA
HB-DMB (s4)	H2B-DMB	GTTCCAGCCAAGAGA[A/G]AATCAGAAGCCWTCT
DMA-BD2 (s1)	DMA-BRD2	TGCCATACTATCACT[A/G]CAAGTAACAAAATTA
BD2-DA (s7)	BRD2-DOA	CCTGTATCCAGTGTA[A/T]TTTCTGTAAACTAGA
RG1 (s2)	RING1	GCTCCATCCGCAGAC[C/T]CCCTTCTCACAGCCG
PN6 (s4)	PFDN6	GTATTCTGTCCCACC[C/T]CTGGCCCCAGCCTGC
RL2 (s1)	RGL2	CTGCGGACTAGCTGC[C/G]GGGGGCGGGGAGCTG
TBP (s1)	TAPBP	CCCGGACGTGGCGGG[G/T]CTCACCTCCCACCGT
MHC class IIa		
A2A (s3)	DQB2-DQA2	GGGACTGGCTTTGGA[C/G]TGGCTTTCTGTGCTG
A3A (s10)	DQA2-DQB1	AGTGAACCCATATGY[A/G]AAATACATACCAAAT
A3C (s7)	DQA2-DQB1	ATCRGATTGTGATTC[C/T]TGAAAAAGGGACATA
A5B (s1)	DQA1-DRB1	AACCAACTGCTATGT[A/G]TTTTGCTTGGATGGC
A6A (s4)	DRB1-DRA	AAAGGAGTTGGATAT[A/G]TTCCGAGAAAGGTGA
A6C (s5)	DRB1-DRA	TCTGCTCCCCACTCC[A/G]TTGCCATTTCCTCCC
A6B (s1)	DRB1-DRA	TGTTCCTCACCAGGA[A/G]TATTCTTAAAAAGTG
DRA (s1)	DRA	ACACACGTAGTTACT[A/G]TAGCGTTTAAGATGA
BL2 (s1)	BTNL2	TCTCCTCTTGGCCTC[C/T]GCTGCCTGAAGATGT
BL2-C10 (s8)	BTNL2-c6orf10	WTTCCCAACTCCCAG[A/G]AAAACTCTCCATCAC
MHC class III*		
TXB	TNXB	CCAGGGAAGAAATCG[A/G]AGCAGAGCCTGCATC
CFB	CFB	TCCATCCATTCTGAG[C/T]TTCTGCAGTGCCCTC
C2	C2	TGGGCCTAGCAAGCT[C/G]TGCCCTGGGCTCACT
MH5	MSH5	TCCTTTTCTGCTCCA[A/G]TTGGTCCATGTCCAC
G6B	G6B	AGACTAGGAGTTGAG[C/T]GACCTACATTCCAGG
BT5	BT5	GTGGGGCTTGGGGAC[A/G]GGGGTACCCTGTAAG
APM	APOM	CAGTGTTGGCAGGAC[A/G]GCCAGCTCATAGGGG
BT2	BT2	AATAAAAACAGCAAG[C/T]CCAAGCCCTAGAGCA
TNF	TNF	TTTGTGCCTCCTTTT[A/G]CTTATGTTTTTTAAA
LTA	LTA	CAGGATGTCCCAGGT[A/G]GGAGRGTATATACTG

** SNPs identified by Qin, (2009)*

5.2.3 Analysis of SNP genotypic data

The panel of 30 SNP markers, 10 known loci from each of the class IIa, IIb and III regions of the ovine MHC, were typed for both sheep Cohorts 1 and 2. Allele frequencies, Hardy Weinberg proportions and genetic differentiation were performed using the Genepop population genetics software application (*http://genepop.curtin.edu.au/*). Heterozygosities and linkage disequilibrium, were performed using the SNPstats software package (*http://bioinfo.iconologia.net/index.php?module=Snpstats*). SNPstats estimates haplotype frequencies using an implementation of the expectation maximization (EM) algorithm previously written for the *haplo.stats* application (Sinnwell and Schaid, 2005).

5.3 Results

5.3.1 Genotyping of individual loci and allele frequencies

The genotyping method is a robust method that has been widely used in many published studies. Genotyping of several blind duplicates across all loci, and negative controls were included as a quality control measure: no discrepancies were observed.

Allele frequencies for all SNPs were estimated and are summarized in Tables 5.3 and 5.4, as well as represented diagrammatically in Figure 5.1.

Table 5.3: Allele frequencies for 30 SNP loci derived from Cohort 1 genotypes from the Rylington Merino flock.

Locus	Allele 1 Frequency (Red Bars)	Allele 2 Frequency (Blue Bars)
DB-TP2	T (0.15)	C (0.85)
PB8-TP2	T (0.33)	C (0.67)
PB9	T (0.27)	G (0.73)
HB-DMB	G (0.68)	A (0.32)
DMA-BD2	G (0.17)	A (0.83)
BD2-DA	T (0.81)	A (0.19)
RG1	T (0.21)	C (0.79)
PN6	T (0.24)	C (0.76)
RL2	G (0.62)	C (0.38)
TBP	T (0.58)	G (0.42)
A2A	G (0.31)	C (0.69)
A3A	G (0.62)	A (0.38)
A3C	T (0.05)	C (0.95)
A5B	G (0.19)	A (0.81)
A6A	G (0.98)	A (0.02)
A6C	G (0.97)	A (0.03)
A6B	G (0.84)	A (0.16)
DRA	G (0.61)	A (0.39)
BL2	T (0.31)	C (0.69)
BL2-C10	G (0.51)	A (0.49)
TXB	G (0.51)	A (0.49)
CFB	T (0.67)	C (0.33)
C2	G (0.64)	C (0.36)
MH5	G (0.85)	A (0.15)
G6B	T (0.17)	C (0.83)
BT5	G (0.65)	A (0.35)
APM	G (0.86)	A (0.14)
BT2	T (0.81)	C (0.19)
TNF	G (0.58)	A (0.42)
LTA	G (0.81)	A (0.19)

Table 5.4: Allele frequencies for 30 SNP loci derived from Cohort 2 genotypes from the Rylington Merino flock (all family groups).

Locus	Allele 1 Frequency (Red Bars)	Allele 2 Frequency (Blue Bars)
DB-TP2	T (0.18)	C (0.82)
PB8-TP2	T (0.39)	C (0.61)
PB9	T (0.34)	G (0.66)
HB-DMB	G (0.69)	A (0.31)
DMA-BD2	G (0.15)	A (0.85)
BD2-DA	T (0.89)	A (0.11)
RG1	T (0.2)	C (0.8)
PN6	T (0.3)	C (0.7)
RL2	G (0.53)	C (0.47)
TBP	T (0.55)	G (0.45)
A2A	G (0.29)	C (0.71)
A3A	G (0.65)	A (0.35)
A3C	T (0.05)	C (0.95)
A5B	G (0.15)	A (0.85)
A6A	G (0.97)	A (0.03)
A6C	G (0.98)	A (0.02)
A6B	G (0.82)	A (0.18)
DRA	G (0.57)	A (0.43)
BL2	T (0.43)	C (0.57)
BL2-C10	G (0.55)	A (0.45)
TXB	G (0.47)	A (0.53)
CFB	T (0.66)	C (0.34)
C2	G (0.58)	C (0.42)
MH5	G (0.86)	A (0.14)
G6B	T (0.16)	C (0.84)
BT5	G (0.56)	A (0.44)
APM	G (0.88)	A (0.12)
BT2	T (0.84)	C (0.16)
TNF	G (0.48)	A (0.52)
LTA	G (0.88)	A (0.12)

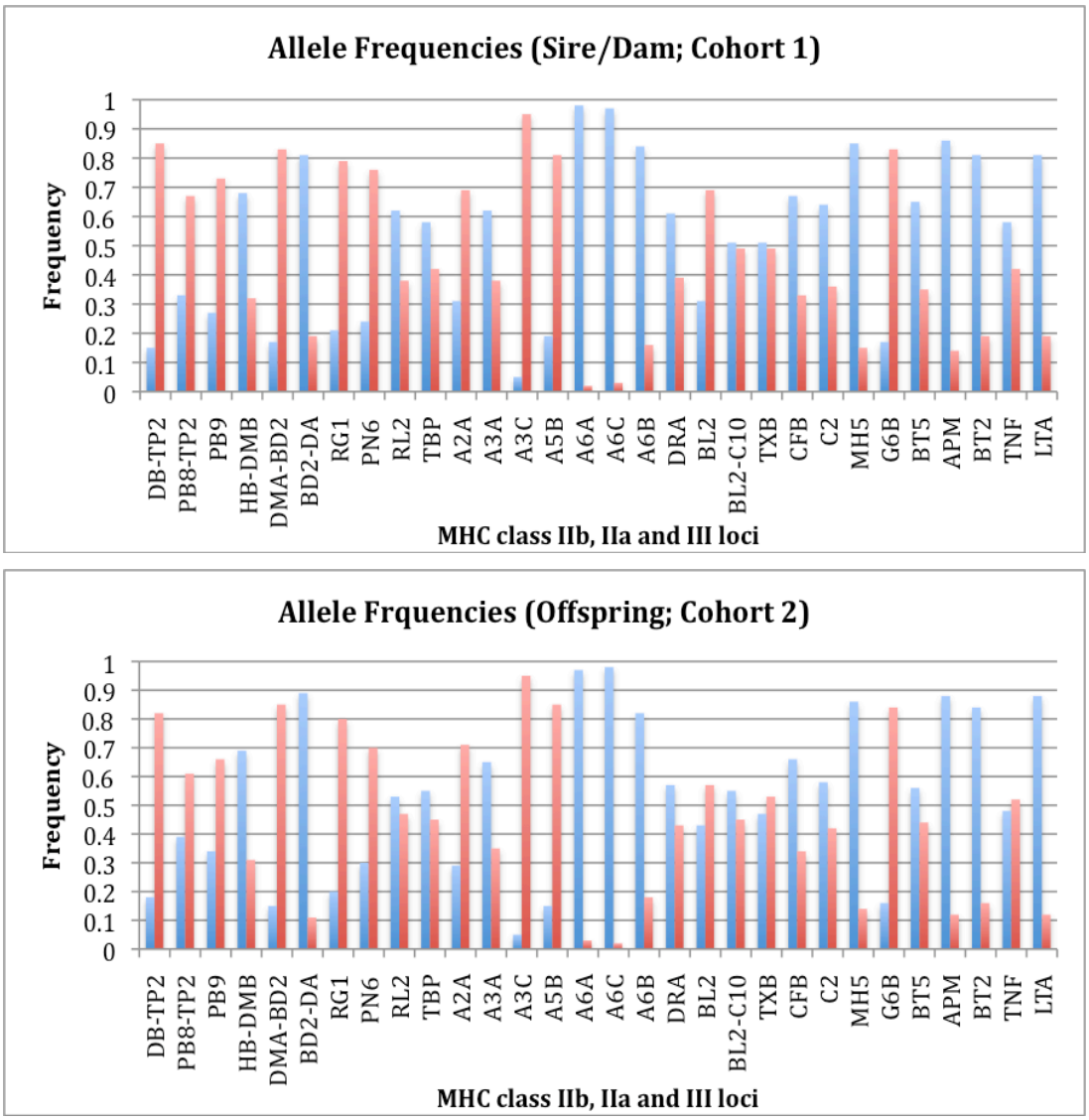


Figure 5.1: Allele frequency distribution of 30 SNPs across the ovine MHC class IIa, IIb and III region in two groups (Cohort 1 and Cohort 2). Red and blue bars represent both alleles for each locus as represented in Table 5.3 and 5.4.

5.3.2 Genetic characterisation of sheep populations

For all 30 SNPs tested the sire/dam sub-population manifested Hardy Weinberg proportions whereas 5 of the 30 loci in the offspring cohort showed departures from Hardy Weinberg proportions at the 5% probability level (Table 5.5). This result is consistent with a higher degree of kinship in Cohort 2 than in Cohort 1.

Table 5.5: Hardy Weinberg Equilibrium Exact Test (Genepop) for sheep comprising Cohorts 1 and 2. (NB: values in red font show SNPs which did not manifest Hardy Weinberg proportions – only present in Cohort 2).

	Cohort 1		Cohort 2	
Locus	**p-value**	**Standard Error**	**p-value**	**Standard Error**
DB-TP2	1.0000	0.0000	0.6152	0.0029
PB8-TP2	0.2799	0.0036	0.0667	0.0037
PB9	0.7616	0.0018	0.3273	0.0057
HB-DMB	0.7802	0.0018	0.0611	0.0028
DMA-BD2	1.0000	0.0000	0.2626	0.0037
BD2-DA	0.1074	0.0018	1.0000	0.0000
RG1	0.7208	0.0017	0.8205	0.0017
PN6	1.0000	0.0000	0.1120	0.0036
RL2	0.4446	0.0032	0.4696	0.0062
TBP	0.4486	0.0035	0.5517	0.0055
A2A	0.3863	0.0038	0.0000	0.0000
A3A	0.2043	0.0034	0.0160	0.0014
A3C	0.1497	0.0018	0.0000	0.0000
A5B	0.6771	0.0018	0.0000	0.0000
A6A	1.0000	0.0000	1.0000	0.0000
A6C	1.0000	0.0000	1.0000	0.0000
A6B	1.0000	0.0000	0.2089	0.0034
DRA	0.7982	0.0018	0.0365	0.0024
BL2	0.7709	0.0019	0.3763	0.0063
BL2-C10	0.8092	0.0017	0.5578	0.0024
TXB	1.0000	0.0000	0.4634	0.0064
CFB	1.0000	0.0000	0.7475	0.0033
C2	0.5916	0.0031	0.1440	0.0043
MH5	1.0000	0.0000	0.1354	0.0027
G6B	0.6748	0.0017	0.1084	0.0025
BT5	0.7893	0.0016	0.0556	0.0025
APM	0.3399	0.0023	0.4748	0.0025
BT2	1.0000	0.0000	0.2855	0.0035
TNF	0.6043	0.0034	0.2423	0.0057
LTA	0.1007	0.0018	0.7216	0.0019

(Genepop analysis: H_0=Random union of gametes)

5.3.3 Analysis of SNP heterozygosity in the ovine MHC region

Heterozygote frequencies for each locus varied across the class II and III region, with several loci just over 50% heterozygosity. Under Hardy Weinberg proportions, the maximum heterozygosity level should be 0.5 and the observed values of 0.51-0.55 are due to sampling errors. A region of very low heterozygosity was observed in the class IIa subregion encompassing the DQB1, DQA1 and DRB1 genes (loci A3C, A5B, A6A and A6C) (Figure 5.2). This region of low heterozygosity was observed in both cohorts. Heterozygosity levels for each locus across the three sub-regions for both cohorts of animals is displayed in Table 5.6.

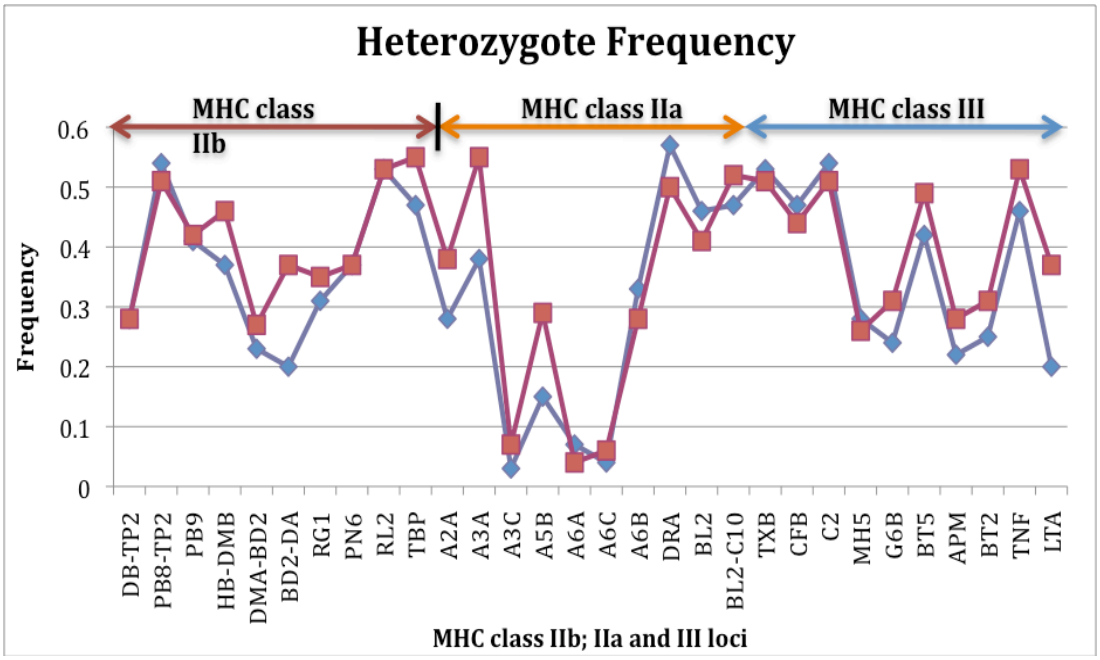


Figure 5.2: Comparison of heterozygosities of 30 SNPs across the ovine MHC class IIa, IIb and III region for two groups of animals [Cohort 1 red line and Cohort 2 blue line]. A black vertical line shows the location of the predicted 15 cM DNA localized chromosomal inversion that is present in the cattle MHC and separates the class IIb and IIa subregions.

Table 5.6: Summary of heterozygoties for each locus across the ovine MHC class IIb, IIa and III region.

			Cohort 1 (sire/dam)			Cohort 2 (offspring)		
MHC region	**Locus**	**Location**	**Number of homozygotes**	**Number of heterozygotes**	**Obs. Heterozygosity Ho**	**Number of homozygotes**	**Number of heterozygotes**	**Heterozygosity Ho**
Class IIb	DB-TP2	*DOB-TAP2*	47	18	0.28	137	53	0.28
Class IIb	PB8-TP2	*PSMB8-TAP2*	33	34	0.51	88	104	0.54
Class IIb	PB9	*PSMB9*	37	27	0.42	108	75	0.41
Class IIb	HB-DMB	*H2B-DMB*	35	30	0.46	118	68	0.37
Class IIb	DMA-BD2	*DMA-BRD2*	48	18	0.27	145	44	0.23
Class IIb	BD2-DA	*BRD2-DOA*	42	25	0.37	152	39	0.2
Class IIb	RG1	*RING1*	42	23	0.35	132	59	0.31
Class IIb	PN6	*PFDN6*	43	25	0.37	119	69	0.37
Class IIb	RL2	*RGL2*	32	36	0.53	91	102	0.53
Class IIb	TBP	*RPBP*	30	36	0.55	96	86	0.47
Class IIa	A2A	*DQB2-DQA2*	41	25	0.38	132	51	0.28
Class IIa	A3A	*DQA2-DQB1*	30	37	0.55	118	71	0.38
Class IIa	A3C	*DQA2-DQB1*	63	5	0.07	180	6	0.03
Class IIa	A5B	*DQA1-DRB1*	44	18	0.29	163	28	0.15
Class IIa	A6A	*DRB1-DRA*	64	3	0.04	179	13	0.07
Class IIa	A6C	*DRB1-DRA*	64	4	0.06	181	8	0.04
Class IIa	A6B	*DRB1-DRA*	46	18	0.28	124	60	0.33
Class IIa	DRA	*DRA*	32	32	0.5	82	108	0.57
Class IIa	BL2	*BTNL2*	38	26	0.41	105	88	0.46
Class IIa	BL2-C10	*BTNL2-c6orf10*	32	35	0.52	101	91	0.47
Class III	TXB	*TNXB*	33	34	0.51	90	101	0.53
Class III	CFB	*CFB*	37	29	0.44	100	87	0.47
Class III	C2	*C2*	33	34	0.51	88	105	0.54
Class III	MH5	*MSH5*	50	18	0.26	139	53	0.28
Class III	G6B	*G6B*	47	21	0.31	147	46	0.24
Class III	BT5	*BT5*	33	32	0.49	109	80	0.42
Class III	APM	*APOM*	49	19	0.28	150	43	0.22
Class III	BT2	*BT2*	46	21	0.31	141	47	0.25
Class III	TNF	*TNF*	31	35	0.53	103	86	0.46
Class III	LTA	*LTA*	42	25	0.37	150	38	0.2

Heterozygosities for each locus in the ovine MHC class II and III region were also compared for the offspring of each family group. Each family group has an average WEC measurement; Family 1 has the highest average WEC and Family 2 has the lowest average WEC (Figure 5.3 and Appendix 5A.1). Heterozygosities for Families 1 and 2 are shown in Figure 5.3. Obvious differences in heterozygosities were observed in different segments of the ovine MHC class II and III regions, especially in the segments spanning the PB9 to BD2-DA, TBP to A6B and BT5 to TNF loci. Heterozygosities for each locus for Family 1 and 2 were also compared with the average for Cohort 2 (Figures 5.4 and 5.5).

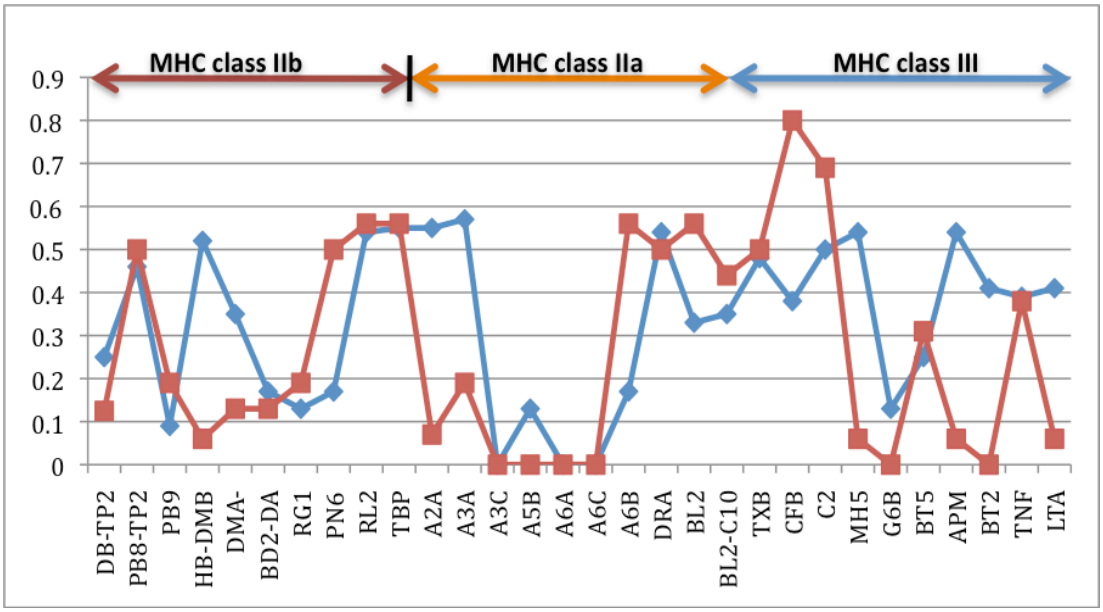


Figure 5.3: Heterozygosities of each locus across the ovine MHC class IIa, IIb and III regions in two family groups [Family 1 (blue line) and Family 2 (red line)]. A black vertical line shows the location of the predicted 15 cM DNA localized chromosomal inversion that is present in the cattle MHC and separates the class IIb and IIa subregions.

