

**ANALYSIS OF HAPLOTYPE STRUCTURE IN THE BOVINE MAJOR
HISTOCOMPATIBILITY COMPLEX**

A Dissertation

by

KRISTA L. FRITZ

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2009

Major Subject: Genetics

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Approved by:

Chair of Committee,	Loren C. Skow
Committee Members,	Bhanu P. Chowdhary
	Chistine G. Elsik
	James E. Womack

Intercollegiate Faculty Chair,	Craig J. Coates
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ABSTRACT

Analysis of Haplotype Structure in the Bovine Major

Histocompatibility Complex. (December 2009)

Krista L. Fritz, B.S., University of Illinois

Chair of Advisory Committee: Dr. Loren C. Skow

The goal of this project was to identify and characterize polymorphic markers spanning regions of the bovine major histocompatibility complex (BoLA) to analyze patterns of genetic variation and haplotype structure across diverse cattle breeds with various breed histories and selection pressures. Genetic markers that demonstrated sufficient levels of polymorphism, locus specificity, Mendelian inheritance, and the accurate typing of alleles across diverse haplotypes were chosen to define separate haplotype structures for the BoLA IIb and BoLA IIa-III-I regions and to evaluate breakpoints in linkage disequilibrium within the regions surrounding BoLA IIa-III-I. A total of 23 microsatellites, two SNPSTRs, 62 SNPs, and the alleles of three class IIa genes were selected for use in this study. These markers revealed eleven recombination events, low levels of recombination in BoLA IIa-III-I, a sharp break in haplotype structure in the region centromeric to class IIa, prolonged linkage disequilibrium in the extended class I region, strong conservation of BoLA IIa-III-I haplotype structure, BoLA IIa-III-I homozygous haplotype identity across seven different breeds of cattle, and a small number of common BoLA IIa-III-I haplotypes within the Angus and Holstein breeds. This work demonstrated that 52 SNPs from the Illumina 50K SNPchip could accurately predict BoLA IIa-III-I haplotypes. These 52 SNPs represent tagSNPs that can predict BoLA IIa-III-I genetic variation and could offer a cost-effective means for screening large sample sizes for haplotype/disease association studies in the future.

DEDICATION

This work is dedicated to all the family, friends, and teachers that have encouraged me to pursue a higher education. You have been an invaluable source of optimism and support, and I could not have done this without you!

None of us got where we are solely by pulling ourselves up by our bootstraps. We got here because somebody - a parent, a teacher, an Ivy League crony or a few nuns - bent down and helped us pick up our boots. - Thurgood Marshall

An educated man should know everything about something, and something about everything. - C.V. Wedgwood

*If you want immediate change, higher education is not the place for you.
- Laura Saunders*

We must remember that intelligence is not enough. Intelligence plus character - that is the goal of true education. - Martin Luther King, Jr.

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Thank you to everyone who has given me DNA samples for this project, including Dr. Clare Gill and the Bovine HapMap Consortium for the Bovine HapMap Project DNA samples, Dr. Robert Schnabel and Dr. Jerry Taylor of the University of Missouri for the BoLA homozygous DNA samples, Dr. James Derr and Dr. Natalie Halbert for Yellowstone National Park Bison DNA samples, Dr. Waithaka Mwangi for the DRB3*1101 defined Holstein DNA samples, Dr. James Womack, Elaine Owens, and Jan Elliott for DNA samples from the Bovine International Reference Families and L1 Dominette, and Dr. Pia Untalan, Dr. Pat Holman, and Dr. Penny Riggs for the DNA samples from the Lone Star tick resistance study.

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CHAPTER I

INTRODUCTION

Project Rationale

The major histocompatibility complex (MHC) is associated with more human diseases than any other region of the genome, and genetic variation within this region explains a substantial proportion of differential immune responsiveness between individuals (Graham et al. 2007; Ovsyannikova et al. 2006). Likewise, genetic variation in the bovine MHC (BoLA) has been associated with numerous disease susceptibilities and immune responses in cattle (Glass et al. 2000; Juliarena et al. 2008; Maillard et al. 2003) and represents an important genomic target for manipulation to improve the health and productivity of cattle. The goal of this project is to identify and analyze polymorphic markers spanning regions of BoLA to augment the markers discovered by the bovine genome sequencing project and provide for a rigorous characterization of the patterns of genetic variation and haplotype structure across diverse cattle breeds with various breed histories and selection pressures. Knowledge of BoLA haplotype structure will enable selection of tagSNPs to predict common genetic variation and will offer a cost-effective means for screening large sample sizes for haplotype/disease association studies. This should prove especially valuable in cattle, where the nature of polymorphisms has made the typing of classical BoLA genes laborious and time consuming. This work will ultimately aid future marker-assisted selective breeding programs for animals with superior disease resistance and productivity, as well as providing for improved vaccine development in livestock.

The Major Histocompatibility Complex

Discovery of the MHC and Its Functional Properties

The major histocompatibility complex (MHC) was discovered at a time when scientists were investigating the biological basis of tissue graft rejection. Little and

This dissertation follows the style of Mammalian Genome.

Tyzzler (Little and Tyzzler 1916) demonstrated that several dominant Mendelian factors influenced the resistance of mice to tumor grafts, suggesting for the first time that a genetic basis was responsible for transplant rejection. This hypothesis was further supported in 1927 when Bover (Bover 1927) demonstrated that skin transplants between identical twins were not rejected, and in the 1950s when a patient with end stage renal failure recovered after a successful kidney transplant from his identical twin (Hume et al. 1955).

Around the same time that properties of the human MHC were being discovered, studies in mice were demonstrating a similar genetic system controlled tissue transplantation in rodents. Peter A. Gorer, a young pathologist working at the Lister Institute for Preventative Medicine in London, identified the first major histocompatibility complex in 1936 while performing transplantation studies in mice (Gorer 1936). Around the same time, George Snell working at the Jackson Laboratory in Bar Harbor, Maine also discovered the mouse MHC in his own transplantation studies using strains of congenic mice (Snell 1948; Snell and Higgins 1951). Gorer had referred to the MHC as “Antigen II” while Snell had called it the “Histocompatibility Locus” or the “H locus.” The terms were combined, and the mouse MHC was given the name “Histocompatibility Two,” abbreviated as “H-2” (Gorer et al. 1948). The H-2 designation is still used for the mouse MHC today (Ellis et al. 2006).

The MHC has since been subdivided into three regions - class I, class II, and class III - based on gene content and function. Serological typing of MHC class I molecules in mice began late in the 1930s (Gorer 1936), and similar research in the human MHC began decades later (Van Rood et al. 1968). The name of the human major histocompatibility complex, the human leukocyte antigen (HLA), was derived from the observation that human white blood cells, leukocytes, became agglutinated after the transfusion of blood from one person to another (Chalmers et al. 1959; Dausset et al. 1954). Hence human leukocyte antigens were originally identified as donor antigens that were rejected by transplant recipients.

Jean Dausset first described an MHC antigen, “MAC”, which is known today as HLA-A2 (Dausset 1958), and Baruj Benacerraf linked the role of immune response genes to the MHC (Benacerraf 1981). The HLA-A2 allele identified by Dausset was later used

to describe the three-dimensional structure of a class I molecule (Bjorkman et al. 1987b) and the interaction of class I molecules with T cells (Bjorkman et al. 1987a). It was subsequently discovered that the cytotoxic function of CD8⁺ T cells is restricted by MHC class I molecules (Zinkernagel and Doherty 1975), and similar MHC restriction occurs with CD4⁺ T cells and MHC class II molecules (Stern et al. 1994; Swierkosz et al. 1978). “MHC restriction” means that a T cell cannot recognize and appropriately respond to an antigen unless the antigen is bound to an MHC molecule that matches the T cell receptor.

The discovery of MHC restriction was an important milestone in MHC research because it broadened the understanding of the role of the MHC from transplantation to a critical part of the acquired immune response (Doherty and Zinkernagel 1975b). Nonetheless, realizing of the role of the MHC in rejection of transplants has made a significant impact in modern medicine. Beginning the 1960s, doctors have been matching HLA alleles in donors and recipients for successful organ transplantation (Terasaki 1991). For their accomplishments contributing to the understanding of histocompatibility, Snell, Dausset, and Benacerraf were awarded the Nobel Prize in Medicine or Physiology in 1980.

The Evolution of the Major Histocompatibility Complex

It is probable that the MHC emerged with the adaptive immune system during vertebrate evolution. The MHC is not found in the primitive chordate amphioxus, the closest living invertebrate relative of vertebrates (Castro et al. 2004). However, human orthologs of MHC genes without adaptive immune functions were found in the amphioxus species, *Branchiostoma floridae* (Abi-Rached et al. 2002; Vienne et al. 2003). These well-conserved genes between the human MHC and amphioxus are referred to as “anchor genes.” Amphioxus cosmids containing anchor genes were mapped to a single chromosome, indicating only one set of anchor genes preceded the origin of vertebrates (Castro et al. 2004). Curiously, nine MHC anchor genes have paralogs at three other chromosomal locations on human chromosomes 1, 9, and 19 (Abi-Rached et al. 2002). These MHC paralogs may be ancestral remnants of vertebrate segmental, chromosomal, or genome duplications. Phylogenetic analysis suggests that all duplication events would have occurred over the same period of time, between 528 and 766 million years ago

(Vienne et al. 2003). It is plausible that the ancestor of jawed vertebrates experienced major duplication events or was even a polyploid. Ohno proposed that one or more whole genome duplications took place in the evolutionary lineage leading to mammals (Ohno 1970). The en bloc duplications probably occurred in several genomic regions, including the region containing the MHC anchor genes, before the evolution of jawed vertebrates (Abi-Rached et al. 2002). This implies that the MHC arose from ancient chromosomal duplications in a common ancestor of jawed vertebrates.

It is hypothesized that the MHC originated with relatively few genes that subsequently duplicated, diversified, and recruited other nearby genes to function in ways that work coherently with the MHC. The high levels of linkage disequilibrium observed in the MHC (Chen et al. 2009) may have resulted after genes within the MHC region evolved to coherently function with each other. Some genes may have been directly co-opted for a new function, whereas others may have acquired the new function in one duplicated copy and retained the original function in the second copy. The vertebrate immune system appears to have recruited proteasomes toward a new biochemical pathway that generates MHC class I epitopes (Niedermann et al. 1997). Proteasomes homologous to the proteasome utilized by the MHC can be found in *Drosophila* and yeast (Niedermann et al. 1997). Phylogenetic analysis demonstrates that the duplication of PSMB5-8-like, PSMB6-9-like, and PSMB7-10-like ancestral genes gave rise to PSMB5 and PSMB8, PSMB6, and PSMB9, PSMB7, and PSMB10 genes after the separation of jawed and jawless vertebrates (Abi-Rached et al. 2002; Clark et al. 2000). After the duplication, PSMB5, PSMB6, and PSMB7 may have retained their ancestral function of protein degradation, while the PSMB8, PSMB9, and PSMB10 genes evolved a more specialized function that produces specific peptides for MHC presentation (Danchin et al. 2004). Several cathepsins seem to have also been co-opted multiple times throughout evolution for the MHC class II peptide presentation process (Uinuk-ool et al. 2003).

A TAP-family gene, ABCB9, is found in the lamprey genome (Uinuk-ool et al. 2003). The lamprey ABCB9 gene is probably co-orthologous to vertebrate antigen processing genes TAP1/TAP2 and ABCB9 genes. An ancestral ABCB9-like gene duplication could have given rise to a gene that retained the ancestral function, ABCB9, and a second mutated copy with a new function, TAP1/2. A second duplication event

would have been required to generate TAP1 and TAP2 (Danchin et al. 2004). A third duplication event would have occurred in the bovine MHC where another TAP gene, TAP2.1, seems to have arisen by duplication of TAP2 (Childers et al. 2006). The ABCB9-like gene duplication event would have occurred after the separation of jawed and jawless vertebrates, at a time when large-scale duplication events are described for the whole genome (Abi-Rached et al. 2002). It is also possible that ABCB9 was not linked to proteasome function until after the duplication event when the protein molecule behavior shifted (Danchin et al. 2004). In either case, this is an example of MHC genes obtaining a new function by duplication and divergence.

Comparative Arrangement of MHCs in Divergent Species

The arrangement of genes within the MHC varies among different vertebrate species. The genes of the MHC are usually clustered together, but their functions do not appear to be altered when their grouping is disrupted. The previously described amphioxus MHC anchor genes correspond to the current positions of eutherian MHC class II and class III genomic regions. The MHC class I genes were translocated recently in eutherian evolution away from their ancestral position (Ohta et al. 2002) to be arranged as class I-III-II regions along a single chromosome. The chicken has a 92 kb “minimal essential MHC” with the class III region positioned outside of the class I and II regions (Kaufman et al. 1999). In fact, the class I and II regions are adjacent to one another in all non-mammalian species with the exception of teleost fish whose class I and II regions are on separate chromosomes (Sato et al. 2000). The MHC structure of teleost fish demonstrates that a specific organization of the class II and class I regions is not necessary for the normal function of class I and class II loci. The MHC class I and class II genes of sharks are located together, though they are evolutionarily older than teleost fish (Ohta et al. 2002) indicating that the organization of the teleost fish MHC is a derived characteristic. Comparison among three teleost species - the medaka, zebrafish and pufferfish - revealed that MHC class I gene content is conserved, while gene order and transcriptional orientation is not. This suggests that a strong selective pressure exists to conserve the linkage of certain MHC class I genes despite recurrent genetic rearrangements (Matsuo et al. 2002).

The bovine, swine, monotreme, and marsupial MHCs also lend support to the idea that class I and class II genes can vary in arrangement without compromising their functions. An ancestral inversion transposed a large portion of the BoLA class II region to a pericentric location approximately 20 Mb away from the remainder of BoLA (Childers et al. 2006). The transposed region, termed BoLA IIb, is about 450 kb in length and contains genes homologous to the HLA classical class II and extended class II regions with some exceptions (Childers et al. 2006). The centromere of swine chromosome 7 separates the class II region of the swine leukocyte antigen complex from the remainder of the MHC (Smith et al. 1995). Characterization of the marsupial *Monodelphis domestica* has shown that class I genes are interspersed within the class II region, as opposed to the class II and class I regions being distinctly separated by the class III region as in eutherians (Belov et al. 2006). An extreme exception to this marsupial arrangement is the tammar wallaby, whose class I genes are spread across six different chromosomes (Deakin et al. 2007). The MHC of monotremes shares a region of interspersed class I and II loci with the marsupial, although the monotreme MHC has been reported to lie within the pseudoautosomal region of two different pairs of sex chromosomes (Dohm et al. 2007). The class I and class II genes were probably located close together in a common mammalian ancestor of eutherians, monotremes, and marsupials. In support of this idea, class I pseudogenes are found in the human class II region and both functional and non-functional class I genes are found in the rodent class II region (Hurt et al. 2004).

The opossum class I region has “framework genes” that are present in homologous locations in eutherians (Belov et al. 2006). But whereas eutherian MHC class I loci expand and diversify around these framework genes, the opossum MHC class I loci are not interspersed among them (Amadou 1999; Belov et al. 2006). Class I framework genes have not been identified in non-mammalian species, with the exception of some homologous eutherian class I framework genes found near the MHC of teleost fish (Clark et al. 2001; Matsuo et al. 2002). This suggests that the establishment of a block of class I framework genes occurred prior to the translocation of the MHC class I genes from their ancestral position to the class I framework region.

The MHC class II region of eutherians contains antigen processing genes - TAP1, TAP2, PSMB8, and PSMB9 - that process and transport endogenous peptides for presentation on MHC class I molecules. Homologous antigen processing genes are found in the class I region of non-mammals (Kaufman et al. 1999; Ohta et al. 2002). The adjacent class I and II regions of the ancestral MHC may have promoted the antigen processing genes to co-evolve with class I genes, and eventually led to the translocation of both the class I genes and the antigen processing genes to a new location in eutherians (Ohta et al. 2002). The positioning of antigen processing genes TAP and PSMB near class I loci in *Monodelphis domestica* may have restricted the diversification of the class I genes, as evidenced by the presence of a single classical class I gene in *Monodelphis domestica* (Belov et al. 2006).

Genes within the class I region seem to undergo a more rapid birth-and-death process than genes within the class II region (Wan et al. 2009). Shared class I lineages have not been observed between mammals belonging to different orders, but the basic organization of mammalian class II genes most likely became established prior to the divergence of mammalian orders (Takahashi et al. 2000). The DR, DQ, and DP class II genes are common to placental mammals and encode classical class II molecules responsible for presenting exogenous peptides to helper T cells (Wan et al. 2009). Most eutherian class II regions have a single DRA gene, but the feline MHC contains four possible functional DRA genes (Yuhki et al. 2008). The feline MHC also lacks a functional DQ region and has only pseudogene homologs of DP genes (Yuhki et al. 2008). Ruminant MHCs have a functional DYA and DYB gene pair and one or two functional DQ gene pairs (Ballingall et al. 2004a; Sigurdardóttir et al. 1992). Horses may have two or three functional DQ gene pairs, and the giant panda has two functional DQ gene pairs (Fraser and Bailey 1998; Horin and Matiasovic 2002; Wan et al. 2009).

The giant panda contains a class II gene cluster - DOB, TAP2, PSMB8, TAP1, PSMB9, DMB, DMA, BRD2, DOA - that shows conserved gene order with other sequenced mammalian class II regions including humans, cats, dogs, cattle, pigs, and mice (Wan et al. 2009). Genes within this conserved class II region include DO and DM genes, non-classical class II molecules that assist in the loading of peptides onto classical class II molecules; PSMB, a proteasome subunit involved in the cleaving of peptides for

presentation by class I molecules; TAP, a transporter for antigen processing; BRD2, a mitogen-activated nuclear kinase; and BTNL2, a member of the immunoglobulin superfamily (Wan et al. 2009). The human MHC has multiple DR, DQ, and DP genes, and to date the HLA is the only species with two functional DPA and DPB genes (Wan et al. 2009).

The DM genes are the only class II cluster shared between mammals, birds, frogs, and bony and cartilaginous fishes (Kaufman et al. 1999; Kumanovics et al. 2003). The MHC class II DR, DP, DO, and DM genes appear to have arisen before the divergence of marsupials and placental mammals ~150 million years ago (Wan et al. 2009). The DY genes are not specific to ruminants, as a DYB pseudogene has been identified in the giant panda (Wan et al. 2009). It is probable that the MHC class II loci of DY and DQ evolved after the divergence of marsupials and mammals, around 130 million years ago, and the DY genes were subsequently lost in mouse and human lineages (Wan et al. 2009). Orthologous class II loci were not detected between marsupial DCA, DBA, and DBB class II genes and eutherian DPA, DOA, DRB, DQB, and DYB class II genes (Wan et al. 2009). It is assumed that these genes diverged from a common mammalian ancestor, and the orthologous genes have been lost.

The most conserved region of the MHC, the MHC class III region, is found in all mammalian species to date, as well as having homology in echinoderms like the sea urchin (Smith et al. 1998) and protosomes like *D. melanogaster* and *C. elegans* (Danchin et al. 2003; Trachtulec and Forejt 2001). This suggests that genes of the class III region were the original inhabitants of the MHC genomic region and were entrapped by chance during the development of the adaptive immune system. Class I and II MHC genes positioned themselves in the class III region and subsequently duplicated, diversified, and recruited other nearby genes to create the present-day mammalian MHC.

Organization and Function of the HLA as a Model for MHC Research

The human major histocompatibility complex (HLA) is a collection of genes spanning more than 4 Mb on human chromosome 6p21.3 and represents one of the most gene dense and polymorphic regions of the human genome (Mungall et al. 2003; Robinson et al. 2003). Many genes within the HLA play a critical role in the innate and

adaptive immune response by conferring the ability to identify foreign pathogens and distinguish between self and non-self (Ovsyannikova et al. 2006). Accordingly, more diseases have been associated with the HLA than any other genomic region including most known autoimmune conditions (Baschal et al. 2009). Sequence variation within the MHC can influence susceptibility or resistance to disease and underlies disparate levels of immune responsiveness between individuals (Graham et al. 2007; Ovsyannikova et al. 2006). For all of these reasons, the HLA is a prime candidate region for disease association studies and one of the most extensively studied regions in the human genome. The vast amount of HLA research has made the human MHC an excellent comparative resource for the studies of the MHCs in other species.

The HLA has historically been divided into three regions based on function: class II, class III, and class I. All of these regions contain disproportionately high levels of genes with immune function that are often clustered together in functional groups (Traherne 2008) (Figure 1). It may be advantageous for HLA genes to cluster by functional similarity to facilitate coordinated gene expression and rapid diversification by recombination and sequence exchange. However, the clustering of polymorphic genes may also result in an increased level of linkage disequilibrium across the region if particular combinations of alleles function well together and as a result, rarely become separated over evolutionary time (Traherne 2008).

The more centromeric MHC class II region spans about 0.7 Mb and contains the classical class II genes, HLA -DQ, -DR, and -DP (Yuhki et al. 2007). These classical class II genes encode glycoproteins expressed on the surface of antigen presenting cells, such as macrophages and dendritic cells. The primary function of the classical class II molecules is to present short exogenous peptides to CD4⁺ helper T cells to initiate the humoral immune response (Hughes and Nei 1990). The class II classical and nonclassical genes are found in α / β gene pairs, wherein each gene encodes an alpha or beta chain that forms a heterodimeric α / β protein (Wan et al. 2009). Amino acids encoded in the second exons of α / β pairs of class II molecules line the antigen binding site and are highly polymorphic in classical class II genes. The HLA also contains a single monomorphic DRA gene and variable numbers of DRB genes among different HLA haplotypes (Hughes and Nei 1990). The nonclassical HLA -DM and -DO genes are

involved in the proper loading of peptides onto classical class II molecules (Wan et al. 2009). The HLA class II region also contains several pseudogenes; TAP1 and TAP2 transporter genes associated with class I molecules; PSMB8 and PSMB9 proteasome genes; a TAPBP gene encoding tapasin, a chaperone molecule associated with class I antigen presentation; and BTNL2, a member of the immunoglobulin superfamily (Rhodes et al. 2001; The MHC Sequencing Consortium 1999; Wan et al. 2009).

The HLA class III region is sandwiched between the class I and II regions and includes a heterogeneous collection of genes with roles in both innate and acquired immunity as well as genes that encode proteins of no apparent immunologic function (The MHC Sequencing Consortium 1999). The gene content and organization of the class III region is the most conserved of all MHC regions across different species (Xie et al. 2003). The human class III region includes genes involved in the complement cascade, C2, CFB, C4A, and C4B; hormonal synthesis, CYP21; inflammation and cell stress, NFKBIL1, LTA, TNF α , LTB, LST1, NCR3, AIF1; heat shock proteins HSPA1A, HSPA1B, HSPA1L; extracellular matrix organization, TNX; regulatory receptors NOTCH4 and AGER; and the immunoglobulin superfamily, LY6G5B and LY6G6C (Carroll et al. 1984; The MHC Sequencing Consortium 1999). The HLA class III region is the most gene dense section of the entire human genome, containing 60 genes spanning 700 kb, which is about one gene every 12.9 kb (The MHC Sequencing Consortium 1999; Xie et al. 2003). In contrast to other regions of the HLA, pseudogenes are virtually absent from the class III region (The MHC Sequencing Consortium 1999; Xie et al. 2003). The complement genes are the most polymorphic of the class III region, and the number of C4 genes in HLA haplotypes can vary from two to six copies (Schneider et al. 1986; Yang et al. 2007).

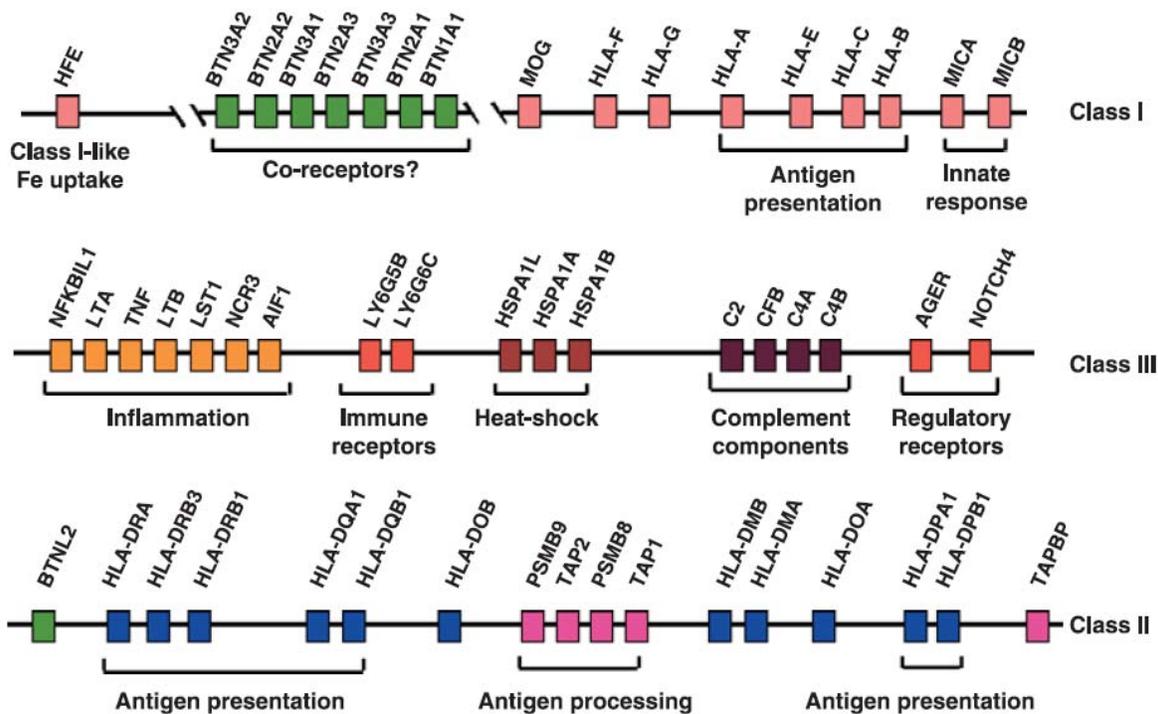


Figure 1. Reduced Gene Map of the Human MHC. Clusters of genes with immune function in the HLA class I, III, and II regions are highlighted. Genes with functional similarity are depicted by boxes of the same color. This figure was reprinted with permission from Blackwell Publishing Ltd: *International Journal of Immunogenetics* (Traherne 2008), copyright 2008.

The more telomeric HLA class I region covers approximately 2 Mb and contains many pseudogenes in addition to protein-coding genes (Geraghty et al. 1992; Shiina et al. 1999; The MHC Sequencing Consortium 1999). The HLA class I molecules are subdivided into class Ia (classical) molecules and class Ib (non-classical) molecules (Shiina et al. 1999; The MHC Sequencing Consortium 1999). Class Ib nonclassical molecules, HLA-E, HLA-F, and HLA-G, present conserved microbial epitopes to T cells, exhibit limited tissue expression, and are not very polymorphic (Geraghty et al. 1990; Koller et al. 1988; McMaster et al. 1995). Both classical class Ia and nonclassical class Ib genes may serve as ligands for natural killer cells (Braud et al. 1998; Sivori et al. 1996). Class Ia classical molecules are very polymorphic and are ubiquitously expressed on the surface of virtually every nucleated cell in the body (Daar et al. 1984). The class Ia molecule forms a heterodimer with the non-MHC encoded β_2 microglobulin (Figure 2)

and presents endogenous antigens to CD8⁺ T cells to illicit a cytotoxic immune response (Bjorkman et al. 1987b). Class Ia molecules play a crucial role in the intrinsic adaptive immune response to viral diseases and cancers by distinguishing recognizing self vs. non-self peptides (Bjorkman et al. 1987b). HLA contains three classical class Ia loci, HLA-A, HLA-B, and HLA-C, that are each present on all haplotypes (Cao et al. 2001; Horton et al. 2008). Other translated genes located within the class I region include MOG, a component of the myelin sheath that mediates the complement cascade (Zai et al. 2004), and MICA and MICB, MHC class I chain-related genes that are considered markers of stress in the epithelia (Bahram et al. 1994). The extended class I region contains HFE, a gene involved in regulating iron levels (Feder et al. 1996), and butyrophilin (BTN) genes, members of the immunoglobulin superfamily that may help stimulate T cells (Rhodes et al. 2001).

A single MHC class I gene encodes an alpha chain, also known as the class I heavy chain, consisting of three extracellular alpha domains, a transmembrane region, and a cytoplasmic domain (Bjorkman et al. 1987b). The class I molecule noncovalently binds with the product of a β_2 microglobulin gene, which is located outside the MHC on human chromosome 15 (Goodfellow et al. 1975). The MHC class II molecule is a heterodimer of an alpha chain and a beta chain encoded by one alpha gene and one beta gene (Stern et al. 1994). Both the alpha and beta chains of class II molecules contain extracellular, transmembrane, and cytoplasmic regions (Stern et al. 1994). Although the protein components of class I and II molecules differ, both have similar molecular shapes, including an antigen binding site where peptides are loaded and presented to T cells (Brown et al. 1993).

The antigen binding site may also be referred to as the peptide-binding region, the peptide-binding groove, the peptide-binding site, the antigen recognition site, or the antigen presentation site (Figure 2). The peptide composition of the antigen binding site determines what epitopes are bound and presented to T cells to effectively illicit an immune response (Doherty and Zinkernagel 1975b; Stern et al. 1994). The exons that encode the antigen binding sites represent the regions of class I and II genes with the highest levels of nucleotide diversity (Hughes et al. 1994; Hughes et al. 1990). The antigen binding site of class I molecules is encoded by exons 2 and 3 of a single class I

gene, and the antigen binding site of class II molecules is encoded by exon 2 of an alpha gene and exon 2 of a beta gene. Peptide epitopes presented by class I molecules are usually nine amino acids long, while peptide epitopes presented by class II molecules can vary from 11 to 17 amino acids in length (Brown et al. 1993). A peptide presented by a class I molecule is limited in size because the ends of the peptide are tucked into the antigen binding site, but length requirements are not as stringent for peptides presented by the class II molecules because the peptides ends can freely extend from both sides of the class II antigen binding site (Brown et al. 1993).

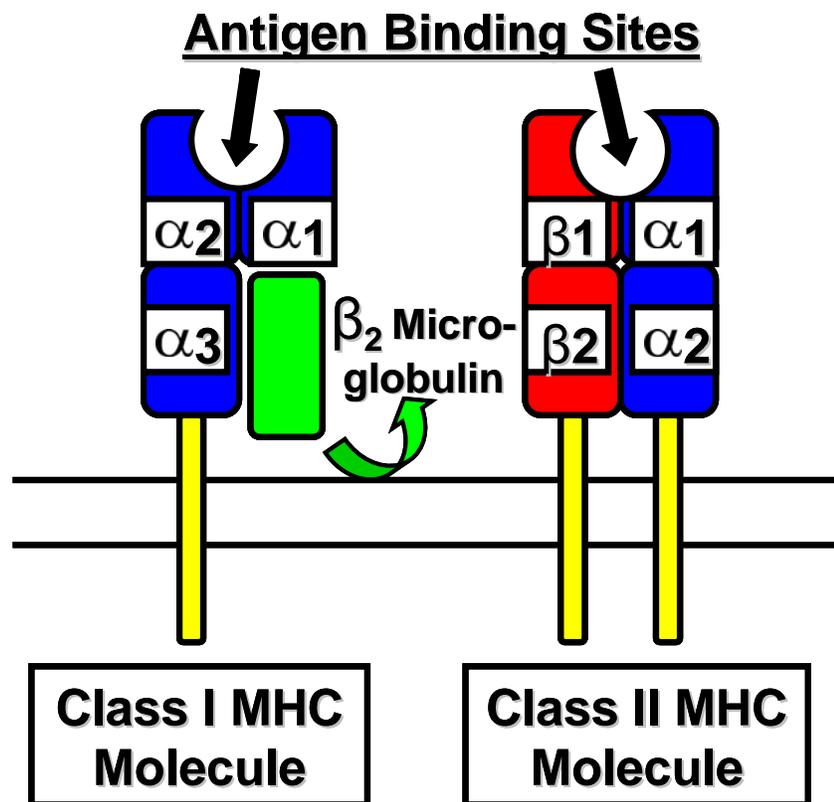


Figure 2. The Structure of MHC Class I and II Molecules. The color blue represents alpha chains, red depicts beta chains, the β_2 microglobulin is green, the transmembrane and cytoplasmic regions are yellow, and the cell membrane is denoted by the two horizontal black lines.

The Nature of Selection at the MHC

Aside from its immunological role, the MHC has also been implicated in mate choice, kin recognition, and reproductive success (Wedekind et al. 1995). Pathogen-driven selection and sexual selection are thought to be the major forces in maintaining MHC polymorphism (Hedrick 2002; Penn and Potts 1999). Zinkernagel and Doherty first proposed a hypothesis for MHC polymorphism suggesting that heterozygosity at the MHC would be advantageous, specifically heterozygosity at the antigen presenting sites, because the individual could recognize a wider variety of pathogens (Doherty and Zinkernagel 1975a). This hypothesis is referred to as the overdominance model and is considered a type of balancing selection (Takahata et al. 1992). Another type of balancing selection is negative frequency dependence, the theory that rare MHC alleles are maintained in a population because pathogens are least likely to evade recognition from rare MHC alleles (Clarke and Kirby 1966).

These models of balancing selection have traditionally been used to explain why species such as the San Nicolas Island Fox (Aguilar et al. 2004), the African Buffalo (Wenink et al. 1998), the North American Bison (Mikko et al. 1997), the Arabian Oryx (Hedrick et al. 2000), the White Tailed Deer (Van Den Bussche et al. 2002), the Asiatic Lion (Sachdev et al. 2005), and the Chinese River Dolphin (Yang et al. 2005) have maintained high levels of MHC diversity despite possessing otherwise low genetic variability. However, it seems probable that there may be additional forces of selection acting upon the MHC. According to the balancing selection models of overdominance and negative frequency dependence, it is unlikely that these species would have maintained high levels of MHC polymorphism if they had experienced insignificant pathogen challenge or had small populations with few mate options (Van Oosterhout 2009). A computer simulation study found that the overdominance model required near-lethal selection against MHC homozygotes (selection coefficient > 0.8) in the bottleneck generations of the San Nicolas Island Fox to account for the observed levels of MHC polymorphism (Van Oosterhout 2009).

Van Oosterhout (2009) faulted the traditional models of balancing selection for disregarding the influence of linkage disequilibrium and epistatic gene-gene interactions. The HLA has markedly high levels of linkage disequilibrium extending beyond the

boundaries of the MHC, and SNPs within the extended regions of the human MHC have been associated many human diseases (De Bakker et al. 2006). The extended linkage disequilibrium of the HLA may promote epistatic interactions between different combinations of alleles at multiple loci to alter disease phenotypes (Gregersen et al. 2006) and facilitate the genetic hitchhiking of nearby functional genes that potentially contain recessive deleterious mutations (Shiina et al. 2006). The MHC may undergo balancing selection on genes with immune function, as observed in overdominant selection, in addition to purifying selection on MHC-linked recessive deleterious mutations (Van Oosterhout 2009). Hence, the increased frequency of haplotypes containing immune genes that confer disease resistance would be counterbalanced by the reduced frequency of haplotypes containing deleterious recessive mutations. This counterbalance may explain why there have been so few demonstrations of host-parasite co-evolution (Woolhouse et al. 2002).

Another model of MHC evolution originated from the discovery that plants sharing the same alleles at the self-incompatibility locus (S-locus) exhibit high levels of seed abortion (Stone 2004). These abortions are triggered by the expression of a “sheltered load” of deleterious homozygous alleles that are otherwise unexpressed due to enforced heterozygosity and recombination suppression around the S-locus (Stone 2004; Uyenoyama 1997). Van Oosterhout (2009) proposed that recessive deleterious mutations accumulate within and around the HLA region, but the characteristically large amount of genetic diversity at the HLA rarely allows for these harmful recessive variants to be expressed as homozygotes. Consequently, the deleterious mutations are not removed through the processes of natural selection and remain preserved within HLA haplotypes. A computer simulation of this hypothesis revealed that haplotypes with relatively few deleterious recessive mutations had the greatest selective advantage and appeared at the highest frequency within a population (Van Oosterhout 2009).

Van Oosterhout (2009) also proposed that recessive deleterious mutations within the same HLA haplotype reinforce linkage disequilibrium and reduce the effective rate of recombination. It is detrimental for recessive deleterious mutations to appear in the homozygous state, so it is selectively advantageous for multiple mutations to remain in the same haplotype rather than spreading throughout many haplotypes and increasing the

probability of homozygosity. This promotes the creation of divergent blocks of DNA with elevated levels of linkage disequilibrium since recombinant haplotypes would have a lower fitness value than non-recombinants because recombinants would combine the deleterious mutations from both parental haplotypes. Consistent with this model, deeply diverged haplotypes of the HLA class II region have been preserved by linkage disequilibrium over tens of millions of years (Raymond et al. 2005). It follows that “recombination hotspots” would be selectively maintained over random recombination events, which would shuffle deleterious recessive mutations throughout the HLA region (Van Oosterhout 2009). The suppression of randomly distributed recombination events would generate high levels of linkage disequilibrium within the HLA.