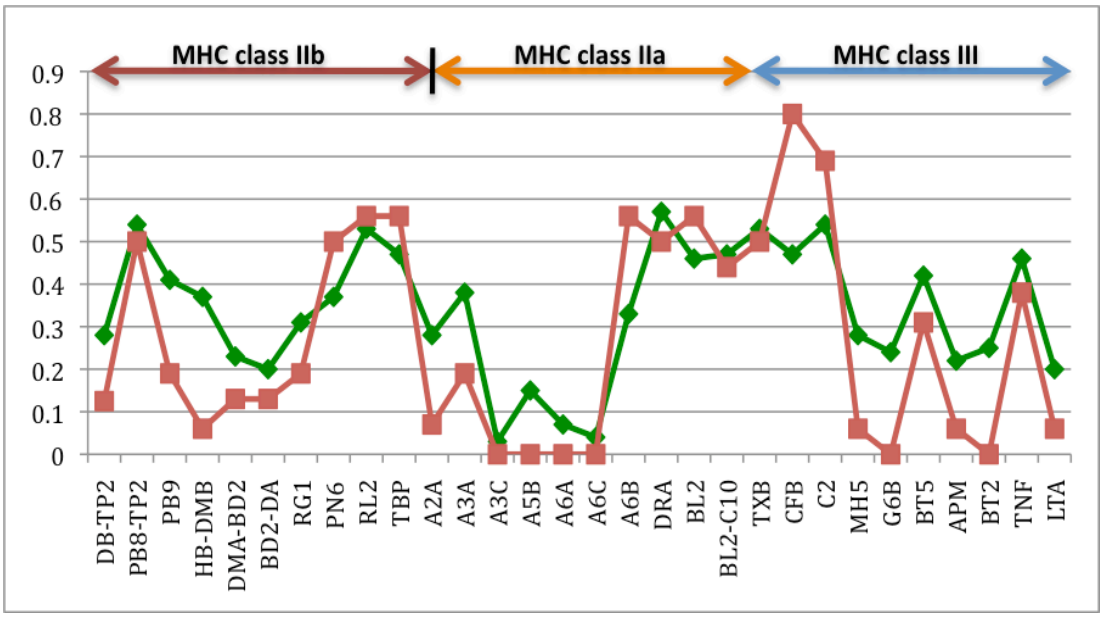


Figure 5.4: Heterozygosities of each locus across the ovine MHC class IIa, IIb and III regions. Family 2 (red line) and the average for all offspring (Cohort 2; green line). A black vertical line shows the location of the predicted 15 cM DNA localized chromosomal inversion that is present in the cattle MHC and separates the class IIb and IIa subregions.

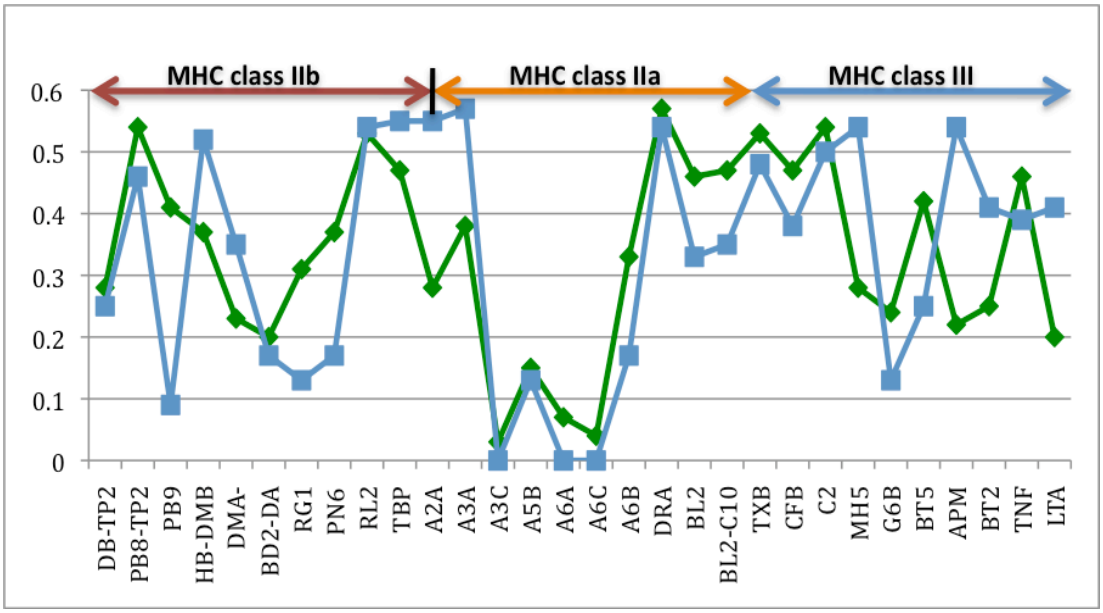


Figure 5.5: Heterozygosities of each locus across the ovine MHC class IIa, IIb and III regions Family 1 (blue line) and the average for all offspring (Cohort 2; green line). A black vertical line shows the location of the predicted 15 cM DNA localized chromosomal inversion that is present in the cattle MHC and separates the class IIb and IIa subregions.

5.3.4 Analysis of regions of linkage disequilibrium in the ovine MHC

The results of the analysis are shown in Tables 5.7 and 5.8 and Figure 5.6. Several chromosomal regions across the class II and III regions manifested relatively high LD and may be indicative of a conserved region resulting from reduced recombination (Figure 5.7). Two disruptions of LD were observed between the loci RG1/BD2-DA and BT2/APM. A large region was also observed in the MHC class IIa spanned by four consecutive SNP markers (A5B, A6A, A6C and A6B) manifesting a relatively low LD.

Table 5.7: D' values of LD analysis for each locus across the ovine MHC class IIb, IIa and III region for Cohort 1.

	DB.TP2	PB8.TP2	PB9	HB.DMB	DMA.BD2	BD2.DA	RG1	PN6	RL2	TBP	A2A	A3A	A3C	A5B	A6A	A6C	A6B	DRA	BL2	BL2.C10	TXB	CFB	C2	MH5	G6B	BT5	APM	BT2	TNF	LTA
DB.TP2	.	0.9986	0.0827	0.6809	0.9982	0.0216	0.9978	0.1083	0.0921	0.4527	0.2233	0.2579	0.9927	0.7804	0.5218	0.9888	0.3991	0.618	0.031	0.495	0.1094	0.3481	0.3623	0.9973	0.1216	0.4028	0.9974	0.0554	0.2727	0.428
PB8.TP2	.	.	0.2466	0.8771	0.1618	0.3485	0.522	0.3526	0.1453	0.461	0.0815	0.2914	0.0843	0.1019	0.0458	0.9923	0.3108	0.0225	0.043	0.0482	0.147	0.3524	0.4606	0.2069	0.2957	0.548	0.2698	0.2655	0.4145	0.6131
PB9	.	.	.	0.6247	0.9988	0.0725	0.0532	0.1588	0.2711	0.233	0.1845	0.1733	0.2739	0.0559	0.2594	0.9937	0.4362	0.7202	0.149	0.0373	0.1188	0.4936	0.3708	0.0116	0.2748	0.0814	0.0555	0.2751	0.192	0.9987
HB.DMB	.	.	.	.	0.2636	0.9991	0.9992	0.8137	0.2234	0.2695	0.3657	0.4191	0.5571	0.0236	0.0775	0.3137	0.7835	0.0902	0.0963	0.0339	0.1147	0.1338	0.0162	0.2999	0.3051	0.3117	0.4851	0.2236	0.1968	0.377
DMA.BD2	.	.	.	.	.	0.2626	0.1623	0.6055	0.5448	0.9992	0.0809	0.1033	0.075	0.3679	0.5239	0.3462	0.0262	0.0701	0.0846	0.2592	7E-04	0.1052	0.0082	0.0853	0.0198	0.1551	1E-04	0.0123	0.0635	0.085
BD2.DA	.	.	.	.	.	.	0.0331	0.4799	0.71	0.099	0.7137	0.0516	0.6774	0.1085	0.4687	0.2106	0.1248	0.2881	0.1666	0.0324	0.1824	0.6421	0.7061	0.274	0.505	0.5577	0.964	0.1582	0.5221	0.0523
RG1	.	.	.	.	.	.	.	0.999	0.4086	0.5553	0.2131	0.5305	0.3516	0.1547	0.985	0.587	0.215	0.0222	0.1644	0.0355	0.2607	0.3447	0.4384	0.4172	0.8303	0.3212	0.4546	0.8818	0.2062	0.7367
PN6	.	.	.	.	.	.	.	.	0.9995	0.9995	0.2389	0.316	0.9959	0.226	0.9959	0.9929	0.267	0.0344	0.1188	0.0566	0.0264	0.151	0.1514	0.2678	0.041	0.1551	0.4565	0.0328	0.0145	0.2645
RL2	.	.	.	.	.	.	.	.	.	0.5586	0.0219	0.0197	0.997	0.0313	0.995	0.9955	0.0298	0.064	0.2018	0.2478	0.2261	0.2169	0.1921	0.3524	0.0259	0.1146	0.4658	0.115	0.0941	0.2337
TBP	.	.	.	.	.	.	.	.	.	.	0.0924	0.1007	0.4851	0.1099	0.9946	0.1591	0.3235	0.0441	0.0873	0.0181	0.1917	0.0895	0.1525	0.2133	0.2452	0.2109	0.3589	0.0219	0.2158	0.2165
A2A	.	.	.	.	.	.	.	.	.	.	.	0.8223	0.1255	0.9993	0.99	0.8002	0.7442	0.0848	0.4713	0.1968	0.1002	0.1869	0.1053	0.1788	0.4987	0.773	0.2826	0.5418	0.5793	0.5191
A3A	.	.	.	.	.	.	.	.	.	.	.	.	0.997	0.9994	0.9918	0.9955	0.9988	0.1455	0.4233	0.1459	0.027	0.1518	0.107	0.3481	0.7005	0.7959	0.4617	0.9195	0.5497	0.6475
A3C	.	.	.	.	.	.	.	.	.	.	.	.	.	0.9942	0.9397	0.9982	0.9928	0.9971	0.4019	0.5883	0.0169	0.9965	0.3114	0.637	0.428	0.4834	0.6322	0.994	0.4687	0.6774
A5B	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.984	0.9911	0.0919	0.1442	0.4498	0.5515	0.411	0.6431	0.6566	0.1685	0.1168	0.2091	0.2038	0.9988	0.0524	0.9981
A6A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.8944	0.024	0.992	0.9955	0.9936	0.9937	0.9954	0.9952	0.9789	0.0079	0.9912	0.9778	0.9834	0.9925	0.9834
A6C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.989	0.2702	0.9975	0.9965	0.9965	0.9947	0.9943	0.998	0.9898	0.9933	0.998	0.9086	0.9959	0.9086
A6B	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.9988	0.7256	0.6003	0.9991	0.9986	0.9986	0.2625	0.9974	0.5553	0.3036	0.9976	0.4402	0.9976
DRA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.8104	0.4845	0.0799	0.2051	0.2309	0.5961	0.455	0.0353	0.9989	0.4935	0.0036	0.3526
BL2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.9997	0.4769	0.1314	0.1782	0.2914	0.8126	0.252	0.2965	0.9992	0.0381	0.9992
BL2.C10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.192	0.1674	0.1008	0.8133	0.0549	0.0804	0.7895	0.0621	0.3556	0.1953
TXB	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.2197	0.2528	0.7523	0.3859	0.0381	0.7051	0.0327	0.1169	0.4563
CFB	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.9998	0.397	0.9986	0.2121	0.9988	0.0227	0.0937	0.118
C2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.5183	0.9987	0.2244	0.999	0.1138	0.158	0.0078
MH5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.9672	0.9987	0.9995	0.1248	0.9989	0.1248
G6B	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.9987	0.7871	0.4084	0.4915	0.2459
BT5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.9988	0.9993	0.9456	0.6847
APM	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.5741	0.9989	0.5741
BT2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.9994	0.7399
TNF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.9994
LTA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table 5.8: P-values for LD analysis for each locus across the ovine MHC class IIb, IIa and III region for Cohort 1.

	DB.TP2	PB8.TP2	PB9	HB.DMB	DMA.BD2	BD2.DA	RG1	PN6	RL2	TBP	A2A	A3A	A3C	A5B	A6A	A6C	A6B	DRA	BL2	BL2.C10	TXB	CFB	C2	MH5	G6B	BT5	APM	BT2	TNF	LTA
DB.TP2	.	8E-04	0.811	0	0.0326	0.8279	0.0153	0.3521	0.5692	0.0116	0.1113	0.1125	0.2609	0.0766	0.0362	0.4027	0.4185	0.0198	0.9211	0.0205	0.5893	0.2469	0.1917	0.0441	0.1901	0.1537	0.0507	0.577	0.2699	0
PB8.TP2	.	.	0.0152	0	0.564	0.18	0	0.1062	0.3549	0.0016	0.6629	0.0665	0.7452	0.6999	0.9094	0.1621	0.0318	0.8254	0.8201	0.7069	0.2447	0.0508	0.0057	0.155	0.2803	0.0013	0.0719	0.307	0.0052	0.0183
PB9	.	.	.	0.0035	0.0023	0.811	0.8536	0.0975	0.0168	0.0637	0.3975	0.3497	0.6579	0.6304	0.4726	0.2299	8E-04	1E-04	0.1374	0.7911	0.4204	0.0191	0.0564	0.9294	0.0222	0.4562	0.68	0.3643	0.1207	0.001
HB.DMB	.	.	.	.	0.0554	2E-04	1E-04	3E-04	0.1662	0.0729	1E-04	0	0.3067	0.9309	0.8477	0.3675	0.0095	0.39	0.6203	0.7973	0.3629	0.1355	0.9246	0.3266	0.0231	0.076	0.1236	0.0795	0.1971	0.0031
DMA.BD2	.	.	.	.	.	0.0052	0.1116	0.0782	0.0276	0	0.7835	0.6797	0.6536	0.3773	0.0432	0.1215	0.7785	0.7799	0.5322	0.1996	0.9971	0.7113	0.9555	0.3626	0.822	0.5646	0.9993	0.976	0.7861	0.8355
BD2.DA	.	.	.	.	.	.	0.9268	0.1321	0.002	0.5257	0.0089	0.824	0.3817	0.2414	0.0888	0.3756	0.7727	0.053	0.1841	0.8625	0.3228	0.0148	0.0037	0.5281	0.2065	0.0253	0.0313	0.0691	0.016	0.5483
RG1	.	.	.	.	.	.	.	0.001	0.0606	0.0062	0.411	0.016	0.0682	0.102	0.3879	0.0229	0.046	0.9188	0.5274	0.8405	0.1368	0.1686	0.0559	1E-04	0.0288	0.1753	0	0.0144	0.3128	0.0394
PN6	.	.	.	.	.	.	.	.	0	0	0.297	0.1046	0.1257	0.4853	0.0021	0.2539	0.4619	0.7831	0.608	0.7023	0.8648	0.1578	0.1842	0.463	0.703	0.1763	0.2246	0.748	0.9115	0.0096
RL2	.	.	.	.	.	.	.	.	.	0	0.8297	0.8204	0.0331	0.8933	0.0267	0.1118	0.8538	0.4766	0.2259	0.0201	0.0425	0.0277	0.0347	0.1794	0.8625	0.2191	0.085	0.4176	0.3144	0.0996
TBP	.	.	.	.	.	.	.	.	.	.	0.5471	0.4398	0.2651	0.6099	0.0456	0.7847	0.1834	0.734	0.5735	0.8552	0.0568	0.4123	0.1303	0.3824	0.1387	0.0412	0.1537	0.9182	0.0162	0.1684
A2A	.	.	.	.	.	.	.	.	.	.	.	0	0.8216	3E-04	0.2516	0.2827	0.0167	0.425	0.0175	0.1207	0.4365	0.3239	0.5471	0.5669	1E-04	0	0.3798	0	2E-04	0
A3A	.	.	.	.	.	.	.	.	.	.	.	.	0.0351	0	0.1765	0.1159	2E-04	0.1097	0.012	0.1757	0.805	0.1248	0.2415	0.1896	0	0	0.0914	0	0	0
A3C	.	.	.	.	.	.	.	.	.	.	.	.	.	0.2065	0.7014	0	0.2602	0.0354	0.1162	0.1237	0.9631	0.0638	0.5305	0	0.5999	0.3421	0	0.1993	0.2891	0.3817
A5B	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.4204	0.3466	0.3847	0.3339	0.1066	0.0021	0.024	0.0164	0.0079	0.1118	0.2311	0.4103	0.0619	0.0096	0.7392	0.0097
A6A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.785	0.9244	0.1773	0.0121	0.0936	0.0887	0.0135	0.0206	0.4765	0.9755	0.2092	0.49	0.4129	0.1478	0.4129
A6C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.4019	0.67	0.0036	0.0513	0.0479	0.1667	0.1344	0	0.3646	0.1446	0	0.3806	0.0923	0.3806
A6B	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2E-04	0	0.0049	0	9E-04	3E-04	0.0042	0.0285	4E-04	0.0013	0.021	0.0126	0.021
DRA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	0	0.4663	0.0474	0.0148	0.025	0.063	0.7189	3E-04	9E-04	0.9783	0.017
BL2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	3E-04	0.1558	0.0711	0.0423	0.0052	0.0112	0.045	3E-04	0.7352	3E-04
BL2.C10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.0298	0.1979	0.3976	1E-04	0.7675	0.4878	4E-04	0.74	4E-04	0.2934
TXB	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.0863	0.0325	4E-04	0.0408	0.7536	0.0012	0.8553	0.2529	0.0108
CFB	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	0.188	3E-04	0.0254	0.0013	0.9312	0.3832	0.3538
C2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.0627	1E-04	0.0119	5E-04	0.6399	0.1086	0.9541
MH5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.0346	5E-04	0	0.7739	1E-04	0.7739
G6B	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1E-04	0.0952	0	0.0313	0.0073
BT5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	7E-04	1E-04	0	0.006
APM	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.1996	1E-04	0.1996
BT2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	0
TNF	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0
LTA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

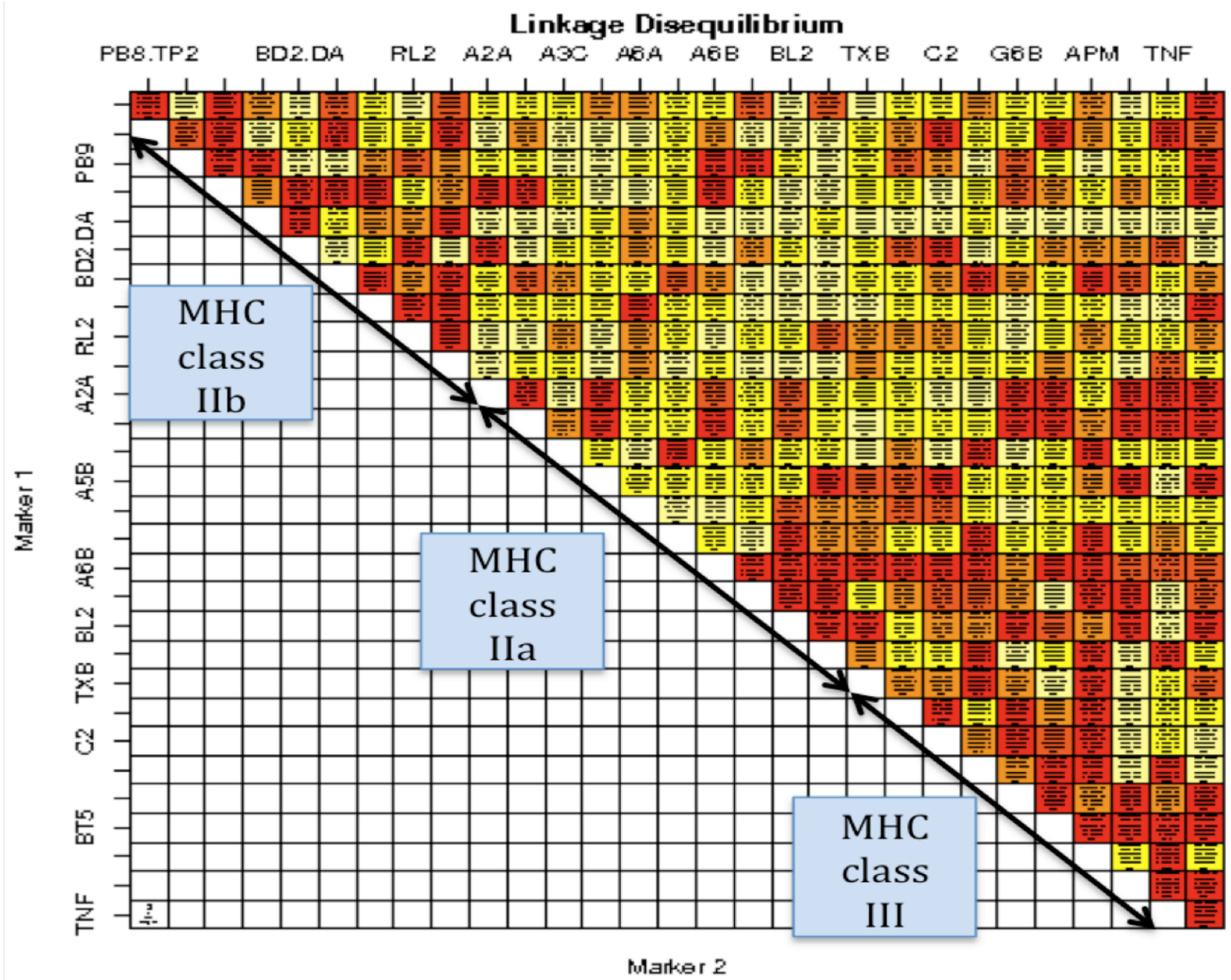


Figure 5.6: LD heat map of each locus in the ovine MHC class IIb, IIa and III regions for Cohort 1 (Boxes highlighted in red are those in high LD and yellow boxes indicate low LD).

5.4 Discussion

This chapter describes the genotyping of individual sheep from two cohorts using the panel of 30 SNP markers described in Chapter 3. Three significant results were obtained as shown below.

1. A SNP LD map spanning the three subregions showed regions of high and low LD within this chromosomal region.

2. SNP heterozygosity varied across the chromosomal regions studied. A region of low heterozygosity was observed in sheep that corresponded with an orthologous region in the human MHC characterised by a dearth of SNPs implying a segment of restricted genetic variation (Traherne *et al.*, 2006).

3. Low heterozygosity in the ovine MHC IIa subregion corresponded with a segment of low LD, one end corresponding to an analogous known recombination hotspot in the human MHC (Jeffreys *et al.,* 2001).

Linkage disequilibrium in the ovine MHC

The LD map covered the ovine MHC class IIb, IIa and III regions for Cohort 1, This analysis is only reported for Cohort 1 (unrelated animals) since animals in Cohort 2 were more likely to be related. Despite the latter concern, the LD maps for both cohorts showed similar regions of high LD between the loci (data not included). A chromosomal segment spanning part of the class IIa and class III regions exhibited relatively high LD. Successive pairwise LD analysis is known to result in the accumulation of type I errors, and therefore the identification of regions of high or low LD would require further investigation using a different data set. The block region of high LD identified by Qin (2009) has been confirmed in this independent study. The test sample used by Qin (2009) is a separate flock from the test animals used in this study, and contains overlaps of animals from one of many family groups. The 10 SNP located in the MHC class III region [identified by Qin (2009)] was in

fact further independently confirmed by the analysis on the animals used in this study. The presence of other segments of high LD is consistent with conserved groups of loci within the sheep MHC analogous to those that exist in the human MHC.

SNP Heterozygosity

Analysis of heterozygote frequency for each locus has demonstrated variable degrees of heterozygosity across the ovine MHC class IIb, IIa and III regions (Figure 5.3). Some regions had loci with observed heterozygosities around 0.5, in contrast to some regions of low heterozygosity (Figures 5.2, 5.3 and 5.4; Ho ≈0.05). The high heterozygosity values are similar to those reported for SNP loci within the human MHC (Miretti *et al.* 2005) and are consistent with the accepted notion that the MHC has a relatively high level of heterozygosity since this facilitates more diverse immunological responses against a wide variety of pathogens. It was noted that several sheep SNP loci had an observed heterozygosity greater than the theoretical maximum value of 0.5 for a diallelic locus in a population manifesting Hardy Weinberg equilibrium (range 0.51- 0.55). This anomaly is explained by sampling errors due to the small population size of Cohort 1. The ovine MHC class IIa region showed low heterozygosities for four loci, from the DRB1-DRA loci to the DQB1-DQA2 loci (Figure 5.3). The heterozygosities in Cohort 2 were very similar to those of Cohort 1 even though sheep in Cohort 2 were more closely related than for Cohort 1.