Computer simulations demonstrated that lower selection coefficients were required in the model proposed by Van Oosterhout than in the overdominance model (Van Oosterhout 2009). Van Oosterhout’s model also showed novel haplotypes coexisting with parental haplotypes for a very short period of time before the novel haplotypes became eliminated. If a new haplotype did replace another haplotype, it would replace its own parental haplotype so all of the recessive deleterious alleles shared between the new haplotype and its parental haplotype would remain conserved. This is directly opposed to overdominant or negative frequency dependent selection, wherein haplotypes with higher disease resistance quickly become dispersed within a population and have equal opportunity to eliminate any other haplotype (Van Oosterhout 2009). Haplotypes within the overdominance model demonstrate a higher turnover rate and a reduced maximum persistence time, but new haplotypes generated in Van Oosterhout’s model do not eliminate or reduce the persistence time of divergent haplotypes (Van Oosterhout 2009). The haplotype lineages evolve virtually independent of population genetic processes like genetic drift, mutation, and selection. This results in the maintenance of divergent haplotypes with long genealogical branch lengths, similar to what is seen in trans-species polymorphism (Bos and Waldman 2006; Van Oosterhout 2009). Trans-species polymorphism, where alleles are shared between long-diverged species, has frequently been noted within the MHC (Figuroa et al. 1988; Graser et al. 1996; Lawlor et al. 1988; Mayer et al. 1988; McConnell et al. 1988).

Gene Maps and Haplotypes of the Human MHC

The relationship between polymorphism, selection, and linkage disequilibrium at the HLA can be further understood through the maps and sequences available from various HLA haplotypes. Campbell and Trowsdale (1993) compiled a map of the HLA by using a combination of physical mapping and DNA sequence data. This map included established HLA genes and many previously undescribed genes and pseudogenes (Campbell and Trowsdale 1993). In 1999, the first contiguous 3.6 Mb sequence-based map of HLA was published (The MHC Sequencing Consortium 1999). The annotated sequence described 224 genes within the 3.6 Mb region of HLA, and 128 (57%) of them were thought to be expressed. Unfortunately this sequence was derived from a number of individuals and represented a mosaic MHC haplotype, so the composite sequence does not represent a true MHC haplotype.

Five years later, approximately 4.75 Mb of two common HLA haplotypes, HLA-A3-B7-Cw7-DR15-DQ6 from the PGF cell line and HLA-A1-B8-Cw7-DR3-DQ2 from the COX cell line, were sequenced in homozygous individuals (Stewart et al. 2004). Each of these haplotypes is estimated to exist in 10% of the northern European population and is associated with common diseases, such as type 1 diabetes and multiple sclerosis (Stewart et al. 2004). These were the first two haplotypes to be sequenced and annotated for the MHC Haplotype Project, which later sequenced an additional six homozygous HLA haplotypes (Horton et al. 2008). Comparison of the eight homozygous HLA haplotypes revealed over 44,000 sequence variations, including nucleotide substitutions, deletions, and insertions (Horton et al. 2008). Coding substitutions were identified in 122 genes, and 97 of these were non-synonymous (Horton et al. 2008). The haplotype derived from the PGF cell line was designated as the reference sequence and incorporated into the human genome assembly (Horton et al. 2008). The haplotype, HLA-A26-B18-Cw5-DR3-DQ2, sequenced from the QBL cell line shared 158 kb of the class II region containing DQ and DR genes with the otherwise divergent haplotype from the COX cell line (Traherne et al. 2006b). Another study analyzed the same HLA class II region containing DQ and DR genes in 21 HLA haplotypes and found deeply divergent class II haplotypes that demonstrated high levels of linkage disequilibrium (Raymond et al. 2005). It was estimated that these class II haplotypes have been independently

evolving for tens of millions of years. Such a high level of haplotype divergence is unprecedented in the human genome and is greater than the divergence of human and primate sequences (Raymond et al. 2005).

The first description of linkage disequilibrium extending beyond the 3.6 Mb boundaries of the HLA was reported by Malfroy et al. (1997). The regions surrounding the previous 3.6 Mb boundaries of the HLA are referred to as the extended human MHC (Horton et al. 2004; Malfroy et al. 1997) (Figure 3). The extended class I subregion (Yoshino et al. 1997) and class II subregion (Stephens et al. 1999) were found to have conserved synteny in the human and mouse. The extended human MHC spans 7.6 Mb and was sequenced concurrently with human chromosome 6 (Mungall et al. 2003). About 33% of the loci within the extended HLA, 139 loci in total, were classified as pseudogenes based on similarity to known proteins and the presence of a premature stop codon. At least four of the pseudogenes, PPP1R2P1 and three olfactory-receptor genes, appear to be functional genes in other haplotypes (Ehlers et al. 2000; Stewart et al. 2004). Within the extended HLA, 252 (60%) of the 421 genes are classified as expressed on the basis of cDNA and/or EST evidence, and approximately 28% of the expressed transcripts have a potential role in immunity (Horton et al. 2004). According to Horton et al., the 28% of expressed transcripts with immune function fall into the following categories: antigen processing/presentation, immunoglobulin superfamily, inflammation, leukocyte maturation, complement cascade, non-classical MHC class I receptor family, immune regulation, and stress response (Horton et al. 2004).

Figure 3. Extended Gene Map of the Human MHC. This figure is color-coded to show the gene content of the five subregions of the extended HLA. Linkage disequilibrium extends throughout these five subregions. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews. Genetics (Horton et al. 2004), copyright 2004.

The extended HLA encompasses immune system genes as well as large families of olfactory receptor genes, zinc-finger genes, tRNA genes, and histone genes (Horton et al. 2004). One of the human genome's longest blocks of linkage disequilibrium spans about 540 kb and is located within the extended class I olfactory-receptor gene cluster (Miretti et al. 2005). The largest clusters of histone and tRNA genes in the entire human genome are found in the extended HLA (Horton et al. 2004). It may be beneficial for histone and tRNA genes to cluster together to facilitate rapid transcription, as many transcripts of tRNA and histone genes are needed for normal cellular function. Transcription of tRNA genes accounts for about 80% of all cellular eukaryotic transcription (Horton et al. 2004). Genes of the MHC may be promoting increased levels of their own transcription by hitchhiking with tRNA and histone gene clusters (Horton et al. 2004). At least eight genes within the HLA have splice site variation among different haplotypes, which may affect their expression at the post-transcriptional level (Horton et al. 2008; Královicová et al. 2004; Traherne et al. 2006a). A more complete understanding of the HLA transcriptome may provide new insights into the causal elements of diseases associated with the HLA.

Transcriptional repression is associated with DNA methylation, a type of epigenetic modification. The methylation profile for about 2.5% of HLA regions was investigated as a pilot study for the Human Epigenome Project (Rakyan et al. 2004). The HLA exhibited a bimodal pattern of methylation with regions of DNA being either hypomethylated or hypermethylated, and methylation profiles varied between different tissues and individuals (Rakyan et al. 2004). Methylation-associated SNPs from the Human HapMap Project demonstrated a positive correlation with human meiotic recombination at a regional level (Sigurdsson et al. 2009). Methylated sites may be preferential for recombination events, or the methylation of DNA after a recombination event may be used to hinder future recombination in the region (Sigurdsson et al. 2009).

Recombination at the MHC

It is estimated that 80% of all recombination events in the human genome occurs within only 10-20% of the DNA sequence (Myers et al. 2005). Regions of DNA with high recombination frequencies typically span less than 4 kb and are known as

“recombination hotspots” (Crawford et al. 2004). One centimorgan corresponds to a meiotic recombination rate of 1% per hundred meioses, and the sex averaged human recombination rate is 1.1 centimorgans (cM) per megabase (Mb) (Kong et al. 2002). A recombination hotspot is identified when the rate of recombination is significantly higher than the genome average rate and is fivefold higher than the rate of recombination within 10 kb of the flanking sequence (Myers et al. 2005). More than 25,000 recombination hotspots have been defined in the human genome, yielding approximately one recombination hotspot every 50 kb (Myers et al. 2005).

Interestingly, the number of defined recombination hotspots in the human genome is analogous to the number of protein-coding genes (Myers et al. 2005). Recombination hotspots appear to preferentially occur within 50 kb of the open reading frames of protein-coding genes (Myers et al. 2005). In the human, mouse, and rat genomes, recombination rates increase with GC and gene content (Spencer et al. 2006). However, human chromosome 19 has the highest gene density and exhibits a relatively low number of recombination hotspots (Myers et al. 2005). Recombination events in the human genome show greater variability on fine-scales than large-scales (Myers et al. 2005). Recombination hotspots vary between human populations and individuals, and they are not conserved between humans and chimpanzees (Myers et al. 2005; Winckler et al. 2005). Humans and chimpanzees share about 99% of their nucleotide sequence, so it appears that recombination hotspots are evolving at a faster rate than the nucleotide sequence (Winckler et al. 2005).

Recombination hotspots are considered to be breakpoints of linkage disequilibrium. Low linkage disequilibrium within the HLA class II region was shown to be predictive of recombination hotspots (Jeffreys et al. 2001). Familial recombination events have been identified in the HLA class II region between the intervals of DPB1-TAP1-TAP2 and DOB-DQB1, but no recombination was observed between the DRB1 and DQB1 genes (Cullen et al. 1997). A recombination hotspot has been localized to intron 2 of the human TAP2 gene (Cullen et al. 1995; Jeffreys et al. 2000). Female meiosis more frequently demonstrated recombination events at the TAP2 hotspot than male meiosis (Jeffreys et al. 2000). The homologous region of the human TAP2 gene did not contain a recombination hotspot in chimpanzees (Ptak et al. 2004). A total of 48

polymorphic markers spanning 3.3 Mb of the HLA were used to identify 325 recombinant chromosomes in 20,031 sperm from 12 individuals (Cullen et al. 2002). Recombination rates and distributions across the HLA were discovered to vary significantly between individuals, but intense recombination hotspots were observed at least every 0.8 Mb. The intervals from DRB1-DRA and CAT75X-RNF9 showed only rare instances of recombination, but high frequencies of recombination were observed in the intervals from DPB1-BRD2, DQB3-DQB1, BAT2-LTA, and the region telomeric to HLA-F, which led to the hypothesis the HLA consists of many strongly associated haplotype blocks that are smaller than 100 kb in length (Cullen et al. 2002).

Since Cullen et al. (2002), the Human HapMap Project and the MHC Haplotype Project have increased the number of polymorphic markers within the HLA to exceed 36,000 SNPs (Miretti et al. 2005). This dense SNP coverage, averaging about one SNP every 1.9 kb, has been used to generate a map of HLA linkage disequilibrium within U.S. pedigrees of northern and western European ancestry (Miretti et al. 2005). Linkage disequilibrium was shown to be higher in the extended class I region than the region encompassing the classical class I genes (Miretti et al. 2005). Little linkage disequilibrium was identified within the class II region (Miretti et al. 2005), which is consistent with previous descriptions of recombination hotspots in the HLA class II region (Cullen et al. 1997; Jeffreys et al. 2001; Jeffreys et al. 2000). Miretti et al. (2005) confirmed the class II recombination hotspots previously identified by sperm-typing experiments (Cullen et al. 2002; Jeffreys et al. 2001) to be located within TAP2, DMB, and BRD2-DOA and also discovered novel hotspots in the class I and II regions. A total of 29 recombination hotspots were identified, averaging one hotspot every 150 kb across the HLA (Miretti et al. 2005). Approximately 90% of the recombination hotspots correlated with breakpoints in linkage disequilibrium.

Excluding the extended HLA class I and II regions, the average recombination rate for HLA was calculated to be 0.7852 cM / Mb (Miretti et al. 2005). This was lower than the human genome average recombination rate of 1.1 cM / Mb (Kong et al. 2002); however, recombination rates within the HLA region varied up to four orders of magnitude between individuals (Miretti et al. 2005). Evolutionarily successful HLA recombination events spanning multiple pedigrees apparently occur infrequently, hinting

that specific combinations of MHC genes are under strong selection pressures over long periods of time. Various explanations may be offered for why specific combinations of alleles at HLA genes have been maintained over evolutionarily long periods of time (Raymond et al. 2005), as opposed to allowing recombinant haplotypes to persist. Divergent HLA haplotypes may suppress recombination events and promote the extension of haplotypes, especially if sequence inversions have occurred (Traherne 2008). Alternatively, specific allelic combinations may be maintained to confer an immunological advantage, deter the expression of deleterious recessive alleles, or they may have undergone recent positive selective sweeps that have not yet had time to degrade (De Bakker et al. 2006; Miretti et al. 2005; Traherne 2008).

Recombination rates have also been researched within the MHCs of other species. The mouse MHC contains at least two recombination hotspots located within the class III region and four within the class II region (Cullen et al. 1997). Recombination events in the mouse MHC were found to be influenced by their MHC haplotype and gender, with elevated recombination rates in female meiosis (Cullen et al. 1997). A linkage study of BTA23 identified a reduced rate of recombination across the BoLA IIa-III-I region (Schnabel et al., in prep) in comparison to the rest of the chromosome (Figure 4).

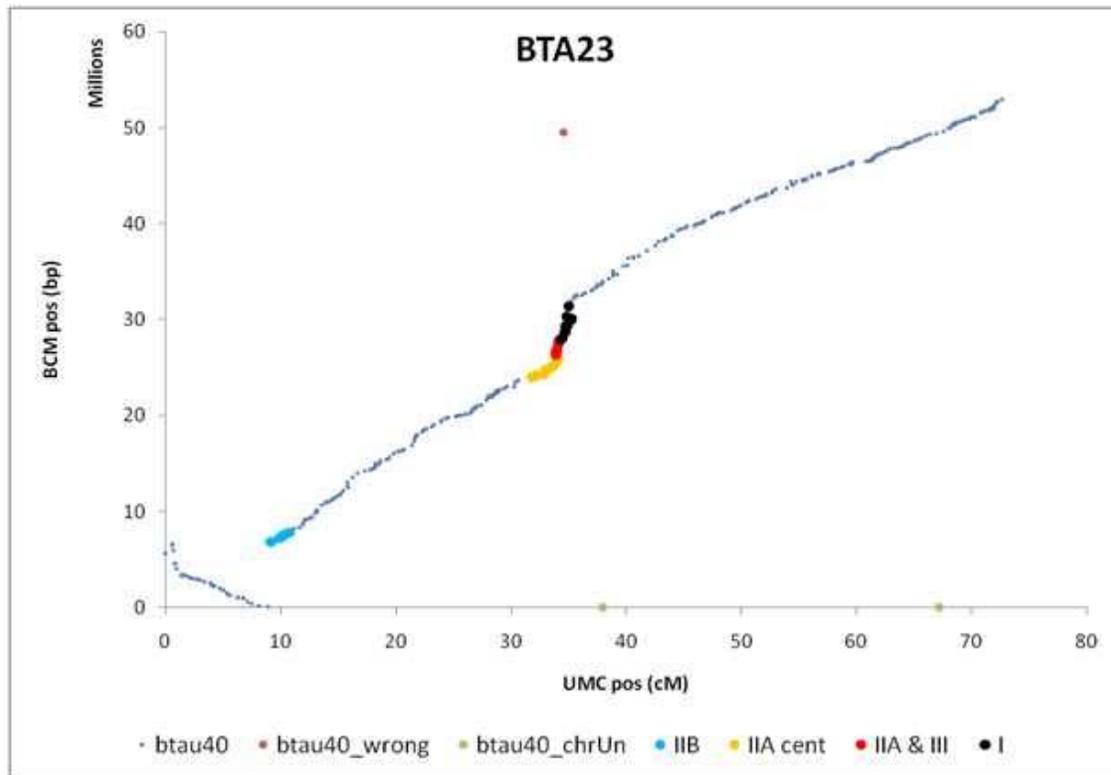


Figure 4. Reduced Recombination Rate in BoLA IIA-III-I (Schnabel et al., in prep). Blue dots display the level of recombination observed within the regions surrounding BoLA on BTA23, yellow represents the region centromeric to class IIA, red depicts class IIA and class III regions, and the class I region is black. The more vertical slant along the class IIA-III-I region is indicative of a reduced rate of recombination. Note that the regions centromeric to class IIA (yellow) and class IIB (bold light blue) exhibit a slope similar to the rest of BTA23 (blue), indicating there is not a reduced recombination rate within these regions.

One study reported an increased rate of recombination within the bovine MHC (BoLA), but the conclusions that were reached may not be reliable as the BoLA marker order was incorrect and at least one of the microsatellite primer pairs was not locus specific (Weimann et al. 2003). It is critically important to have correct marker order and locus specific primers when evaluating recombination rates and haplotype structure. The recent release of the bovine genome assembly has helped improve BoLA marker order, sequence placement, and gene annotation (Brinkmeyer-Langford et al. 2009; The Bovine Genome Sequencing and Analysis Consortium et al. 2009). The human MHC has also been a useful reference for characterizing the bovine MHC, but the organization and gene content of the bovine MHC is different than the human MHC in several respects.

The Bovine Major Histocompatibility Complex

The bovine genome assembly has predicted 154 genes within the BoLA regions located on chromosome 23 (The Bovine Genome Sequencing and Analysis Consortium et al. 2009). These include 60 genes within the class I region, 38 within the class IIa and class IIb regions, and 56 within the class III region (The Bovine Genome Sequencing and Analysis Consortium et al. 2009). A distinguishing organizational feature of BoLA in comparison to HLA is the transposition of a large portion of the BoLA class II region to a pericentric location approximately 20 Mb away from the remainder of BoLA (Childers et al. 2006). The transposed class II region, termed BoLA IIb, is about 450 kb in length and contains genes homologous to the HLA classical class II and extended class II regions, with the exception of a single histone H2B gene (Childers et al. 2006). The BoLA IIb region also contains divergent class II loci *DYA* and *DYB*, a novel *DSB* gene, a *DPB* fragment, and a duplicated transporter 2 gene designated *TAP2.1* (Childers et al. 2006). It is likely that the gene content and location of the class IIb region is characteristic of all Pecoran ruminants (Childers et al. 2006). The other class II genes, notably the *DQ* genes and *DR* genes, are found in the remainder of BoLA within a region known as BoLA class IIa (Brinkmeyer-Langford et al. 2009). Genes of the BoLA class III region are located between the class IIa and class I regions and seem well conserved in organization and function with class III genes of the HLA (Brinkmeyer-Langford et al. 2009).

Within the BoLA class IIa region, cattle have three very polymorphic classical class II genes - *DQB*, *DQA*, and *DRB3* (Sigurdardóttir et al. 1988). At least 104 alleles have been described for the *DRB3* gene, 49 for the *DQB* gene, and 47 for the *DQA* gene (Glass 2007). The *DRB3* gene pairs with the relatively monomorphic *DRA* gene, and the gene products of *DQA* and *DQB* join to form functional heterodimeric class II molecules (Sigurdardóttir et al. 1988; Zhou et al. 2007). The number of BoLA class II *DQ* genes differs among haplotypes in both number and composition (Glass et al. 2000). About half of the common BoLA class II haplotypes appear to have duplicated *DQ* genes in which both sets of *DQ* genes are expressed. Functional intrahaplotype and interhaplotype pairings can occur between the proteins encoded by duplicated *DQA* and *DQB* and may provide an immunological advantage by recognizing a wider variety of pathogens (Glass et al. 2000; Norimine and Brown 2005). Duplicated *DQ* genes have also been found in

the North American bison (Traul et al. 2005), the Chinese river dolphin (Yang et al. 2005), the baleen whale (Baker et al. 2006), the finless porpoise (Xu et al. 2007), the horse (Horin and Matiasovic 2002), and the giant panda (Wan et al. 2009). However, research has not yet shown that the DQ gene duplications are expressed and functional in all of these species. The basic organization of the mammalian MHC class II genes is thought to have been established prior to the divergence of mammalian orders (Takahashi et al. 2000), so it is possible that duplicated DQ genes were derived from a common mammalian ancestor.

In contrast to humans, cattle are thought to have six or more classical class I loci (Birch et al. 2006). The number and composition of BoLA classical class I genes varies between haplotypes, and there does not appear to be a single class I gene consistently expressed on all haplotypes (Birch et al. 2006). Cattle also show more potentially functional NK receptors, which may be capable of binding to MHC class I molecules, than other species (Birch and Ellis 2007; Dobromylskyj and Ellis 2007). Taking into account the variable nature of cattle MHC class I haplotypes, this may reflect a system that ensures sufficient NK receptor/ligand interaction in all individuals. Products of MIC genes may also serve as ligands to activate natural killer cells (Birch et al. 2008b). Three MIC genes have been identified in cattle, and at least one MIC gene has been found on all the class I haplotypes that have been analyzed (Birch et al. 2008b). A number of class I pseudogenes exist within the class I region of cattle, as well as nonclassical class I genes (Birch et al. 2008a). Three nonclassical class I genes have been identified in cattle near MIC genes and one nonclassical class I gene was identified near a class I gene (Birch et al. 2008a). The number of nonclassical class I genes also varies depending on the BoLA haplotype (Birch et al. 2008a).

Splice variants were identified within class IIb for TAP2, PSMB8, and RXRB genes (The Bovine Genome Sequencing and Analysis Consortium et al. 2009). Pseudogenes and gene duplications within the reference sequence of L1 Dominette made the class I and IIa regions difficult to annotate. Nevertheless, the annotated reference haplotype shows three classical class I loci and three MIC genes within the class I region (The Bovine Genome Sequencing and Analysis Consortium et al. 2009). The class IIa region was annotated with the following gene order: DQA2, DQA2-1, DQB, DQA,

DRB3, DRA, and the reference animal, L1 Dominette, was identified as homozygous for the DRB3*1002 allele (The Bovine Genome Sequencing and Analysis Consortium et al. 2009). The results of the BoLA sequence annotation hypothesized that L1 Dominette may be heterozygous at the MHC (The Bovine Genome Sequencing and Analysis Consortium et al. 2009), but work done for this project strongly suggests that she has a homozygous Hereford haplotype that spans the MHC and extended MHC regions (see results section).

The two most recent bovine genome assemblies, Btau3.1 and Btau4.0, differ considerably in their sequence organization of the BoLA regions (Brinkmeyer-Langford et al. 2009). The Btau4.0 assembly was produced with the intention of improving the previous Btau3.1 assembly by integrating data from fingerprint contig maps and BAC end sequences (The Bovine Genome Sequencing and Analysis Consortium et al. 2009). A high resolution radiation hybrid map of BoLA was generated to independently validate the improved accuracy of the Btau4.0 assembly over the Btau3.1 assembly (Brinkmeyer-Langford et al. 2009). Mapping was performed on the 12,000_{rad} radiation hybrid panel by amplifying the following regions of BoLA with seventy-seven primer pairs: 14 primers amplified within the class I and extended class I regions, 19 primers within the class III region, 27 primers within the class IIa and class IIa extended regions, and 17 primers within the class IIb region (Brinkmeyer-Langford et al. 2009). Homologous sequences were identified in the HLA for all but two markers used on the 12,000_{rad} radiation hybrid panel (Brinkmeyer-Langford et al. 2009). Gene order appears to be conserved between the HLA and BoLA, which supports the hypothesis that a single ancestral inversion created the BoLA class IIb region (Brinkmeyer-Langford et al. 2009).

The BoLA radiation hybrid map revealed problems with the Btau3.1 assembly that included sequence inversions within the class IIb region and the incorrect placement of markers within the class I, class IIa, and extended class IIa regions (Brinkmeyer-Langford et al. 2009). The Btau4.0 assembly retained the same BoLA IIb sequence inversion as the Btau3.1 assembly when compared to the radiation hybrid map. An independent BoLA IIb sequence assembly (Childers et al. 2006) validated the correct positioning of the BoLA IIb sequence within the Btau4.0 assembly and the incorrect placement of BoLA IIb markers in the radiation hybrid map (Brinkmeyer-Langford et al.

2009). Overall, the radiation hybrid map and the Btau4.0 assembly were in strong agreement for the assembly of all other BoLA regions. There were some minor discrepancies, but skimmed BAC sequences validated the placement of the markers in the Btau4.0 assembly over their order in the radiation hybrid map (Brinkmeyer-Langford et al. 2009). Some markers that were positioned within BoLA regions on the radiation hybrid map resided in the unassigned contigs of the Btau4.0 assembly (Brinkmeyer-Langford et al. 2009). Regardless of this, Btau4.0 is clearly the superior BoLA assembly and was used in this study of BoLA haplotype structure. An alternate assembly is available for the bovine genome (Zimin et al. 2009), but the UMD assembly of BoLA was also found to be inferior to the Btau4.0 assembly. The Btau4.0 assembly should prove useful for future BoLA disease association studies. Disease association studies are critical to understand the role genetic variants play in disease outbreaks within the modern cattle industry.

The Value of MHC Disease Association Studies

The Importance of Cattle Disease Resistance

Today the cattle industry is an important part of United States agriculture and economy. As of January 1, 2008, the United States had 96.7 million head of cattle (<http://www.ers.usda.gov/news/BSECoverage.htm> 2008). The United States is also the world's leading beef producer with a total retail equivalent value of beef amounting to \$74 billion in 2007, including \$2.175 billion generated from beef exports (<http://www.ers.usda.gov/news/BSECoverage.htm> 2008). As of June 2, 2008 there were 995,838,000 cattle distributed across the world (<http://www.cattlenetwork.com/Content.asp?contentid=226025> 2009) (Figure 5).

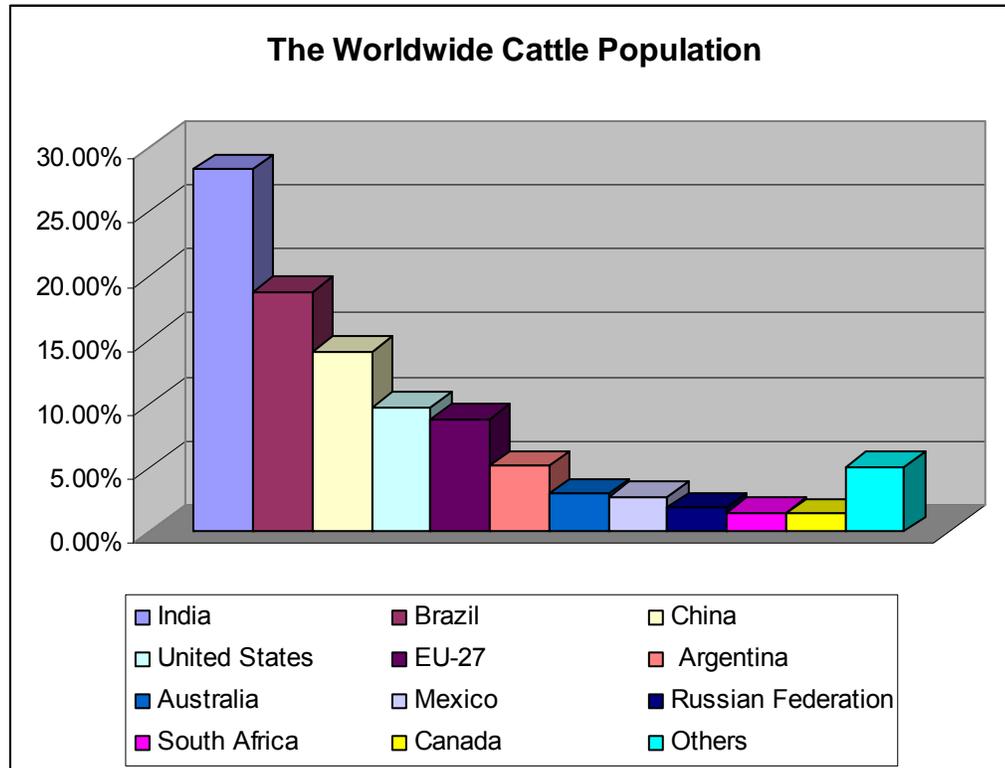


Figure 5. The Worldwide Cattle Population. This graph illustrates the distribution of cattle across the world. India has the most cattle with over 281 million, followed by Brazil with over 187 million, China with over 139 million, and the United States with over 96 million (<http://www.cattlenetwork.com/Content.asp?contentid=226025> 2009).

Disease outbreaks in cattle affect animal welfare and productivity, increase the demand for antibiotic use, place the food supply at risk, and have negative international trade implications (Thompson et al. 2002). Such diseases include those caused by bacteria and viruses, internal and external parasites, feed-borne toxins, and genetic disorders. The outbreak of infectious disease has increasingly become an international concern as the large-scale growth of livestock production and trade has diminished previously local boundaries that would have limited the spread of infectious disease.

Drug treatments are generally effective in preventing disease outbreaks, but alternate methods have been pursued as consumers are becoming increasingly concerned about animal products retaining chemical residues and pathogens acquiring resistance to commonly used drugs. Selectively breeding cattle to be genetically resistant to disease would help create a “chemical free” environment. It is estimated that 50% of all

antibiotics administered in the world are used for veterinary purposes (Teuber 2001). Antibiotics are used in the cattle industry to treat infections and increase overall health, growth, and feed efficiency (Teuber 2001). There is a growing public fear that antibiotic resistance will evolve in bacteria of livestock and spread to the human population, ultimately rendering antibiotics ineffective in treating human disease (Teuber 2001). In addition to bacteria, some parasites of livestock are becoming resistant to traditional drug treatments. Nematodes have become resistant to anthelmintics in sheep, and it has been reported that nematodes of cattle are becoming resistant to the same drugs (Anziani et al. 2001). It has also been reported that the cattle tick, *Boophilus microplus*, has developed a resistance to acaricide (Li et al. 2005).

Many non-genetic factors may influence the strength of an animal's immune response including herd management, animal husbandry, administration of vaccinations and veterinary care, temperature of environment, and quality of diet (Frisch 1981). However, a considerable portion of variability in response to pathogen challenge is genetically inherited. According to the Online Mendelian Inheritance in Animals (<http://omia.angis.org.au/> 2009), there are 379 heritable disorders and traits in cattle, and 80 of these disorders are linked to a single causative locus (<http://omia.angis.org.au/> 2009). Genes within the MHC have been associated with many diseases in vertebrates, and more MHC disease associations have been researched in humans than in any other species (Stewart et al. 2004). Conclusions from research on HLA-linked diseases may be used to improve the design of BoLA disease association studies.

HLA Disease Association Studies

The HLA maintains some of the oldest recognized genetic associations with disease, and one of the first diseases to be associated with the HLA was Hodgkin's lymphoma in 1967 (Amiel 1967). The list of diseases linked to the HLA continues to grow (Shiina et al. 2004), as evidenced by more than 20,000 papers published in the last 30 years describing diseases associated with the HLA (Stewart et al. 2004). Disorders associated with the HLA region encompass immune, cardiovascular, neurodegenerative, psychiatric, metabolic, infectious, dermatological, and ontological diseases (Shiina et al. 2004). Addictive behaviors, such as smoking, have also been linked to specific haplotypes of the

HLA region (Santos et al. 2008). The classical class I and II genes alone are implicated in more than 100 human diseases, including diabetes, rheumatoid arthritis, psoriasis, asthma, and nearly all autoimmune conditions (Shiina et al. 2004).

A strong association was observed between alleles of polymorphic HLA genes and SNPs throughout the HLA region (De Bakker et al. 2006). It is feasible that a subset of SNPs, or tagSNPs, could capture common HLA variation across multiple loci and offer a cost-effective means of screening large sample sizes for disease association studies (De Bakker et al. 2006). The use of tagSNPs carries some loss of power for detecting rare variants, but many genetic risk variants at the MHC have been identified within common haplotypes (Traherne 2008). Many individuals appear to have genetic risk factors for complex diseases within their HLA haplotypes, but most people remain healthy because the expression of deleterious alleles occurs at a low frequency (Traherne 2008). The infrequent expression of diseases associated with the HLA may be attributed to their complex etiologies, which involve multiple genetic, epigenetic, and environmental factors (Vyse and Todd 1996).

Although many diseases have been associated with the HLA region, it has been difficult to identify the specific casual genetic elements that contribute to disease phenotypes (Stewart et al. 2004). The characteristic features of the HLA - high levels of polymorphism, noted linkage disequilibrium, and clustering of genes with similar function - tend to convolute the correlation of disease phenotypes with specific genetic elements, so it is difficult to distinguish whether a genetic variant is casual or merely segregating with the casual genetic element (Fernando et al. 2008). Most published HLA disease association studies have limited the amount of genetic variation in their research projects by investigating less than 20 HLA genes within small cohorts of individuals that have been predominantly European in origin (Fernando et al. 2008).

Most HLA disease association studies have analyzed a subset of the following HLA genes: the classical HLA class I and II loci, HLA-A, HLA-B, HLA-C, DRB, DQA, DQB, DPA, DPB, the TAP genes, the MIC genes, and the class III genes TNF, LTA, LTB, C2, C4, and CFB (Fernando et al. 2008). These experimental designs may have overlooked potential disease associations within the 421 annotated genes and 252 expressed genes of the extended HLA (Horton et al. 2004). Epistatic interactions among

HLA loci, where the function of one HLA locus is altered or masked by another, may also hinder the search for susceptible alleles (Traherne 2008). Such epistatic interactions will be better understood by sequencing and characterizing the genetic content of entire HLA haplotypes. Further analysis of epigenetic modifications and regulatory elements, such as microRNAs, within the HLA will also shed light on the factors contributing to HLA-linked diseases (Traherne 2008).

BoLA Disease Association Studies

Similar to the human major histocompatibility complex, the bovine MHC is a candidate region for many disease association studies. Genetic polymorphisms of BoLA have been investigated for associations with several infectious diseases of cattle. Examples of these diseases include mastitis (Park et al. 2004), dermatophilosis (Maillard et al. 2003), and persistent lymphocytosis (Juliarena et al. 2008). Polymorphisms of BoLA have also been implicated in host resistance to the Lone Star tick (Untalan et al. 2007) and vaccination response to epitopes of various infectious diseases (Garcia-Briones et al. 2000).

Mastitis, or inflammation of the mammary gland, is the most common infectious disease of dairy cattle, and it presents a major economic problem for the dairy industry (Heringstad et al. 2000). Mastitis is a multi-factorial disease with a complex etiology that involves both genetic and non-genetic factors (Heringstad et al. 2000). Many management practices can influence an animal's susceptibility to mastitis including hygiene, housing, climate, milking equipment and procedures, treatment of teat injuries, and quality of feed (Hameed et al. 2008). Mastitis infections range from mild sub-clinical to severe clinical and may be caused by a variety of microorganisms. Clinical infections alter the composition and quantity of milk, increase somatic cell counts, and have the potential to become fatal (Heringstad et al. 2000). Contagious pathogens usually establish mild clinical infections that last for long periods of time (Hameed et al. 2008). The major contagious pathogens causing mastitis are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium bovis*, and *Mycoplasma bovis* (Hameed et al. 2008). The major environmental pathogens that cause mastitis are gram-negative bacteria, typically coliforms, that include *Escherichia coli*, *Klebsiella sp.*, *Enterobacter*

sp., *Citrobacter sp.*, *Serratia*, *Pseudomonas sp.*, *Proteus*; and environmental streptococci that include *Streptococcus uberis*, *Streptococcus dysgalactiae*, and *Streptococcus equines* (Hameed et al. 2008). Environmental pathogens lead to varying degrees of clinical infections and are relatively short in duration (Hameed et al. 2008).

The relationship of BoLA gene variants and mastitis susceptibility has been the focus of many bovine disease association studies. Little association was found between BoLA class I haplotypes and sub-clinical mastitis (Aarestrup et al. 1995), but mastitis resistance may be strengthened by increasing the number of DQ alleles through heterozygosity and copy number. Cows with only one pair of DQ genes were more susceptible to mastitis (Park et al. 2004), and the homozygosity of DQA1*0101 and DQA1*10011 alleles increased susceptibility to mastitis caused by *Streptococci* and *Escherichia*, respectively (Takeshima et al. 2008). The polymorphic classical BoLA class II gene, DRB3, has been a candidate gene for many mastitis disease association studies. A significant ($P < 0.05$) association was found between DRB3*2701-2707 and susceptibility to severe clinical mastitis, predominantly caused by coliforms (Sharif et al. 1998). Susceptibility was shown to be associated with amino acids at a specific position within the antigen binding site of the DRB3 gene (Sharif et al. 2000). No relationship was identified between the alleles DRB3*1501-1502 and DRB3*2701-2707 and sub-clinical mastitis caused by *Staphylococcus aureus*, but an association was found between DRB3*2701-2707 and increased susceptibility to sub-clinical mastitis caused by *Streptococcus dysgalactiae* (Hameed et al. 2008). Low somatic cell count, implying mastitis resistance, was significantly associated ($P < 0.05$) with DRB3*1501-1502 in Holstein cows (Sharif et al. 1998); however, conflicting reports have been published about the association of DRB3*1501-1502 with resistance to clinical mastitis (Dietz et al. 1997; Kelm et al. 1997; Sharif et al. 1998; Starckenburg et al. 1997). Such contradictions may be resolved by sampling larger populations, taking the species of the causal microorganism into account, and considering the entire BoLA haplotype rather than the alleles of a single locus.

Dermatophilosis is an infection of the skin caused by *Dermatophilus congolensis*, a type of actinomycete bacteria associated with *Amblyomma variegatum* ticks (Maillard et al. 2003). Cattle afflicted with dermatophilosis usually live in tropical environments,

show reduced productivity, and have a 15% average mortality rate (Maillard et al. 2003). A BoLA class II haplotype containing the alleles DRB3*0301-0302 / DRB3*3401-3402 and DQB*1804 was strongly associated with susceptibility to dermatophilosis ($P < 0.0001$) in a Brahman cattle population ($n=568$) living on Martinique Island in the French West Indies (Maillard et al. 2003). The cattle were all raised under the same environmental conditions with the same exposure to ticks and regular treatment with acaricide, but infections of dermatophilosis within the herd ranged from benign to fatal (Maillard et al. 2003). Regardless of the severity of their dermatophilosis infection, cattle with the susceptible class II haplotype, DRB3*0301-0302 / DRB3*3401-3402 and DQB*1804, were systematically culled (Maillard et al. 2003). The dermatophilosis disease prevalence had reached 0.76 before the cattle were selectively culled, and the disease prevalence was reduced to 0.2 two years later (Maillard et al. 2003). Four years after the animals had been culled, the disease prevalence had fallen to 0.02 (Maillard et al. 2003). At the end of this five year period, a dermatophilosis-specific ELISA test detected the average antibody prevalence within the population to be 0.98, indicating that the cattle were still being challenged by the dermatophilosis disease (Maillard et al. 2003). This cattle population continued to maintain the low 0.02 level of disease prevalence for at two more years, up until the publication of this research study (Maillard et al. 2003). By selecting against susceptible BoLA haplotypes within a population of cattle, individuals at the highest risk of contracting the disease were eliminated. These results may not have been as successful if the authors had chosen to select against an allele of a single locus rather than a BoLA class II haplotype.

The Bovine Leukemia Virus (BLV) is an oncogenic retrovirus of cattle that infects B lymphocytes of blood and milk (Udina et al. 2003). About 30% of all BLV infections lead to the benign sub-clinical stage of proliferating BLV-infected B cells, known as persistent lymphocytosis (PL). Only about 10% of BLV infections develop to the fatal clinical stage of lymphosarcoma, but the frequency of lymphosarcoma is about 1/3 higher in cattle with PL than cattle that are merely carriers of BLV (Juliarena et al. 2008). There is no vaccine available for BLV, so the retrovirus continues to spread from animal to animal through the transmission of bodily fluids containing infected lymphocytes (Udina et al. 2003). It is estimated that half of all cows belonging U.S.

cattle breeds are infected with BLV (Udina et al. 2003), and they have consequently become lifelong carriers of the retrovirus (Juliarena et al. 2008). The spread of BLV could be controlled if genetically resistant animals were identified and selectively bred within populations (Juliarena et al. 2009). Early PL disease association studies identified a correlation with BoLA classical class I alleles, but these were later discovered to be weak associations across breeds and at the population level (Juliarena et al. 2008). The association with alleles of classical class I genes was attributed to prolonged BoLA haplotypes that spanned the entire length of the MHC into the class II region (Udina et al. 2003). After considering allele segregation and BoLA haplotype structure, PL disease association was investigated in genes of the BoLA class II region (Juliarena et al. 2008). Haplotypes were subsequently defined at the class II region for associations with PL susceptibility and resistance in Holstein cattle (Zanotti et al. 1996). The DRB3*0902 and DRB3*1701 alleles have been associated with PL resistance and the DRB3*1501 or DRB3*1503 allele has been correlated with PL susceptibility (Juliarena et al. 2009). Ayrshire and Black Pied cattle from Russia showed an association between PL resistance the allele DRB3*0201, which has a deletion of codon 65 in DRB3 exon 2 that changes the conformation of the antigen binding site (Udina et al. 2003). Polymorphisms in the promoter region of the BoLA class III TNF α gene may also contribute to the progression of BLV infection (Konnai et al. 2006).

The susceptibility of cattle to tick infestations is thought to have a heritable component, as different breeds of cattle are known to be inherently more resistant to ticks than others, but the causal genetic elements remain unknown (Untalan et al. 2007). Tick infestations can expose cattle to many disease causing-pathogens and decrease their production value, so understanding the casual genetic basis for tick resistance would aid in breeding hosts for parasite resistance and developing effective vaccines (Untalan et al. 2007). Untalan et al. analyzed three BoLA microsatellites and exon 2 of DRB3 for association with tick resistance in a herd of cattle that had been phenotyped for susceptibility to the Lone Star tick, *Amblyomma americanum* (Untalan et al. 2007). The herd of cattle was comprised of three sires - Bull 12, Bull 13, and Rogers bull – and 72 dams including five F1 progeny, and 117 calves including five F2 progeny (Untalan et al. 2007). Bulls 12 and 13 are full siblings from an embryo transfer that used Simmental

semen and Red Poll oocytes (Untalan et al. 2007). The Rogers bull belonged to the Simbrah breed, and the dams were bred with *Bos indicus* and *Bos taurus* influence (Untalan et al. 2007). Calves with a second engorgement weight larger than 13.646g were designated susceptible, and calves with a second engorgement weight less than 4.302 were considered resistant (Untalan et al. 2007). All of the 25 most resistant calves and 16 of the 25 most susceptible calves were sired by Bull 12 or Bull 13 (Untalan et al. 2007). Bulls 12 and 13 had identical BoLA haplotypes and BoLA recombination events were rarely seen in their progeny; however, an association was made between tick resistance and the class II region, particularly the *DRB3*4401* allele shared by Bulls 12 and 13 (Untalan et al. 2007).

The BoLA region is a candidate for the genetic basis of variation between individuals in vaccine response. BoLA class I genes are important for responding to intracellular pathogens like bovine respiratory syncytial virus (Gaddum et al. 2003), *Brucella abortus* (Newman et al. 1996), *Theileria annulata* (Preston et al. 1999), and *Theileria parva* (McKeever et al. 1999). Effective vaccines for intracellular pathogens require epitopes that can be successfully presented by class I molecules and recognized by cytotoxic T cells; however, the extreme variability of BoLA class I genes makes the design of epitopes challenging (Glass 2007). BoLA class II genes may provide a better alternative. Cattle with alleles *DRB3*0201* and *DRB3*3301*, which both have a deletion of codon 65 in exon 2 of the *DRB3* gene, have shown a higher response to the commercial cattle tick vaccine (TickGARD) (Sitte et al. 2002). A vaccine for *Theileria parva* was successful in cattle with the *DRB3*2703* allele ($P = 0.027$), and unsuccessful in protecting against *Theileria parva* in cattle with the *DRB3*1501* allele ($P = 0.013$) (Ballingall et al. 2004b). The effectiveness of vaccines developed from the Foot and Mouth Disease virus (FMDV) was shown to correlate with *DRB3* polymorphisms (Garcia-Briones et al. 2000), and FMDV peptides were also successfully presented by *DQA* allele 22021 and *DQB* allele 1301 (Gerner et al. 2009). Glass et al. has demonstrated that FMDV peptides may be presented by *DR* and *DQ* molecules, and the number of FMDV peptides an animal may present is increased by the interhaplotype pairing of duplicated *DQA* and *DQB* genes (Glass et al. 2000). In conclusion, BoLA haplotypes have been correlated with resistance and susceptibility to a variety of

pathogens. A thorough understanding of the genetic history of various cattle breeds will determine if specific BoLA haplotypes have been selectively maintained or are simply a consequence of population history.

The Genetic History of Domestic Cattle

The Influence of Cattle Domestication on Human Cultural and Genetic Evolution

Cattle domestication is closely associated with the transition of human civilizations from the nomadic practice of hunting and gathering to a more settled way of life sustained by agriculture. Around the time of the last ice age, as the climate became warmer and more seasonal, human agricultural practices appear to have emerged independently in nine areas of four continents between the years of 8500 and 2500 B.C. (Diamond 2002). The domestication of animals, including cattle, occurred early in this period. The development of agriculture during this short 6,000 year time span in human history is attributed to technological advances that allowed for the processing and storage of wild food, competition between different human societies, and the need to feed and sustain an increasing population size (Diamond 2002).

Domestic cattle provided products such as meat, dairy, and leather, maintained grasslands, and served as draft animals for many human societies. In addition to influencing the transformation of human culture, the domestication of cattle has also placed certain selective genetic pressures on human populations. The human diet has changed considerably since the emergence of agriculture and cattle domestication 10,000 years ago, and it is probable that humans are still evolving to adjust to dietary changes such as an increased quantity of red meat with a higher fat content (Naughton et al. 1986) and the adult consumption of milk (Tishkoff et al. 2007).

Virtually all humans are born with the ability to digest lactose, the primary carbohydrate of milk, into glucose and galactose because the enzyme lactase is expressed at high levels in the small intestine (Tishkoff et al. 2007). Levels of this enzyme normally decrease as an infant matures and no longer needs to digest milk from their mother. Evidence suggests that the convergent evolution of two different haplotypes in human populations in Europe and Africa prolonged the expression of the lactase enzyme and, therefore, gave adults the ability to digest lactose (Tishkoff et al. 2007). The

haplotypes conferring the ability to digest lactose throughout adulthood were found in highest frequency in human populations that had a history of raising domestic dairy cows and lowest frequency in the non-pastoralist populations of Asia and Africa (Beja-Pereira et al. 2003; Tishkoff et al. 2007). The observance of high levels of linkage disequilibrium surrounding the lactase-persistent alleles is consistent with the notion of selective sweeps in human populations at the time when cattle domestication was incorporated into their culture (Tishkoff et al. 2007).

The Domestication of Wild Aurochs

The now extinct wild auroch, *Bos primigenius*, is considered the founder of modern cattle breeds. Aurochs depicted in Paleolithic rock and cave paintings show that they were an important source of food for humans and may have also played a role in rituals (Gotherstrom et al. 2005). The auroch was aggressive and much larger than modern domestic cattle. Julius Caesar contributes the following description of the aurochs he encountered within the Hercynian (Black) Forest of Germany in 53 B.C., “They are a little below the elephant in size, and of the appearance, color, and shape of a bull. Their strength and speed are extraordinary; they spare neither man nor wild beast which they have espied. These the Germans take with much pains in pits and kill them. The young men harden themselves with this exercise, and practice themselves in this kind of hunting, and those who have slain the greatest number of them, having produced the horns in public, to serve as evidence, receive great praise (Caesar 1869).”

Fossil evidence suggests that aurochs evolved about 1.5 to 2 million years ago in India from a precursor species, *Bos acutifrons*, and later spread to Europe and North Africa (Hassanin and Douzery 1999; Pilgrim 1939; Pilgrim and Hopwood 1947). Analysis of genetic variation among modern cattle breeds suggests that aurochs had a very large effective population size (Gibbs et al. 2009). Three genetically discrete subspecies of aurochs have been identified across diverse geographical areas including Europe, Asia, and Africa: *Bos primigenius primigenius*, *Bos primigenius namadicus*, and *Bos primigenius opisthonomus* (Loftus et al. 1994). *Bos primigenius primigenius* survived the longest, living into the Medieval times in Europe and allegedly dying in Poland in 1627 (Gotherstrom et al. 2005). Most large mammalian species survived on

the continent of Eurasia after the Late-Pleistocene extinctions in Australia and the Americas, and Eurasia subsequently became the primary location of mammalian domestication (Diamond 2002).

Because aurochs covered such a large geographical area over a considerably long period of time, it is plausible that they were domesticated in more than one time and place. It is widely believed that there were at least two separate domestication events that occurred in two different auroch subspecies that gave rise to modern *Bos indicus* and *Bos taurus* cattle (Loftus et al. 1994). *Bos indicus* and *Bos taurus* cattle are often described as two subspecies because they are able to hybridize and produce fully fertile male and female offspring (Gray 1972). *Bos indicus* cattle are also referred to as “zebu” and have characteristic humps over the top of their shoulders and neck that probably evolved to adapt to the arid climate during a time when their ancestors lived on the edge of the Great Salt Desert of Iran (Loftus et al. 1994). *Bos indicus* cattle often have horns that turn upward, large ears that hang downward, dewlaps with a large amount of excess skin, and well-developed sweat glands that freely perspire to increase their tolerance for heat and their ability to repel insects (OSU 1995). *Bos indicus* and *Bos taurus* diverged at least 100,000 ago and possibly as long as one million years ago (Bradley et al. 1996; Loftus et al. 1994; MacHugh et al. 1998). This means that the ancestors of *Bos indicus* and *Bos taurus* separated long before the occurrence of any livestock domestication events. More insight into the complex history of modern domestic cattle has been achieved through evolutionary genetic research.

Mitochondrial DNA Analysis of Domesticated Cattle and Aurochs

Mitochondrial DNA (mtDNA) is ideal for evolutionary studies because it is maternally inherited, non-recombining, generally has a higher mutation rate than nuclear DNA, and each mammalian cell contains up to 10,000 copies of mtDNA as opposed to one or two copies of nuclear DNA (Robin and Wong 1988). The increased copy number of mtDNA is especially useful when studying ancient DNA that has been degraded and consequently has lost much of its original material, such as mtDNA extracted from the bones of extinct aurochs. The mitochondrial displacement loop, or the D-loop, is frequently used in evolutionary studies because it has the highest rate of nucleotide

divergence (Excoffier and Yang 1999). Analysis of mitochondrial D-loop variation has been particularly useful in characterizing the early events of cattle domestication.

With the exception of a few observations of European aurochs transmitting their mtDNA haplotypes to *Bos taurus* cattle (Achilli et al. 2008), mitochondrial D-loop sequences from the extinct European auroch were found to cluster separately from those of modern cattle (Edwards et al. 2007; Troy et al. 2001). Maternal lines of European *Bos taurus* cattle are thought to have come from a lineage of aurochs in the Near East (Troy et al. 2001). The earliest archeological remains of domesticated cattle date to 5800 B.C. and were found in Anatolia, a cultural center of the Near East known to be the largest preliterate site in Asia in the 6th and 7th millennium B.C. (Perkins 1969). A comparatively large amount of ancestral variation is expected to be retained in the area where cattle domestication first occurred, and it was demonstrated that breeds within the Middle East and Anatolia show higher mitochondrial haplotype diversity than breeds outside the area (Troy et al. 2001). This data supports the widely held view that *Bos taurus* cattle were domesticated from the auroch subspecies *Bos primigenius primigenius* in civilizations of the Near East about 8,000 - 10,000 years ago.

A scientific consensus has not yet been reached regarding the total number and location(s) of cattle domestication events. It is speculated that the domestication of the auroch subspecies, *Bos primigenius namadicus*, occurred in modern-day Pakistan and gave rise to *Bos indicus* cattle (Loftus et al. 1994; MacHugh et al. 1998). Bradley et al. demonstrated that *Bos indicus* mitochondrial sequences were distinct from both European and African *Bos taurus* breeds (Bradley et al. 1996). Analysis of mtDNA in cattle breeds native to Japan, Mongolia, Korea, and China prompted the suggestion of another independent cattle domestication event in East Asia (Lai et al. 2006; Mannen et al. 2004).

The origins of African cattle are complex as they have emerged from nomadic and pastoral migrations and consecutive introductions of animals from the Middle East, Arabia, and the Indian subcontinent (Loftus et al. 1994). *Bos indicus* cattle were probably brought to Africa about 4,000 years ago and became widespread around 700 A.D. with Arabic migrations to North and East Africa (MacHugh et al. 1998). It is possible that there was an additional domestication event in Africa that created modern African *Bos taurus* cattle (MacHugh et al. 1998). In support of this hypothesis, variations

in mtDNA were identified and associated with population expansions in Europe in 5000 B.C. and Africa in 9000 B.C. that would have followed two separate domestication events and the advent of cattle herding (Bradley et al. 1996). It was also noted that mitochondrial DNA sequences from most African *Bos taurus* cattle breeds cluster around a haplotype that is absent from European *Bos taurus* breeds (Troy et al. 2001).

Mitochondrial sequences analyzed by Loftus et al. fell into two distinct lineages that did not correspond to the *Bos taurus* and *Bos indicus* classification, but all European and African *Bos taurus* and *Bos indicus* breeds belonged to one lineage, and all Indian *Bos indicus* breeds belonged to the other (Loftus et al. 1994). One explanation for absence of *Bos indicus* mtDNA in African cattle is that rare mitochondrial lineages were lost in the frequent famines and epidemics that have afflicted Africa. It has been demonstrated that populations that have gone through bottlenecks can lose extensive mitochondrial genetic diversity while retaining their nuclear variability (Wilson et al. 1985).

A more popular explanation for why Loftus et al. found only taurine mitochondrial genomes in the surveyed African breeds is that male *Bos indicus* cattle crossbred with their female *Bos taurus* ancestors, and the male *Bos indicus* influence is therefore not detected in maternally inherited mtDNA (Loftus et al. 1994). A study using genomic microsatellites to evaluate the admixture of African cattle populations concluded that there had been male introgression of zebu-specific alleles in African *Bos taurus* cattle (MacHugh et al. 1998). This would explain why many African *Bos taurus* breeds share morphological similarity with *Bos indicus* cattle. African *Bos taurus* breeds also demonstrate a greater tolerance to heat and disease than European *Bos taurus* breeds (Bradley et al. 1996; Freeman et al. 2004). The tsetse fly is a large biting fly of mid-continental Africa that is also a vector for trypanosomes and is responsible for the spread of trypanosomiasis, also known as sleeping sickness, among most cattle breeds. However, West African *Bos taurus* breeds can thrive in tsetse fly-infested areas because they have developed a resistance to trypanosomiasis (Freeman et al. 2004). This unique inherent resistance, known as trypanotolerance, is not found in any of the *Bos indicus* or European *Bos taurus* breeds (MacHugh et al. 1998). So although African cattle have

more mtDNA similarity to European *Bos taurus* breeds than *Bos indicus* breeds, modern African *Bos taurus* breeds are distinguishable from European *Bos taurus* breeds.

Y Chromosome DNA Analysis of Domesticated Cattle and Aurochs

Since evolutionary studies utilizing mtDNA are not informative for male inheritance, the Y chromosome is useful for phylogenetic studies because it is paternally inherited and undergoes a limited amount of recombination. Despite fewer cellular copies of the Y chromosome in comparison to mtDNA, analysis of markers on the Y chromosome has been possible on ancient DNA samples (Gotherstrom et al. 2005).

Two Y chromosome haplotypes were identified in modern European cattle that exhibited a distinct geographical north-south boundary (Gotherstrom et al. 2005). The haplotype of Y chromosomes in Northern European cattle matched the Y chromosome haplotype of the ancient European auroch, *Bos primigenius primigenius*, while the Y chromosome haplotype in cattle from Southern Europe differed from the ancient haplotype but was found in cattle from the Near East (Gotherstrom et al. 2005). *Bos taurus* and *Bos indicus* cattle had different Y chromosome haplotypes, supporting the theory that two genetically distinct auroch subspecies gave rise to modern *Bos taurus* and *Bos indicus* cattle (Gotherstrom et al. 2005).

Analysis of Y chromosome haplotypes revealed that European aurochs left a paternal imprint on modern cattle by mating with early domesticated female cows. It is unlikely that a genetic imprint would remain from a female auroch crossed with a domestic bull because the calf would not have stayed with the domestic herd. Interbreeding may have been difficult to prevent because wild aurochs co-existed with early domesticated cattle. Another possibility is that cross-breeding with wild ancestors was encouraged by early cattle owners to improve their domestic stock. In any case, studies of Y chromosome haplotypes have proven that the origins of domestic cattle are far more complicated than what was shown by mtDNA studies alone.

It is doubtful that the high level of bovine autosomal sequence diversity is fully accounted for by a small number of domestication events, so this also lends support to the idea of early domestic cattle locally backcrossing with wild ancestors (Vila et al. 2005). It seems that there were multiple cattle domestication events of different auroch

subspecies followed by continued backcrossing with wild ancestors. It is unlikely that aurochs were the only feral bovids capable of successfully interbreeding with early domestic cattle, as modern domestic cattle are capable of hybridizing with various members of the Bovini Tribe (Gray 1972).

Phylogeny of the Bovini Tribe

Ruminants are characterized by a specialized digestive system with three or four stomach compartments that employ microbial fermentation to effectively convert complex plant carbohydrates into volatile fatty acids, which are a major source of energy (Tellam et al. 2009). Ruminants represent the largest group of ungulates, or hoofed mammals, with a history of expansion and diversification that has created over 190 ruminant species distributed across the world (Hassanin and Douzery 2003). The phylogenetic classification of ruminants has been challenging because different ruminant families have developed the same traits in parallel after several evolutionary radiations. Morphological similarities make it difficult to distinguish one divergent species from another in the fossil record. However, genetic studies have substantiated the classification of families within the suborder Ruminantia to include Antilocapridae (pronghorns), Bovidae (cattle, bison, yaks, water buffalo, sheep, goats, gazelles, and antelopes), Cervidae (deer, elk, moose), Giraffidae (giraffes and okapis), Moschidae (musk deer), and Tragulidae (chevrotains) (Hassanin and Douzery 2003).

The Bovidae family is comprised of 128 species and 45 genera that are further grouped into 14 tribes (Allard et al. 1992). Domestic *Bos taurus* and *Bos indicus* cattle belong to the subfamily Bovinae and the tribe Bovini. Other members of the Bovini tribe include *Bos gaurus* (wild gaur), *Bos frontalis* (domestic gaur known as gayal or mithan), *Bos javanicus* (banteng), *Bos mutus* (yak), *Bos sauveli* (kouprey), *Bison bison* (North American bison), *Bison bonasus* (European bison or wisent), *Bubalus bubalis* (domestic Asian water buffalo), *Bubalus arnee* (wild Asian water buffalo), *Bubalus depressicornis* (lowland anoa), *Bubalus quarlesi* (mountain anoa), *Bubalus mindorensis* (tamaraw), *Syncerus caffer* (African buffalo) and the extinct *Bos primigenius* (auroch) (Gallagher et al. 1999a; Hassanin and Douzery 1999; Hassanin and Ropiquet 2004; Nguyen et al. 2008; Ritz et al. 2000).

Mitochondrial studies demonstrate that bison, yak, gaur, and banteng are all more divergent than *Bos indicus* and *Bos taurus* are from each other (Loftus et al. 1994). It is estimated that the *Bubalus* and *Syncerus* genera diverged 6.9 to 7.7 million years ago, while the *Bos* and *Bison* genera split between 3.3 and 4.8 million years ago (Hassanin and Douzery 1999). Based on the sequencing results of 15 autosomal genes, these genera have been grouped into the following clades: 1) all buffalo in *Bubalus* and *Syncerus*, 2) all *Bos* excluding domesticated cattle and yak, 3) all domesticated cattle, and a possible fourth clade of bison and yak (Hassanin and Ropiquet 2004; MacEachern et al. 2009). The clustering of yak with bison prompted the suggestion that yak be removed from the *Bos* genus and reassigned to the *Poephagus* genus (Nijman et al. 2008).

Certain polymorphisms of bovine autosomal gene sequences were discovered to be highly conserved between the subtribes of Bubalina (*Bubalus* and *Syncerus*) and Bovina (*Bos* and *Bison*) (MacEachern et al. 2009). The polymorphisms were not linked over long distances, so it is unlikely that they were introduced by recent introgression. It is more likely that the polymorphisms were present in the last common ancestor of Bubalina and Bovina about 5-8 million years ago (MacEachern et al. 2009). MacEachern et al. estimates that the split of Bubalina and Bovina subtribes occurred within a very large and extremely polymorphic population (MacEachern et al. 2009). After the separation of the two subtribes, autosomal sequence polymorphisms probably underwent lineage sorting - the assortment of ancestral genetic variation into different lineages resulting in a biased distribution of alleles in descendant populations (MacEachern et al. 2009). Although specific polymorphisms and sequences may be conserved, there are characteristic karyotypic differences in diverse members of the Bovini tribe.

A fundamental number of 56-58 autosomal chromosome arms is retained by most members of the Bovidae family, but the diploid chromosome number ranges from $2n = 30$ to $2n = 60$ (Chaves et al. 2004; Gallagher and Womack 1992). This variation in chromosome number is attributed to Robertsonian translocations, or centric fusions, that have joined acrocentric chromosomes to form metacentric or submetacentric chromosomes in different bovid species. The species of *Bos* and *Bison* probably possess a set of autosomal chromosomes that is most similar to the ancestral Bovidae (Gallagher et al. 1999a). Both *Bos taurus* and *Bos indicus* cattle have a diploid chromosome number

of $2n = 60$ with 58 acrocentric autosomes (Gallagher et al. 1999a). In contrast with the evolution of autosomal chromosomes, the evolution of sex chromosomes in the Bovini tribe is the result of more complex rearrangements.

The Y chromosomes of the Bovini tribe vary in both size and centromeric index (Gallagher et al. 1999a; Goldammer et al. 1997; Meo et al. 2005). *Bos taurus* cattle, gaur, and banteng have metacentric Y chromosomes, whereas *Bos indicus* cattle, American bison, European bison, water buffalo, African buffalo, and the lowland anoa have acrocentric Y chromosomes (Gallagher et al. 1999a; Goldammer et al. 1997; Meo et al. 2005). A close relationship between the *Syncerus* and *Bubalus* genera is suggested by the presence of X-specific repetitive DNA on both of their Y chromosomes (Gallagher et al. 1999a). As observed in the Y chromosome, the placement of the centromere and the relative size of the X chromosome is variable within the Bovini tribe, though marker order on the X chromosome seems to be conserved (Chaves et al. 2004; Gallagher et al. 1999a). *Bos* and *Bison* genera share submetacentric X chromosomes, while water buffalo, lowland anoa, and African buffalo have acrocentric X chromosomes (Gallagher et al. 1999a; Meo et al. 2005). Acrocentric X and Y chromosomes are considered to be the ancestral state of the Bovidae family (Gallagher et al. 1999a).

Genetic and karyotypic similarity enables several of the domesticated and wild species of the Bovini tribe to interbreed. Interbreeding between separate populations or species of bovids may be genetically advantageous because it reduces the detrimental effects of inbreeding depression and provides hybrids with an increased level of fitness (Frankham and Loebel 1992). Within the Bovini Tribe, males are the heterogametic sex with XY sex chromosomes and females are the homogametic sex with XX sex chromosomes. Haldane's rule states, "When in the F_1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous [heterogametic] sex (Haldane 1922)." In accordance with Haldane's rule, crosses between different species of the Bovini tribe often result in fully fertile female offspring and sterile male offspring. However, future generations of the hybrid may become fully fertile and reproductively fit even if the F_1 generation is not (Arnold and Hodges 1995). Cattle are able to produce fully fertile female offspring and sterile male offspring by interbreeding with banteng, gaur, yak, and bison (Gray 1972).

Domestic bantengs are also referred to as Bali cattle and have largely hybridized with *Bos indicus* cattle in South-East Asia since as early as 500 A.D. (Bradshaw and Brook 2007; Nijman et al. 2003). Hybridization with domesticated cattle is now considered a threat to the genetic purity of the endangered banteng. Unlike the domestic bantengs of Asia, the feral banteng populations in northern Australia do not show any evidence of introgression with domestic cattle (Bradshaw and Brook 2007). However, the wild banteng population has very low levels of genetic diversity because the founder population consisted of only 20 domestic bantengs that were released in 1849 from a failed British military outpost in Australia (Bradshaw et al. 2007). The Madura zebu breed is speculated to be a hybrid of banteng and *Bos indicus* cattle (Lenstra and Bradley 1999).

The kouprey is now thought to be an extinct, but it previously occupied Cambodia and morphologically appeared to be a cross between zebu cattle and the banteng (Galbreath et al. 2006). Mitochondrial haplotypes of the kouprey could be evolutionary derivatives of banteng mitochondrial haplotypes (Galbreath et al. 2006). The debate continues over whether the kouprey was a wild ox with some combination of banteng, *Bos indicus*, and *Bos taurus* ancestry (Galbreath et al. 2006), or a unique species of wild ox without any hybrid introgression (Hassanin and Ropiquet 2004, 2007). Though most accounts of the kouprey describe it as feral, there is evidence that the kouprey may have been domesticated in Cambodia (Hassanin et al. 2006).

The wild gaur, *Bos gaurus*, is the largest, heaviest, and most powerful member of the Bovini tribe (Choudhury 2002). The three wild subspecies of gaur include the most endangered subspecies *Bos gaurus laosiensis* in Southeast Asia, the most populous subspecies *Bos gaurus gaurus* in India and Nepal, and the smallest subspecies *Bos gaurus hubbacki* in Thailand and Malaysia (Nguyen et al. 2007). Wild gaurs were probably domesticated about 2500 years ago in India (Nguyen et al. 2007). Today the domestic gaur, *Bos frontalis*, is commonly known as gayal or mithan and is completely interfertile with the wild gaur. The gayal may be a derivative of a gaur-cattle cross as the gayal is able to breed with domestic cattle, although F1 females are usually the only fertile offspring of this cross (Nguyen et al. 2007). The selembu cattle of Bhutan and India are

created from gayal–zebu crosses. The fertile selembu females are used for dairy while the sterile males are draft animals (Lenstra and Bradley 1999).

Yaks primarily live on the Qinghai-Tibetan Plateau where they were domesticated about 5000 years ago from a single wild population, and both wild and domesticated yaks exist today (Gu et al. 2007; Guo et al. 2006). The distinctive grunt of the yak inspired its scientific name *Bos grunniens*, and *Bos mutus* since wild yaks grunt only during mating season (Lenstra and Bradley 1999). Hybrids of the yak and *Bos taurus* cattle are referred to as yakows and are usually crosses of taurine bulls and yak cows (Lenstra and Bradley 1999). Like gaur-cattle hybrids, only female F1 yakows can produce viable offspring and male hybrids are always sterile (Lenstra and Bradley 1999). Introgression of yak mtDNA was identified in the Diqing cattle breed of China (Yu et al. 1999) and in the Dwarf Lulu cattle breed of Nepal (Takeda et al. 2004).

Bison experienced their highest population level around 30,000 to 45,000 years ago. The bison population size underwent a sharp decline about 10,000 years ago concurrent with dramatic changes in their climate and the first introduction of humans to their environment (Drummond et al. 2005; Shapiro et al. 2004). Around this time, both humans and bison are believed to have crossed the Bering Land Bridge into North America (Shapiro et al. 2004). Bison species that are now extinct include *Bison antiquus*, *Bison latifrons*, *Bison occidentalis*, and *Bison priscus* (Pushkina and Raia 2008; Wilson et al. 2008; Wilson et al. 2009). Two species of bison still exist today - the North American bison, *Bison bison*, and the European bison or wisent, *Bison bonasus* (Shapiro et al. 2004).

The plains bison, *Bison bison bison*, and the wood bison, *Bison bison athabascae*, were the only North American bison subspecies to survive a severe population bottleneck just 200 years ago (Shapiro et al. 2004). North American bison may have been hunted to the point of extinction if it had not been for five private ranches in the U.S. that captured and raised no more than 100 feral North American bison in the late 1800s (Coder 1975). Nearly all contemporary North American bison can trace their ancestry to the bison of these five private herds or the approximately 30 wild bison that managed to survive in Yellowstone National Park (Halbert and Derr 2007).

North American bison, *Bison bison*, and European bison, *Bison bonasus*, share similar Y chromosome sequences and AFLP patterns, and they interbreed to produce fully fertile male and female offspring (Verkaar et al. 2004). However, studies of mtDNA show that the European bison is quite divergent from the North American bison, and the mitochondrial lineage of European bison may have been associated with *Bos indicus* cattle (Verkaar et al. 2004). European bison underwent a severe population bottleneck and almost became extinct in 1922 (Lenstra and Bradley 1999). The current population is thought to have come from only 12 founder individuals that began a rescue breeding program in 1924 (Perzanowski and Olech 2007). Consequently, today's European bison population maintains low levels of genetic diversity that are responsible for high levels of infertility and disease susceptibility (Wojcik et al. 2009).

Human-controlled breeding of *Bos taurus* and *Bison bison* has successfully created fertile female F1 offspring, and the subsequent backcrossing of F1 females to bison bulls generates fully fertile male and female bison-cattle hybrids (Dary 1974; Halbert and Derr 2007). Hybrids of domestic cattle and bison have been referred to as cattalo or beefalo (Dary 1974). The introgression of mitochondrial and nuclear cattle DNA has been identified in most North American bison herds (Halbert and Derr 2007; Halbert et al. 2004; Halbert et al. 2005; Vogel et al. 2007; Ward et al. 2001). Like North American bison, European bison can also reproduce with domestic cattle. A study by Ward et al. detected *Bos indicus* mtDNA in yak, and both African and European *Bos taurus* mtDNA in North American and European bison (Ward et al. 1999). While members of the *Bos* and *Bison* genera can interbreed, karyotypic differences prevent modern domestic cattle from successfully reproducing with members of the *Bubalus* or *Syncerus* genera.

The smallest buffalo in the world is the anoa, which has the approximate size and shape of a goat or deer (Iannuzzi and Di Meo 2009). The anoa lives on the island of Sulawesi in Indonesia and is in danger of extinction (Iannuzzi and Di Meo 2009). The two subspecies of anoa are the lowland anoa, *Bubalus depressicornis depressicornis*, and the mountain anoa, *Bubalus depressicornis quarlesi* (Schreiber et al. 1999). The endangered tamarao, *Bubalus mindorensis*, lives on the Philippine islands and has been frequently confused with the anoa, but it is considered a separate subspecies of the water

buffalo (Kuehn 1986). The domestic water buffalo, *Bubalus bubalis*, contains two major subspecies - river buffalo and swamp buffalo - which differ from each other in phenotype, behavior, and karyotype (Kumar et al. 2007). Despite these differences, the swamp and river buffalo are able to successfully hybridize with each other, and there may even be a zone in Southeast Asia for hybrid buffalo (Kumar et al. 2007; Lei et al. 2007). Two different domestication events probably gave rise to the river and swamp buffalo subspecies (Kumar et al. 2007; MacEachern et al. 2009). River buffalo are located on the Indian subcontinent, the Middle East, and Eastern Europe, while swamp buffalo are found across China, Bangladesh, Southeast Asia, and northeast India (Kumar et al. 2007). The wild Asian water buffalo, *Bubalus arnee*, still exists but is highly endangered (Flamand et al. 2003; Lenstra and Bradley 1999).

The African buffalo is considered dangerous to humans and has never been domesticated (Lenstra and Bradley 1999). The following are three subspecies of African buffalo: the Cape buffalo, *Syncerus caffer caffer*, located on the savannahs of eastern and southern Africa; the forest buffalo, *Syncerus caffer nanus*, living in the rain forests of western and central Africa; and the West African buffalo, *Syncerus caffer brachyceros*, found in the savannahs of Sudan (Van Hooft et al. 2002). The subspecies may interbreed, but the F1 offspring often have reduced fertility due to unbalanced gametes (Iannuzzi and Di Meo 2009). African buffalo populations have suffered from rinderpest epidemics and habitat fragmentation, but this does not appear to have affected their genetic diversity (Van Hooft et al. 2000).

Formation of Diverse Cattle Breeds

A distinct genetic history fashioned every modern cattle breed to acquire its own unique characteristics. The earliest ancestors of each breed were derived from the domestication of a particular auroch subspecies that subsequently adapted to its environment and the artificial selection pressures imposed on it by man. Each breed may have also been influenced by the crossing of their breed stock to ancient wild aurochs and any number of other local bovine species. This has created a diverse array of cattle breeds with a large amount of genetic diversity. A true cattle breed possesses distinguishing and uniform characteristics that are consistently passed on to their

offspring. Most breeds have corresponding breed associations that determine whether or not an individual is qualified for registry within their breed.

There are currently over 800 recognized cattle breeds in the world (OSU 1995). These breeds are commonly classified as *Bos indicus*, European *Bos taurus*, or African *Bos taurus*, and they may also be divided into groups based on their suitability to produce beef or dairy products. Many European *Bos taurus* breeds such as Simmental, Hereford, Red Poll, White Park, Highland, Galloway, Angus, Red Angus, Dexter, Salers, Limousin, Charolais, Maine-Anjou, and Gelbvieh have been bred for quality beef production. A selection of European *Bos taurus* cattle have been bred for excellence in dairy production and include Holstein, Shorthorn, Brown Swiss, Guernsey, Jersey, Normande, Norwegian Red, Kerry, and Ayrshire. *Bos taurus* breeds developed elsewhere include the Romosinuano of Colombia, the Texas Longhorn of the United States, the N'Dama of Western Africa, and the Sheko of Eastern Africa. The Brahman, Gir, Nelore, Sahiwal, and Tharparkar are *Bos indicus* cattle breeds that were formed in India and Pakistan. The Brangus, Beefmaster, and Santa Gertrudis cattle breeds originated in the United States from crossbreeding *Bos taurus* and *Bos indicus* cattle.