Similar observations were reported by Miretti *et al.* (2005) and Gibson *et al.*, (2006), who interrogated HapMap data from the human genome and showed the presence of regions containing consecutive homozygous SNPs. Homozygous tracts of DNA are also known to exist in inbred populations (Gibson *et al*., 2006). Similar regions of SNP homozygosity also occur in normal human populations including those of different ethnic ancestry. Gibson *et al.* (2006) described 1393 homozygous tracts in the human genome (minimum length of 1Mb - the longest containing an uninterrupted run of 3922 homozygous SNPs spanning 17.9 Mb) although none of these were within the MHC region. More recently,

Simon-Sanchez *et al.* (2007) have also shown the existence of regions of contiguous extended homozygosity in the human genome and suggested these regions would be of value in whole genome association studies and the identification of chromosomal abnormalities. Curtis (2007) showed that these homozygous tracts of DNA were inherited from a common ancestor and were not formed as a result of sporadic deletions or uniparental isodisomy.

A particularly interesting study by Traherne *et al.* (2006), compared SNP presence in extended MHC nucleotide sequence haplotypes present in three well characterised homozygous human cell lines [PGF (HLA-A3-B7-Cw7-DR15-DQ6), COX (HLA-A1-B8-Cw7-DR3-DQ2) and QBL (HLA-A26-B18-Cw5-DR3-DQ2)]. These haplotypes have been associated with protection against type I diabetes, susceptibility to type I diabetes and Grave's disease respectively. Sequence comparisons between PGF and QBL haplotypes and between the COX and QBL haplotypes showed different patterns of SNP distribution across the MHC region. In particular, a comparison of the HLA-DR region between the COX and QBL haplotypes (containing the genes HLA-DRB1, HLA-DQA1, HLA-DQB1 and the pseudogenes HLA-DRB2 and MTCOP3P1) revealed only 14 SNPs over 158 kb. Further analysis of this subregion with 12 chromosomes sharing the DR3-DQ2 haplotype, or in 26 chromosomes sharing the most common HLA class II haplotype, showed that the distribution of SNPs in these two groups of chromosomes was respectively 10 times and 200 times lower that the regions directly adjacent to it, thereby defining a "SNP desert". de Bakker *et al*. (2006) have shown that for larger cohort of ethnically defined human populations the SNP desert region identified by Traherne *et al*. (2006) corresponds to a region of low heterozygosity. Although the presence of similar regions of low heterozygosity in the sheep and human MHCs could be serendipitous, it is tempting to speculate that these regions contain functional sequences for which variation is poorly tolerated.

The observation of low heterozygosity within the ovine MHC class IIa subregion could possibly be explained by dissemination of a deletion of a

chromosomal segment in Merino sheep and that this has created null alleles for some loci. Snibson *et al.* (1998) and Keane *et al.* (2007) have reported a deletion occurring within the DQA1 gene, resulting in DQA1*null alleles, in sheep with varying levels of resistance against parasite infection. No deletions have been reported for the other genes, DRB and DQB, located within the MHC class IIa region. In view of this work, evidence for a similar deletion in the sheep used in this study was sought based on PCR amplification of exon 2 of sheep DQA1 using the procedure of Keane *et al* (2007). It was noted that some animals did not yield a PCR product as expected and the possibility that a deletion in the DQA1 gene in some sheep was considered. This is an unlikely explanation since both alleles for each SNP were observed and the SNPs were in Hardy Weinberg proportions.

The region of low heterozygosity in sheep that is similar to the region of low variation identified in the human MHC may be of evolutionary significance. This phenomenon may have occurred before sheep and humans diverged from a common ancestor and imply a functional significance. Current literature has debated the different modes of selection that maintain high degrees of variation in the MHC region. The MHC has become a paradigm for models of selection to maintain adaptively important genetic diversity in natural populations. Balancing selection (negative frequency-dependent selection or symmetrical overdominant selection) or sexual selection have been proposed. These modes of selection have been reviewed in detail in order to explain how MHC heterozygosity confers enhanced resistance to infection and drives the evolution of these highly polymorphic genes (Piertney and Oliver, 2006; Schaschl *et al.*, 2006). In contrast to these explanations, a recent study has shown that MHC heterozygosity reduces the fitness of experimentally infected mice and provides no immunological benefits when loci determining resistance are recessive (Ilmonen *et al.*, 2007).

Comparison of heterozygosity levels for each family group showed that the region of low heterozygosity was relatively conserved. An interesting observation was made when the two family groups representing the two

extremities of levels of parasite resistance were compared. Four regions across the class IIb, IIa and III were identified to be significantly different between Family groups 1 (high average WEC) and 2 (low average WEC); H2B-DMB/DMA-BRD2, DQB2-DQA2/DQA2-DQB1, CFB/MSH5 and APOM/BT2. Interestingly, the region APOM/BT2 corresponded with the predicted recombination hotspot previously reported by Miretti et al., (2006). The differences between Families 1 and 2 may be coincidental and, as for the segment of low heterozygosity itself, will require confirmation in other sheep and breeds of sheep.

The results of this study, together with the arguments included above make a strong case for the conservation of biologically important low heterozygosity regions within genomes and the MHC, although their function(s) is not yet understood.

Linkage disequilibrium and recombination

Variation in linkage disequilibrium was observed across the three chromosomal regions analysed in this study. Changes in LD within the class IIa region were the most pronounced and commenced near the DQA2-DQB1 loci (Figure 5.4). It was noted that the change from relatively high LD to low LD observed within the class IIa region corresponded with a major portion of the region of low heterozygosity within this chromosomal segment that included the four loci **A3C** (DQB1-DQA2), **A5B** (DQA1-DRB1), **A6A** (DRB1-DRA) and **A6C** (DRB1-DRA). A similar observation has been reported for the analogous region of the human MHC. Traherne *et al.* (2006) constructed a high-resolution LD map (with a SNP density of 1 SNP/ 1.8 Kb) across the human MHC class II region (spanning ≈0.8 Mb) and described a 158 kb subregion, characterized by an absence of SNPs, that they referred to as a “SNP desert”. The centromeric end of the “SNP desert” commenced between the DQB1 and DQB3 loci that also corresponded to a region of rapid decrease in LD. The same subregion contains a predicted recombination hotspot that shapes the genealogy of the DR15-DQ6 haplotype (Cullen *et al.*, 1997; Cullen *et al.*, 2002). A more recent study has confirmed the presence of a recombination hotspot with the region bounded by the

HLA-DRA and HLA-DQA2 (Stenzel *et al.*, 2004). The coinciding observations of rapid decrease of LD in the region of low heterozygosity with a known recombination hotspot in human at a similar position, allows for the proposition of a potential hotspot in the ovine MHC. However, this is inconsistent with the report of Gibson *et al*. (2006) who observed that homozygous tracts occurred in regions with high LD and low recombination. Clearly more data is required to resolve this question.

Regions of decreased LD were also observed in the ovine MHC class IIb and class III regions identified by the loci BRD2-DOA/RING1 and BT2/APOM respectively, although the bounding loci for the class III subregion are poorly defined. All three subregions of decreased LD observed in this study corresponded to the subregions that Miretti *et al.* (2005) and Traherne *et al.* (2006) showed coincided with recombination hotspots in the human MHC. The correspondence between regions of low LD and recombination hotspots is further supported by Webb *et al.* (2008) who showed extreme disruption of marker associations in regions of the human genome manifesting increased recombination. Another point of interest is the observation that locations of recombination hotspots are not conserved between species (Ptak *et al*., 2004).

The results described herein show common characteristics between the human and ovine MHCs. Both species share common regions characterized by low heterozygosity that are coincident with, or adjacent to, regions where decreases in LD are observed. Regions of more frequent recombination may well define the boundaries of block-like structures as have been described in humans. Evidence for such structures is described in Chapter 6.

In summary, genetic diversity within the ovine MHC class IIb, IIa and III region has been investigated. Regions of decreased heterozygosity and regions of low LD were observed and decreased heterozygosity in the class IIa subregion coincided approximately with a region where LD decreased abruptly. However, the panel of SNPs used in this study is too

sparse to permit accurate identification of the boundaries of these regions. This sheep region of interest occurs in approximately the same location as a human MHC hotspot of length≈1.5 kbp bu t appears to be of greater length (Arnheim *et al*., 2007).

Chapter 6

Characterisation of ovine MHC haplotypes and association with phenotypic traits

SNP genotyping of sheep families permitted the identification of Mendelian inherited haplotyes covering the class IIb, IIa and III regions of the MHC. Haplotype inference based on the SNPstats implementation of the EM algorithm permitted the prediction of haplotypes that were identical to known inherited haplotypes. Furthermore, analysis of predicted haplotypes for each subregion showed the presence of haplotypic combinations, some of which also occurred in the known haplotypes. This observation is consistent with a block like structure for each of the subregions similar to that known for the human MHC.

6.1 Introduction

6.1.1 Linkage disequilibrium and haplotypic block-like structures

The extensive LD within the human and mouse MHCs was explained by the discovery of conserved regions or "blocks" of loci within which recombination was rare (reviewed Dawkins *et al.*, 1999; Trowsdale, 2002). These blocks were separated by relatively short sequence segments within which recombination occurred. Such regions are now referred to as "hotspots" of recombination (Dawkins *et al.*, 1999; Daly *et al.*, 2001). The human MHC manifests at least four major block like structures across its ≈4 Mb, together with several additional subregion blocks (Yunis *et al.,* 2003; see also Figure 1.2). It is believed that this phenomenon is a general feature of mammalian genomes (Gabriel *et al.*, 2002). The work of Gabriel *et al*. (2002) was based on an extensive panel of SNPs spanning large regions of individual chromosomes whereas much of the earlier work was based on the more highly polymorphic loci characteristic of the MHC. In general terms, the extensive LD within the MHC has frustrated many attempts to identify candidate disease loci that map more broadly to this region. Nevertheless, despite this problem, considerable progress has been made in the identification of disease susceptibility loci, especially in the human MHC (International MHC and autoimmunity genetics network, 2009). The presence of conserved

blocks of loci within MHC haplotypes in theory should permit the more rapid identification of regions within which candidate loci for disease and productivity traits are present. Subsequent sequencing of these regions should then result in identification of the disease susceptibility locus. Furthermore characterization of haplotype blocks will become a powerful analytical tool for studies on the mechanisms of recombination.

6.2 Materials and Methods

SNP genotyping was performed as described in Chapter 5. Sheep haplotypes were deduced from sheep sire, dam, lamb trios according to Mendelian principles.

6.2.1 Analysis of SNP haplotypic data

The panel of 30 SNP markers, each associated with 10 known loci from each of the class IIa, IIb and III regions of the ovine MHC, were typed for both sheep Cohorts 1 and 2. Haplotype inference and haplotypic associated with phenotype were performed using SNPstats software (http://bioinfo.iconologia.net/index.php?module=Snpstats). SNPstats estimates haplotype frequencies using an implementation of the expectation maximization (EM) algorithm previously written for the *haplo.stats* application (Sinnwell and Schaid, 2005).

6.3 Results

6.3.1 Identification of haplotypes in the ovine MHC region

Inspection of the genotypes of the sheep families from the combined Cohorts 1 and 2 permitted the deduction of 54 haplotypes from half sib families comprising 6 sires and 21 dams. Each haplotype consists of 10 SNPs for each of the class IIb, IIa and III subregions. These SNPs were chosen to be well spaced on the basis of the physical maps of the human and cattle MHCs. Haplotypes were also predicted for all 68 and 193 animals present in Cohorts 1 and 2, respectively, using the SNPstats software package implementation of the EM algorithm. Twelve deduced haplotypes for the six sires and predicted haplotypes for Cohorts 1 and 2 was obtained (result not shown).

All 12 sire deduced haplotypes across the three subregions were distinct (Figure 6.1). Note that for the two sire chromosomes of Family 10 (chromosomes 11 and 12), the last two SNPs of the class IIa subregion could not be resolved. Chromosomes 1 and 8, 7 and 10 shared common class IIb subregion haplotypes while chromosomes 5, 11 and 12 shared common class III subregion haplotypes. Since the sires are not first degree relatives, this observation suggests there is some haplotypic conservation for these two subregions. The 10 most frequent predicted haplotypes for all animals in Cohorts 1 and 2 showed a similar pattern. Since cohort 1 also included the 6 sires, another SNPstats analysis was performed on the remaining 62 animals to avoid double counting of haplotypes (Figure 6.1). In this instance, 8 of the 10 most frequent predicted haplotypes (across all three subregions) were distinct and not present in the 12 known sire haplotypes. Two haplotypes (3 and 10) were in common with known sire haplotypes (chromosomes 3 and 8). However, once again, each of the 3 subregion haplotypes showed haplotypes common to those present in the corresponding sire haplotype panel. For example 8 of 10 predicted class IIb haplotypes, which accounted for≈ 25% of the 124 haplotype s in this group, were also present in the sires. Similar results occurred with the class IIa and III haplotypes, although the effect was less pronounced for the class III subregion.

A further SNPstats analysis was then performed in which predicted haplotype frequencies were estimated for each subregion separately. Similar results were obtained (Figure 6.1). For example, for the class IIb region 4 of the 10 most frequent predicted haplotypes were present in the sires, accounting for ≈35% of the 124 haplotypes present in this group.

The remaining 42 haplotypes deduced from the 21 dams were also examined for the presence of conserved blocks for each subregion and compared with the 12 known sire haplotypes (Figure 6.2). For the class IIb subregion 10 sire and 24 dam haplotypes were unique leaving 9

haplotypes that occurred more than once. Similar results were seen with the class IIa and III subregions.

Figure 6.3 shows the predicted haplotypes in 102 lambs from Cohort 2 (offspring directly related to the 6 sires of the known Family trio were removed) that were common to the corresponding sire haplotypes. Further SNPstats analysis predicted haplotype frequencies for each subregion separately showed similar results (Figure 6.3). For example, 3 of the 10 most frequent predicted haplotypes were present in the sires accounting for ≈22% of the 204 haplotypes present in this group.

			MHC class IIb										Freq	MHC class IIa										Freq	MHC class III										Freq
			DB-TP2	PB8-TP2	PB9	HB-DMB	DMA-BD2	BD2-DA	RG1	PN6	RL2	TBP		A2A	A3A	A3C	A5B	A6A	A6C	A6B	DRA	BL2	BL2-C10		TXB	CFB	C2	MH5	G6B	BT5	APM	BT2	TNF	LTA	
Deduced	Family 1	1	C	C	G	A	A	T	C	C	G	T		G	A	C	A	G	G	G	A	C	A		G	C	C	G	C	G	G	C	G	A	
sire		2	C	C	G	A	A	T	C	C	C	T		G	A	C	A	G	G	G	G	T	G		A	T	G	A	C	G	A	T	G	G	
haplotype	Family 2	3	C	C	G	G	A	T	C	T	C	G		C	G	C	A	G	G	G	A	C	A		A	C	C	G	C	A	G	T	A	G	
from known		4	C	C	G	G	A	T	C	C	G	T		C	G	C	A	G	G	G	G	T	G		G	C	C	G	C	A	G	T	A	G	
family sets	Family 4	5	C	C	T	G	A	T	C	C	G	T		C	G	C	A	G	G	A	G	T	G		G	T	G	G	C	A	G	T	A	G	
		6	T	C	T	A	A	T	C	C	C	G		C	A	C	A	G	G	G	G	T	G		A	C	C	G	C	A	G	T	A	G	
	Family 5	7	C	T	T	G	A	T	C	C	G	T		G	A	C	A	G	G	G	A	C	G		G	T	G	G	T	G	G	C	G	G	
		8	C	C	G	A	A	T	C	C	G	T		C	G	C	G	G	G	G	A	C	A		A	T	G	G	C	A	G	T	A	G	
	Family 7	9	C	T	G	G	A	T	T	C	G	T		C	G	C	A	G	G	G	G	T	G		G	C	C	G	C	A	G	T	A	G	
		10	C	T	T	G	A	T	C	C	G	T		C	G	C	A	G	G	G	A	C	G		A	T	G	A	C	G	A	T	G	G	
	Family 10	11	C	T	G	G	A	T	T	C	C	T		C	G	C	A	G	G	A	G	C/T	G/A		G	T	G	G	C	A	G	T	A	G	
		12	T	C	G	G	A	T	C	T	C	G		C	G	C	A	G	G	A	G	C/T	G/A		G	T	G	G	C	A	G	T	A	G	

			MHC class IIb											MHC class IIa											MHC class III										
			DB-TP2	PB8-TP2	PB9	HB-DMB	DMA-BD2	BD2-DA	RG1	PN6	RL2	TBP		A2A	A3A	A3C	A5B	A6A	A6C	A6B	DRA	BL2	BL2-C10		TXB	CFB	C2	MH5	G6B	BT5	APM	BT2	TNF	LTA	
Inferred	Cohort 1	1	C	C	G	G	A	T	C	T	C	G		C	G	C	A	G	G	G	A	C	A		A	T	G	G	C	A	G	T	A	G	0.0484
haplotypes		2	T	C	G	A	A	T	C	C	G	T		C	G	C	A	G	G	G	G	C	G		A	T	G	G	C	G	G	T	G	G	0.0484
by SNPstats	All 3 regions	3	C	C	G	A	A	T	C	C	G	T		C	G	C	G	G	G	G	A	C	A		A	T	G	G	C	A	G	T	A	G	0.0403
62 animals		4	C	C	G	G	A	T	C	C	G	T		C	G	C	A	G	G	G	G	C	A		G	T	G	G	C	G	G	T	A	G	0.0323
		5	C	C	G	G	A	T	C	T	C	G		C	G	C	A	G	G	G	G	T	G		G	T	G	G	C	G	G	T	G	G	0.0323
		6	C	T	G	G	A	T	C	C	G	T		C	G	C	A	G	G	A	G	T	G		A	T	G	G	C	G	G	T	A	G	0.0242
		7	C	C	G	A	A	T	C	C	C	T		G	A	C	A	G	G	G	A	C	A		G	C	C	G	C	G	G	C	G	A	0.0242
		8	C	C	G	A	A	T	C	C	C	T		G	A	C	A	G	G	G	G	C	A		A	T	G	G	C	G	G	T	A	G	0.0161
		9	C	C	G	A	A	T	C	C	C	T		G	A	C	A	G	G	G	G	T	G		G	C	C	G	C	G	G	C	G	G	0.0161
		10	C	C	G	G	A	T	C	T	C	G		C	G	C	A	G	G	G	A	C	A		A	C	C	G	C	A	G	T	A	G	0.0161

			MHC class IIb											MHC class IIa											MHC class III										
			DB-TP2	PB8-TP2	PB9	HB-DMB	DMA-BD2	BD2-DA	RG1	PN6	RL2	TBP		A2A	A3A	A3C	A5B	A6A	A6C	A6B	DRA	BL2	BL2-C10		TXB	CFB	C2	MH5	G6B	BT5	APM	BT2	TNF	LTA	
Inferred	Cohort 1	1	C	C	G	G	A	T	C	C	G	T	0.1102	G	A	C	A	G	G	G	A	C	A	0.1009	A	T	G	G	C	G	G	T	G	G	0.1339
haplotypes		2	C	C	G	A	A	T	C	C	G	T	0.0917	C	G	C	G	G	G	G	A	C	A	0.0967	G	T	G	A	C	G	A	T	G	G	0.129
by SNPstats	Each subregion	3	C	C	G	G	A	T	C	T	C	G	0.0906	C	G	C	A	G	G	G	A	C	A	0.0839	A	C	C	G	C	A	G	T	A	G	0.0902
62 animals		4	C	T	T	G	A	T	C	T	C	G	0.068	G	A	C	A	G	G	G	G	C	G	0.0789	G	T	G	G	C	A	G	T	A	G	0.0793
		5	C	C	G	A	A	T	C	C	C	T	0.0623	G	A	C	A	G	G	G	G	C	A	0.0724	A	T	G	G	C	A	G	T	A	G	0.0756
		6	C	T	G	G	A	T	T	C	G	T	0.0614	C	G	C	A	G	G	A	G	T	G	0.0704	G	C	C	G	C	G	G	T	G	G	0.0679
		7	T	C	G	A	A	T	C	C	G	T	0.0455	C	G	C	G	G	G	G	G	C	A	0.0543	G	T	G	G	C	G	G	T	G	G	0.0414
		8	C	C	G	G	G	A	T	C	G	G	0.0323	C	A	C	A	G	G	G	G	T	G	0.0515	A	T	G	G	C	G	G	C	G	A	0.0406
		9	T	C	G	A	G	T	C	C	G	G	0.0323	C	G	C	A	G	G	G	G	C	G	0.0499	G	C	C	G	C	G	G	C	G	A	0.0406
		10	T	T	G	G	A	A	C	T	C	G	0.0323	C	G	C	G	G	G	A	G	T	G	0.0374	A	T	G	G	T	G	G	C	G	A	0.0399

Figure 6.1: Comparison of deduced sire haplotypes from family trios with predicted haplotypes across all three subregions or for each subregion for Cohort 1 (excluding the 6 known sires) using SNPstats. The top 10 most frequent haplotypes predicted by SNPstats across all three subregion and for each individual subregion are listed. Identical haplotypes are colour coded according to the deduced haplotypes of the 6 sires. Note that for the two chromosomes of the sire of Family 10 (chromosomes 11 and 12), the last two SNPs of the class IIa subregion could not be resolved.