The breed formations of Hereford, Red Poll, White Park, and Shorthorn took place in England. The Hereford breed was founded in England around 1750 with the purpose of efficiently and economically converting local grass to beef during the British industrial revolution (OSU 1995). Today, Hereford cattle are renowned for reaching early maturity and are used across the world for beef production (Gibbs et al. 2009; OSU 1995). The Red Poll breed was created in England during the 18th century by crossing the now extinct Norfolk Red and Suffolk Dun, although Galloway and Devon cattle may have also influenced the development of the breed (OSU 1995). Red Poll cattle are used for both beef and dairy production and are uniformly red in coat color and polled, meaning they are without horns (OSU 1995). White Park cattle are horned and have white coats with darker colored points on their ears, nose, eyes, tongue, teats, and feet (OSU 1995). White cattle with colored points were described 2,000 years ago in Irish sagas, and they were also mentioned in laws written by a series of Welsh rulers from 856 A.D. to 1197 A.D. (OSU 1995). The White Park breed may have been imported to Britain or directly descended from the “Wild White Bull” that inhabited the forests of the

British Isles, but it is genetically most similar to the Galloway and Highland breeds of Scotland (OSU 1995). The Shorthorn breed originated on the northeastern coast of England as early as 1580, but accurate records of the breed were not kept until after 1750 (OSU 1995). Shorthorns bred for milking were brought to the United States in 1783 and were favored by pioneers in Virginia because of their meat, milk, and strength (OSU 1995). Today Shorthorn cattle are red, red-and-white, or roan in coat color and are found in greatest numbers on the British Isles, the United States, and Australia (OSU 1995).

The Highland, Galloway, Angus, Red Angus, and Ayrshire cattle breeds were founded in Scotland. Highland cattle have adapted to the harsh environment of the Scottish Highlands, and in 1884 they were the first cattle breed to be officially registered in a herd book (OSU 1995). The Highland breed is horned, has long hair around its eyes, provides lean yet marbled meat, is docile in temperament, and is also very intelligent (OSU 1995). Highland cattle are hardy and easy to maintain because they have developed the ability to resist disease and physically thrive in very hot or cold temperatures with low quality food and little to no shelter (OSU 1995). Galloway cattle are described in a historical document of 1573 as large oxen living in southern Scotland (OSU 1995). The Galloway breed has a dense hair coat consisting of a long outer coat that may be a range of different colors and a soft insulating and waterproofing undercoat (OSU 1995). Galloway cattle have a long lifespan, calve easily, can utilize coarse grasses to create tender beef, and do not gain excessive layers of outside fat (OSU 1995). The formation of the Angus breed began in Scotland around the last half of the eighteenth century (OSU 1995). Today Angus cattle are found around the world and are known for their black coat color and excellent meat quality (Gibbs et al. 2009). The Red Angus breed was established in 1954 for the sake of Angus cattle that were not eligible for the Angus registry because they exhibited the recessive red coat color instead of the dominant black coloration (OSU 1995). Red Angus cattle have the same ancestry as black Angus cattle and differ only in coat color. The Ayrshire breed was founded in Scotland before the year 1800 and probably has genetic relationships with the Holstein and Shorthorn breeds (OSU 1995). Ayrshire cattle are strong, adaptable, and medium-sized cattle that have red and white coat colors and excel at grazing and producing milk with moderate levels of butterfat (OSU 1995).

Ireland is the native land of the Dexter and Kerry breeds. The Dexter breed developed in southern Ireland where small land owners found Dexter cattle to be ideal because their hardiness and small size gave them the ability to survive on small plots of land with little shelter (OSU 1995). Dexter cattle are usually black in color, 36 to 44 inches tall at the shoulder, have high fertility rates, and may be used for beef or dairy production (OSU 1995). Though the genetic origins are unclear, the Dexter breed may have been derived from a cross of Kerry or Devon cattle (OSU 1995). It is probable that the Kerry breed descended from the Celtic Shorthorn, which was imported to Ireland as early as 2000 B.C (OSU 1995). Kerry cattle are small, fine-boned dairy animals with a long life span and a black coat color (OSU 1995). The Kerry breed is globally sparse, and as of 1983 there were only 200 Kerry cattle worldwide (OSU 1995).

The Salers, Limousin, Charolais, Normande and Maine-Anjou cattle breeds originated in France. The Salers breed was founded in central France and has been depicted in 7,000 year old cave paintings, making Salers one of the oldest and most genetically pure of all European breeds (OSU 1995). Salers cattle have excellent carcass merit, demonstrate maternal efficiency, possess a dark red coat color and resemble ancient Egyptian red cattle (OSU 1995). Another one of the oldest breeds of cattle is the Limousin, which also originated in France and is currently raised for beef in France, the United Kingdom, and North America (Gibbs et al. 2009). Limousin cattle have a golden-red coat color and are sturdy animals, as they have adapted to thrive in an area of rocky soil and harsh climate (OSU 1995). The Charolais breed began in France as early as 878 A.D. and now resides in France, North America, Brazil, and South America (Gibbs et al. 2009; OSU 1995). Charolais cattle are known for their white coat color, large size, substantial muscling, and rapid growth rate (OSU 1995). Viking conquerors brought Normande cattle to Normandy in the 9th and 10th centuries, and the breed evolved to provide meat and milk to residents of northwestern France (OSU 1995). The Normande breed was introduced to South America in 1890, and over four million purebred Normande cattle reside there today (OSU 1995). Normande cattle have a medium sized frame with excellent body depth and a high mass of muscle that produces a quality carcass yield (OSU 1995). They also reach sexual maturity early, have good fertility and mothering ability, and are ample milk producers (OSU 1995). The Maine-Anjou breed

began in southern France early in the 18th century and is the largest of all breeds originating in France (OSU 1995). Maine-Anjou cattle are used in the production of both beef and dairy, and they have characteristic dark red coloration with white markings that may appear on the head, belly, rear legs, and tail (OSU 1995).

The Guernsey and Jersey dairy breeds began on the Channel Islands, located off the coast of France. The Guernsey dairy breed originated around 960 A.D. on the Channel Islands and is now present throughout North America, the United Kingdom, Oceania, and South Africa (Gibbs et al. 2009). Artificial insemination is frequently used in modern Guernsey breeding programs (OSU 1995). Guernsey cattle do not require as much food as other breeds and are known to produce milk containing high amounts of protein, butter-fat, and beta-carotene from grazing on pasture grasses (OSU 1995). The Jersey breed also came from the Channel Islands and is one of the oldest dairy breeds, as it is thought to have been established as a purebred breed for almost six centuries (OSU 1995). Jersey cattle are known for their small size and relatively high yield of milk with a large amount of butterfat (Gibbs et al. 2009; OSU 1995).

The Simmental and Brown Swiss breeds were derived in Switzerland. The Simmental breed is one of the oldest and most widely distributed breeds of cattle in the world (OSU 1995). The breed originated in Switzerland and was exported to Italy as early as the 1400s (OSU 1995). Simmental cattle are known for their rapid growth and development rates, red and white spotted coat color, and the production of beef and dairy products (OSU 1995). The Brown Swiss breed began in Switzerland and became a prominent dairy breed about one hundred years ago (OSU 1995). Brown Swiss cattle are now found throughout Alpine Europe and the Americas (Gibbs et al. 2009).

The Piedmontese and Romagnola cattle breeds are descendants of early *Bos taurus* and *Bos indicus* hybridization events in Italy. The early auroch ancestors of Piedmontese cattle lived in the Piedmonte region of Italy and interbred with *Bos indicus* cattle about 25,000 years ago (OSU 1995). Today the Piedmontese breed is known for its muscularity, double-muscling, and grey-white coat color (Gibbs et al. 2009). The Romagnola breed is thought to have been derived from the cross of the auroch subspecies living on the Italian peninsula, *Bos primigenius podolicus*, and the auroch subspecies that gave rise to *Bos indicus* cattle, *Bos primigenius namadicus* (OSU 1995). Romagnola

cattle were used for draft work and the production of beef (OSU 1995). Adult Romagnola cattle have ivory-grey colored coats, shaped horns, and are found today in Italy, the United States, and Australia (Gibbs et al. 2009).

Other countries of Western Europe gave rise to Gelbvieh, Holstein, and Norwegian Red cattle. The Gelbvieh breed was established in southern Germany during the late 18th or early 19th century (OSU 1995). Gelbvieh cattle are red in coat color and are thought to have outstanding fertility, calving ease, mothering ability, and calf growth rate (OSU 1995). The Holstein breed began in what is now the Netherlands and now has a large worldwide distribution (Gibbs et al. 2009). Holstein cattle are known for having the highest milk yield of any breed, coat coloration of black-and-white or red-and-white, and the extensive use of artificial insemination in breeding programs (OSU 1995). Norwegian Red cattle were given a name designation in 1961, but they are not yet considered a true breed because they do not demonstrate external uniformity (OSU 1995). Norwegian Red cattle are dual purpose animals used in Norway for both beef and dairy, and they are usually red or red pied in coat color (Gibbs et al. 2009).

Europe is the native homeland for many modern *Bos taurus* breeds, but there are plenty of additional *Bos taurus* breeds that have developed in different areas of the world. The Romosinuano breed originated during the late 1800's in Colombia (OSU 1995). Romosinuano cattle are red-brown in coat color, small in size, docile in temperament, polled, and suitable for beef production (OSU 1995). The Texas Longhorn breed arrived in the United States about 500 years ago (OSU 1995). Texas Longhorn cattle survived on primitive ranges in the southwestern portion of the United States without any regulations or restraints by man, so the breed was truly fashioned by natural selection and adaptation to life in North America (OSU 1995). Around 1865, Texas Longhorn cattle were moved to the western part of the United States to occupy the Great Plains after the almost complete destruction of the North American bison population (OSU 1995). Intensive crossbreeding of Texas Longhorn cattle with more "improved" cattle nearly eliminated the Texas Longhorn breed by 1900, but it was saved in 1927 through preservation in wildlife refuges in Nebraska and Oklahoma (OSU 1995). The Texas Longhorn breed possesses unique and untapped genetic variation that could be used in the future to maximize hybrid vigor in the beef industry.

African *Bos taurus* breeds include N'dama and Sheko. The N'dama breed originated in the Fouta-Djallon highlands of Guinea and is currently the most representative *Bos taurus* breed in West Africa (OSU 1995). N'Dama cattle are trypanotolerant, 100 to 120 cm tall at the shoulders with large heads and lyre-shaped horns, generally have fawn colored hair and skin, and are used for both beef and milk production (Gibbs et al. 2009; OSU 1995). The Sheko breed was first identified in 1929 in Ethiopia and is currently raised across East Africa for work and beef production (Gibbs et al. 2009). Sheko cattle are also trypanotolerant, have no horns or only small horns, and possess a brown coat color that may have patches of black and white (Lemecha et al. 2006). The Sheko breed is considered to be endangered because it continues to interbreed with neighboring *Bos indicus* cattle (Dadi et al. 2008).

Zebu, or *Bos indicus*, cattle breeds include the Brahman, Gir, Nelore, Sahiwal, and Tharparkar. The Brahman cattle of India are considered sacred in the Hindu faith and cannot be eaten or sold, so it has been difficult to import them into the United States (OSU 1995). The Brahman breed of the United States was primarily derived from the Guzerat, Nelore, and Gir breeds imported from India in the late 1800's (OSU 1995). The U.S. Brahman breed is intermediate in size, has adapted to a wide range of climates and types of feed, varies in coat color from white to red to black, has black pigmented skin, and demonstrates a high tolerance for heat (OSU 1995). The Gir is major zebu breed of India that originated within the state of Gujerat in southwest India (OSU 1995). Gir cattle have been used in India to locally improve the Red Sindhi and Sahiwal breeds (OSU 1995). Gir cattle produce both dairy and beef, have a domed forehead, and their coat colors range from red to white (OSU 1995). The Indian Ongole breed was the main contributor to the development of the Nelore breed (OSU 1995). Brazil first recognized the Nelore breed in 1868, and today Brazil is the largest breeder of Nelore cattle (OSU 1995). Nelore cattle have a white to gray coat and loose black skin, can thrive in hot climates with low quality food and little water, have a natural resistance to various insects, produce lean meat, and show reproductive efficiency and good maternal disposition (OSU 1995). The Sahiwal breed was founded in the Punjab region on the border of India and Pakistan (OSU 1995). Sahiwal cattle were brought to Australia in the 1950s and influenced the development of the Australian Milking Zebu and the Australian

Fresian Sahiwal (OSU 1995). Sahiwal have the highest milk yield of all zebu breeds, are suitable for slow draft work, have a high resistance to ticks and parasites, are heat tolerant, and have a reddish brown coat color with occasional white markings (OSU 1995). The Tharparkar breed was developed in the Tharparkar district of Sindh in Pakistan and was influenced by the Kankrej, Red Sindhi, Gir and Nagori breeds (OSU 1995). Tharparkar cattle are used for milk production and draft work, have a medium size and strong build, are white or gray in coat color, and require frequent contact with humans to keep from becoming wild (OSU 1995).

The crossbreeding of *Bos taurus* and *Bos indicus* cattle has utilized the principle of hybrid vigor, or heterosis, wherein the breeding value of the offspring is greater than that of their parents. The Brangus, Beefmaster, and Santa Gertrudis cattle breeds were developed in the United States by crossbreeding *Bos indicus* and *Bos taurus* cattle. The American Brangus Breeds Association was formed in 1949 (OSU 1995). Brangus cattle are approximately 3/8 Brahman and 5/8 Angus, solid black in coat color, and polled (OSU 1995). The breeding program for Beefmaster cattle began in 1908 on the Lasater Ranch, but the headquarters have since moved to Texas (OSU 1995). Although the exact pedigree of the Beefmaster breed founders is unknown, it is estimated that modern Beefmaster cattle have less than one-half Brahman and more than one-fourth Hereford and Shorthorn breeding (OSU 1995). The King Ranch of Texas began forming the Santa Gertrudis breed in 1910, and the United States recognized Santa Gertrudis as a purebred breed in 1940 (OSU 1995). Santa Gertrudis cattle are a deep cherry-red color and have approximately 5/8 Shorthorn and 3/8 Brahman breeding (OSU 1995).

The complex genetic history of modern cattle breeds has generated a wide range of diverse phenotypes. Ongoing research continues to uncover the genetic basis underlying phenotypic traits of cattle like metabolism, lactation, fertility, conformation, and disease resistance. Breed histories should be taken into account when designing association studies, as divergent breeds of cattle may have evolved different genetic variants resulting in the same phenotype. The breed history and genetic structure of domestic cattle differs from that of the domestic dog, as all modern dog breeds are thought to have evolved from a single gene pool of grey wolves in East Asia about 15,000 years ago (Savolainen et al. 2002). Recent studies have revealed the gene content

and organization of the bovine genome, as well as levels of genetic variation present among different breeds of cattle.

The Bovine Genome Project

The Bovine Genome Sequence

Domestic cattle represent the first complete, high-coverage genome sequence of a eutherian mammal belonging to the order Cetartiodactyl (Tellam et al. 2009). The bovine genome sequence is important for understanding the history of mammalian evolution and the relationship of genetic variants with complex phenotypic traits in cattle. A comprehensive description of the *Bos taurus* genome sequence was published in 2009 and was accompanied by many papers analyzing specific attributes of the bovine genome (The Bovine Genome Sequencing and Analysis Consortium et al. 2009). Notably, the human genome was discovered to share a greater degree of protein similarity and chromosomal conservation with the cattle genome than with any of the sequenced rodent genomes.

The bovine genome was sequenced at a coverage of 7.1x, and the primary source of DNA sequence was derived a single inbred Hereford cow, known as L1 Dominette (Tellam et al. 2009). A combination of whole-genome shotgun sequences from L1 Dominette and bacterial artificial chromosomes (BACs) from L1 Domino, the sire of L1 Dominette, were used to generate the most recent genome assembly, termed Btau4.0. Cattle radiation hybrid maps, whole genome and chromosome-specific linkage maps, physical maps anchored by BAC end sequences, expressed sequence tags (ESTs), and full length cDNAs were all employed to accurately assemble sequences and orient scaffolds within the bovine genome (Tellam et al. 2009). The Btau4.0 assembly placed and validated 90% of the entire *Bos taurus* genome sequence on all 29 autosomal pairs and the X chromosome (The Bovine Genome Sequencing and Analysis Consortium et al. 2009). The assembled contigs contained 95% of all available EST sequences, and the bovine genome was estimated to be 2.87 Gbp in size (The Bovine Genome Sequencing and Analysis Consortium et al. 2009). The previous bovine genome assembly, Btau3.1, was the sequence source for the manual annotation of over 4,000 genes. The cattle genome is estimated to contain at least 22,000 protein-coding genes, and 1,217 of those

genes appear to be unique to eutherian mammals (The Bovine Genome Sequencing and Analysis Consortium et al. 2009). Compared to the human genome there is a significant reorganization of bovine genes that encode the proteins found in milk, but most of the genes involved in mammalian metabolic pathways are conserved in cattle.

Regions encompassing cattle-specific evolutionary breakpoints were found to have a higher density of segmental duplications and repetitive elements, which implies that repetitive elements and segmental duplications fashioned the current *Bos taurus* karyotype by processes such as nonallelic homologous recombination (The Bovine Genome Sequencing and Analysis Consortium et al. 2009). Segmental duplications were identified within 3.1% of the cattle genome, and 76% contained complete or partial gene duplications (The Bovine Genome Sequencing and Analysis Consortium et al. 2009). Many of these duplicated genes encode proteins that interface with the external environment, such as immune and sensory genes, and an additionally large number of duplicated genes are associated with expression in the ruminant placenta (The Bovine Genome Sequencing and Analysis Consortium et al. 2009). Cattle-specific gene duplications may have been functionally important for the development of distinct ruminant physiology or for rapid adaptation to changes in the surrounding environment. The broad duplication and divergence of genes involved in innate immunity, such as β -defensin and interferon genes, suggests that cattle needed to adapt to a variety of infectious disease agents throughout the course of their evolution. Changes in immune-related genes may also be related to the development of microbial fermentation or the transition to a herd environment with increased rates of disease transmission (The Bovine Genome Sequencing and Analysis Consortium et al. 2009).

An accurately assembled genome sequence provides future research studies with properly ordered genetic markers. Correct marker order is crucial for studies of linkage disequilibrium and haplotype structure. A haplotype is a stable combination of alleles at multiple loci inherited together in a population, and linkage disequilibrium is the underlying principle of haplotype structure. Linkage disequilibrium is the non-random association of alleles at multiple loci on the same chromosome within a population. If alleles at different loci are in linkage disequilibrium with one another, then they will occur together at a higher frequency than would otherwise be expected. Polymorphic

markers, which are inherited characteristics that occur as two or more traits, are used to analyze linkage disequilibrium and haplotype structure. Single nucleotide polymorphisms, microsatellites, SNPSTRs, and repeat elements are examples of different types of polymorphic makers.

Single Nucleotide Polymorphisms

Single nucleotide polymorphisms (SNPs) are sequence sites occupied by more than one of four nucleotide bases in different individuals. The average nucleotide mutation rate in humans is estimated to be $\sim 2.5 \times 10^{-8}$ (Nachman and Crowell 2000). There are expected to be 9-10 million SNPs in the human genome with a minor allele frequency >0.05 (Frazer et al. 2007). Over six million SNPs have been validated in the human genome, with approximately one SNP occurring every kilobase across the autosomes and the X chromosome (http://www.ncbi.nlm.nih.gov/SNP/snp_summary.cgi 2009). SNPs are attractive as genetic markers because they are present in high abundance, have a low mutation frequency, and are easily converted to high throughput, automated genotyping on “SNP chips.” A large proportion of haplotype diversity may be represented by a small number of SNPs, called tagSNPs, which may be selected for placement on a SNP chip (Montpetit et al. 2006).

The International HapMap Consortium was formed in 2002 with the intention of developing a haplotype map of the human genome that would describe patterns of common genetic variation (International HapMap Consortium 2005). The HapMap project was based on the idea that the human genome is comprised of relatively short sections of DNA in strong linkage disequilibrium, known as haplotype blocks, which are separated by recombination hotspots. Approximately 80% of all recombination events in the human genome occur within recombination hotspots, regions around 2 kb in length, that account for 10–20% of the genome (Myers et al. 2005). In addition to recombination hotspots, haplotype blocks are also influenced by mutation, selection, and population history (Wang et al. 2002). Individuals sharing a recent common ancestor are expected to have haplotype blocks that extend over longer distances and span multiple recombination hotspots. Genomic regions that have undergone adaptive evolution and recent positive selection are also predicted to have extended blocks of linkage disequilibrium (Frazer et

al. 2007). The extended block of linkage disequilibrium surrounding the HLA was identified as a region that has undergone adaptive evolution and recent positive selection (Frazer et al. 2007).

The second phase of the human HapMap project genotyped over 3.1 million SNPs, approximately one SNP every 1 kb, across individuals belonging to four diverse populations including 30 parent-offspring trios from Yoruba in Ibadan, Nigeria (YRI), 30 trios of northern and western European ancestry living in Utah (CEPH), 45 unrelated Han Chinese individuals from Beijing (CHB), and 45 unrelated Japanese individuals from Tokyo (JPT) (Frazer et al. 2007). The average human recombination rate is 1 cM per 1,000 kb; however, recombination rates were discovered to vary on a fine scale across the human genome (Myers et al. 2005). At least one recombination hotspot was identified in all human genomic regions larger than 200 kb (Myers et al. 2005). An average block of linkage disequilibrium spanned many SNPs, but each block had a small number of common haplotypes. It is estimated that 1.09 million tagSNPs in the YRI population and 500,000 tagSNPs in CEPH, CHB, and JPT populations are needed to capture human genome-wide variation (Frazer et al. 2007).

The genome of the domestic dog, *Canis familiaris*, has been sequenced and was found to be approximately 2.41 Gb in size (Lindblad-Toh et al. 2005). About 2.5 million SNPs were identified by comparing 6% of the reference boxer genome to ten different dog breeds (Lindblad-Toh et al. 2005). Linkage disequilibrium extends over tens of kilobases across modern dog breeds and spans several megabases within breeds (Lindblad-Toh et al. 2005). The long haplotype blocks observed in the genome of the domestic dog are probably attributed to domestication from a single gene pool and the genetic bottlenecks associated with modern dog breed formation (Savolainen et al. 2002). It is estimated that 10,000 SNPs would be sufficient to capture all the genetic diversity of domestic dogs (Lindblad-Toh et al. 2005). The genetic diversity within and between breeds of cattle is greater than that of domestic dogs, as cattle show more similarity to human genetic diversity (Gibbs et al. 2009).

The Bovine HapMap Project discovered SNPs across the cattle genome by comparing the Hereford reference sequence to random shotgun sequences from these six cattle breeds: Holstein, Angus, Brahman, Limousin, Jersey, and Norwegian Red (Gibbs et

al. 2009). Only one *Bos indicus* breed, Brahman, was used in the SNP discovery phase, so it is not surprising that many of these SNPs are predominantly taurine and have a higher average minimum allele frequency in *Bos taurus* breeds than in *Bos indicus* breeds (Gibbs et al. 2009). The Bovine HapMap Consortium analyzed more than 37,470 SNPs in 497 cattle from 19 diverse breeds (Gibbs et al. 2009). A total of 12 European *Bos taurus* breeds were genotyped in the Bovine HapMap Project including Angus, Brown Swiss, Charolais, Guernsey, Hereford, Holstein, Jersey, Limousin, Norwegian Red, Piedmontese, Red Angus, and Romagnola (Gibbs et al. 2009). Additionally genotyped were two African *Bos taurus* breeds, N'Dama and Sheko; three *Bos indicus* breeds, Brahman, Gir, and Nelore; and two *Bos indicus* x *Bos taurus* hybrid breeds, Beefmaster and Santa Gertrudis (Gibbs et al. 2009). Each breed was represented by at least 24 animals and one or two parent-offspring trios, with the exception of Red Angus which had only 12 animals (Gibbs et al. 2009). Two anoa, *Bubalus quarlesi*, and two water buffalo, *Bubalus bubalis*, were genotyped for comparison as outgroups (Gibbs et al. 2009). The majority of the SNPs that gave a successful genotyping result in anoa and water buffalo were monomorphic (Gibbs et al. 2009).

The frequencies of SNP genotypes were analyzed with InSTRUCT (Gao et al. 2007), and clusters from varying numbers of presumed ancestral populations (K) were found to be consistent with what is known about cattle breed histories (Gibbs et al. 2009). At the presumed ancestral population of two (K=2), the *Bos taurus* and *Bos indicus* breeds were separated, which is consistent with a pre-domestication subdivision, and the crossbred breeds, Beefmaster and Santa Gertrudis, showed signatures of admixture (Gibbs et al. 2009). The African *Bos taurus* breeds, N'Dama and Sheko, clustered separately from European *Bos taurus* breeds at K = 3 (Gibbs et al. 2009). Higher levels of K separated breeds into their own clusters (Gibbs et al. 2009). In addition to analyzing SNPs across the bovine genome, the Bovine HapMap Project sequenced PCR products from five genomic regions of two chromosomes in 18 Angus, 16 Holstein, and five Brahman animals (Gibbs et al. 2009). The Holstein and Angus chromosomes were estimated to have one SNP every 714 bp, and the Brahman chromosomes were estimated to have one SNP every 285 bp (Gibbs et al. 2009). The lower genetic diversity observed within taurine breeds could be the result of low diversity in their ancestral population or

strong genetic bottlenecks during their domestication and breed formation (Gibbs et al. 2009). Alternatively, this could be reflective of the SNPs being predominantly taurine in nature, and the *Bos indicus* animals were not more genetically diverse but simply showed different fixed alleles at those positions. Overall, the Bovine HapMap Consortium concluded that the genetic diversity within cattle breeds is not low, despite the recent decline in effective population size (N_e) for all breeds (Gibbs et al. 2009). This supports the idea of a large ancestral population with a vast amount of genetic diversity that maintained substantial levels of genetic diversity even after going through genetic bottlenecks associated with domestication and selective breeding (Gibbs et al. 2009).

Patterns of linkage disequilibrium varied among the breeds of cattle analyzed by the Bovine HapMap Consortium (Gibbs et al. 2009). Hereford and Jersey breeds had the highest r^2 values across a range of distances, and the N'Dama breed held the highest r^2 value at short distances and the lowest r^2 value at long distances (Gibbs et al. 2009). The explanation given for this was that the N'Dama breed came from a small ancestral population and has not been through narrow population bottlenecks (Gibbs et al. 2009). Patterns of linkage disequilibrium in *Bos indicus* breeds indicated that they originated from a larger and more genetically diverse pre-domestication population than the *Bos taurus* breeds (Gibbs et al. 2009). Linkage disequilibrium declined much more rapidly when multiple breeds were analyzed together, and there was little evidence for the sharing of common haplotypes across breeds. Only breeds with a recent common ancestor, such as Angus and Red Angus, Holstein and Norwegian Red, and Beefmaster and Santa Gertrudis, showed a correlation between SNP alleles separated by distances of 100 – 250 kb. The phase of SNP alleles was shared at distances up to 10 kb within *Bos taurus* and *Bos indicus* breeds, but SNP phases were not shared between *Bos taurus* and *Bos indicus* breeds (Gibbs et al. 2009). This is consistent with the findings of Marques et al., which demonstrated that Angus and Holstein cattle do not share haplotypes at distances longer than 10 kb, and the strongest haplotypes within each breed are below 100 kb (Marques et al. 2008).

Microsatellites, SNPSTRs, and Repeat Elements

Microsatellites are also known as short sequence repeats (SSRs) or short tandem repeats (STRs), and they consist of 2-5 base pairs units of tandemly repeated DNA sequences. Microsatellites are ubiquitously interspersed throughout the nuclear genome of eukaryotes and often demonstrate polymorphisms within a species by varying in the number of their repeat units (Schlotterer and Tautz 1992; Weber and May 1989). Among the fully sequenced eukaryotic genomes, microsatellites are most common in mammalian species (Katti et al. 2001; Tóth et al. 2000). Analysis of the initial draft sequence of the human genome found more than one million microsatellite loci that together make up approximately 3% of the genome (Lander et al. 2001). Of the many possible microsatellite motifs, (AC)_n repeats are the most polymorphic. The proportion of AC repeats in humans is estimated to exceed 90% (Rockman and Wray 2002).

The microsatellite mutation rate in humans has been estimated to be $10^{-2} - 10^{-5}$ per generation (Tautz 1994), which is approximately 10^3 times higher than the mutation rate for single base substitutions (SNPs). Additionally, microsatellites are more informative than SNPs because large numbers of alleles may occur at each microsatellite locus. The primary mechanism of microsatellite mutation is slippage during DNA replication (Schlotterer and Tautz 1992). In human AC repeats, the rate of slippage is highest at a length of ten repeats and then declines roughly linearly until reaching an apparent maximum at about 20 repeats (Sibly et al. 2003). The frequency distributions of microsatellite lengths within a genome may represent a balance between the expansionary tendencies of slippage mutation and the contractions caused by point mutations breaking longer microsatellites into smaller units (Bell and Jurka 1997). Perfect uninterrupted AC microsatellite motifs with a repeat unit number near ten are most likely to be polymorphic in mammalian genomes (Sibly et al. 2003).

Unfortunately, the mutability that makes microsatellites appealing as polymorphic markers also creates challenges in distinguishing between different microsatellite alleles. Homoplasmy occurs when two variants are identical by state but not by descent (Estoup et al. 2002). The concept of homoplasmy was originally developed by evolutionists to describe a trait present in two species but not derived from a common ancestor, so homoplasmy can describe events of convergence, parallelism, reversion, or features that

appear more than once in a cladogram (Sanderson and Donoghue 1989). Genotyping by identifying size variants such as those found in microsatellites differing in repeat unit number is a common technique used in molecular genetics (Oetting et al. 1995), but genotyping can be complicated by size homoplasy, where two alleles are identical in size but not in composition (Ramakrishnan and Mountain 2004). Size homoplasy is not detected by standard methods of genotyping and may result in a reduced number of observed alleles and a lower proportion of heterozygotes than expected. Simulation studies estimate that the probability of two alleles being identical by state but not by descent is as high as 30%, and the highest levels of homoplasy are noted when there is a constraint on allele size (Estoup et al. 2002).

The analytical problems posed by size homoplasy in microsatellite markers can be reduced by incorporating closely linked SNPs into the genotyping procedure. A “SNPSTR” marker contains one or more single nucleotide polymorphisms (SNPs) and exactly one short tandem repeat (STR) within 500bp stretch of DNA (Mountain et al. 2002). These markers provide complementary evolutionary information because of their differing mutation rates. Each independent SNPSTR combines slowly evolving polymorphic SNP(s) with a more rapidly evolving polymorphic microsatellite (Ramakrishnan and Mountain 2004). SNPSTRs can be thought of as small, independently evolving compound haplotypes. These haplotypes are unlikely to be broken up by recombination if their size is less than 500 bp (Mountain et al. 2002), and simulation studies have shown that SNPSTRs are more accurate and show lower levels of homoplasy than experiments solely based on STRs (Ramakrishnan and Mountain 2004). A database has been developed for SNPSTRs found in human, mouse, rat, dog, and chicken (Agrafioti and Stumpf 2007). These SNPSTRs are inferred *in silico* from information available in the Ensembl database (Agrafioti and Stumpf 2007).

During the development of SNPSTRs, the sequence encompassing the STR should be made as large as possible (up to 500bp) within the constraints of primer design and quality sequence reads to increase the likelihood of finding SNPs within the PCR amplicon. Determining the gametic phase for double heterozygotes requires the cloning and sequencing of PCR products. A SNP found in a cloned PCR sequence should be present in the same location on the direct PCR sequence (i.e. the original heterogeneous

PCR product). This verifies the authenticity of the SNP and eliminates any errors caused by the Taq polymerase during the PCR reaction. It has been demonstrated that conventional Taq polymerase creates an error in 30-70% of cloned PCR sequences with one error incorporated per 1421 nucleotides (Ennis et al. 1990). The majority of errors found in cloned PCR products are point substitutions that can easily be misinterpreted as SNPs. The constraints of primer design for SNPSTRs are the same as those for conventional microsatellite genotyping. Primers should be specific within the thermally selected parameters, and variation underlying the primer sequences should be avoided to prevent the occurrence of false “null” alleles. Pedigreed samples should be used whenever possible to verify the Mendelian inheritance of alleles, and placing PCR primers within highly repeated elements should be avoided to ensure locus-specific amplification. Many microsatellite sequences are closely associated with repetitive DNA in mammalian genomes (López-Giráldez et al. 2006).

Microsatellites associated with SNPSTRs and repeat elements have both been utilized in human population studies (Mountain et al. 2002). Two SNPSTR systems were used to show that anatomically modern humans first migrated out of Africa relatively recently (Mountain et al. 2002). Analysis of polymorphic Alu insertions linked to microsatellites produced similar conclusions about the time of human migration out of Africa (Tishkoff et al. 2000). Polymorphic Alu insertions have shown potential value as lineage and linkage markers for the study of human population genetics, genomic diversity, evolution, and disease associations within HLA (Dunn et al. 2005). Utilizing repeat element polymorphisms in addition to SNPs and microsatellites offers a range of mutation rates to draw from in haplotype studies.

Particular microsatellite motifs are often associated with specific types of repeat elements. The extension of retroposon 3' polyadenylation repeats can lead to the generation of A-rich microsatellites (López-Giráldez et al. 2006). The majority of the A-rich tetranucleotide repeats in the BoLA IIB region are located within 40bp of the terminal end of a SINE or LINE. Over 92% (25/27) of microsatellites with (AGTTC)_n and (AACTG)_n motifs in the BoLA IIB region are associated with SINE-ART2 repeat elements. Such pentanucleotide repeats are known to occur at the 3' end of ART2 (Malik et al. 1998). The bovid-specific Bov-A2 repeat element is usually found tailed with

(AGC)_n or (ACG)_n trinucleotide repeats (Kaukinen and Varvio 1992). The microsatellite motif (AGC)_n is 90- fold overrepresented in cattle compared with human and 142- overrepresented in cattle compared to dog (The Bovine Genome Sequencing and Analysis Consortium et al. 2009). In accordance with that, over 94% (33/35) of microsatellites in the BoLA IIb region with (AGC)_n or (ACG)_n motifs are located on the ends of Bov-A2 repeat elements. There are also over 273 Bov-A2 repeat elements with (AGC)_n or (ACG)_n microsatellite tails located in the BoLA IIa/III/I region.

Bov-A2 repeat elements are specific to ruminants and are widely distributed throughout the bovine genome (The Bovine Genome Sequencing and Analysis Consortium et al. 2009). It is estimated that Bov-A2 repeats account for 1.8% of the cattle genome (Lenstra et al. 1993). They usually consist of two monomers, called Bov-A units, connected by a linker sequence (CACTTT)_n (Onami et al. 2007). A unique feature of the Bov-A2 repeat element is that the number of Bov-A units at a particular locus in the bovine genome can vary between individuals (Onami et al. 2007). PCR primers can be designed in the unique regions flanking both ends of the Bov-A2 repeat to detect allelic differences in Bov-A unit number that can easily be distinguished by gel electrophoresis because each Bov-A unit is ~120bp in length (Onami et al. 2007). Onami et al. (2007) described five polymorphic Bov-A2 retroposons in domestic cattle, but not in Bongo, Arabian Oryx, Axis Deer, Reticulated Giraffe, Pronghorn, and Lesser Mouse Deer. Onami et al. (2007) proposed that polymorphism in Bov-A unit number was a consequence genomic instability associated with cattle domestication.

Sequence analysis has revealed that elongation anywhere from two to six Bov-A monomers occurs by unequal crossing-over between the Bov-A units (Onami et al. 2007). It has also been reported that a higher mutation frequency exists in Bov-A2 sequences compared to other noncoding sequences (Damiani et al. 2000), perhaps due to unequal crossing-over between Bov-A units (Onami et al. 2007). The Bov-A2 element usually ends with a 3' (AGC)_n or (ACG)_n microsatellite that varies from 1-9 repeat units (Kaukinen and Varvio 1992), and these microsatellite tails have been used as polymorphic genetic markers in cattle (Band and Ron 1996). Bov-A2 repeat elements are a rich source of SNPs, polymorphic microsatellites, and varying numbers of Bov-A units. Utilizing these different sources of polymorphism within Bov-A2 elements is

conceptually similar to utilizing the independently evolving compound haplotypes of SNPSTRs.

Aims and Goals of This Project

Evaluation of Polymorphic Markers in BoLA

Different types of genetic markers in the BoLA IIa/III/I region and the BoLA IIb region were evaluated for polymorphism across various breeds of cattle. In addition to characterizing new polymorphic microsatellites throughout the BoLA regions, two published BoLA microsatellites were utilized in this study. The amplified sequences surrounding BoLA microsatellites were analyzed for the presence of SNPs to determine if they may serve as SNPSTR markers. Bov-A2 elements throughout the BoLA regions were evaluated for polymorphism in Bov-A unit number, and alleles of DRB3, DRA, and DQB were defined by comparing the exon 2 sequence to known BoLA alleles. Much of the SNP data was obtained from genome-wide SNP chip typing performed in conjunction with the Bovine HapMap Project and the University of Missouri linkage study. All BoLA markers were evaluated for correct order and reliable genotyping results within pedigreed samples. Criteria for marker retention included locus specificity, consistent typing across all haplotypes (absence of null alleles), discrete alleles (absence of homoplasmy), and a sufficient level of polymorphism, depending on marker type.