		MHC class IIb										Freq	MHC class IIa										Freq	MHC class III									
		DB-TP2	PB8-TP2	PB9	HB-DMB	DMA-BD2	BD2-DA	RG1	PN6	RL2	TBP		A2A	A3A	A3C	A5B	A6A	A6C	A6B	DRA	BL2	BL2-C10		TXB	CFB	C2	MH5	G6B	BT5	APM	BT2	TNF	LTA
Family 1	1	C	C	G	A	A	T	C	C	G	T		G	A	C	A	G	G	G	A	C	A		G	C	C	G	C	G	G	C	G	A
	2	C	C	G	A	A	T	C	C	C	T		G	A	C	A	G	G	G	G	T	G		A	T	G	A	C	G	A	T	G	G
Family 2	3	C	C	G	G	A	T	C	T	C	G		C	G	C	A	G	G	G	A	C	A		A	C	C	G	C	A	G	T	A	G
	4	C	C	G	G	A	T	C	C	G	T		C	G	C	A	G	G	G	G	T	G		G	C	C	G	C	A	G	T	A	G
Family 4	5	C	C	T	G	A	T	C	C	G	T		C	G	C	A	G	G	A	G	T	G		G	T	G	G	C	A	G	T	A	G
	6	T	C	T	A	A	T	C	C	C	G		C	A	C	A	G	G	G	G	T	G		A	C	C	G	C	A	G	T	A	G
Family 5	7	C	T	T	G	A	T	C	C	G	T		G	A	C	A	G	G	G	A	C	G		G	T	G	G	T	G	G	C	G	G
	8	C	C	G	A	A	T	C	C	G	T		C	G	C	G	G	G	G	A	C	A		A	T	G	G	C	A	G	T	A	G
Family 7	9	C	T	G	G	A	T	T	C	G	T		C	G	C	A	G	G	G	G	T	G		G	C	C	G	C	A	G	T	A	G
	10	C	T	T	G	A	T	C	C	G	T		C	G	C	A	G	G	G	A	C	G		A	T	G	A	C	G	A	T	G	G
Family 10	11	C	T	G	G	A	T	T	C	C	T		C	G	C	A	G	G	A	G	C/T	G/A		G	T	G	G	C	A	G	T	A	G
	12	T	C	G	G	A	T	C	T	C	G		C	G	C	A	G	G	A	G	C/T	G/A		G	T	G	G	C	A	G	T	A	G
		DB-TP2	PB8-TP2	PB9	HB-DMB	DMA-BD2	BD2-DA	RG1	PN6	RL2	TBP		A2A	A3A	A3C	A5B	A6A	A6C	A6B	DRA	BL2	BL2-C10		TXB	CFB	C2	MH5	G6B	BT5	APM	BT2	TNF	LTA
D2003189		C	T	T	G	A	T	T	C	G			C	G	C		G	G	G		C	A		A	T	G	G	C	A	G	T		G
		C	C	G	G	G	A	C	C	G	G		C	G	C	A	G	G	G	A	C	A		G	T	G	G	C	G	G	C		A
D2005126		C	C	G	G	A	T	C	T	C	G		C	G	C	A	G	G	G	G	C	G		G	T	G	G	C	G	G	T	G	A
		T	C	G	A	A	T	C	C	G	T		C	G	C	G	G	G	G	G	C	A		A	T	G	G	T	G	G	T	A	G
D2004109		C	T	G	A	G	T	C	C	G	G		G	A	C	A		G	G	A	C	A		A		C	G	T	G	G	T	A	G
		C	C	G	G	A	T	C	C	G	T		G	A	C	A	G	G	G	G	C	A		A		G	G	C	G	G	T	G	G
D2004156		T	C	G	A	A	T	C	C				C	G	C	G	G	G	G	G	T	G		A	T	G	G	C	G	G	T	G	G
		C	T	G	G	A	A	C	T				C	G	C	A	G	G	G	A	T	G		A	T	G	G	C	G	G	T	G	G
D2005137		C	T	G	A	G	T	C	C	G	G		G		C	A	G	G	G	G	T	G		G	T	G	G	C	G	G	T	G	G
		C	C	G	G	A	T	C	C	G	T		C	G	C	A	G	G	A	G	T	G		A	T	C	G	C	A	G	T	A	G
D2004174		C	C	T	G	A	T	C	C	G	T		C	G	C		G	G	G	A	C	A		G	T	C	G	C	G	G	T	G	G
		T	C	G	G	G	A	C	C	C	G		C	G	C	A	G	G	A	G	T	G		G	T	G	G	C	A	G	T	A	G
D2005140		C	C	G	A	A	A	C	C	G	T		G	A	C	A	G	G	G	A	C	A		G	C	C	G	C	G	G	C	G	A
		C	T	G	G	A	T	T	C	G	T		C	G	C	A	G	G	G	A	T	G		A	T	G	G	C	G	G	T	G	G
D2002150		C	C	G	A	A	T		C	C	T		G	A	C		G	G	G			A		G	C	C	G	C	G	G	T	G	G
		C	T	G	G	A	T	C	C	G	T		G	G	C	A	G	G	G	A	C	G		G	C	C	G	C	G	G	T	G	G
D2002137		C	C	G	A	G	T	C	T	C	G		C	G	T	A	G	A	G	G		G		G	T	G	A	C	G	A	T	G	G
		C	C	G	G	G	A	T	C	G	G		G	A	C	A	G	G	G	A	T	G		G	C	C	A	C	G	G	C	G	A
D2004188		C	T	G	G	A	A	C	T	C	G		G		C	A	G	G	G	G	C	A		A	T	G	G	C	G	G	C	G	A
		T	C	G	G	A	T	T	C	G	G		C		C	G	G	G	G	A	C	A		A	T	G	G	C	A	G	T	A	G
D2005208		?	C	G	A	G	T	C	C	G	G		G	A	C	A	G	G	G	G	C	G		A	T	G	G	T		G	C	G	A
		C	C	G	A	G	T	C	C	G	G		C	G	C	A	G	G	G	A	T	G		A	C	C	G	C	A	G	T	A	G
D2002121		C	C	G	A	G	T	C	C	G	G		G	A	C	A	G	G	G	G	C	G		A	T	G	G	T	G	G	C	G	A
		T	T	G	G	A	T	T	C	G	T		G	A	C	A	G	G	G	A	C	G		G	T	G	A	C	G	A	T	G	G
D2000133		C	T	G	A	G	T	C	C	G	G		C	G	C	A	G	G	G	G	C	G		A	?	G	A	C	G	A	T	G	G
		C	T	G	G	A	T	T	C	G	T		C	G	C	A	G	G	A	G	T	G		G	T	G	G	C	A	G	T	A	G
D2003316		C	C	G	A		T	C	C	G	T		G	A	C	A	G	G	G	G	C	A		G	T	G	G	C	G	G	T	G	G
		C	C	G	A	A	T	C	C	C	T		C	G	C	G	G	G	G	A	C	A		A	T	G	G	C	A	G	T	A	G
D2004120		C	T	G	G	A	T	T	C	G	T		C	G	T	A	G	A		G	T	G		G	T	G	A	C	G	A	T	G	G
		C	C	G	A	A	T	C	C	G	T		G	A	C	A	G	G	G	A	C	A		G	T	G	G	C	G	G	T	G	G

D2003192	C	C	G	G	A	T	C	T	C	G		G	A	C	A	G	G	G	G	C	A		A	T	G	G	C	G	G	T	G	G
	C	T	T	G	A	T	C	C	G	T		G	G	T	A	G	G	G	A	C	A		A	T	C	G	T	G	G	T	A	G
D2002117	C	C	G	A	A	T	C	C	G	T		C	G	C		G	G	G	A	C	A		A	T	G	G	C	A	G	T	A	G
	C	C	G	G	G	A	C	C	C	G		C	G	T	A	G	A	G	G	T	G		G	T	G	A	C	G	A	T	G	G
D2005288	C	C		G	G	A	T	C	G	G		C	G	C	G	G	G	G	A	C	A		A	T	G	G	C	A	G	T	A	G
	C	C	G	G	A	T	C	T	C	G		C	G	C	A	G	G	G	A	C	A		G	T	G	G	C	A	G	T	A	G
D2005377	C	T	T	A	G	T	C	C	G	G		G	A	C	A	G	G	G	G	C	A		A	T	G	G	C	G	G	T	G	G
	C	C	G	G	A	T	T	C	C	T		C	G	C	G	G	G	G	G	C	A		A	T	G	G	T	G	G	T	A	G
D2005386	C	T	G	A	G	T	C	C	G	G		G	A	C	A	G	G	G	G	C	A		A	T	G	G	C	G	G	T	G	G
	C	C	T	G	A	T	T	C	C	T		C	G	C	G	G	G	G	G	C	A		A	T	G	G	T	G	G	T	A	G
D2003278	C	C	G	A	A	T	C	C	C	T		C	A	C	A	G	G	G	A	C	A		G	C	C	G	C	G	G	T	A	G
	C	T	T	G	A	A	C	C	G	G		C	G	C	G	G	G	G	G	C	A		A	T	G	G	T	G	G	T	A	G

Figure 6.2: Comparison of the deduced sire haplotypes with the predicted dam haplotypes based on the Mendelian family trio information. Identical haplotypes between the sire haplotype and dam haplotype are colour coded according to the sire haplotype. Identical haplotypes within the dam population are also colour coded (colours different from those of the sire*). The coloured font designates SNP alleles for loci for which only one allele could be identified. Note that for the two chromosomes of the sire of Family 10 (chromosomes 11 and 12), the last two SNPs of the class IIa subregion could not be resolved.*

		MHC class IIb										MHC class IIa										MHC class III									
		DB-TP2	PB8-TP2	PB9	HB-DMB	DMA-BD2	BD2-DA	RG1	PN6	RL2	TBP	A2A	A3A	A3C	A5B	A6A	A6C	A6B	DRA	BL2	BL2-C10	TXB	CFB	C2	MH5	G6B	BT5	APM	BT2	TNF	LTA
Family 1	1	C	C	G	A	A	T	C	C	G	T	G	A	C	A	G	G	G	A	C	A	G	C	C	G	C	G	G	C	G	A
	2	C	C	G	A	A	T	C	C	C	T	G	A	C	A	G	G	G	G	T	G	A	T	G	A	C	G	A	T	G	G
Family 2	3	C	C	G	G	A	T	C	T	C	G	C	G	C	A	G	G	G	A	C	A	A	C	C	G	C	A	G	T	A	G
	4	C	C	G	G	A	T	C	C	G	T	C	G	C	A	G	G	G	G	T	G	G	C	C	G	C	A	G	T	A	G
Family 4	5	C	C	T	G	A	T	C	C	G	T	C	G	C	A	G	G	A	G	T	G	G	T	G	G	C	A	G	T	A	G
	6	T	C	T	A	A	T	C	C	C	G	C	A	C	A	G	G	G	G	T	G	A	C	C	G	C	A	G	T	A	G
Family 5	7	C	T	T	G	A	T	C	C	G	T	G	A	C	A	G	G	G	A	C	G	G	T	G	G	T	G	G	C	G	G
	8	C	C	G	A	A	T	C	C	G	T	C	G	C	G	G	G	G	A	C	A	A	T	G	G	C	A	G	T	A	G
Family 7	9	C	T	G	G	A	T	T	C	G	T	C	G	C	A	G	G	G	G	T	G	G	C	C	G	C	A	G	T	A	G
	10	C	T	T	G	A	T	C	C	G	T	C	G	C	A	G	G	G	A	C	G	A	T	G	A	C	G	A	T	G	G
Family 10	11	C	T	G	G	A	T	T	C	C	T	C	G	C	A	G	G	A	G	C/T	G/A	G	T	G	G	C	A	G	T	A	G
	12	T	C	G	G	A	T	C	T	C	G	C	G	C	A	G	G	A	G	C/T	G/A	G	T	G	G	C	A	G	T	A	G

		MHC class IIb										MHC class IIa										MHC class III										Freq
		DB-TP2	PB8-TP2	PB9	HB-DMB	DMA-BD2	BD2-DA	RG1	PN6	RL2	TBP	A2A	A3A	A3C	A5B	A6A	A6C	A6B	DRA	BL2	BL2-C10	TXB	CFB	C2	MH5	G6B	BT5	APM	BT2	TNF	LTA	
Cohort 2	1	C	T	T	G	A	T	C	T	C	G	C	G	C	A	G	G	G	A	C	G	A	C	C	G	C	A	G	T	A	G	0.0343
	2	C	C	G	G	A	T	C	T	C	G	C	G	C	A	G	G	G	G	C	A	A	T	G	G	C	G	G	T	G	G	0.0294
Inferred	3	C	T	G	G	A	T	T	C	C	T	G	A	C	A	G	G	G	A	C	G	G	T	G	G	T	G	G	C	G	G	0.0294
haplotypes	4	C	T	T	G	A	T	C	T	C	G	C	G	C	G	G	G	G	A	C	A	A	C	C	G	C	A	G	T	A	G	0.0294
on 102	5	C	T	G	G	G	A	C	T	C	G	C	G	C	A	G	G	G	A	C	A	A	T	G	G	C	G	G	T	G	G	0.0245
lambs	6	C	C	G	A	A	T	C	C	G	T	C	G	C	A	G	G	G	A	T	G	G	C	C	A	C	G	G	C	G	A	0.0196
	7	C	C	G	G	A	T	C	C	G	T	C	G	C	A	G	G	G	G	C	A	A	T	G	G	C	A	G	T	A	G	0.0196
All 3	8	C	C	T	G	A	T	C	T	C	G	C	G	C	G	G	G	G	A	C	A	A	C	C	G	C	A	G	T	A	G	0.0196
regions	9	C	T	G	G	A	T	T	C	C	T	C	G	C	G	G	G	G	A	C	A	G	T	G	G	C	A	G	T	A	G	0.0196
	10	C	T	G	G	A	T	T	C	C	T	G	A	C	A	G	G	G	G	T	G	A	T	C	G	T	G	G	T	A	G	0.0196

		MHC class IIb										Freq	MHC class IIa										Freq	MHC class III										Freq
		DB-TP2	PB8-TP2	PB9	HB-DMB	DMA-BD2	BD2-DA	RG1	PN6	RL2	TBP		A2A	A3A	A3C	A5B	A6A	A6C	A6B	DRA	BL2	BL2-C10		TXB	CFB	C2	MH5	G6B	BT5	APM	BT2	TNF	LTA	
Cohort 2	1	C	T	G	G	A	T	T	C	C	T	0.0927	C	G	C	A	G	G	G	A	C	A	0.1535	A	C	C	G	C	A	G	T	A	G	0.1777
	2	C	C	G	G	A	T	C	T	C	G	0.083	C	G	C	G	G	G	G	A	C	A	0.1242	A	T	G	G	C	G	G	T	G	G	0.1143
Inferred	3	C	T	G	G	G	A	C	T	C	G	0.0779	G	A	C	A	G	G	G	G	T	G	0.0685	G	T	G	G	T	G	G	C	G	G	0.1029
haplotypes	4	C	C	T	G	A	T	C	T	C	G	0.0719	G	A	C	A	G	G	G	A	C	G	0.0648	G	T	G	G	C	A	G	T	A	G	0.0751
on 102	5	C	T	T	G	A	T	T	C	G	T	0.0627	C	G	C	A	A	G	G	G	T	G	0.0588	A	T	C	G	T	G	G	T	A	G	0.0735
lambs	6	C	C	G	A	G	T	C	C	G	G	0.0593	C	G	C	G	G	G	A	G	T	G	0.0465	G	C	C	G	C	G	G	T	G	G	0.067
	7	T	T	T	A	A	T	C	T	C	G	0.057	C	G	C	A	G	G	G	G	C	G	0.0445	G	T	G	A	C	G	A	T	G	G	0.057
Each	8	C	C	G	A	A	T	C	C	G	T	0.0566	C	A	C	A	G	G	G	G	C	A	0.0377	A	T	G	G	C	A	G	T	A	G	0.057
subregion	9	C	T	T	G	A	T	C	T	C	G	0.0543	C	A	C	A	G	G	G	G	T	G	0.0354	A	T	C	G	C	A	G	T	A	G	0.0548
	10	T	C	G	A	A	T	C	C	G	T	0.0478	C	G	C	A	G	G	G	G	T	G	0.0351	G	C	C	A	C	G	G	C	G	A	0.0308

Figure 6.3: *Comparison of deduced sire haplotypes from family trios with predicted haplotypes across all three subregions and for each subregion for Cohort 2 (all lambs that are not the progeny of the 6 sires of known deduced haplotypes) using SNPstats. The top 10 most frequent haplotypes predicted by SNPstats across all three subregion and for each individual subregion are listed. Identical haplotypes are colour coded according to the deduced haplotypes of the 6 sires. Note that for the two chromosomes of the sire of Family 10 (chromosomes 11 and 12), the last two SNPs of the class IIa subregion could not be resolved.*

6.3.2 Ovine MHC haplotype recombination

Inspection of the patterns of inheritance of the MHC haplotypes in the sheep family trios revealed the presence of recombination between the class IIb and the class IIa subregions. For example, a sire of genotype IIb1-IIa1-III1/ IIb2-IIa2-III2 mated with a dam of genotype IIb3-IIa3-III3/ IIb4-IIa4-III4 produced a lamb with genotype IIb1-IIa2-III2/ IIb3-IIa3-III3. Four recombinations were observed in the 21 family trios and in all cases the recombination event occurred on a chromosome present in the sire. No instances of recombination were observed between the IIa and III subregions.

6.3.3 Haplotype associations with EBVwec and EBVwecwean and resting serum IgA levels

SNPstats software was used to predict haplotypes in the unrelated animals comprising Cohort 1 that were associated with susceptibility or resistance to nematode worms. For this analysis the response variables chosen were the estimated breeding values for WEC and WECweaning (designated EBVwec and EBVwecwean respectively) since more complete data was available from the Rylington Merino project for these variables and they take into account external effects such as sex, environmental factors and weight. They are therefore more informative than WECs themselves (J Greeff, personal communication). SNPstats predicted 18 and 21 haplotypes with statistically significant associations with EBVwec and EBVwecwean respectively. Ten of the predicted haplotypes were common to both response variables (Table 6.1). Haplotype associations for each subregion with EBVwec and EBV wecwean are shown in Tables 6.2, 6.3 and 6.4.

A similar association analysis using total IgA and parasite specific IgA as the response variable was not possible for Cohort 1 due to the unavailability of serum samples. Associations with the IgA variables for Cohort 2 animals were performed (despite their relatedness). No significant haplotypic associations were predicted (data not shown).

Table 6.1: Association analysis of inferred haplotypes across the ovine MHC class II and III region with (A) EBVwec and (B) EBVwecwean using SNPstats. Haplotypes are in order of decreasing frequency and the degree of association is expressed as the odds ratio relative to the most frequent haplotype (highlighted in green).

A

Haplotype association with EBVwec (n=68)																																
	DB.TP2	PB8.TP2	PB9	HB.DMB	DMA.BD2	BD2.DA	RG1	PN6	RL2	TBP	A2A	A3A	A3C	A5B	A6A	A6C	A6B	DRA	BL2	BL2.C10	TXB	CFB	C2	MH5	G6B	BT5	APM	BT2	TNF	LTA	Frequency	Difference (95% CI)	P-value
1	C	C	G	G	A	T	C	T	C	G	C	G	C	A	G	G	G	A	C	A	A	C	C	G	C	A	G	T	A	G	0.0441	0	---
2	C	C	G	A	A	T	C	C	G	T	C	G	C	G	G	G	G	A	C	A	A	T	G	G	C	A	G	T	A	G	0.0441	-93.42 (-94.06 - -92.78)	<0.0001
3	C	C	G	G	A	T	C	C	G	T	C	G	C	A	G	G	G	G	C	A	A	T	G	G	C	G	G	T	G	G	0.0368	30.77 (30.32 - 31.22)	<0.0001
4	C	T	G	G	A	T	C	C	G	T	C	G	C	A	G	G	G	G	T	G	A	T	G	G	C	G	G	T	A	G	0.0294	49.4 (48.97 - 49.83)	<0.0001
5	C	C	G	G	A	T	C	T	C	G	C	G	C	A	G	G	G	G	T	G	G	C	G	G	C	G	G	T	G	G	0.0276	30.95 (30.6 - 31.3)	<0.0001
6	C	C	G	A	A	T	C	C	C	T	G	A	C	A	G	G	G	G	C	A	A	T	G	G	C	G	G	T	G	G	0.0221	80.45 (80.24 - 80.67)	<0.0001
7	C	C	G	A	A	T	C	C	G	T	G	A	C	A	G	G	G	A	C	A	G	T	G	G	C	G	G	T	G	G	0.0221	24.32 (23.97 - 24.67)	<0.0001
8	C	C	G	G	A	T	C	C	G	T	C	G	C	A	G	G	G	G	T	G	G	C	C	G	C	A	G	T	A	G	0.0221	-12.05 (-12.27 - -11.84)	<0.0001
9	C	C	T	G	A	T	C	C	G	T	C	G	C	A	G	G	A	G	T	G	G	T	G	G	C	A	G	T	A	G	0.0221	40.86 (40.58 - 41.14)	<0.0001
10	C	T	G	G	A	T	C	C	G	T	C	G	C	A	G	G	G	A	C	G	G	C	C	G	C	G	G	T	G	G	0.0221	-8.42 (-8.77 - -8.07)	<0.0001
11	C	C	G	A	A	A	C	C	G	T	C	A	C	A	G	G	G	G	C	A	G	T	G	G	T	G	G	C	G	G	0.0147	-21 (-21.1 - -20.9)	<0.0001
12	C	C	G	A	A	T	C	C	C	T	G	A	C	A	G	G	G	G	C	G	G	C	C	G	C	G	G	T	G	G	0.0147	172.25 (172.06 - 172.44)	<0.0001
13	C	C	G	G	A	T	C	T	C	G	C	G	C	A	G	G	G	A	C	A	A	T	G	G	C	A	G	T	A	G	0.0147	31.75 (31.52 - 31.98)	<0.0001
14	C	C	T	G	A	T	C	T	C	G	G	A	C	A	G	G	G	G	C	G	A	T	G	G	T	G	G	C	G	A	0.0147	12.95 (12.85 - 13.04)	<0.0001
15	C	T	T	G	G	T	T	C	G	G	C	G	C	G	G	G	G	G	C	A	A	T	G	G	T	G	G	T	A	G	0.0147	-51.31 (-51.41 - -51.21)	<0.0001
16	T	C	G	A	A	T	C	C	G	T	C	G	C	G	G	G	G	G	C	A	A	T	G	G	C	G	G	T	A	G	0.0147	58.65 (58.42 - 58.88)	<0.0001
17	T	C	T	A	A	T	C	C	C	G	C	A	C	A	G	G	G	G	T	G	A	C	C	G	C	A	G	T	A	G	0.0147	77.57 (77.4 - 77.73)	<0.0001
18	C	C	G	A	A	T	C	C	C	T	G	A	C	A	G	G	G	A	C	A	G	C	C	G	C	G	G	C	G	A	0.0147	13.61 (13.44 - 13.77)	<0.0001

B

Haplotype association with EBVwecwean (n=68)																																
	DB.TP2	PB8.TP2	PB9	HB.DMB	DMA.BD2	BD2.DA	RG1	PN6	RL2	TBP	A2A	A3A	A3C	A5B	A6A	A6C	A6B	DRA	BL2	BL2.C10	TXB	CFB	C2	MH5	G6B	BT5	APM	BT2	TNF	LTA	Frequency	Difference (95% CI)	P-value
1	C	C	G	A	A	T	C	C	G	T	C	G	C	G	G	G	G	A	C	A	A	T	G	G	C	A	G	T	A	G	0.0441	0	---
2	C	C	G	G	A	T	C	T	C	G	C	G	C	A	G	G	G	A	C	A	A	C	C	G	C	A	G	T	A	G	0.0441	-92.32 (-92.32 - -92.32)	<0.0001
3	C	C	G	G	A	T	C	C	G	T	C	G	C	A	G	G	G	G	C	A	A	T	G	G	C	A	G	T	A	G	0.0368	199.62 (199.62 - 199.62)	<0.0001
4	C	C	G	G	A	T	C	T	C	G	C	G	C	A	G	G	G	G	T	G	G	C	G	G	C	G	G	T	G	G	0.0368	211.4 (211.4 - 211.4)	<0.0001
5	C	C	G	A	A	T	C	C	C	T	G	A	C	A	G	G	G	G	C	A	A	T	G	G	C	G	G	T	G	G	0.0221	-80.76 (-80.76 - -80.76)	<0.0001
6	C	C	G	G	A	T	C	C	G	T	C	G	C	A	G	G	G	G	T	G	G	C	C	G	C	A	G	T	A	G	0.0221	217.29 (217.29 - 217.29)	<0.0001
7	C	C	T	G	A	T	C	T	C	G	G	A	C	A	G	G	G	G	C	G	A	T	G	G	T	G	G	C	G	A	0.0221	-63.08 (-63.08 - -63.08)	<0.0001
8	C	T	G	G	A	T	C	C	G	T	C	G	C	A	G	G	G	G	C	G	A	T	G	G	C	G	G	T	G	G	0.0221	249.2 (249.2 - 249.2)	<0.0001
9	T	C	G	A	G	T	C	C	G	G	G	A	C	A	G	G	G	G	C	G	A	T	G	G	C	G	G	T	G	A	0.0221	131.94 (131.94 - 131.94)	<0.0001
10	C	C	G	A	A	A	C	C	G	T	C	A	C	A	G	G	G	G	C	A	G	T	G	G	T	G	G	C	G	G	0.0147	123 (123 - 123)	<0.0001
11	C	C	G	A	A	T	C	C	C	T	G	A	C	A	G	G	G	A	C	A	G	C	C	G	C	G	G	C	G	A	0.0147	285.05 (285.05 - 285.05)	<0.0001
12	C	C	G	A	A	T	C	C	G	T	G	A	C	A	G	G	G	A	C	A	G	T	G	G	C	G	G	T	G	G	0.0147	217.34 (217.34 - 217.34)	<0.0001
13	C	C	G	G	A	T	C	C	G	T	C	G	C	G	G	G	G	G	C	A	G	T	G	G	C	G	G	T	G	G	0.0147	430.29 (430.29 - 430.29)	<0.0001
14	C	C	G	G	A	T	C	T	C	G	C	G	C	A	G	G	G	A	C	A	A	T	G	G	C	A	G	T	A	G	0.0147	121.74 (121.74 - 121.74)	<0.0001
15	C	C	T	G	A	T	C	C	G	T	C	G	C	A	G	G	G	G	C	G	A	T	G	G	C	G	G	T	G	G	0.0147	70.24 (70.24 - 70.24)	<0.0001
16	C	C	T	G	A	T	C	C	G	T	C	G	C	A	G	G	G	G	T	G	G	C	G	G	C	A	G	T	A	G	0.0147	-66.56 (-66.56 - -66.56)	<0.0001
17	C	T	G	G	A	T	C	C	G	T	C	G	C	A	G	G	G	A	C	G	G	C	C	G	C	G	G	T	G	A	0.0147	27.94 (27.94 - 27.94)	<0.0001
18	C	T	T	G	A	T	T	C	G	T	C	G	C	A	G	G	G	G	T	G	A	T	G	A	C	G	A	T	G	G	0.0147	154.49 (154.49 - 154.49)	<0.0001
19	C	T	T	G	G	T	T	C	G	G	C	G	C	G	G	G	G	G	C	A	A	T	G	G	T	G	G	T	A	G	0.0147	450.72 (450.72 - 450.72)	<0.0001
20	T	C	G	A	A	T	C	C	G	T	G	G	C	A	G	G	G	G	T	G	G	C	C	G	C	A	G	T	A	G	0.0147	127.74 (127.74 - 127.74)	<0.0001
21	C	T	G	G	A	T	C	C	G	T	C	G	C	A	G	G	G	G	T	G	G	T	C	G	C	G	G	T	G	G	0.0147	149.79 (149.79 - 149.79)	<0.0001

Table 6.2: Association analysis of predicted haplotypes in the ovine MHC class IIb subregion for EBVwec and EBVwecwean using SNPstats. Haplotypes are in order of decreasing frequency and the degree of association is expressed as the odds ratio relative to the most frequent haplotype (highlighted in green).

Haplotype association with EBVwec (n=68)													
	DB.TP2	PB8.TP2	PB9	HB.DMB	DMA.BD2	BD2.DA	RG1	PN6	RL2	TBP	Frequency	Difference (95% CI)	P-value
1	C	C	G	G	A	T	C	T	C	G	0.1157	0	---
2	C	C	G	G	A	T	C	C	G	T	0.1113	10.16 (-39.35 - 59.66)	0.69
3	C	C	G	A	A	T	C	C	G	T	0.1031	67.42 (21.19 - 113.65)	0.0053
4	C	C	G	A	A	T	C	C	C	T	0.0576	-66.58 (-82.33 - -50.83)	<0.0001
5	C	T	G	G	A	T	T	C	G	T	0.0532	63.94 (41.92 - 85.96)	<0.0001
6	C	T	G	G	A	T	T	C	C	T	0.0441	102.38 (88.5 - 116.25)	<0.0001
7	C	T	T	G	A	T	C	T	C	G	0.0394	92.33 (79.59 - 105.07)	<0.0001
8	C	C	G	G	G	A	T	C	G	G	0.0364	30.64 (25.73 - 35.55)	<0.0001
9	C	T	T	G	A	T	C	C	G	T	0.0318	-11.22 (-17.16 - -5.28)	0.00038
10	T	C	G	A	A	T	C	C	G	T	0.0305	-3.83 (-14.76 - 7.11)	0.49
11	T	C	G	A	G	T	C	C	G	G	0.0294	24.44 (5.45 - 43.44)	0.013
12	C	C	T	G	A	T	C	T	C	G	0.029	30.64 (22.42 - 38.85)	<0.0001
13	C	T	G	A	G	T	C	C	G	G	0.0233	55.99 (47.08 - 64.9)	<0.0001
14	C	C	T	G	A	T	C	C	G	T	0.0224	14.8 (9.49 - 20.1)	<0.0001
15	C	C	G	A	A	A	C	C	G	T	0.0221	-31.56 (-38.34 - -24.79)	<0.0001
16	T	C	T	A	A	T	C	C	C	G	0.0221	81.93 (78.53 - 85.33)	<0.0001
17	T	T	G	G	A	A	C	T	C	G	0.0221	-5.26 (-17.62 - 7.1)	0.41
18	C	T	G	G	A	T	C	C	G	T	0.0219	47.51 (41.18 - 53.83)	<0.0001
19	C	T	T	G	G	T	T	C	G	G	0.0208	177.13 (171.72 - 182.54)	<0.0001
20	C	C	G	G	G	A	C	C	G	G	0.0151	130.28 (126.33 - 134.24)	<0.0001
21	C	T	T	G	A	T	T	C	G	T	0.0151	-18.7 (-23.39 - -14.01)	<0.0001
22	C	T	T	G	A	A	C	C	G	G	0.0148	169.03 (164.64 - 173.41)	<0.0001
23	C	T	T	G	G	A	C	T	C	G	0.0147	166.57 (161.9 - 171.25)	<0.0001
24	T	C	T	A	A	T	C	T	C	G	0.0144	195.67 (191.53 - 199.81)	<0.0001

Haplotype association with EBVwecwean (n=68)													
	DB.TP2	PB8.TP2	PB9	HB.DMB	DMA.BD2	BD2.DA	RG1	PN6	RL2	TBP	Frequency	Difference (95% CI)	P-value
1	C	C	G	G	A	T	C	T	C	G	0.1541	0	---
2	C	C	G	G	A	T	C	C	G	T	0.0948	-152.77 (-162.91 - -142.63)	<0.0001
3	C	C	G	A	A	T	C	C	G	T	0.0704	-20.42 (-27.7 - -13.14)	<0.0001
4	C	C	G	A	A	T	C	C	C	T	0.0607	-52.01 (-58.25 - -45.76)	<0.0001
5	C	T	G	G	A	T	T	C	G	T	0.0543	-170.21 (-175.36 - -165.06)	<0.0001
6	C	T	T	G	A	T	C	C	G	T	0.0498	354.55 (349.99 - 359.11)	<0.0001
7	C	T	G	G	A	T	T	C	C	T	0.0413	118.74 (114.86 - 122.61)	<0.0001
8	T	C	G	A	A	T	C	C	G	T	0.0407	96.03 (92.28 - 99.78)	<0.0001
9	C	C	G	G	G	A	T	C	G	G	0.0364	86.57 (82.89 - 90.24)	<0.0001
10	C	C	T	G	A	T	C	T	C	G	0.0302	-443.72 (-446.29 - -441.14)	<0.0001
11	C	C	G	A	A	A	C	C	G	T	0.0294	159.91 (156.93 - 162.9)	<0.0001
12	T	C	G	A	G	T	C	C	G	G	0.0294	143.92 (141.06 - 146.79)	<0.0001
13	C	T	G	G	A	T	C	C	G	T	0.0248	152.18 (149.8 - 154.57)	<0.0001
14	C	T	G	A	G	T	C	C	G	G	0.023	197.13 (194.77 - 199.48)	<0.0001
15	C	T	T	G	A	T	C	T	C	G	0.0225	-202.55 (-204.53 - -200.57)	<0.0001
16	T	C	T	A	A	T	C	C	C	G	0.0221	-123.56 (-125.72 - -121.41)	<0.0001
17	T	T	G	A	A	A	C	C	G	T	0.0221	292.2 (290.22 - 294.19)	<0.0001
18	C	T	T	G	G	T	T	C	G	G	0.0211	221.52 (219.29 - 223.75)	<0.0001
19	C	C	T	G	A	T	C	C	G	T	0.0192	-176.71 (-178.18 - -175.24)	<0.0001
20	C	C	G	G	G	A	C	C	G	G	0.015	-84.07 (-85.48 - -82.67)	<0.0001
21	C	T	T	G	A	A	C	C	G	G	0.0147	106.33 (104.89 - 107.76)	<0.0001
22	C	T	T	G	G	A	C	T	C	G	0.0147	356.26 (354.73 - 357.79)	<0.0001
23	T	C	T	A	A	T	C	T	C	G	0.0138	234.31 (233.02 - 235.61)	<0.0001
24	C	T	T	G	A	T	T	C	G	T	0.0132	166.54 (165.52 - 167.55)	<0.0001

Table 6.3: Association analysis of inferred haplotypes in the ovine MHC class IIa subregion for EBVwec and EBVwecwean using SNPstats. Haplotypes are in order of decreasing frequency and the degree of association is expressed as the odds ratio relative to the most frequent haplotype (highlighted in green).

Haplotype association with EBVwec (n=68)													
	A2A	A3A	A3C	A5B	A6A	A6C	A6B	DRA	BL2	BL2.C10	Frequency	Difference (95% CI)	P-value
1	C	G	C	A	G	G	G	A	C	A	0.1114	0	---
2	G	A	C	A	G	G	G	A	C	A	0.0932	55.39 (12.77 - 98)	0.012
3	C	G	C	A	G	G	A	G	T	G	0.0736	42.63 (-11.8 - 97.06)	0.13
4	C	G	C	G	G	G	G	G	C	A	0.0714	3.31 (-44.21 - 50.82)	0.89
5	C	G	C	G	G	G	G	A	C	A	0.0708	-68.21 (-115.22 - -21.19)	0.0051
6	C	G	C	A	G	G	G	G	T	G	0.0629	-51.98 (-102.37 - -1.6)	0.045
7	G	A	C	A	G	G	G	G	C	A	0.0553	7.28 (-48.77 - 63.34)	0.8
8	G	A	C	A	G	G	G	A	C	G	0.0462	94.44 (43.4 - 145.47)	0.0004
9	C	G	C	A	G	G	G	G	C	G	0.0455	-3.96 (-64.75 - 56.84)	0.9
10	G	A	C	A	G	G	G	G	C	G	0.0447	-33.57 (-85.19 - 18.05)	0.2
11	C	A	C	A	G	G	G	G	T	G	0.0344	73.84 (23.38 - 124.31)	0.0048
12	C	A	C	A	G	G	G	G	C	A	0.032	10.24 (-17.88 - 38.36)	0.48
13	C	G	C	G	G	G	A	G	T	G	0.0316	-14.66 (-54.48 - 25.15)	0.47
14	C	G	T	A	G	A	G	G	T	G	0.0294	0.29 (-39.16 - 39.74)	0.99
15	C	G	C	A	G	G	A	G	C	A	0.0273	93.56 (42.98 - 144.15)	0.0004
16	C	A	C	A	G	G	G	A	C	G	0.0263	81.05 (41.58 - 120.51)	<0.0001
17	C	G	C	A	A	G	G	G	T	G	0.0221	47.67 (9.62 - 85.72)	0.015
18	C	G	C	A	G	G	G	A	C	G	0.0178	139.6 (118.13 - 161.06)	<0.0001
19	G	G	C	A	G	G	A	G	T	G	0.0167	-44.51 (-61.3 - -27.72)	<0.0001
20	G	G	C	A	G	G	G	G	T	G	0.0147	186.45 (172.72 - 200.18)	<0.0001
21	C	A	C	A	G	G	G	A	C	A	0.0138	52.38 (46.55 - 58.21)	<0.0001
22	G	A	C	G	G	G	A	G	C	G	0.0097	59.78 (56.64 - 62.92)	<0.0001
23	G	A	C	A	G	G	G	A	T	G	0.0079	-69.68 (-72.77 - -66.58)	<0.0001

Haplotype association with EBVwecwean (n=68)													
	A2A	A3A	A3C	A5B	A6A	A6C	A6B	DRA	BL2	BL2.C10	Frequency	Difference (95% CI)	P-value
1	C	G	C	A	G	G	G	A	C	A	0.0996	0	---
2	G	A	C	A	G	G	G	A	C	A	0.0875	196.85 (188.58 - 205.12)	<0.0001
3	C	G	C	G	G	G	G	G	C	A	0.084	239.05 (230.98 - 247.11)	<0.0001
4	C	G	C	A	G	G	A	G	T	G	0.08	9.38 (1.54 - 17.23)	0.02
5	G	A	C	A	G	G	G	G	C	A	0.066	55.45 (49.48 - 61.42)	<0.0001
6	C	G	C	G	G	G	G	A	C	A	0.059	-24.96 (-29.62 - -20.31)	<0.0001
7	C	G	C	A	G	G	G	G	T	G	0.0553	143.37 (138.83 - 147.91)	<0.0001
8	G	A	C	A	G	G	G	A	C	G	0.0522	242.51 (237.68 - 247.33)	<0.0001
9	C	G	C	A	G	G	G	G	C	G	0.0516	73.65 (68.85 - 78.44)	<0.0001
10	G	A	C	A	G	G	G	G	C	G	0.0373	-229.6 (-232.5 - -226.7)	<0.0001
11	C	G	C	G	G	G	A	G	T	G	0.0365	437.65 (434.46 - 440.85)	<0.0001
12	C	A	C	A	G	G	G	G	C	A	0.0329	95.78 (93.15 - 98.41)	<0.0001
13	C	G	T	A	G	A	G	G	T	G	0.0294	-166.19 (-168.78 - -163.6)	<0.0001
14	C	A	C	A	G	G	G	G	T	G	0.0294	207.35 (205.3 - 209.4)	<0.0001
15	G	G	C	A	G	G	G	G	T	G	0.0261	286.05 (283.85 - 288.25)	<0.0001
16	C	G	C	A	G	G	A	G	C	A	0.0254	216.36 (214.17 - 218.55)	<0.0001
17	C	A	C	A	G	G	G	A	C	G	0.0245	391.8 (389.88 - 393.72)	<0.0001
18	C	G	C	A	A	G	G	G	T	G	0.0221	269.21 (267.27 - 271.16)	<0.0001
19	C	A	C	A	G	G	G	A	C	A	0.0198	470.53 (469.02 - 472.03)	<0.0001
20	C	G	C	A	G	G	G	A	C	G	0.0181	43.77 (42.54 - 44.99)	<0.0001
21	G	A	C	A	G	G	G	A	T	G	0.018	43.94 (42.59 - 45.29)	<0.0001
22	G	G	C	A	G	G	A	G	T	G	0.008	280.89 (280.23 - 281.55)	<0.0001
23	G	A	C	G	G	G	G	A	G	C	G	0.0074	474.19 (473.63 - 474.76)	<0.0001

Table 6.4: Association analysis of inferred haplotypes in the ovine MHC class III subregion for EBVwec and EBVwecwean using SNPstats. Haplotypes are in order of decreasing frequency and the degree of association is expressed as the odds ratio relative to the most frequent haplotype (green).

Haplotype association with EBVwec (n=68)													
	TXB	CFB	C2	MH5	G6B	BT5	APM	BT2	TNF	LTA	Frequency	Difference (95% CI)	P-value
1	G	T	G	A	C	G	A	T	G	G	0.1324	0	---
2	A	T	G	G	C	G	G	T	G	G	0.1132	-23.44 (-67.32 - 20.44)	0.3
3	G	T	G	G	C	A	G	T	A	G	0.1056	**-64.41 (-107.11 - -21.72)**	0.0041
4	A	C	C	G	C	A	G	T	A	G	0.0999	**-83.66 (-130.1 - -37.21)**	0.0007
5	A	T	G	G	C	A	G	T	A	G	0.081	**-48.75 (-96.42 - -1.09)**	0.048
6	G	C	C	G	C	G	G	T	G	G	0.0615	51.88 (-0.02 - 103.79)	0.054
7	G	C	C	G	C	A	G	T	A	G	0.0425	-18.48 (-88.97 - 52.01)	0.61
8	A	T	G	G	T	G	G	C	G	A	0.0416	14.15 (-57.01 - 85.32)	0.7
9	G	T	G	G	T	G	G	C	G	G	0.0368	**-79.09 (-139.45 - -18.73)**	0.012
10	G	T	G	G	C	G	G	T	G	G	0.0353	2.13 (-18.19 - 22.46)	0.84
11	A	T	G	G	C	G	G	C	G	A	0.0324	8.33 (-14.12 - 30.77)	0.47
12	A	T	G	G	T	G	G	T	A	G	0.0316	-21.38 (-78.33 - 35.57)	0.46
13	G	C	C	G	C	G	G	C	G	A	0.0276	**46.65 (11.11 - 82.19)**	0.012
14	G	C	C	G	C	G	G	T	A	G	0.0241	-19.88 (-66.56 - 26.8)	0.41
15	A	C	C	G	C	A	G	T	G	A	0.0147	**-75.63 (-85.83 - -65.42)**	<0.0001
16	A	T	C	G	T	G	G	T	A	G	0.0147	**154.7 (146.56 - 162.85)**	<0.0001
17	G	T	C	G	C	G	G	T	G	G	0.0144	**-78.26 (-83.8 - -72.71)**	<0.0001
18	G	T	G	G	C	G	G	T	G	A	0.0088	**-147.57 (-151.15 - -143.99)**	<0.0001
19	A	C	C	G	T	G	G	C	G	A	0.0087	**-102.64 (-105.21 - -100.06)**	<0.0001

Haplotype association with response (n=68)													
	TXB	CFB	C2	MH5	G6B	BT5	APM	BT2	TNF	LTA	Frequency	Difference (95% CI)	P-value
1	G	T	G	A	C	G	A	T	G	G	0.114	0	---
2	A	T	G	G	C	G	G	T	G	G	0.1109	NA (NA - NA)	NA
3	G	T	G	G	C	A	G	T	A	G	0.1061	**-223.03 (-232.15 - -213.91)**	<0.0001
4	A	T	G	G	C	A	G	T	A	G	0.0994	**-333.42 (-342.21 - -324.64)**	<0.0001
5	A	C	C	G	C	A	G	T	A	G	0.0954	**-364.42 (-372.99 - -355.84)**	<0.0001
6	G	C	C	G	C	G	G	T	G	G	0.0615	**-59.51 (-64.48 - -54.55)**	<0.0001
7	A	T	G	G	C	G	G	C	G	A	0.0455	-0.39 (-4.21 - 3.43)	0.84
8	G	C	C	G	C	G	G	C	G	A	0.0419	**-326.62 (-330.16 - -323.08)**	<0.0001
9	G	T	G	G	T	G	G	C	G	G	0.0368	-2.25 (-5.43 - 0.92)	0.17
10	A	T	G	G	T	G	G	T	A	G	0.0368	**-369.04 (-371.89 - -366.19)**	<0.0001
11	G	T	G	G	C	G	G	T	G	G	0.0312	**-302.57 (-305.15 - -299.99)**	<0.0001
12	G	C	C	G	C	A	G	T	A	G	0.0308	**-403.53 (-405.82 - -401.23)**	<0.0001
13	A	T	G	G	T	G	G	C	G	A	0.0296	**-550.98 (-553.51 - -548.46)**	<0.0001
14	G	C	C	G	C	G	G	T	A	G	0.0243	**-144.77 (-146.79 - -142.75)**	<0.0001
15	G	T	G	G	C	G	G	T	G	A	0.0147	**-218.33 (-219.56 - -217.1)**	<0.0001
16	G	T	C	G	C	G	G	T	G	G	0.0147	**-355.63 (-356.93 - -354.32)**	<0.0001
17	A	C	C	G	C	A	G	T	G	A	0.0147	**-403.73 (-404.92 - -402.54)**	<0.0001
18	A	T	C	G	T	G	G	T	A	G	0.0147	**-20.73 (-21.92 - -19.53)**	<0.0001
19	G	C	C	A	C	G	A	T	G	G	0.0109	**-578.77 (-579.17 - -578.36)**	<0.0001
20	G	T	C	A	C	G	A	T	G	G	0.0074	**-304.72 (-305.35 - -304.09)**	<0.0001

6.4 Discussion

This chapter analyses the haplotypic composition of the ovine MHC. Fifty four haplotypes, based on Mendelian anlysis of sheep family trios, were identified. Predicted haplotypes were also obtained using the EM algorithm implemented in SNPstats. Finally, haplotypic associations were sought using SNPstats for two phenotypic variables – EBVwec/EBVwecweaning and serum IgA/parasite specific IgA. Three significant results were obtained as shown below.

1. A higher than expected frequency of shared subregion haplotypes within the ovine MHC was observed.

2. A high frequency of recombination was observed between the class IIb and IIa subregions that is consistent with separation of these two subregions.

3. Significant associations between ovine MHC haplotypes with EBVs (WEC and WECweaning) were observed but not for total resting serum IgA levels or with parasite specific resting serum IgA levels

Sheep SNP Haplotypes

The top 10 most frequent MHC haplotypes predicted by SNPstats for the 62 animals in Cohort 1 (with sires excluded) exhibited considerable similarity with the known sire haplotypes (Figure 6.1). Two complete length haplotypes (across all three subregions) were predicted to occur which were also present in the sires. When each of the three subregions was analysed independently in SNPstats, four class IIb haplotypes accounting for 35% of the 124 haplotypes were predicted that were present also in the known sire class IIb haplotypes. The 102 animals from Cohort 2 that were not directly related to the 6 known sires in Cohort 1 were also analysed (Figure 6.3). Similar results were obtained again although the significance of this finding is more difficult to interpret since some sheep would have been half siblings. A further 42 known haplotypes for 21 dams were also compared with the 12 known sire

haplotypes included in Figures 6.1. These are shown in Figure 6.2. Once again subregion haplotypic similarity was evident; 32 haplotypes were unique and the remaining 22 consisted of 9 haplotypes which occurred at least twice. These counts contrast with the 1024 possible haplotypes generated from a panel of 10 SNPs/subregion.

The presence of common class IIb haplotypes, together with similar observations for the class IIa and III subregions, suggests some degree of conservation of these haplotypic regions (Figures 6.1, 6.2 and 6.3). It was also observed that there were more contiguous haplotypes for the class IIa and III subregions than for the IIb and IIa subregions. If the haplotypic blocks in the ovine MHC are confirmed, the internal structure of the ovine MHC will resemble that known to be present in the human MHC. The SNPs used in this study manifested Hardy Weinberg proportions for Cohort 1 and the 62 animals compared were not closely related. Moreover, the Rylington Merino flock was originally established in 1988 using geographically diverse founding sires and dams. However, all sheep were of the Merino breed and the effect observed could be a consequence of some breed related genetic conservation. Comparisons of MHC haplotypes between breeds should resolve this question and perhaps help identify ancestral haplotypes in this species.

The high frequency of recombination between the class IIb and IIa subregions reported in this study (four recombinations in 21 families) provides clear evidence for physical separation of these two regions. The recombination frequency implies a physical separation similar to that of the cattle MHC where the class II region is subdivided into two subregions (IIb and IIa), resulting from a chromosomal inversion. For cattle, the distance between the two subregions is approximately 15 cM (van Eijk *et al*., 1995; Childers *et al*., 2006). Interestingly, no recombinations were observed between the class IIa and III subregions. This absent (or lower) recombination frequency is consistent with these two regions being adjacent as would be expected from the human (Stenzel *et al.*, 2004) and cattle physical maps. The only other

characterisation of ovine interlocus MHC haplotypes was reported by Miltiadou *et al.*, (2005) who studied haplotypic diversity in the MHC class I region; haplotypic conservation was not described. A recent study by Qin (2009), suggested the presence of a region of high LD in the ovine MHC at the TNF end of the class III region. The findings of the current study confirm the observations of Qin (2009). This region is also similar to a haplotypic block in the human MHC class III region (Dawkins *et al*., 1983).