Characterization of BoLA Haplotype Structure

The haplotype structure of BoLA IIb was analyzed separately from the haplotype structure of BoLA IIa/III/I, as the two are located on separate regions of chromosome 23. The alleles of most markers were phased by pedigree inference or haplotype homozygosity. SNPs were analyzed by PHASE and fastPHASE to determine haplotype structure when it was not possible to do so by pedigree inference or haplotype homozygosity, and the accuracy of PHASE was assessed in a subset of pedigreed animals. The high level of genetic diversity between different breeds of cattle confounds proper PHASE analysis, so PHASE and fastPHASE were performed within each breed rather than across multiple breeds. A new method of haplotype inference was developed that determines heterozygous haplotypes from comparison to known homozygous

haplotypes present at high frequency. Different subsets of SNPs were typed across various DNA samples, but the SNP data was made as consistent as possible for haplotype comparison purposes.

The heterozygosity of SNPs surrounding homozygous BoLA IIA/III/I haplotypes was used to assess breakpoints in linkage disequilibrium, and microsatellite markers were used to determine the locations and frequencies of recombination events in the BoLA IIB and BoLA IIA-III-I regions. Frequencies of SNP-defined homozygous BoLA IIA-III-I haplotypes were calculated in breeds with the largest sample sizes, including Angus and Holstein. The exon 2 sequence of the DRB3 gene was integrated with microsatellite-derived BoLA IIA-III-I haplotypes to evaluate how predictive DRB3 alleles were of BoLA IIA-III-I haplotypes. Additional microsatellites, SNPSTRs, and the exon 2 sequence of DRB3, DQB, and DRA genes were typed on animals that were defined to be homozygous by 52 SNPs located within the BoLA IIA-III-I region to assess how predictive the SNPs were of BoLA IIA-III-I haplotypes.

Phylogenetic Relationships of BoLA Haplotypes

BoLA IIB and BoLA IIA/III/I haplotypes were analyzed separately and investigated for conservation among animals with different breed histories and selection pressures, and separate phylogenetic trees were constructed for the BoLA class IIA-III-I haplotypes and BoLA class IIB haplotypes. Points of divergence between different BoLA haplotypes were estimated based on the identity of alleles across the BoLA regions, and the clustering of BoLA IIA-III-I haplotypes within the phylogenetic tree was examined for evolutionary relationships between different breeds of cattle, as well as other bovines including bison, gaur, and Cape buffalo. Finally, the linkage disequilibrium, recombination rates, frequencies, and conservation of BoLA haplotypes were compared to other regions of the bovine genome to determine if the phylogenetic history of BoLA is similar to the rest of the bovine genome.

CHAPTER II

MATERIALS AND METHODS

Sources of DNA

Many sources of DNA were used in this experiment to allow for analysis of BoLA haplotypes across a diverse range of cattle. The pedigreed families of the International Bovine Reference Family Panel (Barendse et al. 1997) were helpful in determining marker polymorphism, null alleles, and recombination events. The DNA samples from the Bovine HapMap Project (Gibbs et al. 2009) were valuable in calculating microsatellite allele frequencies across diverse breeds of cattle and providing BoLA SNP genotyping data. BoLA homozygous DNA samples obtained from the University of Missouri were key in defining BoLA haplotypes and analyzing the boundaries of BoLA homozygosity. Microsatellite-derived BoLA haplotypes were characterized in pedigreed animals of the Lone Star Tick study (Untalan et al. 2007) and Holsteins with defined DRB3 alleles. Additional bovid DNA samples, such as Yellowstone National Park bison, were important because they served as outgroups for comparison with domestic cattle breeds.

International Bovine Reference Family Panel

The International Bovine Reference Family Panel (IBRP) is a collection of DNA samples from pedigreed animals including full and half sibling progeny that represent diverse breeds of cattle from various parts of the world. The IBRP was originally used to create a medium-density genetic linkage map of the bovine genome (Barendse et al. 1997). DNA samples from the following 210 IBRP individuals were contributed by Dr. James Womack of Texas A&M University for use in this study:

CISRO Reference Families – 103 individuals

101 (Sire: Brahman x Gir/Ind-Brazil) x 1102 (Dam: Brahman) = 19 offspring
 101 (Sire: Brahman x Gir/Ind-Brazil) x 1202 (Dam: Brahman) = 13 offspring
 101 (Sire: Brahman x Gir/Ind-Brazil) x 2102 (Dam: Brahman) = 14 offspring
 501 (Sire: Friesian x Sahiwal) x 502 (Dam: Friesian x Sahiwal) = 12 offspring
 501 (Sire: Friesian x Sahiwal) x 702 (Dam: Friesian x Sahiwal) = 14 offspring
 1401 (Sire: Friesian x Sahiwal) x 1402 (Dam: Friesian x Sahiwal) = 9 offspring
 1401 (Sire: Friesian x Sahiwal) x 1502 (Dam: Friesian x Sahiwal) = 12 offspring

Texas A&M University (Granada) Reference Families – 57 individuals

5252 (Sire: Brangus) x 2308 (Dam: Brangus) = 19 offspring

5252 (Sire: Brangus) x 1158 (Dam: Brangus) = 11 offspring

5252 (Sire: Brangus) x 4046 (Dam: Brangus) = 10 offspring

5191 (Sire: Brangus) x 0981 (Dam: Brangus) = 11 offspring

BOVMAP Reference Family – 18 individuals

8001 (Sire: Holstein) x 8032 (Dam: Holstein) = 9 offspring

8301 (Sire: Charolais) x 8312 (Dam: Charolais) = 5 offspring

USDA MARC Reference Family – 17 individuals896800 (Sire: Gelbvieh x Simmental) x 861029 (Dam: Piedmontese x Hereford)
= 15 offspringILRAD Reference Families - 15 individuals

ND7 (Sire: N'Dama) x 1419 (Dam: N'Dama) = 5 offspring

ND8 (Sire: N'Dama) x 1688 (Dam: N'Dama) = 6 offspring

Bovine HapMap Project

The Bovine HapMap Consortium analyzed SNPs across 19 breeds of cattle (n = 497) from diverse geographical areas (Gibbs et al. 2009). A total of 412 DNA samples were contributed by Dr. Clare Gill of Texas A&M University with permission from the Bovine HapMap Consortium for use in this project (Table 1).

Table 1. Bovine HapMap DNA Samples. The samples contributed by the Bovine HapMap Consortium represent 16 breeds of cattle, and the majority of the breeds are European *Bos taurus*. Trios consisted of one sire, one dam, and one offspring.

Breed Type	Breed Name	Breed Abbreviation	Number of Animals	Number of Trios	Country of Sampling
European <i>Bos taurus</i>	Angus	ANG	27	3	USA and New Zealand
European <i>Bos taurus</i>	Brown Swiss	BSW	21	1	USA
European <i>Bos taurus</i>	Guernsey	GNS	21	0	USA and United Kingdom
European <i>Bos taurus</i>	Hereford	HFD	24	1	USA and New Zealand
European <i>Bos taurus</i>	Holstein	HOL	53	4	USA and New Zealand
European <i>Bos taurus</i>	Jersey	JER	27	3	USA and New Zealand
European <i>Bos taurus</i>	Limousin	LMS	41	1	USA and France
European <i>Bos taurus</i>	Norwegian Red	NRC	20	0	Norway
European <i>Bos taurus</i>	Piedmontese	PMT	24	0	Italy
European <i>Bos taurus</i>	Red Angus	RGU	12	1	USA and Canada
European <i>Bos taurus</i>	Romagnola	RMG	24	3	Italy
African <i>Bos taurus</i>	N'Darna	NDA	25	0	Guinea
African <i>Bos taurus</i>	Sheko	SHK	20	1	Ethiopia
<i>Bos indicus</i>	Brahman	BRM	25	3	USA and Australia
Crossbred (<i>Bos taurus</i> x <i>Bos indicus</i>)	Santa Gertrudis	SGT	24	0	USA
Crossbred (<i>Bos taurus</i> x <i>Bos indicus</i>)	Beefmaster	BMA	24	1	USA

SNP Defined BoLA Homozygotes

Drs. Robert Schnabel and Jerry Taylor, Department of Animal Sciences, of the University of Missouri provided 102 DNA samples from cattle that were typed as homozygous for SNPs across the entire length of the BoLA IIa-III-I region. These animals were part of a whole genome 50K SNP analysis and included DNA samples from 42 Angus, 23 Holstein, 17 Limousin, six Simmental, four North American bison, three Shorthorn, two Gaur, two Hereford, one Finnish Ayrshire, one Romosinuano, and one Brahman. In silico BoLA SNP data from additional animals were also made available for this project.

Study of Host Resistance to the Lone Star Tick

DNA samples from a total of 176 pedigreed animals from a tick resistance experiment conducted at the US Livestock Insect Research Laboratory, USDA ARS, Kerrville, TX were contributed by Dr. Pia Untalan, USDA, and Drs. Pat Holman (Department of Veterinary Pathobiology) and Penny Riggs (Department of Animal Science) of Texas A&M University. The research herd used in the tick experiment included two BoLA identical sires (Bull 12 and Bull 13) from a Red Poll x Simmental cross, a third sire (Rogers bull) of the Simbrah breed, 72 dams with a *Bos taurus* and *Bos indicus* influence, and 101 calves that were phenotyped for tick resistance or susceptibility (Untalan et al. 2007). In that study, association was found between resistance to infestation by *Amblyomma americanum*, the Lone Star tick, and microsatellite markers within BoLA DRB*4401 (DRB3-174; $P = 0.001$) and DRB1 (DRB1-118; $P = 0.023$) (Untalan et al. 2007). Animals were previously genotyped by PCR-RFLP and by sequencing of Exon 2 of the DRB3 gene.

DRB3 Defined Holstein Samples

Dr. Waithaka Mwangi, Department of Veterinary Pathobiology, Texas A&M University, contributed DNA samples from 21 Holstein calves that had been genotyped at the DRB3 locus and phenotyped in vaccine response studies. All of these animals possessed at least one copy of the DRB3*1101 allele. The Holstein animals and their respective DRB3 alleles are the following:

<u>Calf ID #</u>	<u>DRB3 Alleles</u>
81288	DRB3*0101 / DRB3*1101
81821	DRB3*1101 / DRB3*1201
92334	DRB3*1101 / DRB3*1501
92085	DRB3*1001 / DRB3*1101
90549	DRB3*1101 / DRB3*1101
51379	DRB3*0101 / DRB3*1101
82550	DRB3*0201 / DRB3*1101
81803	DRB3*1101 / DRB3*1501
81841	DRB3*1001 / DRB3*1101
82598	DRB3*1101 / DRB3*1101
90625	DRB3*0101 / DRB3*1101
90183	DRB3*0301 / DRB3*1101
91721	DRB3*1101 / DRB3*1501
91054	DRB3*1101 / DRB3*1801
82591	DRB3*1101 / DRB3*1101
90170	DRB3*1101 / DRB3*1401
90180	DRB3*1101 / DRB3*1201
90380	DRB3*1101 / DRB3*1601
51407	DRB3*1101 / DRB3*1601
90175	DRB3*0902 / DRB3*1101
82736	DRB3*1101 / DRB3*1701

Other DNA Samples

Dr. Derr of Texas A&M University provided DNA samples from 20 North American bison from Yellowstone National Park. Additional samples available in Dr. Skow's laboratory included L1 Domino and L1 Dominette, the Hereford source animals for the CHORI 240 BAC library and the bovine genome sequence, respectively, 23 White-Tailed Deer, 20 domestic sheep, 17 elk, two gaur, two river buffalo, one domestic goat, one Slenderhorn gazelle, one Persian gazelle, one Dama gazelle, and one Arabian

Oryx. These animals were important to understand the phylogeny and extent of BoLA haplotypes and polymorphism.

Genotyping and Selection of BoLA Microsatellites

The Sputnik program (<http://espressoftware.com/sputnik/index.html>) was used to mine the BoLA genomic sequences for microsatellites containing repeat motifs of two to five base pairs in length. Preference was given to perfect dinucleotide AC motifs that had more than six repeats, as they were more likely to be polymorphic. To avoid designing primers within repetitive regions, the “-cow” option within the RepeatMasker website (Smit et al. 1996-2004) was employed to identify any bovine interspersed repeats within the flanking sequences of microsatellites. Primers were subsequently designed to amplify the microsatellites by using the Primer3 software (Rozen and Skaletsky 2000). Most of the default Primer3 design parameters were used, except that the primer GC content was set to a range of 45 – 55%, and PCR product size was set as close to 500bp as possible to increase the likelihood of finding SNPs surrounding each microsatellite. Primers were designed within unique flanking sequences of each microsatellite to yield PCR products of 120-500 bp in length. The primers were then screened against the Btau4.0 assembly by UCSC BLAT (Kent 2002) and NCBI GenBank by BLASTn (Altschul et al. 1990) to confirm uniqueness and locus specificity. A total of 48 primer pairs were designed for the BoLA class IIb region (Table 2) and 28 primer pairs for BoLA IIa-III-I region (Table 3).

To allow for efficient and economical fluorescent genotyping, the forward primer was designed to include a 5' M13 tag - 5' TTTCCCAGTCACGACGTTG 3' - complementary to a third PCR primer labeled with one of three fluorescent dyes - NED, 6-FAM, or VIC (Oetting et al. 1995). This eliminated the cost of labeling each specific forward primer with a fluorescent dye and further reduced the cost because three PCR products labeled with different dyes could be co-loaded within a single well and simultaneously resolved on the ABI-3730 Genetic Analyzer (Applied Biosystems; Foster City, CA). A fourth PET dye available for this technique was not used because PCR products labeled with PET were consistently two base pairs longer than the PCR products generated with NED, 6-FAM, and VIC. All PCR products were separated and analyzed

relative to an internal size standard (GS 500 LIZ®, Applied Biosystems; Foster City, CA) and manually scored using GeneMapper™ 3.5 (Applied Biosystems; Foster City, CA).

PCR reactions for genotyping were performed in a total volume of 20uL containing 25ng of DNA, 1.5U of clear Sigma JumpStart Taq Polymerase (Sigma-Aldrich; St. Louis, MO), 0.8mM dNTPs, 1.5mM Epicentre MgCl₂ Master Amp™ PCR Enhancer (Epicentre Biotechnologies; Madison, WI), Sigma 10x PCR Buffer (Sigma-Aldrich; St. Louis, MO), 0.5mM of fluorescently labeled M13 complementary primer (6-FAM, NED, or VIC (Applied Biosystems; Foster City, CA), 0.1mM of the microsatellite specific reverse primer, and 0.033mM of the M13-tailed microsatellite specific forward primer (Sigma-Aldrich; St. Louis, MO). Annealing temperatures varied among the different primer pairs, but a typical PCR program had the following parameters: 5 minutes at 95°C, 30 seconds at 94°C, 30 seconds at annealing temperature + 2°C, 30 seconds at 72°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at annealing temperature, 30 seconds at 72°C, and concluding with a final extension of 5 minutes at 72°C. Each primer pair was optimized for an annealing temperature at 1.5 mM MgCl₂. Genomic DNAs and PCR products were quantified with NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific; Wilmington, DE) and checked for proper amplification by electrophoresis on 1.0% agarose gels containing 0.36 µg/ml ethidium bromide prior to analysis on the ABI-3730.

Table 2. BoLA IIb Microsatellite Primers. All forward genotyping primers had a 19 base pair, 5' M13 tail (Oetting et al. 1995) denoted in this table by bold blue font. Reference microsatellite motifs and allele sizes were derived from L1 Dominette. The positions of BoLA IIb genes and microsatellites were provided by the Childers et al. annotation (Childers et al. 2006) and given relative positions in the Btau4.0 assembly (Kent 2002).

Forward Primer Name	Forward Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Annealing Temperature	Reference Allele Size
Пb 13 M13F	TTTCCAGTCACGACGTTG GTTCATGGGGTTGCTAAGAGT	Пb 13 R	ATCCAGAGTCACAGAGGAAGG	58°C	269 bp
Пb 14 M13F	TTTCCAGTCACGACGTTG ACCTTCTTTACACACCCATGC	Пb 14 R	TGTGGTTTCCTTTCCAACCTA	58°C	308 bp
Пb 17-2 M13F	TTTCCAGTCACGACGTTG CAGAAAGTCAGAACCACACAGG	Пb 17 R	GGATAAATGACCATAGGGGTCT	58°C	324 bp
Пb 41 M13F	TTTCCAGTCACGACGTTG CAGCACAGCAATTCAGTTACA	Пb 41 R	ATGGGGTTTAGGGAGAAAGAA	58°C	234 bp
Пb 42-2 M13F	TTTCCAGTCACGACGTTG CAAGAAGCCAAACCCAAT	Пb 42-2 R	AATACTCCAGGGACCAAGGG	58°C	460 bp
Пb 53 M13F	TTTCCAGTCACGACGTTG GATATTGAGTCCCAGGCCATA	Пb 53 R	GGAGATGGTGAAGGACAGAGA	60°C	468 bp
Пb 55 M13F	TTTCCAGTCACGACGTTG GTCTTGAAAGTTGAGCCCCTA	Пb 55 R	AAGTCCTGGGTATTCTTTGGAA	58°C	332 bp
Пb 56 M13F	TTTCCAGTCACGACGTTG TGGTGATGCTGGGAGACA	Пb 56 R	GTCTGTCCCTTTCCCCATTGTA	58°C	253 bp
Пb 58 M13F	TTTCCAGTCACGACGTTG CGGACATGACTGAAGCAACT	Пb 58 R	CCATTGACAGGCACTTAGGTT	60°C	367 bp
Пb 60 M13F	TTTCCAGTCACGACGTTG CTACAACCTTCCCTGACAACC	Пb 60-2 R	GAGACCACAGAAACAGCACA	58°C	148 bp
Пb 61 M13F	TTTCCAGTCACGACGTTG CAACATGATTTCTTCCAGCAG	Пb 61 R	CGATGCTTTCACCTCCATAGG	58°C	190 bp
Пb 62 M13F	TTTCCAGTCACGACGTTG GAGTATGGTGGCTCAGATGGT	Пb 62 R	GCAACCTAAATGTCCCCTGT	58°C	323 bp
Пb 83 M13F	TTTCCAGTCACGACGTTG TGCAGGATATAGGATGCTTGA	Пb 83 R	GAAGGCTAGTTTCACGTCCAG	58°C	316 bp
Пb 89 M13F	TTTCCAGTCACGACGTTG GCAATGAAGACCAAGCACTGT	Пb 89 R	CTGTATGGCAAAGGGACTCAG	58°C	205 bp
Пb 90 M13F	TTTCCAGTCACGACGTTG GACCTGCCTTCATGTCTAAGC	Пb 90 R	GGTGTGAGGAGATCATTGTT	58°C	308 bp
Пb 97 M13F	TTTCCAGTCACGACGTTG CATGTGGAAAGGCATTTATCT	Пb 97 R	CACTGAAAACCTGGGAACACAA	58°C	212 bp
Пb 98 M13F	TTTCCAGTCACGACGTTG CATCTATGGGGTCGAACAGAG	Пb 98 R	CCTCAGAAGGAAGTGTGGAGT	58°C	120 bp
Пb 104 M13F	TTTCCAGTCACGACGTTG CTGGGGTTACACAGAGTTGG	Пb 104 R	AGGTTGCTTCCGTATCTTGG	58°C	366 bp
Пb 107 M13F	TTTCCAGTCACGACGTTG CAAGGAGGAAGAACGGACA	Пb 107 R	CGCACAGAGTTGGACACAA	58°C	277 bp
Пb 108 M13F	TTTCCAGTCACGACGTTG CACCTGATGCAGAGCTGACT	Пb 108 R	CCACAACCTGGAACCTGGATG	58°C	308 bp
Пb 111 M13F	TTTCCAGTCACGACGTTG GCAAAGAGGTGGACACGAC	Пb 111 R	GGGAACCCCTGCTCAATACTC	58°C	328 bp
Пb 113 M13F	TTTCCAGTCACGACGTTG TCACTTTGCCATACACCTCA	Пb 113 R	GCTCTCTGCGTCTGTGAGTC	58°C	313 bp
Пb 119 M13F	TTTCCAGTCACGACGTTG GCATAGGGTCTTAGCCATCAG	Пb 119 R	CGTCTACGGGGTTTCATAGAG	58°C	199 bp
Пb 122 M13F	TTTCCAGTCACGACGTTG GGTGCTATACAGTGGGTCTT	Пb 122 R	GTTACCAAAGAGGAAAGGTTGG	58°C	136 bp
Пb 123-2 M13F	TTTCCAGTCACGACGTTG CACCAACCTTCTCTTTGGTA	Пb 123-2 R	CTGACTCACTGAAAAAGACTGAATC	58°C	477 bp

Table 2. Continued.

Forward Primer Name	Forward Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Annealing Temperature	Reference Allele Size
Пб 129 M13F	TTTCCAGTCACGACGTTG CCGACGAGAGGACAAGAAGTA	Пб 129 R	CTATGGGGTCTCACAGAGTCG	58°C	213 bp
Пб 130 M13F	TTTCCAGTCACGACGTTG TAGACACAGGGAGGAAGGAGA	Пб 130 R	GAGTCACAGCAAATCCCCT	58°C	305 bp
Пб 135 M13F	TTTCCAGTCACGACGTTG CCCATTCTGGGCATATAACT	Пб 135 R	CAAACCTCTGGGAGGTAGTGGA	58°C	329 bp
Пб 139 M13F	TTTCCAGTCACGACGTTG GGGATGGGAGACTTTGAGG	Пб 139 R	CAGTGTATTTCCTAACAGCAAGG	58°C	179 bp
Пб 143 M13F	TTTCCAGTCACGACGTTG GACATTGAAGGGAGAGAACCA	Пб 143 R	GGAGAGAAAGGAGGATGTGTG	58°C	240 bp
Пб 145 M13F	TTTCCAGTCACGACGTTG ACTCACC AATCACAGCACAGA	Пб 145 R	CGGACACAACCTGAACAACCTGA	60°C	358 bp
Пб 148 M13F	TTTCCAGTCACGACGTTG TCTCCCCAGATGATATTAC	Пб 148 R	GTCACGAAGAGTTGGACATGA	58°C	199 bp
Пб 149 M13F	TTTCCAGTCACGACGTTG CCAAGGCTCAGTTACATCCAG	Пб 149 R	GTCAATGGGGTCCACAGAGT	58°C	184 bp
Пб 153 M13F	TTTCCAGTCACGACGTTG GAATCCCTTGGACAGAGGAG	Пб 153 R	CTTGCTCAGTATCTGGGGAGA	58°C	245 bp
Пб 158 M13F	TTTCCAGTCACGACGTTG TGCATAAGGATTCAGCAACAG	Пб 158 R	CCTTGAGGTCCATCCATGTT	58°C	329 bp
Пб 170 M13F	TTTCCAGTCACGACGTTG CGGACACAACCTCACTTTCAC	Пб 170 R	CAGTCCTCCCTCAACTTCTGA	58°C	309 bp
Пб 171-2 M13F	TTTCCAGTCACGACGTTG GGGAGGCAGTGAGAAAATCTA	Пб 171-2 R	CTTTCCTAAGGGGCTGAATCTT	58°C	317 bp
Пб 174 M13F	TTTCCAGTCACGACGTTG TGGTTAGAGCAAGGCAATAGG	Пб 174 R	CCTGTTCTCAGGCAAACTACC	58°C	198 bp
Пб 187 M13F	TTTCCAGTCACGACGTTG CAAATTGCTTCTGGTGACTTC	Пб 187 R	TCAAAGATGCCCTATCCTGTGAC	58°C	237 bp
Пб 189 M13F	TTTCCAGTCACGACGTTG CCTTACCCCTTAGAGAAGCTGG	Пб 189 R	ATACTCATGGCAGGGACTTGT	58°C	216 bp
Пб 193 M13F	TTTCCAGTCACGACGTTG CACTCCAGTATCCTTGTCTGG	Пб 193 R	GATTCTTCTGTGTGGTCAGGTT	58°C	314 bp
Пб 196 M13F	TTTCCAGTCACGACGTTG TAATCTCTAGGTCCGTCCATGT	Пб 196-2 R	CTTGGCGAAGGAGAAGTATTTG	58°C	220 bp
Пб 200 M13F	TTTCCAGTCACGACGTTG TCCGAGTCACTTCTGAGTCT	Пб 200 R	GGTTGCTAGGAGITGGAGACA	58°C	346 bp
Пб 204 M13F	TTTCCAGTCACGACGTTG GGCCCTTGATATCCCTACC	Пб 204 R	ATGTAGACCCGTGGATGATTC	58°C	257 bp
Пб 207 M13F	TTTCCAGTCACGACGTTG AAGTTCTTCCAGAGAGTCAGG	Пб 207 R	AGGTACACATCCCTGTGG	56°C	218 bp
Пб 208 M13F	TTTCCAGTCACGACGTTG ATGTTGTCCACGGGTGTATGT	Пб 208 R	TGGTAGCCCCACTCCAGTATTC	58°C	291 bp
Пб 211 M13F	TTTCCAGTCACGACGTTG CTCCTTGTCCCTTTCAG	Пб 211 R	CCAATGCACAGGACTTCTCA	58°C	343 bp
Пб 218 M13F	TTTCCAGTCACGACGTTG GGGTGGTTTAGAGGAAGGAGT	Пб 218 R	GGCAGGATTGTCCATAGTCAT	58°C	222 bp

Table 2. Continued.

Forward Primer Name	Reverse Primer Name	MHC Class	Btau4.0 BTA23 Begin	Btau4.0 BTA23 End	Reference Microsatellite Motif	Microsatellite Placement Relative To Genes
Пb 13 M13F	Пb 13 R	Пb	7188887	7189136	(AGC)6	Intergenic - before GCLC
Пb 14 M13F	Пb 14 R	Пb	7197281	7197571	(AT)9	Intergenic - before GCLC
Пb 17-2 M13F	Пb 17 R	Пb	7200278	7200584	(AC)20	Intergenic - before GCLC
Пb 41 M13F	Пb 41 R	Пb	7257268	7257482	(AT)4(AG)1(TA)4	Intronic - DSB
Пb 42-2 M13F	Пb 42-2 R	Пb	7258738	7259179	(AT)12	Intronic - DSB
Пb 53 M13F	Пb 53 R	Пb	7263383	7264772	(AGC)5	Intronic - DSB
Пb 55 M13F	Пb 55 R	Пb	7276385	7276697	(TCAGT)4	Intergenic - DSB-DYA
Пb 56 M13F	Пb 56 R	Пb	7284699	7284931	(AACTG)4 and (AT)2	Intergenic - DSB-DYA
Пb 58 M13F	Пb 58 R	Пb	7264104	7264451	(AGC)6	Intergenic - DSB-DYA
Пb 60 M13F	Пb 60-2 R	Пb	7291816	7291946	(AC)10	Intergenic - DSB-DYA
Пb 61 M13F	Пb 61 R	Пb	7293053	7293223	(GGA)5	Intergenic - DSB-DYA
Пb 62 M13F	Пb 62 R	Пb	7293295	7293580	(AT)16	Intronic - DYA
Пb 83 M13F	Пb 83 R	Пb	7332658	7332976	(AAAT)4	Intergenic - DYB-DOB
Пb 89 M13F	Пb 89 R	Пb	7341230	7341405	(AT)6	Intronic - DOB
Пb 90 M13F	Пb 90 R	Пb	7328911	7329199	(AC)7	Intronic - DOA
Пb 97 M13F	Пb 97 R	Пb	7340278	7340469	(TTA)4	Intergenic - DOB-TAP2
Пb 98 M13F	Пb 98 R	Пb	7342005	7342105	(AGC)4	Intergenic - DOB-TAP2
Пb 104 M13F	Пb 104 R	Пb	7264120	7264469	(AGC)7	Intergenic - DOB-TAP3
Пb 107 M13F	Пb 107 R	Пb	7367687	7367944	(AGC)4	Intergenic - DOB-TAP2
Пb 108 M13F	Пb 108 R	Пb	7376499	7376787	(ACTGA)5	Intergenic - TAP2-PSMB8
Пb 111 M13F	Пb 111 R	Пb	7389741	7390049	(TA)5C(AT)5	Intronic - TAP1
Пb 113 M13F	Пb 113 R	Пb	7396966	7397261	(AC)7(AT)5	Intronic - PSMB9
Пb 119 M13F	Пb 119 R	Пb	7409831	7410011	(AGC)5	Intergenic - PSMB9-H2B
Пb 122 M13F	Пb 122 R	Пb	7412641	7412759	(AC)7	Intergenic - PSMB9-H2B
Пb 123-2 M13F	Пb 123-2 R	Пb	7412736	7413195	(AC)13	Intergenic - PSMB9-H2B

Table 2. Continued.

Forward Primer Name	Reverse Primer Name	MHC Class	Btau4.0 BTA23 Begin	Btau4.0 BTA23 End	Reference Microsatellite Motif	Microsatellite Placement Relative To Genes
Пb 129 M13F	Пb 129 R	Пb	7419634	7419831	(AGC)5	Intergenic - H2B-DMB
Пb 130 M13F	Пb 130 R	Пb	7420753	7421059	(ACTGA)4	Intergenic - H2B-DMB
Пb 135 M13F	Пb 135 R	Пb	7437017	7437328	(AT)10	Intergenic - H2B-DMB
Пb 139 M13F	Пb 139 R	Пb	7441581	7441740	(TAT)5	Intergenic - H2B-DMB
Пb 143 M13F	Пb 143 R	Пb	7450249	7450471	(TC)7	Intergenic - H2B-DMB
Пb 145 M13F	Пb 145 R	Пb	7451158	7451497	(AC)8	Intergenic H2B-DMB
Пb 148 M13F	Пb 148 R	Пb	7453643	7453823	(GAAGT)6	Intergenic - H2B-DMB
Пb 149 M13F	Пb 149 R	Пb	7458767	7458931	(AGC)5	Intronic - DMB
Пb 153 M13F	Пb 153 R	Пb	7460913	7461140	(AC)11	Intergenic - DMB-DMA
Пb 158 M13F	Пb 158 R	Пb	7467432	7467744	(AT)11	Intergenic - DMB-DMA
Пb 170 M13F	Пb 170 R	Пb	7477598	7477891	(AGC)5	Intergenic - DMA-BRD2
Пb 171-2 M13F	Пb 171-2 R	Пb	7478077	7478373	(AC)4(TC)1(AC)7	Intergenic - DMA-BRD2
Пb 174 M13F	Пb 174 R	Пb	7564507	7564683	(AC)3(AT)1(AC)5	Intronic - BRD2
Пb 187 M13F	Пb 187 R	Пb	7589328	7589547	(AC)13	Intergenic - BRD2-DOA
Пb 189 M13F	Пb 189 R	Пb	7592532	7592731	(AC)10	Intergenic - BRD2-DOA
Пb 193 M13F	Пb 193 R	Пb	7600300	7600597	(AC)10	Intergenic - DOA-COL11A2
Пb 196 M13F	Пb 196-2 R	Пb	7602911	7603112	(AC)22	Intergenic - DOA-COL11A2
Пb 200 M13F	Пb 200 R	Пb	7612980	7613307	(AGC)6	Intergenic - DOA-COL11A2
Пb 204 M13F	Пb 204 R	Пb	7618337	7618574	(ATTT)4	Intergenic - DOA-COL11A2
Пb 207 M13F	Пb 207 R	Пb	7625551	7625747	(AC)18	Intronic - COL11A2
Пb 208 M13F	Пb 208 R	Пb	7626989	7627260	(AC)4(GC)1(AC)5	Intronic - COL11A2
Пb 211 M13F	Пb 211 R	Пb	7629950	7630276	(AC)6	Intronic - COL11A2
Пb 218 M13F	Пb 218 R	Пb	7648858	7649060	(TGGA)2(TGGG)1(TGGA)2	Intronic - COL11A2

Table 3. BoLA IIa-III-I Microsatellite Primers. Most of the forward genotyping primers had a 19 base pair, 5' M13 tail (Oetting et al. 1995) denoted by bold blue font, with the exceptions of LA54 F/LA53 R (Ellegren et al. 1993) and DRBP1 F/DRBP1 R (Creighton et al. 1992). These two primer pairs were ordered with the 6FAM dye directly attached to the 5' end so allele sizes would be consistent with previously published alleles. Reference microsatellite motifs and allele sizes were derived from L1 Dominette, and the positions of genes and microsatellites were derived from the UCSC genome browser Btau4.0 assembly (Kent 2002).

Forward Primer Name	Forward Primer Sequence	Reverse Primer Name	Reverse Primer Sequence	Annealing Temperature	Reference Allele Size
1416 M13F	TTTCCCAGTCACGACGTTG CCAAGCTGTCCCATCTACTCTT	1416 R	TGCTCTGCTTTTGCTTAGACT	60°C	471 bp
605 M13F	TTTCCCAGTCACGACGTTG GGCTAGGCTGTATTGCATTCT	605 R	TAACAAAACCTGGCACCTGTCAC	58°C	437 bp
644-2 M13F	TTTCCCAGTCACGACGTTG CAGTCTGTTCACCTGCAACTGT	644-2 R	AAGCTCATTCACTCTGGGAAAG	58°C	515 bp
658 M13F	TTTCCCAGTCACGACGTTG CCTCTCTTCTGTCCAAACATC	658 R	TCATATTCACCCTCTTCATCC	58°C	423 bp
940-2 M13F	TTTCCCAGTCACGACGTTG TTCAACCAGCTAACATCAGGAG	940 R	CAGAATCTCTGCAGGTGTCTCA	58°C	148 bp & 154 bp
995 M13F	TTTCCCAGTCACGACGTTG CTCGGAATCTTTCAGTCAGGTC	995 R	TCCTCGTTAAGTCCAGGCTAAG	58°C	458 bp
1153-4 M13 F	TTTCCCAGTCACGACGTTG CTGAAGAGGCCCTTGCAATG	1153-4 R	TCACTGAATATGCACACACGC	58°C	216 bp
1176-3 M13F	TTTCCCAGTCACGACGTTG TGAGATGTTAGGCAAGAAGCTG	1176-3 R	CTCTGCAGCCTCTATTGGTTTC	58°C	229 bp
41 M13F	TTTCCCAGTCACGACGTTG GCCATAAAGCTTTCCCAGATAC	41 R	CAGGTAAGTGCAGGAGACAGA	58°C	440 bp
6FAM LA54 F	6FAM* GAGAGTTTCACTGTGCAG	LA53 R	CGCGAATCCCAGTGAAGTATCT	58°C	195 bp
6FAM DRBP1 F	6FAM* ATGGTGCAGCAGCAAGGTGAGCA	DRBP1 R	GGGACTCAGTCTCTATCTCTTTG	58°C	119 bp
171 M13F	TTTCCCAGTCACGACGTTG ACTTTCAGCCAACAGGTAATGCT	171-2 R	TGGAGAGGGTTGTTTTTCAGA	58°C	347 bp
198-2 M13F	TTTCCCAGTCACGACGTTG TGGTGTATGCAAATGAGAGGTG	198-2 R	GCCATATATGAGGATGCCTACC	58°C	196 bp
AGER M13F	TTTCCCAGTCACGACGTTG TCCCAATAAGGTAGAGGATGA	AGER R	TCCAAGAGCCAGTTAAGAGTCC	58°C	406 bp
ATF6B M13F	TTTCCCAGTCACGACGTTG ATCAATGTCAGCACCATCTCTG	ATF6B R	ATCTCTTCAGTGTGCCTTAC	58°C	267 bp
CYP21A2 M13F	TTTCCCAGTCACGACGTTG AAGGCCAGGGCACGACCCGCG	CYP21A2 R	GGGACATGATGAGTGACTGAC	58°C	313 bp
1249 M13F	TTTCCCAGTCACGACGTTG CCCCTGAACCTCTATGGTACTGC	1249 R	CAACTTCAGTCTCCTCCGAGAT	58°C	458 bp
1268-2 M13F	TTTCCCAGTCACGACGTTG CTGGAAAAGCCCCACTAAAGTA	1268-2 R	GGCACAACTAACACACTCCAAA	58°C	266 bp
301 M13F	TTTCCCAGTCACGACGTTG GAATCCATCACCTTGTCTCTC	301 R	GAGTAACCCCTCCTGCTAGTCA	58°C	492 bp
312 M13F	TTTCCCAGTCACGACGTTG TCCTGGTCAACCCTGTATTCT	312-2 R	CTACAGTTTGGGAGGTGAAAA	58°C	458 bp
362 M13F	TTTCCCAGTCACGACGTTG AGAGAGTGAAGGGAGAGAGAGCA	362 R	TCTTCACTCCTTCAGGAAGTC	58°C	448 bp
366 M13F	TTTCCCAGTCACGACGTTG ACAAAAGACAGGCAGAGGAAAGA	366 R	TACATACATAGACATCGCCCA	58°C	448 bp
397 M13F	TTTCCCAGTCACGACGTTG ATGCTCAGGAGAACTGAGTCC	397 R	CCTACAATGGTCATAGTGCAGG	58°C	479 bp
415 M13F	TTTCCCAGTCACGACGTTG AGTAGCCCTGGAAGGATACACA	415 R	CAATATCCATGGTTAGGGCTGT	58°C	475 bp
475 M13F	TTTCCCAGTCACGACGTTG AGGGAACCATGAAGACTGTTG	475 R	CAAGTAAAAAGAGGGCACGAGT	58°C	506 bp
1870-2 M13F	TTTCCCAGTCACGACGTTG AAAACAGAGAACACCAGCATCC	1870-2 R	ACAATGGCCCTTCTAGCCCTTC	58°C	172 bp
1778-2 M13F	TTTCCCAGTCACGACGTTG CTTTGAGATGAGGCCAGCAG	1778-2 R	CCAACATCACTGGAAGACAGAA	58°C	258 bp
1687-2 M13F	TTTCCCAGTCACGACGTTG CCTCCACTCAGATAAGGAGTC	1687-2 R	GACAAAACCAGGAGGGAAGATT	58°C	418 bp

Table 3. Continued.