Haplotype Associations with Phenotype

The availability of EBVs for WEC and WECweaning for cohort 1 animals permitted a search for ovine MHC haplotypes associated with these phenotypic variables that identify parasite resistant sheep. From a theoretical viewpoint, the use of haplotypes rather than single loci is preferred for associations in chromosomal regions manifesting high LD. However, identification of haplotypes continue to be a significant problem. Haplotype association analysis was performed for Cohort 1 using SNPstats (Tables 6.1, 6.2, 6.3 and 6.4). Eighteen and 21 haplotypes across all three subregions were predicted by SNPstats to be significantly associated with the EBVwec and EBVwecweaning variables respectively. When analysed individually, all three subregions also manifested significant associations with the two variables.

In comparison, no significant associations were predicted by SNPstats for the resting IgA levels available for sheep in Cohort 2 (described in Chapter 4). The latter observation contrasts with previous work by Davies *et al*., (2006) who have shown the presence of QTLs in the ovine MHC region for parasite resistance, as well as for IgA levels. However comparisons between such studies are difficult due to differing species of parasite and differences in the density and nature of the markers utilised in the work.

In summary, the results described in this chapter provide strong evidence for the existence of haplotypic blocks for each of the three MHC subregions studied, with consequent similarity to the human MHC. This

result has important implications for subsequent disease/productivity gene mapping. Strong evidence for the separation of the class IIb and IIa subregions in sheep based on recombination within haplotypes is also described. Finally, significant associations between phenotypic variables predicting parasite resistance and MHC haplotypes were detected but the extent to which these are real and/or useful is not clear and requires further study in larger cohorts of sheep and different breeds.

Chapter 7
General Discussion and Concluding Remarks

The aims of this project, as initially proposed in Chapter 1, have been achieved with the following main outcomes:

1. Target loci, including known genes, within the ovine MHC class II region were identified and 102 SNPs were discovered. A subset of these was used to generate haplotypes by deduction from sheep pedigrees and by inference using the EM algorithm. Recombination within the class II region showed that is subdivided into two subregions as occurs in the class II region of cattle. Comparisons of haplotypes showed similarities between each of three subregions of the ovine MHC (class IIb, class IIa and class III) that is consistent with conserved 'blocks' as is known to occur within the human MHC.

2. A segment characterised by low heterozygosity within the class IIa subregion was discovered that appears conserved between humans and sheep and may reflect an as yet unknown biological function.

3. Total and parasite-specific serum IgA levels in post weaning sheep not under deliberate challenge with gastrointestinal parasites was a relatively poor predictor of resistance or susceptibility to parasite infestation, although some evidence for the previously reported role for IgA in adaptive immunity to parasites was obtained.

4. Evidence for MHC haplotypic associations and EBVs based on WEC as a measure of parasite resistance was obtained but not for resting serum IgA levels (both total and parasite-specific).

The results supporting the first main outcome all contribute to a better understanding of the internal structure of the ovine MHC, especially the class II region. Over the past decade there has been a more general appreciation that genomic DNA conceals an internal structure manifested by regions of conserved sequence or blocks separated by subregions within which recombination is more frequent. This phenomenon was originally discovered by tissue typing of the human MHC and provided strong evidence for the presence of ancestral MHC haplotypes reflecting the evolutionary origins of this gene complex that controls adaptive immune responses in vertebrates. The presence of haplotypic blocks within genomes has important consequences for constructing haplotype maps for inherited traits and for understanding the evolutionary role of recombination. Recently, van Oosterhout (2009) has proposed that haplotypic blocks resulting from low frequencies of recombination conceal deleterious recessive mutations thereby adding to balancing selection and thus reinforcing epistatic selection against recombinants.

Proof of conserved haplotypic blocks within the MHC which recombine is relatively rare requires genotypic and haplotypic data for many individuals within a species, preferably across geographical or breed like populations. Despite the vast number of HLA genotypes generated by many international histocompatibility workshops over three or more decades, identification of conserved blocks and their evolutionary significance was only accepted slowly. The concept is now widely accepted following several landmark studies, for example Gabriel *et al*., (2002). In general terms, sophisticated statistical analysis of SNP data is required to prove the existence of conserved haplotypes and several methods have been published (Patil *et al*., 2001; Zhang *et al*., 2002). Two results from this project indicate the present of haplotype blocks in Merino sheep. These are the co-occurrence of subregion haplotypes (both deduced and predicted) in sheep from the Rylington Merino Project and the presence of extended sequence segments with high LD thereby implying infrequent internal recombination. The loci used in this study were not mapped sufficiently accurately to permit delineation of the putative boundaries of the blocks described and financial constraints

restricted the number of SNPs that could be genotyped across the cohorts available for this project. Future studies using more extensive SNP typing across both independent sheep populations and other breeds should reveal the internal block structure and their evolutionary history.

An original objective of this project, to identify the putative break point between the sheep class IIa and IIb subregions, would have required considerably more sequencing work and was not pursued due to both resource and time constraints. However, frequent recombination (≈20%) between the two panels of 10 SNPs characteristic of the class II subregions provided strong evidence for a physical separation of these subregions even if their boundaries could not be identified. It is interesting that while the Overgo primer method was successful in a cognate project for the sheep class III region (Qin, 2009) and for the putative class IIb subregion, it did not deliver similar success with the putative class IIa subregion. This may be explained by increased variation in this subregion with a consequent decrease in primer specificity for sheep loci. This problem was obviated to a large extent by the publication of the sheep virtual genome map in 2007 and the complete sequence of a BAC clone containing a major portion of the MHC class IIa region in late 2008. However these latter reports were only available relatively late in the course of this project.

Twenty two of the 103 SNPs discovered were intra-genic with the remainder being inter-genic. Identification of SNPs by comparison of generated sequence with sequences published in the sheep genome project was not attempted because this approach may have led to the inclusion of sequencing errors present in the non-annotated sheep genome project or breed specific polymorphisms. The approximate relative positions of discovered SNPs were deduced by comparison with a published complete sequence of the sheep IIa region in a BAC, and synteny with the published sequence of the cattle class IIb region and to a lesser extent with synteny for the human class II region.

As has been observed in the human MHC, SNP heterozygosity varied across the ovine MHC. A subregion of ≈100 kbp that included the A3C,

A5B, A6A and A6C loci manifested low levels of heterozygosity in both sheep cohorts studied. Furthermore this region coincided approximately with a region of significantly low LD. It is hypothesised that this region contains a likely recombination hotspot in sheep and marks the boundary of two adjacent conserved blocks of loci. It is of interest also that the decline in LD occurring at the DQB2 end of this region coincides with the presence of a recombination hotspot in the human class II region identified by Jeffreys *et al*., (2001). Arnheim *et al*., (2007) have reviewed the distribution and properties of recombination hotspots in the human genome and reports an average size of about 1.5 kbp. If the putative hotspot in the sheep class IIa region is of similar size, then it must constitute only a portion of the ≈100 kbp described above. Clearly, this question will only be resolved when the functions of the sequenced region are analysed further. It will be important that future comparative studies be performed, especially in other ungulates.

Gastrointestinal parasite resistance status in sheep is usually assessed by means of faecal worm egg counts (WEC), which are expensive to measure and have a large variance. Hence, the availability of an easier alternative measure of parasite resistance would be of considerable advantage to the industry. Since it has been shown that serum IgA levels in sheep following challenge with parasites are elevated and negatively correlated with worm length. Therefore by positive correlation, IgA may be associated with worm fecundity (Stear *et al*., 1995; Amarante *et al*., 2005). Resting serum IgA levels being alternative predictors of parasite resistance was considered a possibility. In this project both resting serum total IgA and parasite-specific IgA levels were measured for sheep for which WEC data were available. While a biologically significant negative trend was observed for total IgA and parasite-specific IgA with WEC, no statistically significant correlations between resting WEC and serum total IgA or parasite-specific IgA were observed. Despite the lack of significant correlations, a general linear model predicted that approximately 25% of WEC variation could be significantly attributed to the effects of family groups and parasite-specific IgA. The latter results are consistent with mucosal IgAs having a

role in parasite immunity. Therefore resting serum IgA levels do not offer an alternative, less expensive, method for predicting parasite resistance/susceptibility in sheep.

Haplotype association studies with EBVwec and EBVwecwean using SNPstats predicted 18 and 21 haplotypes respectively that are significantly associated with the corresponding responses. In addition, 10 identical haplotypes were predicted to be associated with both EBVwec and EBVwecwean. This study is the first to provide haplotype association analysis against parasite resistance in sheep across the MHC class II and III region. However, the significance of this haplotype association study requires further investigation to determine the usefulness of these predicted haplotypes as predictors of parasite resistance or susceptibility in sheep. In general terms, if the ovine MHC does consist of conserved haplotypic blocks, association studies with individual SNP genotypes will not advance the identification of disease candidate loci but will minimize the work required to identify the haplotypic region within which the candidate locus resides.

The characterisation of the ovine MHC is an important prelude to further understanding of the adaptive immune response in this economically important species, as well as with the mapping of candidate genes associated with parasite immunity and productivity traits. The identification of ovine MHC haplotypes will provide an important tool for further studies focussed on applications of marker-assisted selection for disease resistance and productivity. However, such studies in sheep are relatively more difficult than equivalent studies in humans due to the lack of information and understanding of the sheep genome as well as the usual lack of well defined pedigrees in sheep flocks. Sheep are often bred using a form of line breeding, entailing non-random mating, and as a result, are more related than for example humans. One way of addressing this problem would be to compare different breeds of sheep, or flocks from different geographical locations that could be expected to be genetically distinct.

In reviewing this project it is clear that further progress will depend on the biological and financial resources available to the sheep industry in Australia and overseas. More extensive panels of SNPs need to be identified to permit higher density LD and physical mapping of the ovine MHC and identification of candidate loci associated with parasite resistance. The parameters used to define parasite resistant animals are relatively laborious to measure and imprecise. In addition, the investigation of IgA and parasite resistance would be better suited to sampling intervals corresponding to WEC measurements. Finally, the sheep populations available for study are limited in number and sometimes lack sufficient background databases of phenotypic and productivity traits. The Rylington Merino flock is an invaluable resource for research of this nature and the high cost of creating and maintaining these animals limits the rate of progress in this field. Progress may be increased by the comparison of distinct breeds of sheep (and even closely related ungulates) with different natural resistance to gastrointestinal parasites.

At the conclusion of this project it is useful to review the underlying hypotheses in context. There is no doubt that genetic association analysis based on polymorphic markers has identified many genes causally linked to disease susceptibility and manifestation as well as other traits. However it is worth keeping in mind that such studies have not always followed a predictable path. For example, the presence of the HLA-B27 MHC class I allele in humans diagnosed with ankylosing spondylitis has an odds ratio in excess of 90 and is the strongest such association recorded to date. Despite many research programs seeking to explain this association, to date its rationale remains elusive. There is a lesson to be learned here. In my opinion, association studies of MHC related diseases should acknowledge the polygenic nature of most disease and productivity traits, and seek out combinations of genetic markers that define combinations each with more moderate odds ratios. Some of these combinations may define haplotypes that may well permit elucidation of the underlying mechanisms that result in the observed

association(s). That is one reason why one of the main objectives of this project was the detection of MHC extended haplotypes. Hence in my view, although strong associations are desirable, reproducible associations of more modest magnitude in distinct populations of animals will also be important outcomes with practical applications.

Prior to the commencement of this study, little was known about the ovine MHC class II region. This work is the first to identify and use SNP genotyping for the class II region of the ovine MHC. Furthermore SNP based haplotyping can complement existing studies of polymorphisms of classical class II genes. The work reported in this thesis has resulted in further significant knowledge of the composition and structure of the ovine MHC class II region. Further studies based on more complete sequence analysis will of course reveal species-specific or even breed-specific differences as well as more accurate mapping of the putative chromosomal inversion. It is hoped this information will provide tangible benefits to those who derive their livelihood from this industry and also contribute to a more complete understanding of the evolution in mammals of this fascinating gene complex.

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Appendix

3A.1: GenBank submissions

GenBank: GQ867665.1

Ovis aries prefoldin 6 (PFDN6) gene, complete cds

Features Sequence

```
LOCUS       GQ867665               1359 bp    DNA     linear   MAM 04-OCT-2009
DEFINITION  Ovis aries prefoldin 6 (PFDN6) gene, complete cds.
ACCESSION   GQ867665
VERSION     GQ867665.1  GI:260081546
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 1359)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine PFDN6 gene
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1359)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (03-SEP-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
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                     /gene="PFDN6"
                     /product="prefoldin 6"
     CDS             join(272..335,576..646,725..849,1100..1229)
                     /gene="PFDN6"
                     /note="subunit of prefoldin"
                     /codon_start=1
                     /product="prefoldin 6"
                     /protein_id="ACX31202.1"
                     /db_xref="GI:260081547"
                     /translation="MAELIQKKLQGEVEKYQQLQKDLSKSMSGRQKLEAQLTENNIVK
                     EELALLDGSNVVFKLLGPVLVKQELGEARATVGKRLDYITAEIKRYESQLRDLEQQSE
                     QQRETLAQLQQEFQRAQAAKAGAPGKA"
ORIGIN
        1 ccagcattct ctatttactt ccgggttcac caatactgaa gtgagagcgt gagtagggga
       61 gcggttcggc tgaggagttc ggggtactgc cactccctga ggctgggggt gagtcgatat
      121 cccggaccag gagagactgc agtgggtgcc ccgaggggct gcctctcaag agggctgtca
      181 cctccgcagg ccagaaaggg gagcccgggt accttccaga gcgtgagacc cagcgccct
      241 ctcccgctgc ccgcaggctt tcgtccccgc catggctgag ctaatccaga agaagctgca
      301 gggggaagtg gagaaatatc aacagctgca gaagggtaag ggagcagggt aggggtgacc
      361 tcgcggcaat tcgctcacaa cctgtctata cccgaataat gtattctgtc ccacctctgg
      421 ccccagcctg cggctccttc cccttctctg tagtcttccc attccctgtg ccgcctgctc
      481 atccttgcct gcttcctcag ctccgccttc ttaccaggac tgtggtggtc gggtggcaca
      541 tgagatgtct ctaaacaaac cttttcatcc ggcagacttg agtaaatcca tgtcggggag
      601 gcagaaacta gaggcacaac taacagaaaa taatatcgtg aaggaggtga gagactgaga
      661 tttgtggggc gaggggggac ctgtcttgaa agtgattctg atgtttttcc cactcctcca
      721 ccaggaactg gccctgctgg atgggtccaa cgtggtgttt aaacttctgg ggcccgtgtt
      781 ggtcaaacag gagctggggg aagctcgggc cacagtgggg aaaaggctgg actacatcac
      841 agctgagatg tgagtcttcc ttccacccca ctgtgttata cttgatccgt tgggaataaa
      901 ggtgctgaga aacctttcta gagtcattct ccagagcagc ccccatttga gcaggttctt
      961 caactccttc ctgtgtcctt caactccttc ccttttcttc atactcccct tagagctcaa
     1021 gtttctgacc aatcttgtct tcagcaccct caagagtaag tctcactaat ttctcatttg
     1081 ctttttgcc cttcctcagt aagcggtatg aatcccagct ccgagaccta gagcagcagt
     1141 cagaacaaca gagggagacc ctggctcagc tgcagcagga gtttcagagg gcgcaggcgg
     1201 caaaggcagg ggctcctggg aaagcctgac cctgtcctgg gggggtggg cgggggagg
     1261 aatgcggcag ctctagggtc tatactgtag ctaataaaat gtgaaaatac ctggcgtgtt
     1321 ctgaccagcc acttctgttg taactgtctc catcctttc
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GenBank: GU056180.1

Ovis aries WDR repeat domain 46 gene, partial cds

Features Sequence

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LOCUS       GU056180                886 bp    DNA     linear   MAM 10-NOV-2009
DEFINITION  Ovis aries WDR repeat domain 46 gene, partial cds.
ACCESSION   GU056180
VERSION     GU056180.1  GI:262478677
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 886)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine WDR46 gene
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 886)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-OCT-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
     source          1..886
                     /organism="Ovis aries"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9940"
     mRNA            join(<40..108,208..402,512..592,726..>838)
                     /product="WDR repeat domain 46"
     CDS             join(40..108,208..402,512..592,726..>838)
                     /note="WDR46"
                     /codon_start=1
                     /product="WDR repeat domain 46"
                     /protein_id="ACY68387.1"
                     /db_xref="GI:262478678"
                     /translation="METVPKPGSNVPPKKDRLQAKKKKPRRYWEQETTGTAAGASPGP
                     PHKRKRSRELRPQKPKRNDTSKKFRISKKPRELKSPGPQRTFSGAQDPFAGPAPVSVE
                     VVRKYCRIDKSKELPHAKTKTRSRLAVAEAQEEETSVRAARSELLLAEEPG"
     variation       357
                     /note="SNP"
                     /replace="a"
ORIGIN
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       61 ggcagtaatg ttccacccaa gaaggacaga ctgcaagcca agaagaaggt agggatctcc
      121 ttggggtgga aaggaagttt ttatttgcgg ggttcgaggg gcggggcgaa gggcagcgga
      181 atacgaggga tcctgctctg ttggtagaaa ccgcggcgat actgggagca agagaccact
      241 gggactgcgg ccggagcttc tccaggtccc cctcataaac ggaagaggag tcgagagctc
      301 cgcccccaga agccaaagag aaatgacacc tcaaagaagt ttcggatctc caagaagccc
      361 cgagaactga agagtccggg gcctcagcgg acctttctg gggtgagcct gggacctccc
      421 ggggtgccga ggcaagtcgc cttgtgcttt cagggtcggg gggttggggt cagctcgagc
      481 ctgtttctta attgcctcct ccctgccgca ggcccaggac ccatttgcag gccccgcccc
      541 tgtcagtgtg gaggtggttc ggaagtactg tcgcattgac aaatccaagg aggtgagggc
      601 tagtcggaga gtggggagcg gttgcaggag ggagtgtctg ggtgagttga atgggaggcg
      661 gggctctcct gtgacccccg catcccttgg tccttctctc acttcatctc tcaccgctgt
      721 tttagctacc acatgcgaag accaagacac gaagccgact tgcggtggct gaagcacaag
      781 aagaggaaac aagtgtcaga gctgctcgat ctgagcttct gcttgctgaa gaacctgggt
      841 gagtggaccc tgatgtgggc ctccccaatc cctgatttat ggggct
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GenBank: GQ131519.1

Ovis aries butyrophilin-like 2 (BTNL2) gene, partial sequence

Features Sequence

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LOCUS       GQ131519                403 bp    DNA     linear   MAM 17-JUN-2009
DEFINITION  Ovis aries butyrophilin-like 2 (BTNL2) gene, partial sequence.
ACCESSION   GQ131519
VERSION     GQ131519.1  GI:239912256
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 403)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine MHC class IIa region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 403)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-MAY-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
     source          1..403
                     /organism="Ovis aries"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9940"
                     /chromosome="20"
     gene            <1..>403
                     /gene="BTNL2"
                     /note="butyrophilin-like 2; coding region not determined"
     variation       64
                     /gene="BTNL2"
                     /note="SNP"
                     /replace="t"
     variation       121
                     /gene="BTNL2"
                     /note="SNP"
                     /allele="a"
     variation       223
                     /gene="BTNL2"
                     /note="SNP"
                     /replace="t"
ORIGIN
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       61 ctccgctgcc tgaagatgtc tctagggcaa gttagaatct cccttgacta cacctctgga
      121 ggcctctcct tttataactt gaacaagtgc cctatttatg gtgcatgagt gctcggctgt
      181 aaattttagc gctgtgccac ctgggaagcc ttatctatac ttccccagtt ttcttatctg
      241 aaattctgca ccccccttctt tctttgatct ccgagtgtag gttcttctga atctttcctg
      301 cacccaagga ttctcagcca cacctccaag gagaacaatt accgcattct ggaagccagg
      361 gttatattct aagtcccagt tcttttctct tttcaccaac tac
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GenBank: GQ131518.1

Ovis aries MHC class IIb region between HKE6 and RING1 genomic sequence

Features Sequence

```
LOCUS       GQ131518                501 bp    DNA     linear   MAM 17-JUN-2009
DEFINITION  Ovis aries MHC class IIb region between HKE6 and RING1 genomic
            sequence.
ACCESSION   GQ131518
VERSION     GQ131518.1  GI:239912255
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 501)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine MHC class IIb region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 501)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-MAY-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
     source          1..501
                     /organism="Ovis aries"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9940"
                     /chromosome="20"
                     /map="between HKE6 and RING1"
     misc_feature    1..501
                     /note="MHC class IIb region between HKE6 and RING1"
     variation       44
                     /note="SNP"
                     /replace="g"
     variation       99
                     /note="SNP"
                     /replace="c"
     variation       146
                     /note="SNP"
                     /replace="t"
ORIGIN
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       61 aacttactgc ctttcactag acggctccat ccgcagactc ccttctcaca gccgcttttc
      121 cacttttggc ccctttttcca cccagcttct cactccctta ccctgctttg gaatcacttt
      181 tcacatttag ctcgtttaat cctcaaaact gacgtgccag agaggtagag ccaagtatta
      241 ttgtcttcac ttgaaagata gtaaacaggc tgaaatgaac ttccctggtc ttcagttccg
      301 catcataagt gtgtgcattt aagactcccc ttgggagtgc attaaaaatg gaattctggg
      361 ccggatctca cacacacacc gtccccacac acacacacta acagtaatgg gatgggaccc
      421 cagactctgc atttgtagca tgcaccataa gggattctga tataggcagt cccaggaccg
      481 cactggaaga aattctgttg a
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GenBank: GQ131517.1

Ovis aries MHC class IIb region between TAP2 and PSMB8 genomic sequence

Features Sequence

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LOCUS       GQ131517                670 bp    DNA     linear   MAM 17-JUN-2009
DEFINITION  Ovis aries MHC class IIb region between TAP2 and PSMB8 genomic
            sequence.
ACCESSION   GQ131517
VERSION     GQ131517.1  GI:239912254
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 670)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine MHC class IIb region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 670)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-MAY-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
     source          1..670
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                     /mol_type="genomic DNA"
                     /db_xref="taxon:9940"
                     /chromosome="20"
                     /map="between TAP2 and PSMB8"
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     variation       307
                     /note="SNP"
                     /replace="t"
     variation       322
                     /note="SNP"
                     /replace="t"
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       61 ttcatctccg cggggctatt tgaggtaaat ggggctgtgt tcacaattcc cgcacacatg
      121 tgaggaggaa acgtgttcct cccattctaa cgcagttaaa gacaggtgtt ccaagggccc
      181 tcctccaaca tatccaaacc attaacaagc ggccacacaa gagcctcctg gttgagagcc
      241 cgcagaaagg accctaacct aggctaaact gcgcacgggt gtccacagac aacacggagc
      301 cttccagcgg tcctggagga acgagagtgg gagcccagga gaaggatttg gtgagcccca
      361 tttcttaagc aggggtttct ctcgtctcag gaaaaatctg tttctacttc ctgtcccctc
      421 cccccagcca ccccagccct gagcccacag ccacaacaca cagcttttat aaccgttttc
      481 ctttattgtg cttagcgggg catccaggag gttggggtgg gggtgacgtt cattgagaca
      541 gagaatacac aaagggataa actctgtgct gccttccagt ggccactggt tctccatctc
      601 tgggccaggt caggccgcct tctcccagag tgcgatccgg cccaggtccc tgagtcagcc
      661 aggcttcccc
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GenBank: GQ131516.1