Forward Primer Name	Reverse Primer Name	MHC Class	Btau4.0 BTA23 Begin	Btau4.0 BTA23 End	Reference Microsatellite Motif	Microsatellite Placement Relative To Genes
1416 M13F	1416 R	Centromeric to IIa	24180523	24180976	(AC)10	Intergenic - TFAP2B-IL17A
605 M13F	605 R	Centromeric to IIa	24240199	24240619	(AC)6	Intergenic - TFAP2B-IL17A
644-2 M13F	644-2 R	Centromeric to IIa	24348422	24348916	(AC)6	Intergenic - TFAP2B-IL17A
658 M13F	658 R	Centromeric to IIa	24369054	24369457	(AC)9	Intergenic - TFAP2B-IL17A
940-2 M13F	940 R	Centromeric to IIa	24931349	24931480	(AC)10	Intergenic - TFAP2B-IL17A
995 M13F	995 R	Centromeric to IIa	25049980	25050422	(AC)5	Intergenic - TFAP2B-IL17A
1153-4 M13 F	1153-4 R	Centromeric to IIa	25462357	25462572	(AC)17	Intergenic - PAQR8-TMEM14A
1176-3 M13F	1176-3 R	Centromeric to IIa	25510736	25510945	(AC)7	Intergenic - PAQR8-TMEM14A
41 M13F	41 R	Centromeric to IIa	26063328	26063752	(AC)5(AG)1(CA)3	Intronic - ELOVL5
6FAM LA54 F	LA53 R	IIa	26336439	26336633	(TC)10(AC)22	Intronic - DRB3
6FAM DRBP1 F	DRBP1 R	IIa	26374724	26374843	(TC)9(AC)12	Intronic - DRBP1
171 M13F	171-2 R	IIa	26382160	26382489	(AC)6	
198-2 M13F	198-2 R	IIa	26443036	26443212	(AC)14	Intronic - DRA
AGER M13F	AGER R	III	27029590	27029978	(AC)10	Intronic - AGER
ATF6B M13F	ATF6B R	III	27079046	27079293	(AC)14(TC)8	Intronic - ATF6B
CYP21A2 M13F	CYP21A2 R	III	27119005	27119298	(AC)21	Intronic - CYP21
1249 M13F	1249 R	III	27403361	27403803	(AC)5	3' UTR - LY6G6F
1268-2 M13F	1268-2 R	III	27449326	27449571	(AC)10	Intronic - BAT3
301 M13F	301 R	I	27914404	27914879	(AC)5	Intronic - TCF19
312 M13F	312-2 R	I	27947815	27948257	(AC)13	Intronic - CDSN
362 M13F	362 R	I	28097911	28098341	(AC)5	Intronic - DDR1
366 M13F	366 R	I	28107883	28108367	(AC)9	Intronic - DDR1
397 M13F	397 R	I	28182049	28182512	(AC)6	Intergenic - DDR1-IER3
415 M13F	415 R	I	28215985	28216444	(AC)6	Intronic - FLOT1
475 M13F	475 R	I	28419385	28419872	(AC)7	Intergenic - GNL1-LOC512672
1870-2 M13F	1870-2 R	Extended I	30408769	30408924	(AC)17	Intronic - ZSCAN12
1778-2 M13F	1778-2 R	Extended I	30648137	30648378	(AC)9(CG)1(AC)4(TA)1(AC)5	Intergenic - ZSCAN16-HIST1H2BN
1687-2 M13F	1687-2 R	Extended I	30902731	30903134	(AC)25	Intergenic - ZSCAN16-HIST1H2BN

Observed Recombination Events

Recombination events were detected by comparing parental microsatellite haplotypes to the microsatellite haplotypes of their offspring. All recombination events were assigned to an interval between flanking microsatellite markers based on where the alleles of the offspring deviated from the expected parental haplotype. The offspring found to have recombinant haplotypes were genotyped at least two times across all microsatellite markers to ensure the recombination events were identified accurately. Recombination events were investigated within the sample sets representing the largest number of meioses, including the sires and dams of the International Reference Families and the sires of the resistance to the Lone Star tick experiment.

Additional Genetic Markers

Identification and Characterization of SNPSTRs

Polymorphic microsatellites can provide additional information if they are surrounded by single nucleotide polymorphisms (SNPs). A “SNPSTR” contains one or more SNPs and exactly one polymorphic microsatellite, or short tandem repeat (STR), located within 500bp of each other (Mountain et al. 2002). SNPSTRs in the BoLA region were initially discovered by sequencing amplicons from a small subset of diverse cattle breeds, including 26 parents from the International Bovine Reference Family Panel, and SNPSTRs with the highest minimum allele frequencies were chosen for analysis across a larger number of individuals. Amplicons were cloned and sequenced in both the forward and reverse directions to determine the gametic phase in heterozygous animals. Each SNP identified in a cloned PCR sequence was validated by its presence at the same location in the direct PCR sequence (i.e. the original heterogeneous PCR product). This verified that the SNP was real and not an artifact of Taq polymerase error during the PCR reaction (Ennis et al. 1990).

PCR reactions were carried out with 25ng of genomic DNA as template, 1 μ L of Sigma 10x buffer (Sigma Aldrich), 0.3 pmol of each primer, 0.2 mM dNTPs, 1.5 mM MgCl₂, 0.25 U JumpStart REDTaq DNA polymerase (Sigma Aldrich), and the reaction was brought to a total volume of 10 μ L with autoclaved double distilled water. PCR products were cloned with the TOPO TA Cloning Kit for Sequencing (Invitrogen;

Carlsbad, CA). Before PCR products were cloned into the TOPO vector, 1.5U of Sigma JumpStart Taq Polymerase (Sigma-Aldrich; St. Louis, MO) was added to the PCR product and incubated at 72°C for 15 minutes to ensure single deoxyadenosine (A) were overhanging on the 3' ends of PCR products. The TOPO cloning reaction was performed with 4µL of fresh PCR product, 1µL of salt solution, and 1µL of TOPO vector. The mix was incubated for 30 minutes at room temperature, placed on ice, and 2µL of the mix was added to one vial of chemically competent E.coli (Invitrogen; Carlsbad, CA) to be incubated on ice for 5 minutes. The cells were heat shocked at 42°C for 30 seconds, immediately placed on ice, given 250µL of S.O.C. medium, and then incubated with shaking at 200rpm for one hour at 37°C. A total of 50µL of the transformed cells were spread on prewarmed plates containing 50µg/mL kanamycin and incubated at 37°C overnight. Single well-isolated colonies were selected for analysis and grown for about one hour in LB media containing 50µg/mL kanamycin. Cells pellets were collected by centrifugation and sent to SeqWright for plasmid purification and sequencing (SeqWright; Houston, TX).

PCR products for direct sequencing were purified with QIAquick Purification Kit (Qiagen; Valencia, CA). The purified PCR products were sequenced in reactions with 2µL of Big Dye (Applied Biosystems, Foster City, CA), 2µL of Half Big Dye (Genetix; Boston, MA), 0.5µL of Master Amp PCR Enhancer (Epicentre; Madison, WI), 1µL of 10µM forward or reverse microsatellite primer, the amplified PCR product, and the reaction was brought to a total volume of 10µL with autoclaved double distilled water. The amount of PCR product added to the reaction followed the rule of 10ng per every 100bp of PCR product. The sequencing reaction consisted of 2 minutes at 96°C; 35 cycles of 96°C for 30 seconds, 50°C for 15 seconds, and 60°C for 4 minutes; and a final extension of 60°C for 5 minutes. Each sequencing reaction was then cleaned with Spin-50 spin columns (BioMax; Odenton, MD) to remove excess primers and nucleotides, dried down, and eluted with formamide before sequencing on the ABI 3730 (Applied Biosystems; Foster City, CA) in the laboratories of Dr. Derr (Department of Veterinary Pathobiology) or Dr. Chowdhary (Department of Veterinary Integrative Biosciences) of Texas A&M University. Some direct PCR products were also sequenced at SeqWright (SeqWright; Houston, TX). All PCR products were resolved by electrophoresis on 1.0%

agarose gels containing 0.36 µg/ml ethidium bromide to ensure proper amplification before the sequencing reaction, and all sequences were analyzed with Sequencher 4.1 (Gene Codes Corporation; Ann Arbor, MI).

Sequencing of BoLA IIa Alleles

One goal of this project is to generate genetic markers that will be predictive of alleles at BoLA genes. Previous studies of the BoLA region defined alleles of class IIa genes by PCR amplification and sequencing across the polymorphic exon 2. This project utilized published primers to sequence exon 2 of the BoLA class IIa genes DRB3 (Baxter et al. 2008; Miltiadou et al. 2003), DRA (Zhou et al. 2007), and DQB (Russell 2000). The alleles of class IIa genes will be investigated for their association with BoLA polymorphic genetic markers, including microsatellites, SNPSTRs, and SNPs. The following primers were used for the direct PCR amplification of class IIa genes from genomic DNA:

DRB3FRW = 5' - CGCTCCTGTGA (C/T) CAGATCTAT CC - 3'
 DRB3REV = 5' - CACCCCCGCGCTCACC - 3'
 DRA F = 5' - TCTTCCTCTCCTGGTTCCCAC - 3'
 DRA R = 5' - GCTACAATGCTACAAT - 3'
 DQBEX2fwd = 5' - GGGCC (T/A) GTGTTA (C/T) TTCAC (C/T) AA - 3'
 DQBEX2rev1 = 5' - TTGT (G/T) TCTGCACACC (C/G) TGTCC - 3'
 DQBEX2rev2 = 5' - TTGT (G/T) TCTGCACACC (C/G) TGTCC - 3'

The DRB3FRW/DRB3REV and DRAF/DRAR primers were used at 1.5mM MgCl₂ and an annealing temperature of 58°C, while DQBEX2fwd/DQBEX2rev primers were used with a concentration of 1.5mM MgCl₂ and an annealing temperature of 60°C. PCR reactions were performed with 25ng of genomic DNA as template, 1µL of Sigma 10x buffer (Sigma Aldrich), 0.3 pmol of each primer (with the exception of DQBEX2rev consisting of 0.15pmol of DQBEX2rev1 and 0.15pmol of DQBEX2rev2), 0.2 mM dNTPs, 1.5 mM MgCl₂, 0.25 U JumpStart REDTaq DNA polymerase (Sigma Aldrich), and the reaction was brought to a total volume of 10µL with autoclaved double distilled water. The thermal profile was 5 minutes at 95°C, 30 seconds at 94°C, 30 seconds at annealing temperature +2°C, 30 seconds at 72°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at annealing temperature, 30 seconds at 72°C, and a final extension of 5

minutes at 72°C. All PCR products were resolved by electrophoresis on 1.0% agarose gels containing 0.36 µg/ml ethidium bromide to ensure proper amplification before sequencing. All sequences were analyzed with Sequencher 4.1 (Gene Codes Corporation; Ann Arbor, MI). Most PCR products were sent SeqWright for direct PCR product sequencing (SeqWright; Houston, TX), but some sequencing was done in house following the same sequencing protocol as described for SNPSTRs. Sequencing primers were the same as PCR primers, and all PCR products were sequenced in both forward and reverse directions.

Characterization of Bov-A2 Retroposons

Bov-A2 retroposons were investigated for their suitability as polymorphic markers in the BoLA regions. Bov-A2 elements are prevalent in the bovine genome, and a subset of Bov-A2 retroposons are polymorphic in Bov-A unit number (Lenstra et al. 1993). These size polymorphisms vary in intervals of approximately 120 base pairs per Bov-A unit, so they are easily distinguished by PCR and gel electrophoresis (Onami et al. 2007). Bov-A2 retroposons were identified in the BoLA IIb and BoLA IIa-III-I regions by submitting genomic BoLA sequence into the “-cow” option within the RepeatMasker website (Smit et al. 1996-2004). Primers were designed in locus specific regions flanking the Bov-A2 repeats using Primer3 software (Rozen and Skaletsky 2000). Most of the default Primer3 design parameters were used, with exceptions of setting the range of the primer GC content to 45 - 55 %. A total of 90 primers were used to investigate Bov-A unit number polymorphism (Table 4).

In addition to analyzing Bov-A2 polymorphisms in BoLA, this study also investigated the phylogenetic history of Bov-A2 polymorphism. Bov-A2 elements described in the Onami et al. 2007 study were used to evaluate Bov-A unit number polymorphism across domestic cattle and their phylogenetic relatives. Primers from the Onami et al. 2007 study and three pairs of redesigned primers were used to amplify the five Bov-A2 elements in our study (Table 5).

Table 4. BoLA Bov-A2 Primers. Bov-A2 primers were developed around Bov-A2 retroposons located in the BoLA regions within the Btau4.0 assembly. The expected size listed for each Bov-A2 is the amplicon size created by the primers in UCSC Blat (Kent 2002). Polymorphic Bov-A2 retroposons will vary in size increments of approximately 120 bp.

Bov-A2 Name	BoLA Class	Btau4.0 BTA23 Begin	Btau4.0 BTA23 End	Left Primer Sequence	Right Primer Sequence	Annealing Temperature (°C)	Expected Size (bp)
Bov-A2 BoLA IIb 2	IIb	7167831	7168670	GCACAATGAAGGAAGGGTGT	TGAGAGTCAGCAGTCCTTATGC	62	839
Bov-A2 BoLA IIb 3	IIb	7171934	7172374	CCAGAGTGTCTCCTGAGTCCT	CTGTGAGCAAACATAAGCATCC	64	441
Bov-A2 BoLA IIb 13 - 2	IIb	7188719	7189139	CTGAACCAGACCCACTCTCTAA	GCAATCCAGAGTCACAGAGGA	60	424
Bov-A2 BoLA IIb 16	IIb	7199826	7200227	TCCCAGAGGAAAGTCACTATCTAGC	TTGGGTCCCAGTTACTCTTCTC	64	399
Bov-A2 BoLA IIb 29	IIb	7223281	7223665	TTGAGGGCTTGTGTGAAC TAGA	TTATTTACCCCTTCCTCCATCC	64	383
Bov-A2 BoLA IIb 40	IIb	7256235	7256774	TGGATACTTGCCCTCTTTAGGA	CATCCCTTAAACGTTTTCAGAG	64	540
Bov-A2 BoLA IIb 91	IIb	7332656	7333054	CAAGCAATGTTCTCATGTTTCC	TTTGTGACCCGCTATCTTTCTT	64	399
Bov-A2 BoLA IIb 94 - 2	IIb	7336408	7336427	CTATGCAGGGCGAATGGTT	CAGGATTGACCCCATGTTTC	60	491
Bov-A2 BoLA IIb 98	IIb	7341717	7342105	ATGGATGTTCCCTCTCACTCAGC	CCTCAGAAGGAAGTGTGGAGTT	64	386
Bov-A2 BoLA IIb 119	IIb	7409693	7410089	TTGTTTCTCTCAGAAGCCGAAT	TGATATTGTTGGTTACTGCGATT	64	397
Bov-A2 BoLA IIb 121 - 2	IIb	7410895	7411717	ACCACAGCGGTGTCTAAGTG	GTGTAATCAGCCAGCCAAC	66	818
Bov-A2 BoLA IIb 124	IIb	7413382	7414220	GGGAGAAAGTAAAGTGGAGAACTGTC	GCTGTCCCAATAACTAGAGTGACG	64	834
Bov-A2 BoLA IIb 149	IIb	7458845	7459231	GGAGTCTCTGCAAGTCTGATT	TTGTGCTCCATGCTGAGTCTT	64	387
Bov-A2 BoLA IIb 170 - 2	IIb	7477364	7478204	GGCTGGGAAACACACACTTT	GGGGCTGTTTACTGCTGCT	66	839
Bov-A2 BoLA IIb 200	IIb	7613090	7613480	GGTTTAGTTCGTCTGGAAGCA	GTTGCTGTGATCGTGTATCTG	64	391
Bov-A2 1194	Centromeric to IIa	22035502	22035882	GATGTCGCCCTCAAAGTGAT	GCCAGCCTTTGTCAAAGTACC	60	381
Bov-A2 1397 - 2	Centromeric to IIa	24147100	24147719	CTACCCAGTGAAGCAAAGCA	TGCCTAATAGGGGCAGCTAA	60	620
Bov-A2 1403	Centromeric to IIa	24154186	24154562	TGACCTTTGACCTTGGGACT	CTGGGATCAAGGCGAATCTA	64	377
Bov-A2 604 - 2	Centromeric to IIa	24236454	24236980	CAAGCTAGGTGAAGGGGATT	CACCCAGTCAAAGCTAGCATT	56	527
Bov-A2 633	Centromeric to IIa	24309685	24310084	TGGGGTAGCAATCCTAAATGAC	GCTTGGCACTTACCCCTTCTA	66	400
Bov-A2 635	Centromeric to IIa	24315878	24316259	GGGTAGGCAAACTGTCTGATA	TAAGTGTACTGAGGCTGGCACT	66	382
Bov-A2 640	Centromeric to IIa	24335941	24336314	AGATCGGCATAAAACGTGCT	GTTGCCTAAAGTTCAGTGCTC	66	374
Bov-A2 705	Centromeric to IIa	24446231	24446726	TCCCTCCTGACACTGTGAAA	GCCTAACAGGCCAGAGTTC	64	496
Bov-A2 708	Centromeric to IIa	24456633	24456652	ACAAGGTTGGCAGAAGCAGT	AATCAACCCTGACTCACAGC	66	494
Bov-A2 710	Centromeric to IIa	24459407	24459833	TGTTGGTCTTATGTTGGCTCTC	AGCCAAAGAGATGGCTTCA	62	427
Bov-A2 728	Centromeric to IIa	24493803	24494100	TACTGGCATGAACAGGGACA	CAGGCTGTGAACAAGACAGAG	64	298
Bov-A2 740	Centromeric to IIa	24514071	24514470	GCCGACCAGAGAAGTGTGTT	GAGGCCATGAGCACAGGATA	68	400
Bov-A2 770	Centromeric to IIa	24591819	24592218	TATCCAGCAGAGGGATGGT	GCTTTGTTCCCTCTGTGGA	62	400
Bov-A2 830	Centromeric to IIa	24721602	24721985	TCCCAGATACCCAGGACACT	TCTTGAGGGAGATGGGACAC	66	384
Bov-A2 852	Centromeric to IIa	24757824	24758116	GACTGAACCAGGAAAGATGG	GACTACTGCTACTGCCCAAAA	64	293
Bov-A2 957	Centromeric to IIa	24967766	24968241	CAGCTTCCACTTCAATAGCA	CTTTGGGCATCTCCTTTCTG	64	476
Bov-A2 961 - 2	Centromeric to IIa	24984710	24985282	CCTCTGTATGCAAGGCACAA	CTGGGTCACAACAAGGGATT	56	573
Bov-A2 992 - 2	Centromeric to IIa	25043339	25043700	GCTTGTCACTCTGATTGACTCTG	GATCCTCTCATCTGGCCAAC	56	381
Bov-A2 1009	Centromeric to IIa	25086562	25086951	TTGCCTCTGGTGAAGCTGTA	CTGGCAAAGCAACAGAGCTA	64	390
Bov-A2 1067	Centromeric to IIa	25221873	25221892	GTGCTCCACCTTTTGAAGC	TATGGAAAGCAGGAGAGGTG	66	348
Bov-A2 1068 - 2	Centromeric to IIa	25225364	25225385	GAAAGTCTCCAGGACTCACTGT	CCAGATGACAGCCATTITCC	62	540
Bov-A2 1068	Centromeric to IIa	25225854	25225873	CCCCTGCATGTGTTTTT	GATCAACGTATCTGCCTGCT	60	600
Bov-A2 1071	Centromeric to IIa	25238013	25238491	CATTTTGGGCCTTAGGGTCT	TTAACACACCTCCCCCTGAG	64	479
Bov-A2 1113	Centromeric to IIa	25332834	25333221	TGCCTGGACATCTTCTACCC	TTTCCAAGTGACCCCTGAAC	64	388
Bov-A2 1128	Centromeric to IIa	25385599	25385946	TACGTCCAGTGTGGCTCCT	ACTCCAATCACGTGGGCACT	66	348
Bov-A2 1156	Centromeric to IIa	25473531	25473922	TGCTTTGACAGGTGAGTTGG	ATTCAACAGTCCCTTGCAG	64	392
Bov-A2 1168	Centromeric to IIa	25497602	25497988	TGAAGCTGTCTCCTGTGTTG	GGGACAATTTGGTCTCTGA	64	387

Table 4. Continued.

Bov-A2 Name	BoLA Class	Btau4.0 BTA23 Begin	Btau4.0 BTA23 End	Left Primer Sequence	Right Primer Sequence	Annealing Temperature (°C)	Expected Size (bp)
Bov-A2 37	Class IIa	26056075	26056322	TAACCTTGGCTCAGTGTGCT	GCTGGTGACTATGCACTCATCT	68	248
Bov-A2 38	Class IIa	26057709	26057937	ATGTAGGGCACCTACCATCACT	GTATGGCAGCTCCTTCTGTT	66	229
Bov-A2 39	Class IIa	26059655	26060153	CTTCTCCAGTCCCCTTCTACT	TTTAGGGTACCTCTGCTTCAGG	60	499
Bov-A2 40	Class IIa	26060369	26060724	GTGTGGCTCCAATTAGGTCTGT	TTGTCAACAGTTTACCCTGTC	66	356
Bov-A2 98	Class IIa	26181026	26181385	GACCACAGACAGAACCTTAGCC	TCTTGAATCCCACTCAAGTCA	56	360
Bov-A2 149-2	Class IIa	26321910	26322405	TGAGAAAAGGACGGGTTATG	GGCTTTCAGAGATGCTCAA	56	496
Bov-A2 164	Class IIa	26364994	26365214	GAAGGAGATTAGTAGGGAGCTATGAG	CAGCATTGTTAGTCTCCTCTGG	58	221
Bov-A2 1221	Class III	27330785	27331005	GGCACCTTTAACCTTTAGCC	CCCCAGCTTAACCTGAAACA	64	221
Bov-A2 1226 - 2	Class III	27353408	27353995	CAAGTCCAGGGCAAGAGGTA	CACACCAAGTTCCTCATCTT	66	588
Bov-A2 1242	Class III	27387319	27387682	CACCGAGAAACAGGGACAAT	CGTCGATCCTATCCATCCTT	64	364
Bov-A2 298	Class I	27899472	27899919	ACAAGGAGTCCCAACCACTG	CCCAGTCTCACTACCCATTCA	60	448
Bov-A2 320	Class I	27974408	27974707	AGTGGGAATGTCCTGACG	TAGCAAAGTTCCTCCAGGA	56	300
Bov-A2 321	Class I	27976352	27976826	CAAGTGAGTGTGCTTGGTGA	GCCGATATTCACACCAGGTT	60	475
Bov-A2 330	Class I	28017157	28017446	GCTTGTGCTTTTCCCTCTA	GGGGAAGAAAACCACATCCT	60	290
Bov-A2 357	Class I	28080362	28080860	GGCAAGTAACAGGGAAGAGG	CATACAGCCCCAGTACGTT	60	499
Bov-A2 370	Class I	28115261	28115949	GGGCTCATTAGGGCGAAATA	GCTGAGACACTCCAATCCAA	60	689
Bov-A2 373	Class I	28120235	28120713	AGCCTTGGGGCTATCTCTGT	CTTCACAGGTGGGATACACG	60	479
Bov-A2 400	Class I	28185780	28186269	CCTTCCACAAGATCAACTGC	TGTCAGAGTTCCTCGTTTGC	60	490
Bov-A2 471	Class I	28405227	28405931	CAGCACAGGAATATGCTGGA	CGGAAAAGCCCATAAATGTCT	60	705
Bov-A2 490	Class I	28470152	28470748	TCCTCACAGAGTGTGGGCTA	GAGGATGCATGTGGTCTGA	60	597
Bov-A2 514	Class I	28531178	28531660	CACCCAAATGGGAGTGAGAAC	GGGAGCAATCAGGACAAAAGT	64	483
Bov-A2 538	Class I	28612487	28612981	CGCCCTCCCATTGCAAAAAT	CCGCCACTGAGACAAAATGTA	56	495
Bov-A2 539	Class I	28614064	28614449	AGGGGACCAGGACAAAGACT	ACTTATGGGTCAGCCCGTTT	56	386
Bov-A2 558	Class I	28662878	28663328	GTACCTAAAGGCCAGACA	ATTTGTGGGCTCAAGTCAG	56	451
Bov-A2 564	Class I	28675610	28676071	TACATGGCTTCCCTCTCAC	ATCTCGGCTCCTGTACAAA	56	462

Table 4. Continued.

Bov-A2 Name	BoLA Class	Btau4.0 BTA23 Begin	Btau4.0 BTA23 End	Left Primer Sequence	Right Primer Sequence	Annealing Temperature (°C)	Expected Size (bp)
Bov-A2 1903	Extended Class I	30325085	30325417	GGGAGGGGATTGGACTAAAA	CTTCAGTTTCTGGGCCTCTG	60	333
Bov-A2 1902	Extended Class I	30328863	30329351	GACTCTTGCCATGGAGTCCT	GGAATAGGAACCTGGGCAGT	56	489
Bov-A2 1877	Extended Class I	30396515	30396997	CGTTTCCACCTTCTACAGTTCC	CAGGAGGTTTTCCCATACC	60	483
Bov-A2 1876	Extended Class I	30396592	30397011	GCGGTTTCTGCTTACGTTTC	GTTGTTGCTGCTGCTGTTGT	60	420
Bov-A2 1867	Extended Class I	30414021	30414399	GAGCTGAGGCCTAGGTCAAAA	GAGGAGGCCATTGTACCTC	60	379
Bov-A2 1845	Extended Class I	30470136	30470664	ATACTGGGAACAGGCCACTG	CAGAAGGTCAGGTGACAACG	60	529
Bov-A2 1843	Extended Class I	30474887	30475384	GTGGGAAGATCGCCAAGTAA	AACACCCCTCATGTTGCACAG	60	498
Bov-A2 1833	Extended Class I	30506338	30507037	CAAGGAAATCCCTCTCCACTC	CATCCAGGGGAAACAGTCAG	60	700
Bov-A2 1832	Extended Class I	30510112	30510602	CTTAGGAGCGAATCTGTGGA	GGGTTTTCAAGCCACTGTCA	60	491
Bov-A2 1802	Extended Class I	30578911	30579404	CAACAGAGAAGCCATCCTGAG	GCTTTGCCACACTCATGACA	60	494
Bov-A2 1750	Extended Class I	30723291	30723656	ATGGCATAAGGGTGTCCAGAG	AGTTGCAGCAGCAAGAGTGA	56	366
Bov-A2 1660	Extended Class I	30972756	30973228	CTGGTGATAGGAAAGCAGGTG	ACGGTTCTACCCGGAGATT	60	473
Bov-A2 1649	Extended Class I	30991083	30991532	TGTAATCTCACCCCTCCA	TTAGGCAAGGTAGCCTATGAGC	60	450
Bov-A2 1641	Extended Class I	31003088	31003547	AGCTCTCCATTGCTCAAAGC	CATCCTAAGCCCCAGACTGA	60	460
Bov-A2 1617	Extended Class I	31084783	31085281	GCCTCCGTCCATAACCAGAT	GTGAAGAACGTCACAACGTG	60	499
Bov-A2 1616	Extended Class I	31086932	31087390	AGATTAGCGGTTTGCTGCT	GGACATATTGGGCTGTCACTG	60	459
Bov-A2 1610	Extended Class I	31112224	31112745	CGTCCAGACAGCTTTTCGTT	CTCTACCTATTGCCCCTCA	60	522
Bov-A2 1609	Extended Class I	31115793	31116288	TCATCGCGTCTTACCACAC	GACCATCAGAAGGGAGGTTT	60	496
Bov-A2 1607	Extended Class I	31117738	31118220	AACCTAGAAGACTGGCGGGACT	TGAACTCTCCTGTGACCTACC	60	483
Bov-A2 1598	Extended Class I	31155391	31155410	TCCTGAATTGGCAGTCCTC	ATCTAGCAGCCCAACTCGTC	64	488
Bov-A2 1593	Extended Class I	31174245	31174818	GATCGGGTTGGGAATTACT	CCCAACTCACTGCACACACT	64	574
Bov-A2 1548	Extended Class I	31295345	31295735	GCTCCACAAGAGAACATTGC	TCTTTCCAGCTTGACCCTA	62	391
Bov-A2 1545	Extended Class I	31298557	31298942	GCGTGAAAGAAGCGATTACCT	CTCAGCATCCCACTGTCAAG	64	386

Table 5. Onami et al. 2007 Primers. Primers used in the Onami study were CO1, BAAA21, BE1, Bf 6, and Basix. Primers redesigned for this study were BE1-2, Bf 6-2, and BASix-4. Annealing temperatures used in this study for CO1, BAAA21, BE1-2, Bf 6-2, BASix-4 were 64°C, 64°C, 68°C, 66°C, and 55°C respectively.

Primer Name	Forward Primer	Reverse Primer	Chromosome Location	Btau4.0 BTA23 Begin	Btau4.0 BTA23 End
CO1	CTGTCCTGTGGCTCTATCCT	TTCTCCCATAGGTAGTGC	4	53948455	53949277
BAAA21	ATCTTGCCACTTTCATTTC	GGAGAGGGTAGAAGGGAAGA	28	41329175	41329973
BE1	AGAGCGCCAGTCAGCTCAAC	AGATCCCACATGCCTGGGAG	6	74541353	74542344
Bf 6	CTGTGTCAGTTTTGGGCAG	GGTCCTACTGACACACAAGG	28	36549329	36550158
Basix	AGAATACTGAGGTAAAAAT	TGAGGTCATAGAGAGTCCGA	5	83082236	83084086
BE1 - 2	GAAGAGGGAAGCTTAGGGAGTTTC	CACCTAGACGTCTCTGACTTAGCAC	6	74541380	74542371
Bf 6 - 2	GGCAGCTGTTCTTTATAAGGTCTG	GGTCCTACTGACACACAAGGGAGATTG	28	36549343	36550164
BASix - 4	CTGAGGTAAAAATTGGGAGTGCC	TGTTTCATGTGATGTGGCTTGTG	5	83082088	83084080

Bov-A2 retroposons were amplified in cycles of one 30 second denaturation step at 94°C; 1 cycle of 94°C for 30 sec, annealing temperature + 2°C for 30 sec, 72°C for 1 min and 30 sec; 35 cycles of 30 sec at 94°C, 30 sec at annealing temperature, 1 min and 30 sec at 72°C; and a final extension for 10 min at 72°C. PCR products were resolved by electrophoresis in 1.0% agarose gels containing 0.36 µg/ml ethidium bromide. Alleles were scored manually by comparison to 0.5ug of 100bp DNA Ladder (New England BioLabs) or 0.5ug of 1kb DNA Ladder (New England BioLabs). Both direct PCR product sequencing and cloned PCR product sequencing was utilized to analyze polymorphisms in Bov-A2 retroposons.

Analysis of BoLA Single Nucleotide Polymorphisms

Data from the genotyping of various breeds of cattle on genome-wide SNP chips (Matukumalli et al. 2009) in conjunction with the Bovine HapMap Project and University of Missouri linkage project was made available for use in this study. Haploview was used for the visualization of linkage disequilibrium and haplotype blocks (Barrett et al. 2005). FigTree was used to construct phylogenetic trees from the SNP data (<http://tree.bio.ed.ac.uk/software/figtree/> 2007). Different subsets of BoLA SNPs were used in each of the projects, but the data was made as consistent as possible for the analysis in this study. A total of 85 BoLA SNPs were analyzed in the Missouri samples, 114 HapMap SNPs were analyzed by PHASE, and 59 HapMap SNPs by fastPHASE (Table 6). The BoLA IIa region was represented by the smallest number of informative SNPs in all data sets.

Table 6. BoLA SNP Positions. Each SNP evaluated in an analysis is designated by an “X.”

BoLA Class	BTA23 Position in Btau4.0	University of Missouri	HapMap PHASE	HapMap fastPHASE	BoLA Class	BTA23 Position in Btau4.0	University of Missouri	HapMap PHASE	HapMap fastPHASE
IIb	7228956		X		I	28390649		X	X
IIb	7229152	X	X		I	28410906		X	
IIb	7280210	X	X	X	I	28436700	X	X	X
IIb	7339117	X	X	X	I	28542478	X	X	
IIb	7384619		X	X	I	28573563		X	
IIb	7398492	X	X	X	I	28752297	X	X	
IIb	7453429		X	X	I	28753058			X
IIb	7453668		X		I	28782231	X	X	
IIb	7455564		X	X	I	28803585	X	X	
IIb	7458121	X	X	X	I	28828816	X	X	
IIb	7479927	X	X		I	28890490	X	X	
IIb	7562289	X	X		I	28914694		X	X
IIb	7611266	X	X	X	I	28929062			X
IIb	7632049	X	X		I	28943537	X	X	
IIb	7666392	X	X		I	28969968		X	
IIa	26336469	X	X		I	29024454			X
IIa	26359831	X	X		I	29058544	X	X	
IIa	26404894	X			I	29068878		X	
IIa	26442664		X		I	29074525			X
IIa	26446938			X	I	29094169	X		X
IIa	26797818	X			I	29230537		X	
IIa	26873151	X	X		I	29230690			X
III	27014916	X	X		Extended I	29305663	X	X	
III	27037837		X		Extended I	29323427			X
III	27076772		X	X	Extended I	29347180	X	X	
III	27078160	X			Extended I	29368535	X	X	
III	27114828	X	X		Extended I	29395930	X	X	
III	27162533	X	X		Extended I	29440989	X	X	
III	27196973	X	X	X	Extended I	29471875	X	X	
III	27236662		X		Extended I	29516693			X
III	27285019	X	X	X	Extended I	29706065		X	
III	27286587	X	X		Extended I	29745302	X	X	
III	27289228		X	X	Extended I	29811117			X
III	27330080	X	X		Extended I	29844484			X
III	27349190		X		Extended I	29846761			X
III	27368932	X	X		Extended I	30021312			X
III	27407104		X	X	Extended I	30066821	X	X	
III	27429820	X	X		Extended I	30091550	X	X	
III	27471183	X	X		Extended I	30117027	X	X	
III	27541551		X	X	Extended I	30213365	X	X	
III	27542578	X	X		Extended I	30238291	X	X	X
I	27573173		X	X	Extended I	30238489			X
I	27580795	X	X		Extended I	30241269			X
I	27719069	X	X		Extended I	30273732	X	X	X
I	27737134	X	X	X	Extended I	30305546	X	X	
I	27745730	X	X		Extended I	30357570	X		
I	27895932			X	Extended I	30385066	X	X	
I	27911886	X	X		Extended I	30408757	X	X	
I	27913274			X	Extended I	30418559			X
I	27913734		X	X	Extended I	30439115	X	X	
I	27939837	X	X		Extended I	30480833			X
I	27977781	X	X		Extended I	30481353	X	X	
I	28007939	X	X		Extended I	30502690			X
I	28043179	X	X	X	Extended I	30503059			X
I	28064091	X			Extended I	30515461	X	X	
I	28085366		X		Extended I	30527398		X	X
I	28112250	X	X		Extended I	30540529	X	X	
I	28140134	X	X		Extended I	30546712		X	X
I	28145846		X	X	Extended I	30562383	X	X	X
I	28165417	X	X		Extended I	30596918	X	X	
I	28206725	X	X		Extended I	30640326	X	X	
I	28229903		X		Extended I	30661700	X	X	X
I	28268180	X	X		Extended I	30687916	X	X	
I	28312087	X	X		Extended I	30712904		X	X
I	28315507		X	X	Extended I	30758291		X	X
I	28315515		X	X	Extended I	30758467		X	X
I	28315653		X	X	Extended I	30870913	X	X	X
I	28315841		X	X	Extended I	30892545	X		
I	28315966		X	X	Extended I	30917451	X	X	
I	28338172		X	X	Extended I	30958975	X	X	
I	28341860	X	X		Extended I	30980996	X		

Haplotypes Inferred from BoLA Heterozygotes

Most SNPs from the University of Missouri were homozygous throughout the BoLA IIA-III-I region, but SNPs from the Bovine HapMap Project were both homozygous and heterozygous at BoLA. The SNPs from the Bovine HapMap Project were analyzed with PHASE (Stephens et al. 2001) by Dr. John Huber, School of Rural Public Health, Texas A&M University, and fastPHASE (Scheet and Stephens 2006) by Dr. Clare Gill, Department of Animal Science, Texas A&M University to identify haplotypes. Default parameters were used for PHASE, and animals were analyzed in PHASE groups by breed to increase the accuracy of haplotype inference. The fastPHASE analysis was performed with the subpopulation option, and only samples with >90% completion rates and SNPs with >0.05 minimum allele frequencies were retained for analysis by fastPHASE. Both PHASE and fastPHASE analyzed SNPs of BoLA IIB separately from those of BoLA IIA-III-I.