Ovis aries proteasome subunit beta type 9 (PSMB9) gene, partial sequence

Features Sequence

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LOCUS       GQ131516                586 bp    DNA     linear   MAM 17-JUN-2009
DEFINITION  Ovis aries proteasome subunit beta type 9 (PSMB9) gene, partial
            sequence.
ACCESSION   GQ131516
VERSION     GQ131516.1  GI:239912253
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 586)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine MHC class IIb region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 586)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-MAY-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
     source          1..586
                     /organism="Ovis aries"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9940"
                     /chromosome="20"
     gene            <1..>586
                     /gene="PSMB9"
                     /note="proteasome subunit beta type 9; coding region not
                     determined"
     variation       43
                     /gene="PSMB9"
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                     /replace="t"
     variation       123
                     /gene="PSMB9"
                     /note="SNP"
                     /replace="a"
     variation       202
                     /gene="PSMB9"
                     /note="SNP"
                     /replace="t"
     variation       237
                     /gene="PSMB9"
                     /note="SNP"
                     /replace="t"
ORIGIN
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       61 catatagcac agggaactta ttcaaaattc tgtgataaac cataatggaa aagaatatga
      121 aagaaagtat atatgtataa ctgaatcaac tttgctatac agtagaaatt aacacagcac
      181 tgcaaatcaa cgatacttaa agttttttta attggcaact ctgatgctga tgacttcgca
      241 ctgcttgctg acagggaggc ggtggtgaac cgagtgtttg acaagctctc ccccctgcac
      301 cagcgcatct actgcgctct ctccggctca gctgctgacg ctcaggccat agcggacatg
      361 gccgcctacc agctggagct ccatgggtat gaaatggtgg gctcccccgt ccaggccgcc
      421 tagaactccc ccactcagtg aacgccaaca ccgtgtccta ttccaggggc gctaccgtgg
      481 aaaaggaaaa cttcttgctt tgccttcagc tttcatttct ctttgaactg gcactcgact
      541 ctctcaaagc tggtttctgc atctgtaaag cagagataac taatag
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GenBank: GQ131515.1

Ovis aries MHC class IIa region between BTNL2 and C6orf10 genomic sequence

Features Sequence

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LOCUS       GQ131515                571 bp    DNA     linear   MAM 17-JUN-2009
DEFINITION  Ovis aries MHC class IIa region between BTNL2 and C6orf10 genomic
            sequence.
ACCESSION   GQ131515
VERSION     GQ131515.1  GI:239912252
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 571)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine MHC class IIa region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 571)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-MAY-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
     source          1..571
                     /organism="Ovis aries"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9940"
                     /chromosome="20"
                     /map="between BTNL2 and C6orf10"
     misc_feature    1..571
                     /note="MHC class IIa region between BTNL2 and C6orf10"
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                     /replace="c"
     variation       72
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                     /replace="a"
     variation       154
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                     /replace="a"
     variation       162
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                     /replace="t"
     variation       182
                     /note="SNP"
                     /replace="t"
     variation       186
                     /note="SNP"
                     /replace="t"
     variation       205
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                     /replace="t"
     variation       220
                     /note="SNP"
                     /replace="a"
     variation       277
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                     /replace="a"
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     variation       363
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                     /replace="t"
     variation       449
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                     /replace="t"
     variation       535
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                     /replace="a"
     variation       541
                     /note="SNP"
                     /replace="a"
     variation       562
                     /note="SNP"
                     /replace="c"
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      121 gagacagcga agagagaggg ctggtttgta gaagctccag agaagaattt ttgggttttg
      181 ggacacgagg aaggaaaagt cataattccc aactcccagg aaaactctcc atcacttagg
      241 cagcagcccc acaggatagg agttttcctg gactgggaag ctgggaacct gtccttttat
      301 aacatggccg acgggtccca catctattct ttcactggca tcaccttctg tgggaccctt
      361 ttcccttatt ttagccttcg gggtactggc gcatctttga ccatctgctc aacttcagat
      421 caccctgaga attgtcctga ttcttctcca aagacctctc taactcattt aagtagttgt
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      541 gctgcctctg ctcaggcttc ttacaggtat g
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GenBank: GQ131514.1

Ovis aries ral guanine nucleotide dissociation stimulator-like 2 (RGL2) gene, partial sequence

Features Sequence

```
LOCUS       GQ131514                720 bp    DNA     linear   MAM 17-JUN-2009
DEFINITION  Ovis aries ral guanine nucleotide dissociation stimulator-like 2
            (RGL2) gene, partial sequence.
ACCESSION   GQ131514
VERSION     GQ131514.1  GI:239912251
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 720)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine MHC class IIb region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 720)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-MAY-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
     source          1..720
                     /organism="Ovis aries"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9940"
                     /chromosome="20"
     gene            <1..>720
                     /gene="RGL2"
                     /note="ral guanine nucleotide dissociation stimulator-like
                     2; coding region not determined"
     variation       260
                     /gene="RGL2"
                     /note="SNP"
                     /replace="g"
     variation       680
                     /gene="RGL2"
                     /note="SNP"
                     /replace="a"
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       61 ccaagggcca aggaaaggcc tggaggaggg gaagaggaaa agcctagagg ctctgagtcc
      121 cgggggaagg gaatggcaag aaacagtttc tcgtgggagc ctcctcttcc tactccggag
      181 gttcctgtca ctgtgacaac actaataaac cagacactac ctgagctcta ctcctgccct
      241 caggctgcgg actagctgcc gggggcgggg agctggctga aggcagatcc cgtgggaaaa
      301 ggtgggccaa ccgcatctat cttcaaaagc tggatgcaag ggcttggtgg gaacagtttc
      361 ctcagaatgg tttatgcctc cccagcaacc tccccacccc tggtcctgtt attggctctt
      421 tcgtttttct cctcagttgt tacacaccca cgtcaagtac ccaattaaag ccatcagtat
      481 gaaaaacacc cttggctctg aattcctaag acaatgttag gacaaagaaa tggaagaacc
      541 acttcgtgga caatgtacaa acttggaggt ctcagaatat cacaatgcat tatgtgctgg
      601 gatatgaaat ccaatttaag ctacatttaa acaaagtcta ggaagaaaag agactaaaaa
      661 gaactaagat gccagggctg ttctgaggat gacaagaaat catagcaaaa gaggattacc
//
```

GenBank: GQ131513.1

Ovis aries MHC Ovar-DR-alpha (DRA) gene, partial sequence

Features Sequence

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LOCUS       GQ131513                378 bp    DNA     linear   MAM 17-JUN-2009
DEFINITION  Ovis aries MHC Ovar-DR-alpha (DRA) gene, partial sequence.
ACCESSION   GQ131513
VERSION     GQ131513.1  GI:239912250
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 378)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine MHC class IIa region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 378)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-MAY-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
     source          1..378
                     /organism="Ovis aries"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9940"
                     /chromosome="20"
     gene            complement(<1..>378)
                     /gene="DRA"
                     /note="MHC Ovar-DR-alpha; coding region not determined"
     variation       175
                     /gene="DRA"
                     /replace="a"
ORIGIN
        1 ggcatcttac tctcagcgta agtaaaggac tgggaaatgc tgaagcatgg ttgtcttcag
       61 tgaggtgaac agttggagca ttgccaggtt tgtaaagcta ttaaggcaga aatatcttca
      121 ttgatcttct ctctaacaaa gtccgttacc tgtagcagaa cacacgtagt tactgtagcg
      181 tttaagatga atattttgag ctcttatctt cagattcctt actcttactc cttaacccct
      241 gactttggaa aagcctctta atatttgtgc gacctctcat aaatgcaacc tttccaacat
      301 gaactgctat ttcctacctt ccctgaaaga atgagggatc aggccatgtt tcactttctc
      361 ttagatcttc tgacaccc
//
```

GenBank: GQ131512.1

Ovis aries MHC class IIa region between DQA1 and DRB1 genomic sequence

Features Sequence

```
LOCUS       GQ131512                686 bp    DNA     linear   MAM 17-JUN-2009
DEFINITION  Ovis aries MHC class IIa region between DQA1 and DRB1 genomic
            sequence.
ACCESSION   GQ131512
VERSION     GQ131512.1  GI:239912249
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 686)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine MHC class IIa region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 686)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-MAY-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
     source          1..686
                     /organism="Ovis aries"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9940"
                     /chromosome="20"
                     /map="between DQA1 and DRB1"
     misc_feature    1..686
                     /note="MHC class IIa region between DQA1 and DRB1"
     variation       228
                     /note="SNP"
                     /replace="a"
ORIGIN
        1 ggtatgcact ccagtgaatt gttcatacag gaacataaca ataaattgtt ttggatggtg
       61 tgaagtcaca tagctataag atcatatgat agatagataa aaagaataca gtcaagacta
      121 gagatagtgt aattaagaaa agcagtagca ctgaaggaat tgtagacagc taaacacttc
      181 tggtgtttca ttcatgttct gaaaggaaaa taaaccaact gctatgtgtt ttgcttggat
      241 ggcagaaaca atctggtagg actgattctt gtcatgcttt tgcatttccc atagcttaga
      301 atttttattc agtaaaaagc aacaagtatt tgtcattcct ggagaagctt attttgaata
      361 ttcattatgt tatgttctgc tatgattatt aaaaggcatt tttctcttgt tattgatgta
      421 ggcatggttt agatgatttt tcaaattaac tggccatttc cttcttattt taacagttta
      481 ctgaacttct ttctcttctg aaagtttttt tcaaggtcag attccaaatt ctgaattaca
      541 gagattgaca agcatttttg cacctggatc acctttagca tctctggatg ggtattaaat
      601 agcaccaagt tttcctcctt taccttgctt gtgtaacata tgaaatgaac atatgactta
      661 aaagtggatg ctagcaataa ttactg
//
```

GenBank: GQ131511.1

Ovis aries MHC class IIb region between H2B and DMB genomic sequence

Features Sequence

```
LOCUS       GQ131511                434 bp    DNA     linear   MAM 17-JUN-2009
DEFINITION  Ovis aries MHC class IIb region between H2B and DMB genomic
            sequence.
ACCESSION   GQ131511
VERSION     GQ131511.1  GI:239912248
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 434)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine MHC class IIb region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 434)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-MAY-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
     source          1..434
                     /organism="Ovis aries"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9940"
                     /chromosome="20"
                     /map="between H2B and DMB"
     misc feature    1..434
                     /note="MHC class IIb region between H2B and DMB"
     variation       107
                     /note="SNP"
                     /replace="a"
     variation       150
                     /note="SNP"
                     /replace="t"
     variation       151
                     /note="SNP"
                     /replace="t"
     variation       184
                     /note="SNP"
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     variation       196
                     /note="SNP"
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     variation       202
                     /note="SNP"
                     /replace="t"
     variation       289
                     /note="SNP"
                     /replace="a"
     variation       423
                     /note="SNP"
                     /replace="a"
ORIGIN
        1 gccttggttt gtgcaatagt tgcttctctt atacgttacc ctctgagcct cacacccaca
       61 tctgtcacct taggaagggt tacttaggaa ggagggatgt gggcaaggga agctcttatc
      121 agaccggggc aatcaggagc catccaaggc gccttaagca ggaccagagt tccagccaag
      181 agagaatcag aagccttctc ccttcctcta ctcaaaaccc attttgctac cttcttgtaa
      241 tcatggacac ggttgctcca taattcaaac tagtaaagaa atttaaggga aaaaaaaccc
      301 taagttgaac gtcttctgaa tatcatattt ctaactgaaa tgtcagtgat cagaaatatc
      361 tccacacaca tgggataaat agtattgtag atatttattc tatacatagt cctgaaagta
      421 aatatattaa cttc
//
```

GenBank: GQ131510.1

Ovis aries MHC class IIa region between DRB1 and DRA genomic sequence

Features Sequence

```
LOCUS       GQ131510                606 bp    DNA     linear   MAM 17-JUN-2009
DEFINITION  Ovis aries MHC class IIa region between DRB1 and DRA genomic
            sequence.
ACCESSION   GQ131510
VERSION     GQ131510.1  GI:239912247
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 606)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine MHC class IIa region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 606)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-MAY-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
     source          1..606
                     /organism="Ovis aries"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9940"
                     /chromosome="20"
                     /map="between DRB1 and DRA"
     misc_feature    1..606
                     /note="MHC class IIa region between DRB1 and DRA"
     variation       94
                     /note="SNP"
                     /replace="c"
     variation       97
                     /note="SNP"
                     /replace="a"
     variation       198
                     /note="SNP"
                     /replace="a"
     variation       350
                     /note="SNP"
                     /replace="a"
     variation       485
                     /note="SNP"
                     /replace="a"
     variation       549
                     /note="SNP"
                     /replace="t"
ORIGIN
        1 aatgttgcct ttaaaccaga atgaggtgat aagttgaagg ggacagagat gaaagctgga
       61 cttttccaaa taaatcttat ttaatatttt aacgaggtat ttttgaatat tttgtgtaat
      121 aaaaatttaa ataatatgta aaaggtgata tcagaaacta aaaacaagct gtaatgattg
      181 aaacagactt gtcaaaagta tatgatgaga aacaagctag aaaagtaatt ttttactttt
      241 ctttttttagc atttttaac tcttgcatag aacattgtgg aactataatt gtaaatcagc
      301 atttatttat aaattccata gacttcagtt gtataaagga gttggatatg ttccgagaaa
      361 ggtgactaca tttgataagc agatagtggg ttttgctttt atattccttc tgaagagtca
      421 gctttaaatg taagtcaaat ctaaagagta gatagtagat tcagaagtaa taaatctgaa
      481 actcgtttca gagtaatggt aatttgaata caatttctcc atagtcagaa gcaactatta
      541 ttttgtttcc aaccatagtc acaatttatt ttatgcatat gttgattatg tgtgaaacag
      601 gttatt
//
```

GenBank: GQ131509.1

Ovis aries MHC class IIa region between DQA2 and DQB1 genomic sequence

Features Sequence

```
LOCUS       GQ131509                635 bp    DNA     linear   MAM 17-JUN-2009
DEFINITION  Ovis aries MHC class IIa region between DQA2 and DQB1 genomic
            sequence.
ACCESSION   GQ131509
VERSION     GQ131509.1  GI:239912246
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 635)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine MHC class IIa region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 635)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-MAY-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
     source          1..635
                     /organism="Ovis aries"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9940"
                     /chromosome="20"
                     /map="between DQA2 and DQB1"
     misc_feature    1..635
                     /note="MHC class IIa region between DQA2 and DQB1"
     variation       19
                     /note="SNP"
                     /replace="t"
     variation       66
                     /note="SNP"
                     /replace="t"
     variation       100
                     /note="SNP"
                     /replace="a"
     variation       157
                     /note="SNP"
                     /replace="a"
     variation       217
                     /note="SNP"
                     /replace="a"
     variation       266
                     /note="SNP"
                     /replace="c"
     variation       285
                     /note="SNP"
                     /replace="a"
     variation       301
                     /note="SNP"
                     /replace="t"
     variation       486
                     /note="SNP"
                     /replace="t"
     variation       487
                     /note="SNP"
                     /replace="a"
ORIGIN
        1 tagtttcttc attctgtaca ttaactcctt tgtttacctc ttggtccact ggcaagcgat
       61 ggcaaccata gtcaaatttt attaaaatct tactgtgtcg ttttgcagaa gcatttctct
      121 tccaggaatc aggacatcta aattgggaga acccaagttt ataggaacag ttagcattaa
      181 ttccacaact aagtttcata gtaagaggag tcactccgtc ttggtttaca tccttagttt
      241 gagactgata tcacatctta gaggagagtg tcctccagtc aacactgttc aatcattcta
      301 ctgggtagat acatctttat aatgggccat gttgcccaat gcttttaccc ctttgttctt
      361 aaatgggttc tgtgatctga ggcaatactg tggctgagta ttatgccaat aaatatgatg
      421 tatcatgaat ccttggatgg taataatatt ggaggcactg tgggcaaaaa tagtgaaccc
      481 atatgcgaaa tacataccaa atctaggaag gaaaaatcac ttacctacca aggtggaagg
      541 ttttgatata gttagtctgc tgccaattaa cttgatcttc ccaacgaatg atgctatatt
      601 aaaagttcag tgtcattctc tatttttggt atgtt
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GenBank: GQ131508.1

Ovis aries MHC class IIa region between DQB2 and DQA2 genomic sequence

Features Sequence

```
LOCUS       GQ131508                600 bp    DNA     linear   MAM 17-JUN-2009
DEFINITION  Ovis aries MHC class IIa region between DQB2 and DQA2 genomic
            sequence.
ACCESSION   GQ131508
VERSION     GQ131508.1  GI:239912245
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 600)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine MHC class IIa region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 600)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-MAY-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
     source          1..600
                     /organism="Ovis aries"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9940"
                     /chromosome="20"
                     /map="between DQB2 and DQA2"
     misc_feature    1..600
                     /note="MHC class IIa region between DQB2 and DQA2"
     variation       19
                     /note="SNP"
                     /replace="c"
     variation       24
                     /note="SNP"
                     /allele="c"
     variation       44
                     /note="SNP"
                     /allele="c"
     variation       64
                     /note="SNP"
                     /replace="t"
     variation       100
                     /note="SNP"
                     /allele="a"
     variation       107
                     /note="SNP"
                     /replace="t"
ORIGIN
        1 caagttcctg ttctctccgt ggcttacagg gactggcttt ggagtggctt tctgtgctgc
       61 tttgtagttg tctgccagag ctcactcttg ctgctgtcag gagtgcacac agggagctga
      121 ctacagtgtg gagcgtgcat gggtgaggtg catgtactgt taggagtgcc tgggctagtt
      181 gacgggattc aatgatgagg cctccccagt ggattgttag tagacttctt gaggaatctg
      241 tgccttttc agatcttcct agcgttctat ctaccacatt accagcctca tcccacccca
      301 gttatgtagc tcatgacata gtactctgga tagggtaaga gagaaacgaa tctcttcggc
      361 agtttcctgc acagttgagg aagacagatg ttccctcaca agttctcact ttcccctcca
      421 ggataattca tgggttgaga aggtccttcc tggcaccaat ctgtgctgcc ttggggagga
      481 gtgaggtctt cctcctactc tttccaatgt gtccaaatta tatttctttg ctccagtgat
      541 gtccttgaag ttatccactg gaaagttaga cttccacaaa gtgtttaaaa ttgttctcca
//
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GenBank: FJ999659.1

Ovis aries DOB-TAP2 intergenic region genomic sequence

Features Sequence

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LOCUS       FJ999659                618 bp    DNA     linear   MAM 17-JUN-2009
DEFINITION  Ovis aries DOB-TAP2 intergenic region genomic sequence.
ACCESSION   FJ999659
VERSION     FJ999659.1  GI:239837542
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 618)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine MHC class IIb region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 618)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAY-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
     source          1..618
                     /organism="Ovis aries"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9940"
                     /chromosome="20"
                     /map="between DOB and TAP2"
     misc_feature    <1..>618
                     /note="DOB-TAP2 intergenic region; MHC class IIb region"
     variation       163
                     /replace="a"
     variation       201
                     /replace="a"
     variation       253
                     /replace="c"
     variation       284
                     /replace="a"
     variation       423
                     /replace="t"
     variation       486
                     /replace="c"
     variation       534
                     /replace="t"
ORIGIN
        1 gaaactggaa aaaaatattt gataagtaag aagagcaaga cttctttttc tccaaagtga
       61 tctataagtg tattattact taatttgcct ctcgatccat ggcccaatat tgtgaatcct
      121 gcaagatgaa acagacatgt ttcttgtcca caagaaacgt aagatttagg tggagtgaca
      181 aagaaacaat tcactataat gtcaggaaga atcaaatgag ctttaaagct tgggcacaca
      241 agaaaattca agtcgtctag aaagagttca tgatgaagct gatcaagttg atatttcaca
      301 gaagtggcca acaggaaatg ttataattcg gaatacccag tgcatgggga caacagagaa
      361 ggaataaaca gtgaaacaaa aaatgtgcca ggaattcctt aaaaattact gtttggccac
      421 aacaaatata tgtatgcaca ctaattcact gaatgggaga agaacacagt agaatttttt
      481 tctttttcac ttcctaaga gaacacatgt tttgatgggt gtgtaaggtg agactgtaat
      541 ggtactagac ccagagagtc tggaatattt gagttacgtg ttaccgaaca tgctgagagg
      601 ccctcagctg cccacaaa
//
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GenBank: FJ999658.1

Ovis aries BRD2-DOA intergenic region genomic sequence

Features Sequence

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LOCUS       FJ999658                654 bp    DNA     linear   MAM 17-JUN-2009
DEFINITION  Ovis aries BRD2-DOA intergenic region genomic sequence.
ACCESSION   FJ999658
VERSION     FJ999658.1  GI:239837541
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 654)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine MHC class IIb region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 654)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAY-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
     source          1..654
                     /organism="Ovis aries"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9940"
                     /chromosome="20"
                     /map="between BRD2 and DOA"
     misc_feature    <1..>654
                     /note="BRD2-DOA intergenic region; MHC class IIb region"
     variation       22
                     /replace="a"
     variation       81
                     /replace="a"
     variation       133
                     /replace="a"
     variation       216
                     /replace="t"
     variation       285
                     /replace="t"
     variation       453
                     /replace="a"
ORIGIN
        1 catagacaca ctcacaaaca cgcacacttt atgatgcaaa tcccaaataa gaaaatccag
       61 aaaaagcaca aagaatactt gactacctgt gcactggttt aacaactatt aagattttcc
      121 catatttatt gccactgtgt gtgtgtgtgt atctacatcc tattcaaaat aaagacatca
      181 tggcatttta cctttaaata cttaaaactg tcttgctaca taaccacagg aaagtaatcg
      241 tgctgaacaa agttgaaaat aatttcctaa tatcattgat tatccggtct atttcttcat
      301 ctatctcgaa agtatcttta tatctgtttt cccccaaaag tgttgccaaa aatcaccctc
      361 taaatttgat tgtaatgtct atgcagtctc tcttaatccc cccttctcct tttttatatg
      421 atttttcaag aggtcaagcc agttgcctta tagaatatct catattctgt gtttgtatgg
      481 cggttttctt cctggcttca tttgacttat tcctgtatcc agtgtatttt ctgtaaacta
      541 gaactttgag caaaaggctt tattagattc cgattaaaga tttttcacag taattctgca
      601 tacgtgatgc cgtgcacttt tcctctcatc aagacgtgtg tgatgacggt gctc
//
```

GenBank: FJ999657.1

Ovis aries DRB1-DRA intergenic region genomic sequence

Features Sequence

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LOCUS       FJ999657                615 bp    DNA     linear   MAM 17-JUN-2009
DEFINITION  Ovis aries DRB1-DRA intergenic region genomic sequence.
ACCESSION   FJ999657
VERSION     FJ999657.1  GI:239837540
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 615)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine MHC class IIa region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 615)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAY-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
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                     /organism="Ovis aries"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:9940"
                     /chromosome="20"
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     misc_feature    <1..>615
                     /note="DRB1-DRA intergenic region; MHC class IIa region"
     variation       49
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     variation       133
                     /replace="t"
     variation       507
                     /replace="t"
     variation       515
                     /replace="t"
     variation       551
                     /replace="g"
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      121 tgacaatact tacgatgctt tctcatttta ccccattctc tgtcatttca tagcctgtat
      181 aggatttatt ttccaatatc ctccaaaaag gacttttga gctctgttca ttaccctcat
      241 tcttgcactt gttcattaaa tggattagct aaatgtgtgc agaggacact ggagtttcag
      301 gcacggtgtc tctctccttg ttctttattt tctattgcca aatccagaaa cttgatttac
      361 caatgatcct cagaagccag gggggaaggg agaggtgtgg agacagaaaa acaagacatt
      421 acctttcaga aacaaccaga tgtctcacgc ataagggtga ctcatctgtt gaggttgttc
      481 agttactcag tcatgtccag taatgtctaa aacagcgcat ttttgttact cattaatata
      541 tacaacacat agaacttaag ttgtagtaat tgattcattc tttttaaaaa taaattagta
      601 tgatttgtaa attgt
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GenBank: FJ999656.1