We have developed a method of matching all possible combinations of known BoLA haplotypes to identify the haplotypes present in unknown heterozygotes. However, unlike PHASE and fastPHASE, this method requires prior knowledge of BoLA haplotypes and the haplotype frequencies within breeds. Common haplotypes are seen most frequently in homozygous BoLA haplotypes, so identifying and counting homozygotes is an easy method of quantifying common haplotypes. The known BoLA haplotypes are listed in order according to their frequency within a particular breed. For example, a cattle breed may be known to contain the following BoLA haplotype frequencies, and the BoLA heterozygote may have the following unphased SNP alleles:

	SNP1	SNP2	SNP3	SNP4	SNP5	Haplotype Frequency
Haplotype 1	A	G	T	A	A	25 %
Haplotype 2	C	A	T	G	G	15 %
Haplotype 3	C	G	T	A	A	10 %
Haplotype 4	A	A	G	A	A	8 %
Haplotype 5	C	G	G	G	A	2 %
Unknown:	C/C	A/G	T/T	G/A	A/G	

The matching technique begins at the top of the known haplotype list with the most frequent haplotype, and the unknown haplotype is compared in the following order to these combinations of haplotypes:

1 st comparison:	Haplotype 1 and Haplotype 2
2 nd comparison:	Haplotype 1 and Haplotype 3
3 rd comparison:	Haplotype 1 and Haplotype 4
4 th comparison:	Haplotype 1 and Haplotype 5
5 th comparison:	Haplotype 2 and Haplotype 3
6 th comparison:	Haplotype 2 and Haplotype 4
7 th comparison:	Haplotype 2 and Haplotype 5
8 th comparison:	Haplotype 3 and Haplotype 4
9 th comparison:	Haplotype 3 and Haplotype 5
10 th comparison:	Haplotype 4 and Haplotype 5

The matching function collects all possible combinations of haplotypes that would create the unknown heterozygote's allele combinations. In this example, the only combination of known haplotypes that matches the unknown heterozygote is the combination of Haplotypes 2 and 3. The program can be set to choose the most probable haplotype combination based on known haplotype frequencies, or the program can create an output of all possible haplotype combinations and allow the user to decide how to proceed. In the case of no haplotype combination being found to match the unknown heterozygote, the program will search for a single haplotype match within the heterozygote. For example, no suitable combination is found for the following unknown heterozygote:

	SNP1	SNP2	SNP3	SNP4	SNP5
Unknown Heterozygote	A/C	G/G	T/G	T/A	A/A

However, one of the unknown haplotypes matches Haplotype 1. That would make the unknown second haplotype for SNP1 / SNP2 / SNP3 / SNP4 / SNP5 equal C / G / G / A / A. This new haplotype would be added to the bottom of the known haplotype list because it is present at the lowest frequency. In this way, the list of known haplotypes grows as the analysis continues.

CHAPTER III

RESULTS

Genotyping and Selection of BoLA Microsatellites

Genotyping Parents of the International Bovine Reference Family Panel

Microsatellites were characterized in this study to analyze the haplotype block structure, linkage disequilibrium, and recombination rates of the BoLA regions. The high mutation rate of microsatellites makes them appealing as genetic markers; however, they must be developed carefully to ensure reliable scoring of alleles and successful amplification across divergent haplotypes. BoLA microsatellites were initially genotyped on members of pedigreed families to evaluate polymorphism, locus specificity, and normal Mendelian inheritance.

The following parents of the International Bovine Reference Family Panel were used to characterize 76 BoLA microsatellites: 101 sire (Brahman x Gir/Indu), 1102 dam (Brahman), 1202 dam (Brahman), 2102 dam (Brahman), 501 sire (Friesian x Sahiwal), 502 dam (Friesian x Sahiwal), 702 dam (Friesian x Sahiwal), 1401 sire (Friesian x Sahiwal), 1402 dam (Friesian x Sahiwal), 1502 dam (Friesian x Sahiwal), 5252 sire (Brangus), 2308 dam (Brangus), 1158 dam (Brangus), 4046 dam (Brangus), 5191 sire (Brangus), 0981 dam (Brangus), 8001 sire (Holstein), 8032 dam (Holstein), 8301 sire (Charolais), 8312 dam (Charolais), 861029 dam (Gelbvieh x Simmental), 896800 sire (Piedmontese x Hereford), ND7 sire (N'Dama), 1419 dam (N'Dama), ND8 sire (N'Dama), 1688 dam (N'Dama). Microsatellites were genotyped across offspring of the International Reference Families only if they exhibited locus specificity and polymorphism among the parents.

Microsatellites were often closely linked with repetitive elements, so designing primers with robust locus specificity was a very important criterion for developing robust markers. Null alleles were recognized by non-Mendelian inheritance of microsatellite alleles in the offspring, and homoplasmy was discovered when more than one polymorphic element was present in the sequence of a microsatellite PCR product. Null alleles usually occurred due to biased amplification in heterozygotes because SNPs at the primer binding site resulted in preferential amplification of specific alleles. This problem was frequently

overcome by redesigning primers, but it was not always possible to redesign locus specific primers around microsatellites and avoid the problem of null alleles. Null alleles can generate an inaccurate picture of haplotype structure because they make heterozygous individuals appear homozygous. Polymorphic microsatellites with locus specific amplification were retained for use in this study only if they did not demonstrate homoplasy or null alleles. Out of 76 microsatellites characterized, 53 did not meet the necessary criteria to be used in this study. Of the 23 microsatellites used in this study, LA54 and DRBP1 have been characterized in previous studies of BoLA (Creighton et al. 1992; Ellegren et al. 1993). Although null alleles were found in both of these microsatellite primer pairs, the primers were retained in this study to make results and allele genotyping sizes consistent with the previous BoLA research.

Many different microsatellite motifs were investigated for polymorphism within the BoLA IIb region, but microsatellites containing (AC) motifs with more than five repeats were found to be the most polymorphic. In light of this, most microsatellites selected for characterization in the BoLA IIa-III-I region were (AC)₅₊ motifs. Perfect microsatellite motifs - microsatellite motifs without any nucleotide interruptions - were found to be more polymorphic than imperfect microsatellites. Homoplasy was most often observed in compound microsatellites when the amplicon contained more than one polymorphic microsatellite motif. None of the (AGC)_n microsatellites associated with Bov-A2 retroposons were retained for use in this study for various reasons, including nonspecific primer amplification, too much size variation, or not showing any polymorphism in size. Monomorphism, homoplasy, null alleles, and nonspecific amplification were the most common reasons why certain microsatellites were not chosen for use in this study (Table 7). Of the BoLA microsatellites that were selected, nine were located in the BoLA class IIb region, three within the region centromeric to class IIa, four in the class IIa region, two in the class I region, and three in the extended class I region (Table 8).

Table 7. BoLA Microsatellites Not Chosen for This Study. This table lists the name of the microsatellite, the location of the forward microsatellite primer on BTA23 in the Btau4.0 genome assembly, the BoLA class of the microsatellite position, the microsatellite reference motif from L1 Dominette, and the reason why the microsatellite was not selected for use in this study

Microsatellite Name	Btau4.0 BTA23 End	MHC Class	Microsatellite Motif	Reason Not Used
IIb 13	7188887	IIb	(AGC)6	Too Much Size Variaton, Primer in Bov-A2
IIb 41	7257268	IIb	(AT)4(AG)1(TA)4	Monomorphic
IIb 42	7258738	IIb	(AT)12	Nonspecific Amplification
IIb 53	7263383	IIb	(AGC)5	Too Much Size Variaton, Primer in Bov-A2
IIb 58	7264104	IIb	(AGC)6	Monomorphic, Primer in Bov-A2
IIb 104	7264120	IIb	(AGC)7	Nonspecific Amplification, Primer in Bov-A2
IIb 55	7276385	IIb	(TCAGT)4	Monomorphic
IIb 56	7284699	IIb	(AACTG)4 and (AT)2	Virtually Monomorphic
IIb 61	7293053	IIb	(GGA)5	Monomorphic
IIb 62	7293295	IIb	(AT)16	Nonspecific Amplification
IIb 90	7328911	IIb	(AC)7	Monomorphic
IIb 83	7332658	IIb	(AAAT)4	Virtually Monomorphic
IIb 97	7340278	IIb	(TTA)4	Monomorphic
IIb 89	7341230	IIb	(AT)6	Monomorphic
IIb 98	7342005	IIb	(AGC)4	Monomorphic, Primer in Bov-A2
IIb 107	7367687	IIb	(AGC)4	Monomorphic, Primer in Bov-A2
IIb 108	7376499	IIb	(ACTGA)5	Monomorphic
IIb 111	7389741	IIb	(TA)5C(AT)5	Monomorphic
IIb 113	7396966	IIb	(AC)7(AT)5	Homoplasly
IIb 119	7409831	IIb	(AGC)5	Null Alleles, Primer in Bov-A2
IIb 122	7412641	IIb	(AC)7	Nonspecific Amplification
IIb 129	7419634	IIb	(AGC)5	Monomorphic, Primer in Bov-A2
IIb 130	7420753	IIb	(ACTGA)4	Monomorphic
IIb 135	7437017	IIb	(AT)10	Nonspecific Amplification
IIb 139	7441581	IIb	(TAT)5	Nonspecific Amplification
IIb 143	7450249	IIb	(TC)7	Monomorphic
IIb 148	7453643	IIb	(GAAGT)6	Monomorphic
IIb 149	7458767	IIb	(AGC)5	Monomorphic, Primer in Bov-A2
IIb 158	7467432	IIb	(AT)11	Homoplasly
IIb 170	7477598	IIb	(AGC)5	Monomorphic, Primer in Bov-A2
IIb 174	7564507	IIb	(AC)3(AT)1(AC)5	Monomorphic
IIb 189	7592532	IIb	(AC)10	Homoplasly
IIb 193	7600300	IIb	(AC)10	Null Alleles
IIb 196	7602911	IIb	(AC)22	Null Alleles
IIb 200	7612980	IIb	(AGC)6	Virtually Monomorphic, Primer in Bov-A2
IIb 204	7618337	IIb	(ATTT)4	Monomorphic
IIb 208	7626989	IIb	(AC)4(GC)1(AC)5	Homoplasly
IIb 211	7629950	IIb	(AC)6	Nonspecific Amplification
IIb 218	7648858	IIb	(TGGA)2(TGGG)1(TGGA)2	Monomorphic

Table 7. Continued.

Microsatellite Name	Btau4.0 BTA23 Begin	MHC Class	Microsatellite Motif	Reason Not Used
1416	24180523	Centromeric to IIa	(AC)10	Monomorphic
644	24348422	Centromeric to IIa	(AC)6	Null Alleles
658	24369054	Centromeric to IIa	(AC)9	Monomorphic
995	25049980	Centromeric to IIa	(AC)5	Monomorphic
1153	25462357	Centromeric to IIa	(AC)17	Null Alleles
41	26063328	Centromeric to IIa	(AC)5(AG)1(CA)3	Virtually Monomorphic
ATF6B	27079046	III	(AC)14(TC)8	Homoplasmy
CYP21A2	27119005	III	(AC)21	Nonspecific Amplification
1249	27403361	III	(AC)5	Monomorphic
301	27914404	I	(AC)5	Monomorphic
362	28097911	I	(AC)5	Monomorphic
366	28107883	I	(AC)9	Monomorphic
397	28182049	I	(AC)6	Monomorphic
475	28419385	I	(AC)7	Monomorphic

Table 8. Allele Ranges of Selected Microsatellites. Nine microsatellites were selected within the BoLA IIb region, three within the region centromeric to class IIa, four within class IIa, two in class III, two in class I, and three in the extended class I region. Most of these microsatellites were (AC)5+ motifs with more than four alleles.

Microsatellite	Within Gene	MHC Class	Reference Motif	Allele Range (bp)
IIb 14		IIb	(AT)9	306 - 312
IIb 17		IIb	(AC)20	320 - 338
IIb 60		IIb	(AC)10	138 - 152
IIb 123		IIb	(AC)13	461 - 487
IIb 145		IIb	(AC)8	356 - 368
IIb 153		IIb	(AC)11	246 - 254
IIb 171		IIb	(AC)4(TC)1(AC)7	317 - 333
IIb 187		IIb	(AC)13	231 - 241
IIb 207	COL11A2	IIb	(AC)18	212 - 222
605		Centromeric to IIa	(AC)6	435 - 443
940	PKHD1	Centromeric to IIa	(AC)10	142 - 158
1176		Centromeric to IIa	(AC)7	229 - 249
LA54	DRB3	IIa	(TC)10(AC)22	149 - 227
DRBP1	DRB1 (psuedo)	IIa	(TC)9(AC)12	117 - 137
171		IIa	(AC)6	343 - 363
198	DRA	IIa	(AC)14	182 - 218
AGER	AGER	III	(AC)10	404 - 414
1268	BAT3	III	(AC)10	242 - 280
312	CDSN	I	(AC)13	450 - 484
415	FLOT1	I	(AC)6	473- 487
1870		Extended I	(AC)17	158 - 186
1778		Extended I	(AC)9(CG)1(AC)4(TA)1(AC)5	246 - 282
1687		Extended I	(AC)25	388 - 436

Observed Recombination Events

Recombination Events in BoLA IIb

In addition to providing valuable information about microsatellite polymorphism, genotyping pedigreed families also resolved recombination events. Two recombination events were observed out of 368 total meioses within the BoLA IIb region in parents of the International Reference Families (Table 9). One recombination event took place during male meioses and the other during female meioses.

Table 9. BoLA IIb Recombination Events. The number of recombination events, microsatellite markers flanking the events, and examples of genes within the interval of recombination are given in this table. Blue indicates that one recombination event took place during male meioses and pink shows the other occurred in female meioses.

Animal ID	Number of Meiosis Events	Number of Recombinants	Intervals of Recombination Events
101 sire	46	0	
1102 dam	19	0	
1202 dam	13	0	
2102 dam	14	0	
501 sire	26	0	
502 dam	12	0	
702 dam	14	0	
1401 sire	21	0	
1402 dam	9	0	
1502 dam	12	0	
5252 sire	40	0	
2308 dam	19	0	
1158 dam	11	0	
4046 dam	10	0	
5191 sire	11	0	
0981 dam	11	0	
8001 sire	9	1	DMB (IIb 145 - IIb 153)
8032 dam	9	0	
8301 sire	5	0	
8312 dam	5	0	
861029 dam	15	1	TAP 2 (IIb 60-2 - IIb 123-2)
896800 sire	15	0	
ND7 sire	5	0	
1419 dam	5	0	
ND8 sire	6	0	
1688 dam	6	0	
Total	368	2	

One of the BoLA IIB recombination events took place within an interval containing the genes *DYA*, *DYB*, *DOB*, *TAP2.1*, *TAP2*, *PSMB8*, *TAP1*, and *PSMB9*, while the other recombination event occurred near the *DMB* gene (Figure 6). A total of two recombinants from 368 meioses over a distance of approximately 450 kb calculate to 0.543 cM, which is consistent with the average rate of recombination in the bovine genome.

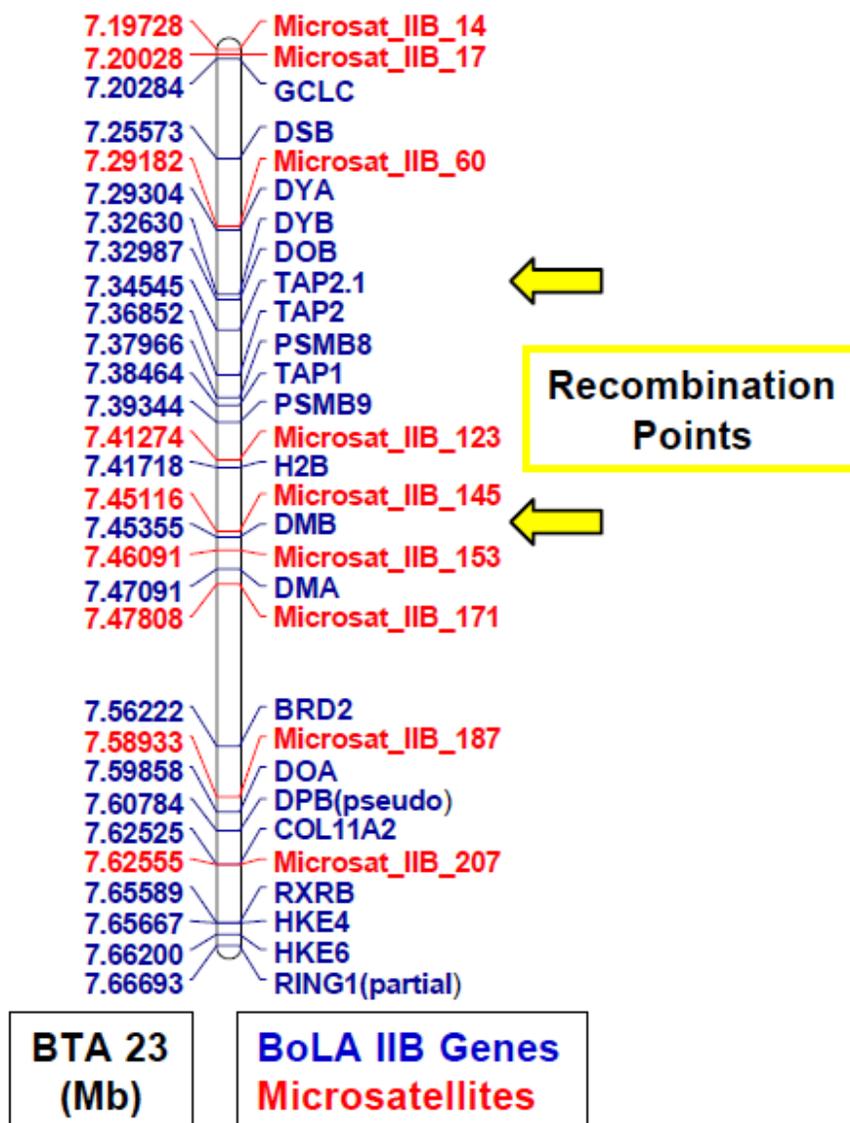


Figure 6. Intervals of BoLA IIB Recombination Events. Arrows (yellow) indicate intervals where the BoLA IIB recombination events occurred in relation to the positions of genes (blue) and microsatellites (red). The positions of BoLA IIB genes and microsatellites are listed on the left by their megabase position on chromosome 23.

Recombination Events in BoLA IIa-III-I

Nine recombination events were identified within the larger BoLA region out of 406 total meioses in parents of the International Reference Families and sires of the Lone Star Tick resistance study (Table 10). Four recombination events were observed in the region centromeric to BoLA class IIa, three within the BoLA IIa-III-I region, two in the extended class I region (Figure 7), and three took place during male meioses and six during female meioses. All recombination events were observed within parents of the International Reference Families, and there were no recombinants among the 50 informative meioses in the sires of the Lone Star Tick resistance study.

Table 10. BoLA IIa-III-I Recombination Events. The number of recombination events and microsatellite markers flanking the events are given in this table. Blue indicates three took place during male meioses and pink shows six occurred in female meioses.

Animal ID	Number of Meiosis Events	Number of Recombinants	Intervals of Recombination Events
101 sire	46	1	Centromeric to IIa (940 - 1176)
1102 dam	19	0	
1202 dam	13	0	
2102 dam	14	1	Class III - Class I (312 - 1268)
501 sire	26	0	
502 dam	12	0	
702 dam	14	0	
1401 sire	21	0	
1402 dam	9	0	
1502 dam	12	1	Class IIa - Class III (AGER - 198)
5252 sire	40	0	
2308 dam	19	3	One Centromeric to IIa (1176 - LA54) Two Extended Class I (415 - 1870)
1158 dam	11	0	
4046 dam	10	0	
5191 sire	11	0	
0981 dam	11	0	
8001 sire	9	1	Centromeric to IIa (1176 - LA54)
8032 dam	9	1	Class III - Class I (312 - 1268)
8301 sire	5	0	
8312 dam	5	0	
861029 dam	15	0	
896800 sire	15	1	Centromeric to IIa (940 - 1176)
ND7 sire	5	0	
1419 dam	5	0	
Bull 12 / 13	41	0	
Roger Bull	9	0	
Total	406	9	

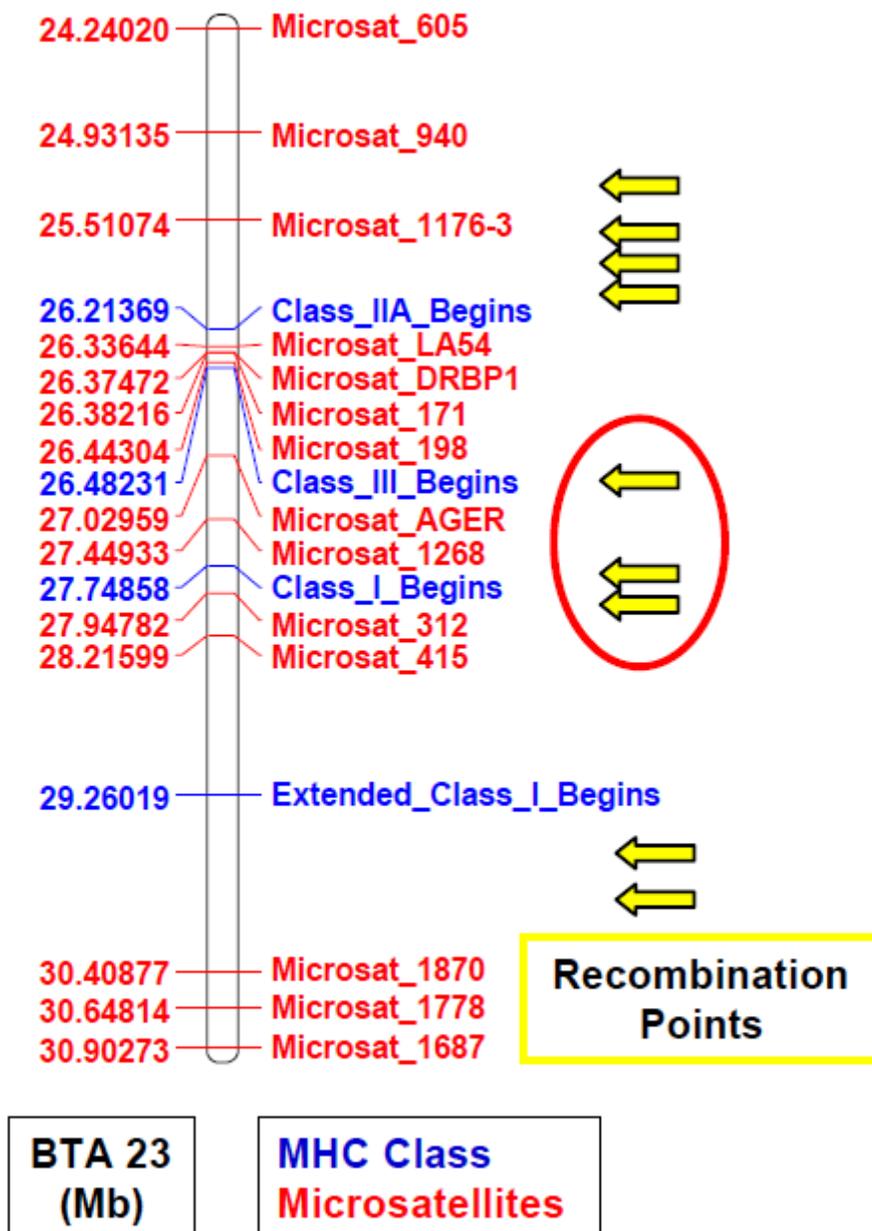


Figure 7. Intervals of BoLA IIa-III-I Recombination Events. Arrows (yellow) indicate intervals where the recombination events occurred in relation to the positions of BoLA classes (blue) and microsatellites (red). The positions of genes and microsatellites are listed on the left by their megabase position on chromosome 23. The arrows encompassed by a red circle are depicting the three recombination events that took place within the BoLA IIa-III-I region.

Haplotype Blocks of BoLA IIA-III-I

The number of observed recombination events within the BoLA IIA-III-I region was used to estimate a BoLA recombination rate. Three recombinants out of 406 total meioses spanning about 4 Mb of the BoLA IIA-III-I region calculates to be about 0.18 cM / 1 Mb. This is a much lower rate of recombination compared to the bovine genome wide average of 1.25 cM / 1 Mb (Arias et al. 2009), but it is consistent with the reduced recombination levels of BoLA IIA-III-I observed by Schnabel et al. (unpublished results). A larger study is needed to validate the hypothesis of a lower recombination rate in BoLA, but this evidence suggests that large blocks of linkage disequilibrium exist within the BoLA IIA-III-I region and are infrequently fragmented by recombination. In support of this, 21 Holsteins sharing at least one copy of the DRB3*1101 allele showed strong conservation of BoLA IIA-III-I microsatellite alleles. Microsatellite 605, located in the centromeric to IIA region, exhibited a different allele in one Holstein animal that may be evidence of a recombination event centromeric to BoLA class IIA. But out of 24 haplotypes containing the DRB3*1101 allele, all animals genotyped to have identical microsatellite alleles across the BoLA IIA-III-I region. When the Holstein animals were selected for the presence of the DRB3*1101 allele, they were inadvertently selected for a shared haplotype across the entire BoLA IIA-III-I region. Animals that had been typed for BoLA microsatellites were also typed for DRB3 alleles to see if more instances could be observed of DRB3 alleles predicting entire BoLA haplotypes. Parents of the International Reference Families were typed for DRB3 alleles to investigate whether their DRB3 alleles correlated with their microsatellite-derived haplotypes of BoLA IIA-III-I (Table 11).

Table 11. Alleles of DRB3 and BoLA IIa-III-I Microsatellites. Haplotypes sharing DRB3 alleles are highlighted in the same color, and breaks in allele identity are depicted by the disappearance of color. The names of the microsatellites and their BoLA classes are listed at the top of the table. Animals listed in this table are parents of the International Reference Families, and their microsatellite haplotypes were determined by genotyping offspring.

		IIA	IIA	IIA	IIA	IIA	III	III	I	I	Ext. I	Ext. I	Ext. I
Breed	Animal ID	DRB3 Allele	LA54	DRBP1	171	198	AGER	1268	312	415	1870	1778	1687
Brangus	5252 sire	DRB3*0101	185	119	347	196	408	266	454	475	166	260	422
Brangus	5191 sire	DRB3*0101	185	119	347	196	408	266	454	475	166	260	422
Brangus	4046 dam	DRB3*0201	193	129	347	204	406	270	456	475	168	258	418
Brangus	5191 sire	DRB3*0201	193	129	347	204	406	270	456	475	168	258	418
Friesian x Sahiwal	501 sire	DRB3*0201	193	129	347	204	410	278	476	475	168	272	408
Friesian x Sahiwal	502 dam	DRB3*0201	193	129	347	204	410	278	476	475	168	272	408
Friesian x Sahiwal	1401 sire	DRB3*0201	193	129	347	204	410	278	476	475	168	272	408
Brangus	5252 sire	DRB3*0501	189	125	347	196	406	266	472	475	166	248	394
Brahunan	1202 dam	DRB3*0901	195	121	343	194	410	266	472	475	170	260	388
Brahunan	2102 dam	DRB3*0902	187	131	345	194	406	266	458	473	170	260	418
Friesian x Sahiwal	502 dam	DRB3*0902	187	133	345	202	410	278	456	475	170	260	426
Brangus	2308 dam	DRB3*1001	null	125	343	202	410	276	456	475	172	260	400
Brangus	1158 dam	DRB3*1001	null	125	343	202	410	276	456	475	172	260	400
Friesian x Sahiwal	702 dam	DRB3*1001	null	125	345	188	406	266	462	475	168	260	422
Friesian x Sahiwal	1402 dam	DRB3*1001	null	125	345	188	406	266	462	475	168	260	406
Holstein	8001 sire	DRB3*1001	null	125	345	188	406	266	462	475	170	260	426
Holstein	8032 dam	DRB3*1001	null	125	345	188	406	266	462	475	170	260	426
Brangus	0981 dam	DRB3*1101	187	119	347	196	408	266	456	475	178	248	390
Holstein	8032 dam	DRB3*1201	205	125	345	200	408	270	482	475	168	260	436

Table 11. Continued.

		IIA	IIA	IIA	IIA	IIA	III	III	I	I	Ext. I	Ext. I	Ext. I
Breed	Animal ID	DRB3 Allele	LA54	DRBP1	171	198	AGER	1268	312	415	1870	1778	1687
Holstein	8001 sire	DRB3*1501	193	119	347	196	406	270	456	475	168	252	428
Friesian x Sahiwal	501 sire	DRB3*1703	177	119	345	218	406	270	480	475	168	272	408
Brahman	2102 dam	DRB3*1801	183	119	347	188	408	276	456	475	172	260	434
Brangus	1158 dam	DRB3*1801	183	123	347	194	408	276	458	475	170	246	412
Piedmontese x Hereford	896800 sire	DRB3*1801	183	123	347	194	408	278	456	475	172	248	394
Friesian x Sahiwal	1401 sire	DRB3*1901	181	125	345	192	414	270	456	475	172	266	388
Friesian x Sahiwal	1402 dam	DRB3*1901	181	125	345	192	410	274	460	473	176	252	428
Friesian x Sahiwal	1502 dam	DRB3*2002	183	121	357	196	406	264	456	475	170	258	424
Gelbvieh x Simmental	861029 dam	DRB3*2101	179	127	345	188	408	276	458	475	168	260	426
Friesian x Sahiwal	1502 dam	DRB3*2201	187	133	345	182	414	270	456	475	172	266	414
Brahman x Gir/Indu	101 sire	DRB3*2501	177	129	345	206	404	266	458	475	178	248	390
Friesian x Sahiwal	702 dam	DRB3*2705	161	121	343	188	406	270	458	475	168	248	430
Brahman	1102 dam	DRB3*3001	173	null	345	204	406	264	458	475	168	258	394
Brangus	2308 dam	DRB3*3001	173	null	345	204	406	268	458	475	170	260	428
Gelbvieh x Simmental	861029 dam	DRB3*3201	185	135	347	196	406	264	456	475	170	260	388
Piedmontese x Hereford	896800 sire	DRB3*3301	151	125	345	190	410	270	456	475	170	258	416
Brahman	1202 dam	DRB3*3601	183	117	343	206	406	270	456	475	170	260	392
Brangus	4046 dam	DRB3*3601	183	117	343	206	406	276	476	475	176	252	388
Brahman x Gir/Indu	101 sire	DRB3*3601	183	117	343	206	406	276	476	475	170	260	388
Brahman	1102 dam	DRB3*3601	183	117	343	206	406	276	476	475	170	260	388
Brangus	0981 dam	DRB3*4501	185	121	343	194	408	270	456	475	166	258	414

While some animals shared haplotypes across the entire BoLA IIA-III-I region, others showed strong haplotype blocks surrounding the DRB3 allele and extending only through the class IIA region. It is clear that the DRB3 allele is not always predictive of the entire BoLA haplotype, as was the case with the DRB3*1101 Holstein animals. The same DRB3 allele may appear on the background of different BoLA IIA-III-I haplotypes. The accurate identification of BoLA haplotypes will require markers to be genotyped across the entire BoLA IIA-III-I region. Typing markers only within the class IIA region is not sufficient to predict the entire BoLA haplotype, but it may be predictive of alleles at class IIA genes. It was previously demonstrated that alleles of the LA54 microsatellite are predictive of many alleles at the DRB3 gene, but the microsatellite cannot distinguish between all DRB3 alleles (Ellegren et al. 1993). Adding additional markers may help to more accurately predict alleles of DRB3 and other important class IIA genes, such as DQA and DQB.

Additional Genetic Markers

Identification and Characterization of SNPSTRs

Additional polymorphic markers, including SNPSTRs and Bov-A2 retroposons, were sought in the BoLA IIA-III-I region to more comprehensively characterize breakpoints in BoLA linkage disequilibrium. SNPSTRs were identified by sequencing BoLA microsatellite amplicons of parents of the International Reference Families, and several SNPs with minimum allele frequencies greater than 5% were identified within the amplicons of polymorphic BoLA microsatellites (Table 12). The animals sequenced for the discovery of SNPSTRs belonged to the breeds of Brahman, Brahman x Gir/Indu, Friesian x Sahiwal, Holstein, Brangus, Charolais, Gelbvieh x Simmental, Piedmontese x Hereford, and N'Dama. The SNPs found to have minimum allele frequencies below 5% among these breeds may exhibit higher minimum allele frequencies in other breeds. Two of the SNPs found in microsatellite amplicons 605 and 415 were tri-allelic, and at least 17 SNPs were identified within the 171 class IIA microsatellite amplicon.

Table 12. BoLA SNPSTRs. This table describes the total number of SNPs found within each microsatellite amplicon, the total number of individuals sequenced for each amplicon, the total number of observed SNPs, and the number of those with a minimum allele frequency higher than 0.05. The microsatellites with the highest number of surrounding SNPs are highlighted yellow.

Microsatellite Name	BoLA Class	Number of Individuals Sequenced	Total Number of SNPs	Number of SNPs >5% MAF
605	Centromeric to IIa	27	4	4 (one is tri-allelic)
940	Centromeric to IIa	23	5	4
171	Class IIa	27	20	17
198	Class IIa	26	4	3
1268	Class III	27	1	0
312	Class I	25	4	4 (one is tri-allelic)
415	Class I	27	8	7
1870	Extended Class I	25	5	3
1778	Extended Class I	27	8	4
1687	Extended Class I	27	7	4

Characterization of Bov-A2 Retroposons

A typical Bov-A2 repeat element consists of two monomers, called Bov-A units, connected by a conserved linker sequence (CACTTT)_n. Bov-A2 retroposons are unique in that the number of Bov-A units at a particular locus can vary between individuals. Each Bov-A unit is approximately 120 bp in length, making segmental polymorphisms easily distinguishable by agarose gel electrophoresis (Figure 8). Bov-A2 retroposons located in BoLA were investigated for polymorphism in Bov-A unit number across nine breeds of cattle and two North American Bison from Yellowstone National Park. Out of 90 Bov-A2 elements analyzed, only 11 were found to be polymorphic in Bov-A unit number. It was difficult to design primers to amplify the polymorphic Bov-A2 elements across all breeds. The increased nucleotide mutation rate of the repeat element probably hindered the binding of primers and created many null alleles. This property does not make Bov-A2 markers suitable for use in characterizing BoLA haplotype structure; however, the discovery that North American Bison show polymorphism in Bov-A unit number was significant. It contradicted the hypothesis that Bov-A unit number polymorphism arose because genome instability was instigated by the cattle domestication process (Onami et al. 2007). Since the process which generates Bov-A

unit number polymorphism was found in bison and is not unique to domestic cattle, it was decided to further investigate Bov-A2 polymorphisms in additional species.

Revisiting the Study of Onami et al. 2007

Onami et al. 2007 proposed that Bov-A2 length polymorphisms were a consequence of genomic instability resulting from the domestication and selective breeding of cattle. Onami et al. 2007 analyzed five polymorphic Bov-A2 repeat elements in *Bos taurus* breeds of cattle, one bongo (*Tragelaphus euryceros*), one Arabian Oryx (*Oryx leucoryx*), one Axis deer (*Axis axis*), one Reticulated Giraffe (*Giraffa camelopardalis*), one Pronghorn (*Antilocapra Americana*), and one Lesser mouse deer (*Tragulus javanicus*). The limited sampling of distant cattle relatives was not a robust experimental design for the Onami et al. 2007 study. The purpose of this experiment was to rigorously assess the hypothesis that polymorphisms in Bov-A unit number are specific to domestic cattle breeds by evaluating Bov-A2 polymorphisms within species more closely related to cattle. Alleles of the five polymorphic Bov-A2 loci used by Onami et al. 2007 were evaluated in Yellowstone National Park bison, Texas Longhorn cattle, Florida Scrub cattle, and various other wild bovids and ruminants (Table 13). The alleles were easily distinguishable by agarose gel electrophoresis, as each Bov-A unit is about 120 bp in length (Figure 8). Bov-A2 alleles were scored in gel electrophoresis and subset of the polymorphic Bov-A2 PCR products was sequenced.