Ovis aries DMA-BRD2 intergenic region genomic sequence

Features Sequence

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LOCUS       FJ999656                550 bp    DNA     linear   MAM 17-JUN-2009
DEFINITION  Ovis aries DMA-BRD2 intergenic region genomic sequence.
ACCESSION   FJ999656
VERSION     FJ999656.1  GI:239837539
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 550)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine MHC class IIb region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 550)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAY-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /db_xref="taxon:9940"
                     /chromosome="20"
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     misc_feature    <1..>550
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     variation       91
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       61 taaattttcc ttaactgcca tactatcact gcaagtaaca aaattaaaaa tgactgctta
      121 acttaaccca gtcttgtgtt cattttcccc cctgcctatc tcaaaaatgc ctaagagaaa
      181 gtttgaaata gaaagcactc ttgtggtttt caaattgtgc ttttatttct taaaactact
      241 ttcctatttt acatgatgtc tcataaacct gttaaatgtt cagtaatgaa tttttaggca
      301 aattaagttc aggaattact acactgaatg tacttctatc tcaaaatcta caaaactaac
      361 ctcaccagct tcccttccca gcatgatcca gctcttgtat tcaagagctc agtgtgtagg
      421 ctgactgtct acccaatcag atgagtcaca aatgtgggag tcaagtttga caacttcatc
      481 ttcctcaaac catcttgccc agttgttcac tatcgatcct acctcttccg catctcctga
      541 atctgtcctc
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GenBank: GQ144417.1

Ovis aries DQA2-DQB1 intergenic region genomic sequence

Features Sequence

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LOCUS       GQ144417                603 bp    DNA     linear   MAM 15-JUN-2009
DEFINITION  Ovis aries DQA2-DQB1 intergenic region genomic sequence.
ACCESSION   GQ144417
VERSION     GQ144417.1  GI:239785638
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 603)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine MHC class IIa region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 603)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-MAY-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
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                     /db_xref="taxon:9940"
                     /chromosome="20"
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     variation       237
                     /note="SNP"
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     variation       323
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      121 gatagagtaa ttaataacag acatcacctg ctgatgttaa caactagaaa acaggaaaaa
      181 tatataaggt gattgttttc aaatactgga caacaaatcc tatcggattg tgattcctga
      241 aaaagggaca taagcaagag gaacactatg attcgtttga atttctgcct gaagtatgtt
      301 ttcaaagcat ggcacacagg agaaagtctc aagtggaaca gaatgaattt gatgagctga
      361 agagaaagtg atttgagttt gggaacgcta agaaggataa actttgaaag gcagatatca
      421 gagaaaagca aaagctgaag agctccagaa atctacatag tgccccttg ggtctatagc
      481 ttaatatcaa atggaaattc attaaccaca aagggacgct atgctctttc tgattaaaac
      541 ttctattcca ctcagtgtgt gtcctctagg aactcttctg attacagctg ttggattact
      601 ctt
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GenBank: GU056181.1

Ovis aries MHC class IIa region between DRB1 and DRA genomic sequence

Features Sequence

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LOCUS       GU056181                591 bp    DNA     linear   MAM 10-NOV-2009
DEFINITION  Ovis aries MHC class IIa region between DRB1 and DRA genomic
            sequence.
ACCESSION   GU056181
VERSION     GU056181.1  GI:262478679
KEYWORDS    .
SOURCE      Ovis aries (sheep)
  ORGANISM  Ovis aries
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Laurasiatheria; Cetartiodactyla; Ruminantia;
            Pecora; Bovidae; Caprinae; Ovis.
REFERENCE   1  (bases 1 to 591)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Identification of polymorphisms in the ovine MHC class IIa region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 591)
  AUTHORS   Lee,C.Y., Wetherall,J.D., Munyard,K.A. and Groth,D.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-OCT-2009) Biomedical Sciences, Curtin University,
            Kent Street, Perth, WA 6102, Australia
FEATURES             Location/Qualifiers
     source          1..591
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                     /mol_type="genomic DNA"
                     /db_xref="taxon:9940"
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                     /note="MHC class IIa region between DRB1 and DRA"
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     variation       79
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     variation       81
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                     /replace="a"
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                     /note="SNP"
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     variation       118
                     /note="SNP"
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       61 tgatgtataa attctgttgt gtgtaaataa atactcctct attctgctcc ccactccgtt
      121 gccatttcct ccccaccatc tgtcttaccc cttatcagag gccaaaccct aggctttcag
      181 attcactcaa ctgaaggtgc aatatttcat tgacctggag aatcaagaac caaaaccaaa
      241 ttctgaacct agagctaagt agagatctcc caacaaacaa acactgctct aggtttttcc
      301 agatgagaac tggaccacag caactggact gtttgcagat cctctgaatg agactgagcc
      361 ctgataaact ggaactttat ttggaaagtt agctgcctaa gaagatggca ggctagtgcc
      421 tgaaaataac catcttgttg gggcctgaat gttgggttct tttcagagat gggtggagat
      481 gaggaaacaa agtaaaaaga ccatttaatt cttgcaaata ctcctagaat ggcaagcctc
      541 cagcaggaaa tacgttgttt cacttcttta cagcccttca cagtgggcaa g
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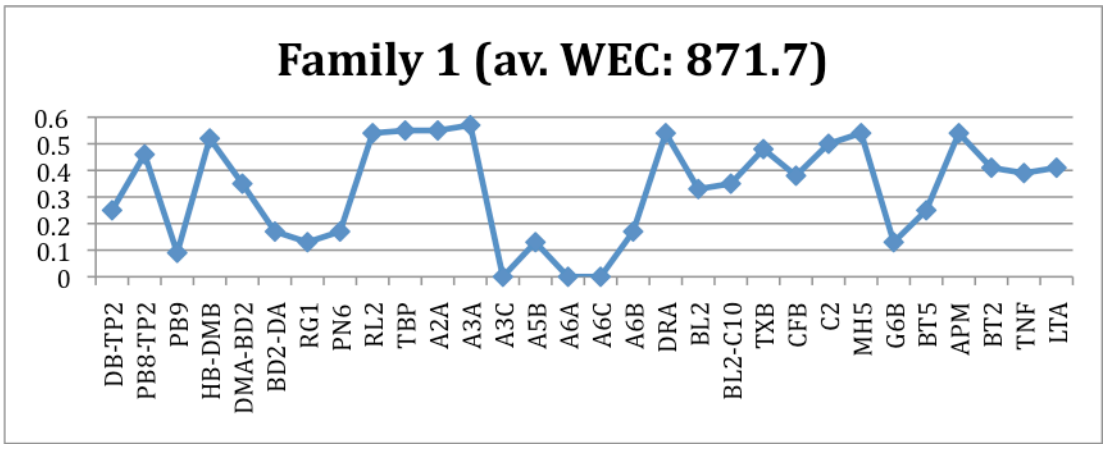
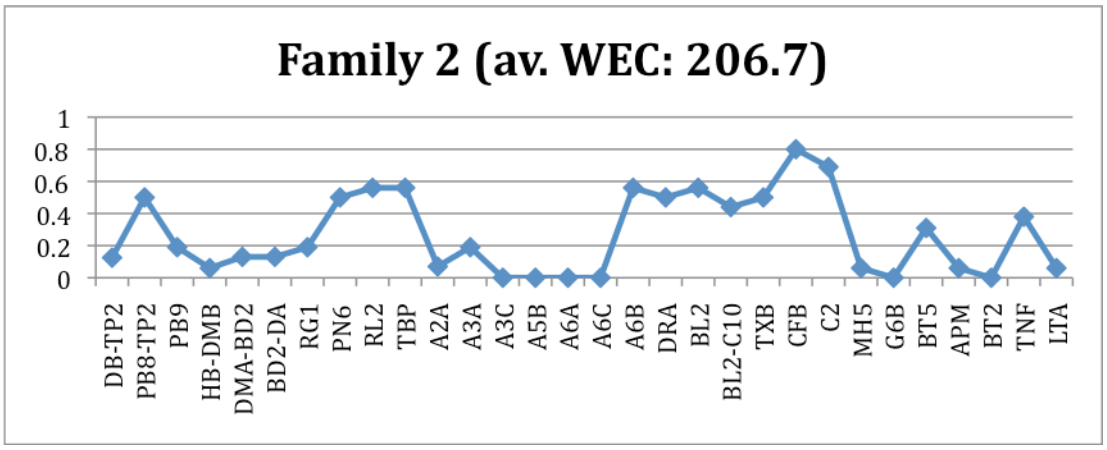
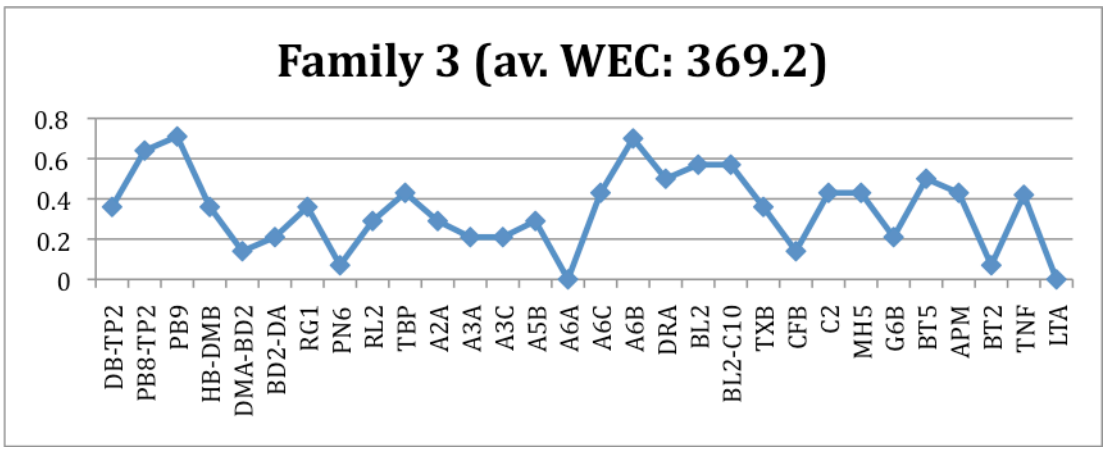
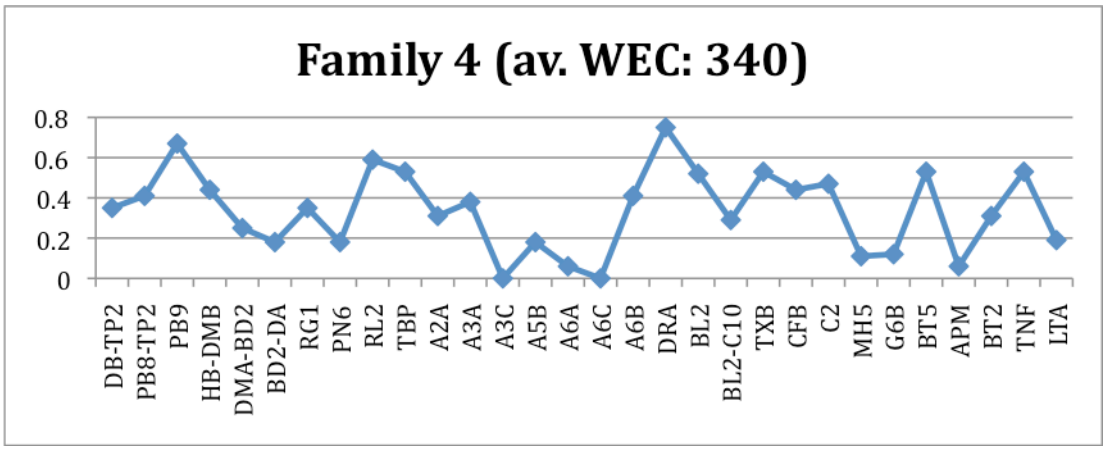
5A.1: Heterozygosities across the ovine MHC class IIb, IIa and III subregions for the offspring of each Family group.





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0.4
0.2
0
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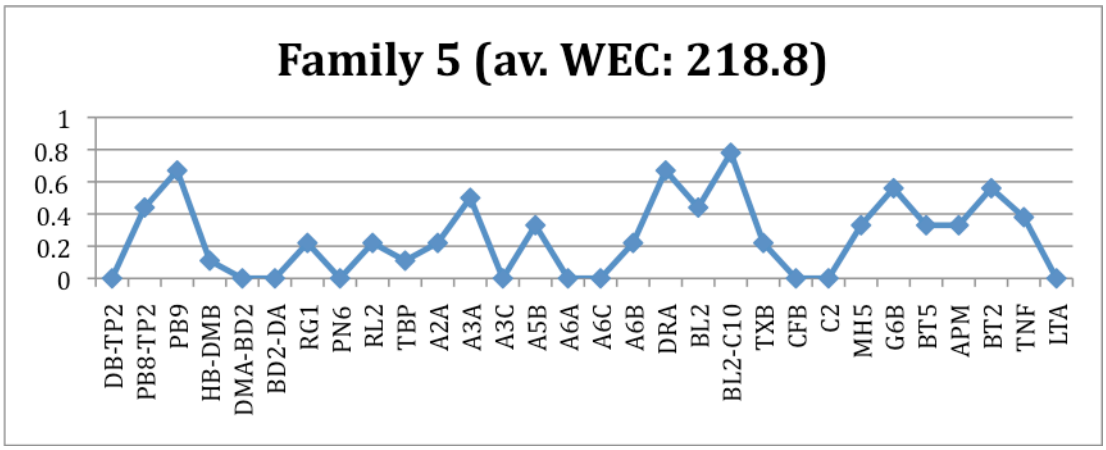
PB8-TP2
PB9
HB-DMB
DMA-BD2
BD2-DA
RG1
PN6
RL2
TBP
A2A
A3A
A3C
A5B
A6A
A6C
A6B
DRA
BL2
BL2-C10
TXB
CFB
C2
MH5
G6B
BT5
APM
BT2
TNF
LTA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0.4
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PB8-TP2
PB9
HB-DMB
DMA-BD2
BD2-DA
RG1
PN6
RL2
TBP
A2A
A3A
A3C
A5B
A6A
A6C
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DRA
BL2
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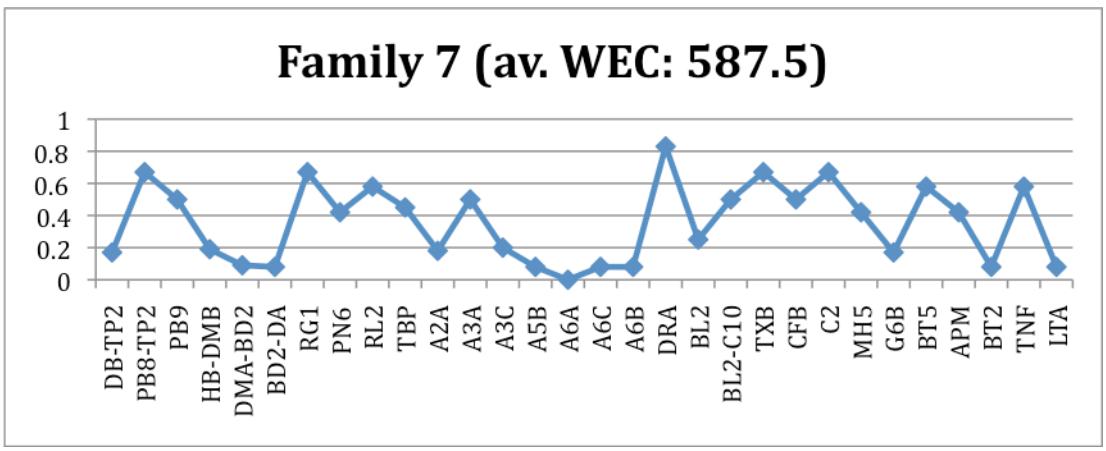
CFB
C2
MH5
G6B
BT5
APM
BT2
TNF
LTA


Family 7 (av. WEC: 587.5)
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0.4
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0
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PB8-TP2
PB9
HB-DMB
DMA-BD2
BD2-DA
RG1
PN6
RL2
TBP
A2A
A3A
A3C
A5B
A6A
A6C
A6B
DRA
BL2
BL2-C10
TXB
CFB
C2
MH5
G6B
BT5
APM
BT2
TNF
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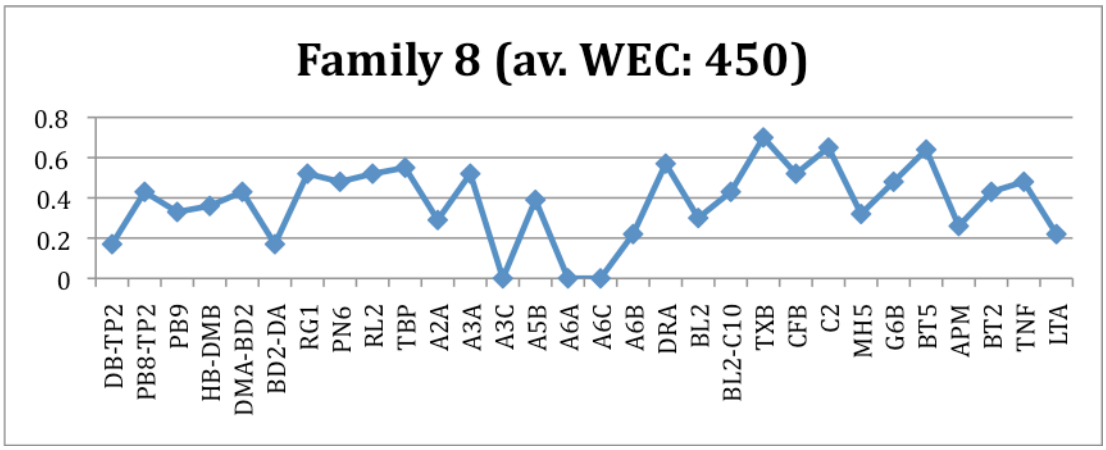
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PB8-TP2
PB9
HB-DMB
DMA-BD2
BD2-DA
RG1
PN6
RL2
TBP
A2A
A3A
A3C
A5B
A6A
A6C
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BL2-C10
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BT2
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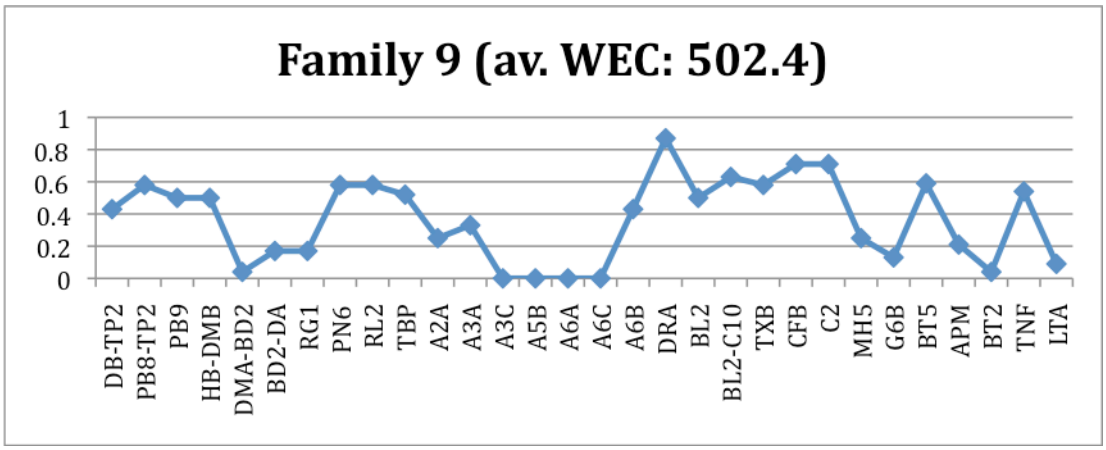
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PB8-TP2
PB9
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DMA-BD2
BD2-DA
RG1
PN6
RL2
TBP
A2A
A3A
A3C
A5B
A6A
A6C
A6B
DRA
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BT2
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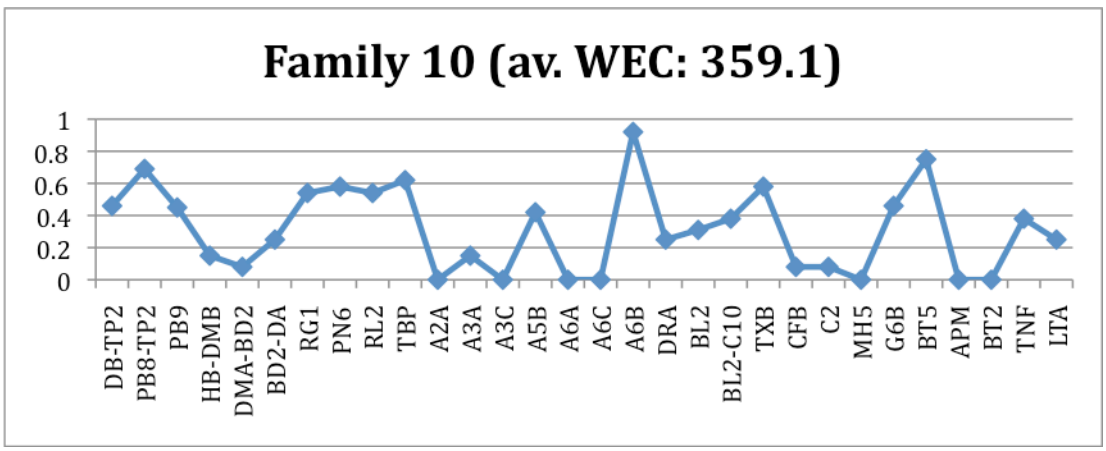
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PB8-TP2
PB9
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DMA-BD2
BD2-DA
RG1
PN6
RL2
TBP
A2A
A3A
A3C
A5B
A6A
A6C
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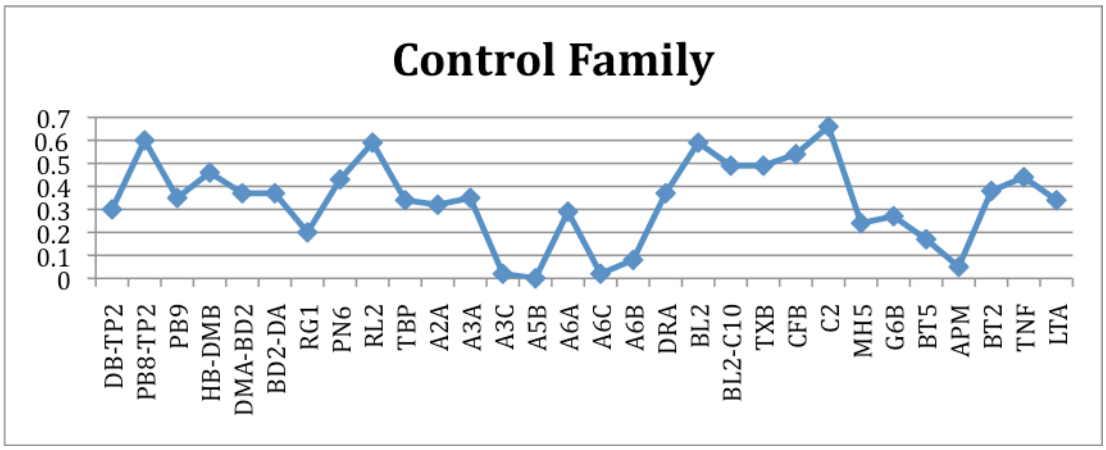
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PB8-TP2
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HB-DMB
DMA-BD2
BD2-DA
RG1
PN6
RL2
TBP
A2A
A3A
A3C
A5B
A6A
A6C
A6B
DRA
BL2
BL2-C10
TXB
CFB
C2
MH5
G6B
BT5
APM
BT2
TNF
LTA