The Bov-A2 retroposon CO1 was inserted in all bovines; BAAA21 exhibited up to three Bov-A monomers in domestic cattle, feral cattle, gaur, banteng, and bison; Bf6 exhibited Bov-A3 in gaur, Bov-A4 in bison, and Bov-A5 in bison and Florida Scrub; BE1 showed Bov-A3 in domestic and feral cattle, gaur, and bison, Bov-A4 in domestic and feral cattle and banteng, and Bov-A5 in banteng; Basix maintained Bov-A3 in domestic cattle and bison, Bov-A4 in gaur, Bov-A5 in Florida Scrub, and Bov-A6 in domestic cattle and Florida Scrub. The results of this experiment demonstrate that polymorphisms in Bov-A unit number are present in both wild and feral bovids of the *Bos* and *Bison* genera (Table 14); therefore, Bov-A2 polymorphisms do not appear to be a consequence of genomic instability associated with domestication.

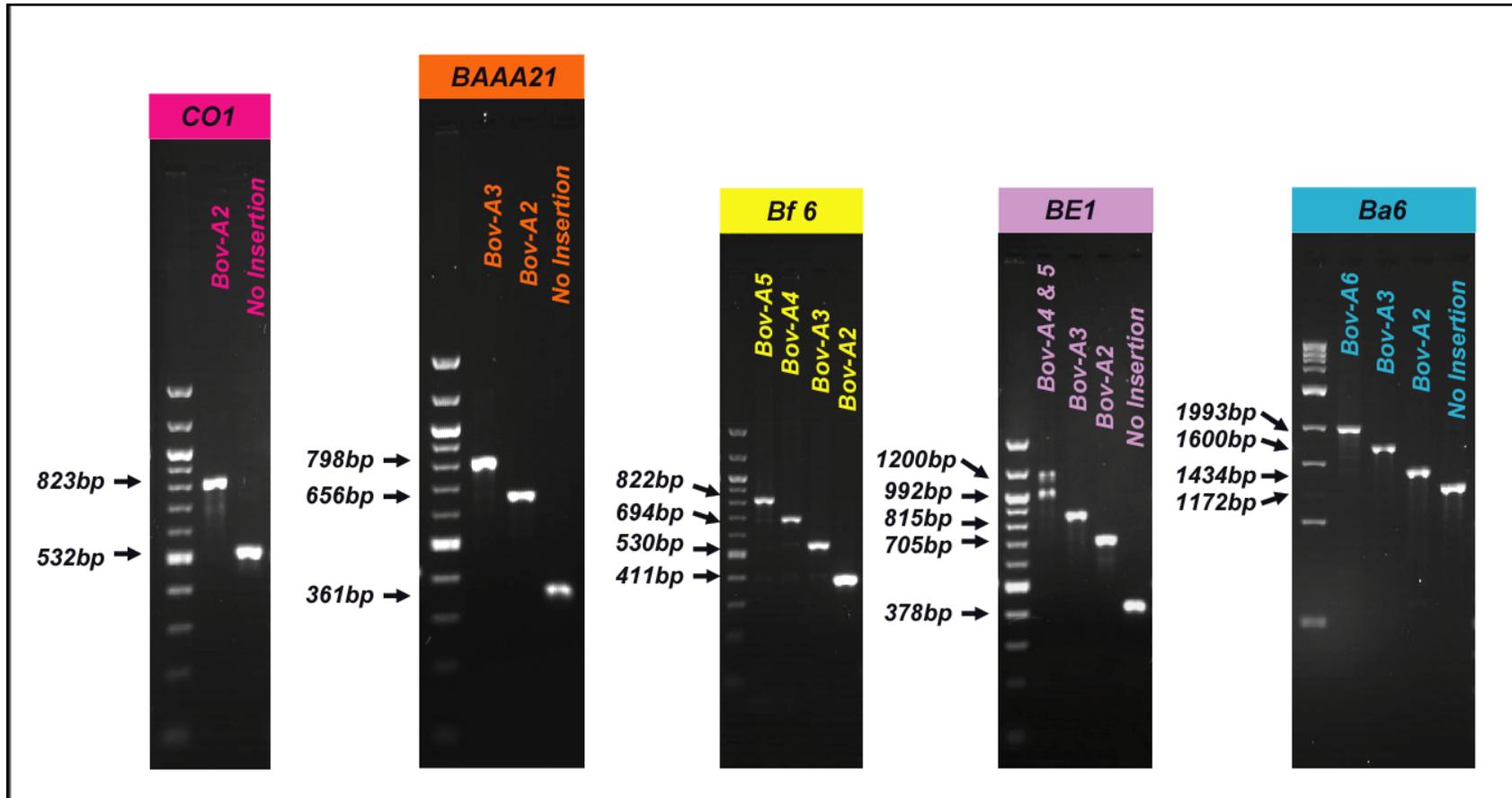


Figure 8. Agarose Gel Images of Bov-A2 PCR Products. Five sets of primers were used to amplify the five Bov-A2 retroposons described by Onami et al. 2007. There were two observed alleles for the primer pair CO1, three alleles for BAAA21, five alleles for Bf6, five alleles for BE1, and five alleles for Ba6. “No insertion” of a Bov-A2 element was counted as an allele.

Table 13. DNA Samples Used to Evaluate the Onami et al. 2007 Hypothesis. A variety of DNA samples from domestic cattle breeds, feral cattle, wild bovines, bovids, and cervids were utilized to amplify Bov-A2 elements from the Onami et al. 2007 study. Several trios (sire, dam, and offspring) were included in the analysis to ensure that the primers were amplifying properly without null alleles.

<u>Sources of Samples</u>	<u>Common Name</u>	<u>Scientific Name</u>	<u>n</u>
Domesticated Cattle (n = 30)	Holstein	<i>Bos taurus</i>	3 (1 trio)
	Charolais	<i>Bos taurus</i>	3 (1 trio)
	Hereford	<i>Bos taurus</i>	1
	Normande	<i>Bos taurus</i>	1
	Gelbvieh x Simmental	<i>Bos taurus</i>	1
	N'Dama	<i>Bos taurus</i>	4 (2 parent/offspring)
	Brangus	<i>Bos taurus x indicus</i>	6 (2 trios)
	Piedmontese x Hereford	<i>Bos taurus x indicus</i>	1
	Friesian x Sahiwal	<i>Bos taurus x indicus</i>	6 (2 trios)
	Brahman x Gir/Indu-Brazil	<i>Bos indicus</i>	3 (1 trio)
	Nelore	<i>Bos indicus</i>	1
Feral Cattle (n = 31)	Texas Longhorn	<i>Bos taurus</i>	11
	Florida Scrub	<i>Bos taurus</i>	20
Wild Bovines (n = 3)	Gaur	<i>Bos gaurus</i>	2
	Banteng	<i>Bos javanicus</i>	1
Wild Bovines (n = 25)	Bison	<i>Bison bison</i>	21
	Cape Buffalo	<i>Syncerus caffer</i>	1
	Water Buffalo	<i>Bubalus bubalis</i>	4
Other Bovids (n = 25)	Domestic Goat	<i>Capra aegagrus</i>	1
	Feral Sheep	<i>Ovis aries</i>	20
	Slender-horned Gazelle	<i>Gazella leptoceros</i>	1
	Persian Gazelle	<i>Gazella subgutturosa</i>	1
	Dama Gazelle	<i>Gazella dama</i>	1
	Arabian Oryx	<i>Oryx leucoryx</i>	1
Cervids (n = 38)	White Tailed Deer	<i>Odocoileus virginianus</i>	22
	Elk	<i>Cervus canadensis</i>	17

Table 14. Polymorphisms of Onami et al. 2007 Bov-A2 Retroposons. The columns of this table represent alleles of specific Bov-A2 retroposons, and the rows represent the group of animals genotyped. Each cell lists the number of individuals with an particular allele over the total number of individuals that successfully amplified with that Bov-A2 primer pair. Cells with one or more alleles are highlighted with a color specific to the Bov-A2 primer pair, and cells without any allelic representation are without color.

Figure 3: Bov-A2 Alleles Observed / Total Number of Alleles at Five Loci (CO1, BAAA21, BF 6, BE1, and Ba6)																					
Common Name	CO1		BAAA21			BF 6					BE1					Ba 6					
	No Insert	Bov-A2 (dimer)	No Insert	Bov-A2 (dimer)	Bov-A3 (trimer)	No Insert	Bov-A2 (dimer)	Bov-A3 (trimer)	Bov-A4 (tetramer)	Bov-A5 (pentamer)	No Insert	Bov-A2 (dimer)	Bov-A3 (trimer)	Bov-A4 (tetramer)	Bov-A5 (pentamer)	No Insert	Bov-A2 (dimer)	Bov-A3 (trimer)	Bov-A4 (tetramer)	Bov-A5 (pentamer)	Bov-A6 (hexamer)
Holstein	0/4	4/4	0/4	0/4	4/4	0/4	4/4	0/4	0/4	0/4	0/4	3/4	0/4	1/4	0/4	0/6	0/6	0/6	0/6	0/6	5/6
Charolais	0/4	4/4	0/4	0/4	4/4	0/4	4/4	0/4	0/4	0/4	0/4	3/4	0/4	1/4	0/4	0/4	2/4	0/4	0/4	0/4	2/4
Hereford	0/2	2/2	0/2	0/2	2/2	0/2	2/2	0/2	0/2	0/2	0/2	0/2	0/2	2/2	0/2	0/2	2/2	0/2	0/2	0/2	0/2
Normande	0/2	2/2	0/2	0/2	2/2	0/2	2/2	0/2	0/2	0/2	0/2	1/2	0/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	2/2
Gelbvieh x Simmental	0/2	2/2	0/2	0/2	2/2	0/2	2/2	0/2	0/2	0/2	0/2	1/2	0/2	1/2	0/2	0/2	1/2	0/2	0/2	0/2	1/2
FDama	0/6	6/6	0/6	0/6	6/6	0/6	6/6	0/6	0/6	0/6	0/6	3/6	0/6	3/6	0/6	0/6	3/6	2/6	0/6	0/6	1/6
Brangus	0/8	8/8	0/8	0/8	8/8	0/8	8/8	0/8	0/8	0/8	0/8	3/8	0/8	5/8	0/8	0/8	3/8	1/8	0/8	0/8	4/8
Piedmontese x Hereford	0/2	2/2	0/2	0/2	2/2	0/2	2/2	0/2	0/2	0/2	0/2	1/2	0/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	2/2
Friesian x Sahiwal	0/8	8/8	0/7	5/7	2/7	0/8	8/8	0/8	0/8	0/8	0/8	3/8	1/8	4/8	0/8	0/8	1/8	1/8	0/8	0/8	6/8
Brahman x Gir/Indu-Brazil	0/4	4/4	0/4	2/4	2/4	0/4	4/4	0/4	0/4	0/4	0/4	3/4	0/4	1/4	0/4	0/4	2/4	0/4	0/4	0/4	2/4
Nelore	0/2	2/2	0/2	0/2	2/2	0/2	2/2	0/2	0/2	0/2	0/2	1/2	0/2	1/2	0/2	0/2	2/2	0/2	0/2	0/2	0/2
Texas Longhorn	0/22	22/22	0/22	0/22	22/22	0/22	22/22	0/22	0/22	0/22	0/22	10/22	4/22	8/22	0/22	0/22	22/22	0/22	0/22	0/22	0/22
Florida Scrub	0/40	40/40	0/40	0/40	40/40	0/40	30/40	0/40	0/40	10/40	0/40	26/40	3/40	11/40	0/40	0/40	24/40	0/40	0/40	4/40	12/40
Gaur	0/4	4/4	0/4	0/4	4/4	0/4	0/4	4/4	0/4	0/4	0/4	0/4	4/4	0/4	0/4	0/4	0/4	4/4	0/4	4/4	0/4
Banteng	0/2	2/2	0/2	0/2	2/2	0/2	2/2	0/2	0/2	0/2	0/2	0/2	0/2	1/2	1/2	0/2	2/2	0/2	0/2	0/2	0/2
Bison	0/42	42/42	0/42	2/42	40/42	0/42	6/42	0/42	7/42	29/42	0/42	23/42	19/42	0/42	0/42	0/20	0/20	20/20	0/20	0/20	0/20
Cape Buffalo	0/2	2/2	0/2	2/2	0/2	0/2	2/2	0/2	0/2	0/2	2/2	0/2	0/2	0/2	0/2	0/2	2/2	0/2	0/2	0/2	0/2
Water Buffalo	0/8	8/8	0/8	8/8	0/8	0/8	8/8	0/8	0/8	0/8	8/8	0/8	0/8	0/8	0/8	8/8	0/8	0/8	0/8	0/8	0/8
Domestic Goat	2/2	0/2	2/2	0/2	0/2	2/2	0/2	0/2	0/2	0/2	2/2	0/2	0/2	0/2	0/2	0/2	2/2	0/2	0/2	0/2	0/2
Feral Sheep	40/40	0/40	0/40	40/40	0/40	40/40	0/40	0/40	0/40	0/40	40/40	0/40	0/40	0/40	0/40	0/40	40/40	0/40	0/40	0/40	0/40
Slender-horned Gazelle	2/2	0/2	0/2	2/2	0/2	2/2	0/2	0/2	0/2	0/2	2/2	0/2	0/2	0/2	0/2	0/2	2/2	0/2	0/2	0/2	0/2
Persian Gazelle	2/2	0/2	0/2	2/2	0/2	2/2	0/2	0/2	0/2	0/2	2/2	0/2	0/2	0/2	0/2	0/2	2/2	0/2	0/2	0/2	0/2
Dama Gazelle	2/2	0/2	0/2	2/2	0/2	2/2	0/2	0/2	0/2	0/2	1/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Arabian Oryx	2/2	0/2	0/2	2/2	0/2	2/2	0/2	0/2	0/2	0/2	1/2	1/2	0/2	0/2	0/2	0/2	2/2	0/2	0/2	0/2	0/2
White Tailed Deer	44/44	0/44	42/42	0/42	0/42	40/44	4/44	0/44	0/44	0/44	44/44	0/44	0/44	0/44	0/44	0/26	26/26	0/26	0/26	0/26	0/26
EK	18/18	0/18	20/22	2/22	0/22	23/34	11/34	0/34	0/34	0/34	26/26	0/26	0/26	0/26	0/26	0/16	16/16	0/16	0/16	0/16	0/16

It is likely that domestic cattle maintain substantial levels of genetic diversity because they were derived from a large and genetically diverse ancestral population (Gibbs et al. 2009), and not because domestication and selective breeding have produced genomic instability. Bov-A2 elements may be useful for phylogenetic studies of Bos and Bison, but they are not robust genetic markers for genotyping across different breeds of domestic cattle.

Analysis of BoLA Single Nucleotide Polymorphisms

Analyzing Homozygotes at the BoLA IIA-III-I-Extended I Region

During the course of this project, we were able to utilize data from a large whole genome SNP project (J. Taylor and S. Moore; USDA NRI 2006-35616-16697) that used the Illumina 50K SNPchip to genotype more than 13,000 animals from twenty six breeds of domestic cattle and three different species of ruminants (Gaur, Bison, and Cape buffalo). One of the objectives of this project was to use dense whole genome SNP analysis as a linkage experiment to validate the different sequence assemblies of the bovine genome. As such, all the domestic cattle are fully pedigreed for high resolution linkage mapping. Among the thousands of animals genotyped, 796 animals were identified to be homozygous across the entire span of the BoLA IIA-III-I region. This resource was extremely valuable in examining the relationships between BoLA haplotypes and the distribution of BoLA haplotypes among various breeds, and it allowed for the integration of SNP-defined BoLA haplotypes with the new polymorphic markers identified in this project.

We obtained 115 DNA samples representing 53 different haplotypes from Drs. Jerry Taylor and Bob Schnabel of the University of Missouri, Department of Animal Sciences. These samples represented a subset of the 109 haplotypes from the total 796 animals typed as homozygous across 52 SNPs spanning about 3 Mb of the BoLA IIA-III-I region. This was an excellent resource for characterizing BoLA haplotype structure because none of the SNPs required alleles to be phased, which is needed to identify haplotypes in heterozygous individuals. Homozygous haplotypes avoided errors of incorrectly phasing SNP alleles and generating false haplotypes.

The boundaries of BoLA homozygosity were analyzed by calculating the heterozygote frequency of SNPs flanking the homozygous BoLA regions in 796 animals (Figure 9). Boundaries of homozygosity are important to identify because haplotype homozygosity can be predictive of linkage disequilibrium (Sabatti and Risch 2002). BoLA homozygosity expands through part of the extended class I region, but the homozygosity abruptly ends at the boundary of class IIa. The region centromeric to BoLA IIa is relatively devoid of genes, whereas the extended class I region houses many gene families with homology to the extended class I region of HLA. The linkage disequilibrium expanding into the HLA extended class I region may be attributed to genes of the extended class I region hitchhiking with genes of BoLA, or vice-versa. Linkage disequilibrium extending beyond the class I region has been observed in humans (Horton et al. 2004), and it is probable that cattle have similar patterns of linkage disequilibrium.

SNPs were selected for analysis of haplotype structure based on the boundaries of homozygosity observed in Figure 9. There were no SNPs selected beyond the class IIa region, but several SNPs in the extended class I region were chosen for analysis with SNPs-derived haplotypes of the BoLA class IIa-III-I region. A total of five SNPs in class IIa, 12 SNPs in class III, 26 in class I, and nine SNPs in the extended class I region were analyzed in homozygous haplotypes of 796 animals (Table 15). These animals included cattle breeds Angus, Holstein, Hereford, Limousin, Simmental, Brahman, Brown Swiss, Belted Galloway, Dexter, Finnish Ayrshire, Gir, Guernsey, Jersey, Japanese Black, Kerry, Maine Anjou, MARC (representing many breeds used in a linkage study), Nelore, Norwegian Red, Red Poll, Romagnola, Romosinuano, Santa Gertrudis, Shorthorn, Salers, Scottish Highland, and White Park. Also included in analysis of BoLA homozygous haplotypes are North American bison, gaur, and African buffalo.

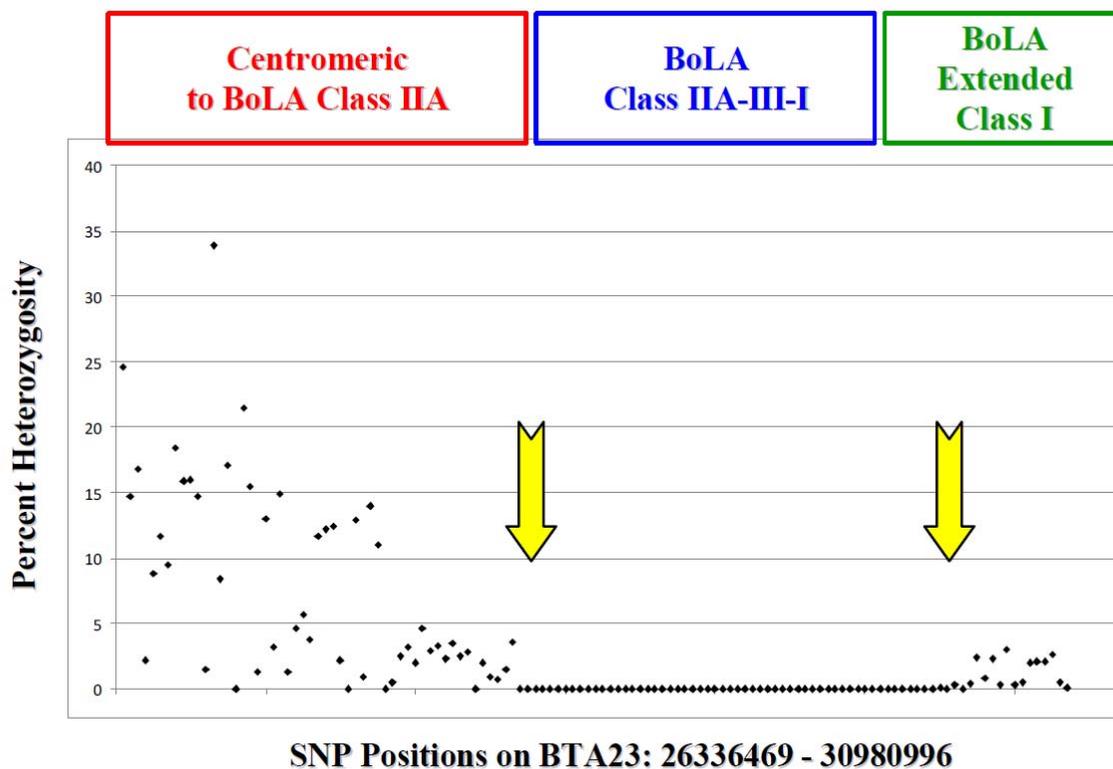


Figure 9. Breakpoints in BoLA Homozygosity. The position of the region centromeric to class IIa is depicted by a red box, the position of the BoLA class IIa-III-I by a blue box, and the position of the extended class I region by a green box. 52 SNPs were analyzed in the region centromeric to IIa, five in class IIa, 12 in class III, 26 in class I, and 31 in the extended class I region. SNPs are represented by black dots and plotted by their percent heterozygosity. The 796 individuals analyzed were all homozygous for SNPs within the BoLA IIa-III-I region, so the SNPs in that region are plotted at zero. Yellow arrows indicate the approximate breakpoints of homozygosity.

Table 15. Continued.

Total Number of Haplotypes Per Breed	Haplotype Count	Haplotype Name	Breed ID BT A 23 Position																																																							
			263386460	263598311	264054824	267978118	26871161	27014916	270781160	27114828	27162531	27190973	27248019	27286587	27330090	27368932	27429820	27471183	27524578	27580795	27719069	27747730	27914866	27939837	27977781	28007939	28043179	28064001	28112250	28140134	28165417	28200725	28268100	28312087	28341800	28349700	28342478	28732297	28782211	28807585	28828816	28899400	28923517	29045824	29004160	291656061	29247100	29368535	29495930	29440990	29474875	29745302	30066821	30091550				
Limousin = 62	10	LMS 1	G	A	A	G	A	C	A	G	C	G	A	A	G	A	C	A	A	G	A	C	G	C	A	G	A	A	A	A	A	A	A	G	G	A	A	A	A	A	G	G	A	G	C	A	A	A	C									
	10	LMS 2	G	A	A	A	G	C	G	G	C	G	A	A	G	A	A	G	A	A	G	G	C	G	A	A	G	A	A	A	A	A	A	A	G	C	G	A	A	A	A	A	A	A	A	A	A	A	A	G	G	C	G	A	A	C		
	10	LMS 3	G	A	G	A	G	C	A	G	C	A	A	G	A	A	G	A	A	G	A	A	G	C	C	A	A	A	A	A	A	A	A	A	G	G	C	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C			
	6	LMS 4	G	A	G	A	G	C	G	G	C	G	A	A	G	A	A	A	A	A	A	G	C	G	A	A	A	A	A	A	A	A	A	A	G	G	C	G	A	G	A	G	A	A	A	A	A	A	A	A	A	C	C					
	4	LMS 5	G	A	A	A	A	C	A	G	C	A	G	G	A	A	G	A	A	A	A	G	G	C	A	C	G	G	A	A	A	A	A	A	G	A	C	G	A	G	A	G	C	A	A	A	A	A	A	A	A	G	G	C	G	A	A	C
	4	LMS 6	G	A	A	G	A	G	A	G	A	A	A	A	A	A	A	A	A	A	A	A	G	C	G	C	G	A	A	A	A	A	A	A	G	G	C	G	A	G	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C			
	2	LMS 7	G	A	A	G	G	C	G	A	C	G	G	G	A	A	G	A	A	A	A	G	A	C	G	G	A	A	A	A	A	A	A	A	G	A	G	C	G	G	G	G	G	C	G	A	G	G	G	C	G	A	C	C				
	2	LMS 8	G	A	A	G	A	C	A	A	A	A	A	A	A	A	A	A	A	A	A	G	G	C	G	C	A	G	A	A	A	A	A	A	A	C	G	A	G	G	G	A	G	G	G	A	G	G	C	G	A	C	C					
	2	LMS 9	G	A	G	A	G	C	G	A	C	A	A	A	A	A	A	A	A	A	A	A	G	A	G	C	A	A	A	A	A	A	A	A	G	C	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C			
	2	LMS 10	G	A	A	G	A	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	G	C	G	C	A	A	A	A	A	A	A	A	A	G	C	G	G	A	G	A	A	A	A	A	A	A	A	A	A	A	C	C				
	2	LMS 11	G	G	G	A	G	C	A	A	C	G	A	A	A	A	A	A	A	A	A	A	G	C	G	A	A	G	G	C	A	A	A	A	A	G	C	G	G	A	G	A	A	A	A	A	A	A	A	A	A	A	A	C	C			
	2	LMS 12	G	A	G	A	G	C	G	A	A	G	A	A	A	A	A	A	A	A	A	A	G	C	G	A	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C			
	2	LMS 13	G	A	G	A	G	C	A	G	C	A	A	A	A	A	A	A	A	A	A	A	G	A	G	C	G	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C			
	4	LMS 14	G	A	A	G	G	C	A	G	C	G	A	C	G	A	A	A	A	A	A	A	A	G	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C			
Simmental = 16	6	SIM 1	G	A	G	A	A	C	A	G	C	G	G	G	A	C	G	G	G	C	A	C	A	A	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C					
	2	SIM 2	G	A	G	A	G	C	G	A	C	A	G	A	A	A	A	A	A	A	A	G	C	G	A	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C			
	2	ANG 3	G	A	G	A	G	A	G	A	C	A	G	G	A	A	A	A	A	A	A	A	G	C	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C			
	2	SIM 4	G	A	G	G	G	C	G	A	A	G	A	A	A	A	A	A	A	A	A	A	G	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C			
	2	ANG 1	G	A	G	A	A	C	G	A	A	G	A	A	A	A	A	A	A	A	A	A	G	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C				
2	ANG 4/HOL 5	G	A	A	G	G	C	G	A	C	A	A	A	A	A	A	A	A	A	A	A	A	G	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C				
Brahman = 16	14	BRM 1	G	A	G	G	G	C	G	A	C	G	A	A	A	A	A	A	A	A	A	G	C	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	A				
	2	BRM 2	G	A	G	A	G	C	A	A	C	G	A	A	A	A	A	A	A	A	A	A	G	C	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C			
Brown Swiss = 8	4	BSW 1	G	A	G	A	G	C	A	G	C	G	A	A	A	A	A	A	A	A	A	A	G	C	G	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C			
	2	BSW 2	G	A	G	G	G	A	G	A	C	A	A	A	A	A	A	A	A	A	A	A	A	C	A	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C		
	2	ANG 3	G	A	G	A	G	A	G	A	C	A	A	A	A	A	A	A	A	A	A	A	G	C	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C		
Belted Galloway = 2	2	BTG 1	G	G	G	A	G	A	G	A	C	A	G	G	A	C	A	G	A	G	C	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C				
Dexter = 2	2	DEX 1	G	A	G	A	G	C	G	A	C	A	A	A	A	A	A	A	A	A	A	A	G	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C			
Finnish Ayrshire = 18	8	FAY 1	G	A	G	A	G	C	G	A	C	A	G	A	A	A	A	A	A	A	A	A	G	C	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C			
	2	FAY 2	G	A	G	A	G	C	G	A	C	A	G	G	A	A	A	A	A	A	A	A	G	C	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C		
	2	HOL 12	G	A	G	A	G	C	G	A	C	A	A	A	A	A	A	A	A	A	A	A	G	C	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C		
	2	FAY 4	G	G	G	G	C	G	A	C	A	A	G	G	A	A	A	A	A	A	A	A	A	G	C	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C		
	2	FAY 5	G	A	G	G	C	G	A	C	A	G	G	A	A	A	A	A	A	A	A	A	A	G	C	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C		
	2	FAY 6	G	A	G	A	G	A	C	A	A	A	G	G	A	A	A	A	A	A	A	A	A	G	C	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C		
Gir = 2	2	GIR 1	G	A	A	G	G	C	G	A	C	A	A	A	A	A	A	A	A	A	A	A	G	C	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C			
Guernsey = 2	2	GNS 1	G	A	G	G	G	C	A	A	C	A	G	G	A	C	A	A	A	A	A	A	G	C	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C			
	Jersey = 10	4	JER 1	G	A	G	A	G	C	A	A	C	A	G	G	A	C	A	A	A	A	A	A	G	C	A	C	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C		
		2	JER 2	G	A	G	G	C	A	A	C	A	G	G	A	C	A	A	A	A	A	A	A	A	G	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C		
		2	JER 3	G	A	G	A	G	C	A	G	C	G	G	A	C	G	G	A	A	A	A	A	A	G	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C	
2	JER 4	G	A	A	G	C	A	A	C	A	A	G	G	A	C	A	A	A	A	A	A	A	G	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	C	C		
Japanese Black = 6	4	JPB 1	G	A	A	G	C	G																																																		

One of the surprising results of this analysis was the observation that seven entire BoLA IIA-III-I haplotypes were conserved among different *Bos taurus* breeds of cattle (Table 16), indicating that these haplotypes have been stable over hundreds of years since cattle domestication. All shared haplotypes were found among *Bos taurus* breeds in spite of significantly different breed histories and selection pressures. We failed to find shared haplotypes among *Bos indicus* breeds, but this is likely an artifact of sampling as not many *Bos indicus* cattle were analyzed in the 50K SNP study. One haplotype was shared between the crossbred Santa Gertrudis breed and the *Bos taurus* Maine Anjou breed, but this haplotype was assumed to be taurine in origin.

Table 16. BoLA Haplotypes Shared Across Breeds. The haplotype name is listed in the first column of this table, followed by the breeds that were identified with that particular haplotype. Each row represents a different shared haplotype, and the rows are colored to correspond with the haplotypes of Table 15.

HAPLOTYPE	SHARED ACROSS BREEDS		
ANG_1	ANGUS	SIMMENTAL	
ANG_3	ANGUS	SIMMENTAL	BROWN SWISS
ANG_4/HOL_5	ANGUS	HOLSTEIN	SIMMENTAL
ANG_7/HOL_2	ANGUS	HOLSTEIN	
HOL_12	HOLSTEIN	FINNISH AYRSHIRE	
LMS_14	LIMOUSIN	KERRY	SHORTHORN
MAO_2	MAINE ANJOU	SANTA GERTRUDIS	

The SNP-defined BoLA IIA-III-I haplotypes are not clustered in similarity by breed or breed type. Divergent haplotypes are found within and between breeds. No clear distinction is seen between BoLA haplotypes derived from *Bos indicus* cattle and those derived from *Bos taurus* cattle. A phylogenetic tree shows that *Bos indicus* BoLA haplotypes are intermingled with *Bos taurus*, but there is a clear separation of cattle haplotypes from African buffalo, bison, and to some extent gaur (Figure 10).

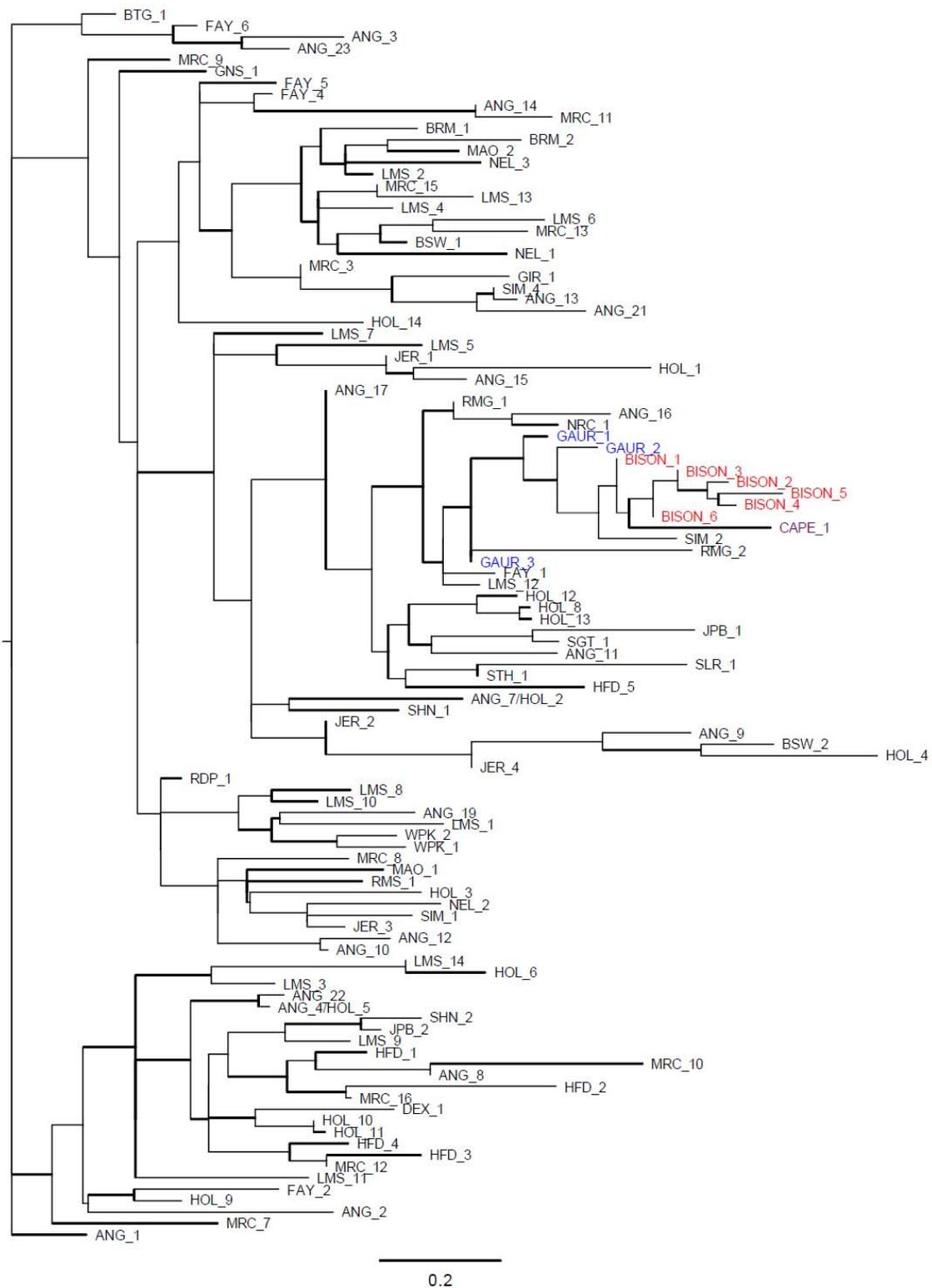


Figure 10. Phylogenetic Tree of BoLA IIa-III-I Haplotypes. Bison haplotypes are red in font, gaur are blue, and Cape buffalo are purple. This tree was constructed with FigTree software (<http://tree.bio.ed.ac.uk/software/figtree/>).

Of the 796 animals homozygous at the BoLA IIA-III-I region, 394 were also homozygous for haplotypes defined by ten SNPs in the BoLA class IIb region (Table 17). These homozygous BoLA IIb haplotypes were identified among various cattle breeds and North American bison.

Table 17. Genotypes of 27 Homozygous BoLA IIb Haplotypes. The name of the BoLA IIb haplotype is listed in the first column, and the SNP positions are listed across the top.

Haplotype Name and Position on BTA23 Brau4.0	7229152	7280210	7339111	7399492	7458121	7479927	7562289	7611266	7632049	7666392
IIB_ANG_1	G	A	A	G	C	G	A	A	G	G
IIB_ANG_2	G	G	A	A	A	A	G	G	G	G
IIB_ANG_3	G	A	G	G	C	G	G	A	G	G
IIB_ANG_4	G	A	A	G	C	G	A	G	G	A
IIB_ANG_5	G	A	G	A	A	G	G	G	G	G
IIB_ANG_6	G	G	A	A	C	G	G	G	G	G
IIB_ANG_7	G	A	G	G	C	G	G	A	G	A
IIB_HOL_9	G	A	G	G	C	G	A	A	G	G
IIB_HOL_15	G	A	A	G	A	A	A	A	G	G
IIB_HOL_18	G	A	A	A	C	G	G	A	G	G
IIB_HOL_27	G	A	G	G	A	A	A	A	G	G
IIB_HOL_28	G	G	A	A	C	G	A	A	G	G
IIB_HOL_29	G	G	A	G	C	G	G	G	G	G
IIB_HOL_44	G	G	A	G	A	A	A	A	G	A
IIB_HFD_1	A	A	G	A	C	G	G	G	G	A
IIB_HFD_2	G	G	A	A	C	G	G	G	G	A
IIB_LMS_1	G	G	G	G	C	G	G	A	G	G
IIB_LMS_3	G	A	G	G	A	A	G	A	G	G
IIB_STH_1	G	A	A	A	C	G	G	G	G	G
IIB_RMS_1	G	A	G	G	A	G	G	A	G	G
IIB_FAY_1	G	A	G	G	C	G	G	G	G	G
IIB_DEX_1	G	A	A	A	C	A	G	G	G	G
IIB_BTG_1	G	A	G	G	C	G	A	A	G	A
IIB_JPB_1	G	G	A	G	A	A	A	A	G	G
IIB_JPB_2	G	G	A	G	C	G	G	A	G	G
IIB_NEL_1	G	A	A	G	A	G	A	A	G	G
IIB_BISON_1	G	A	A	G	A	A	G	A	G	G

A phylogenetic tree of BoLA IIb haplotypes (Figure 11) shows different a relationship among BoLA IIb haplotypes when compared to the relationships described by the BoLA IIA-III-I haplotype tree. BoLA IIb haplotype relationships are more simplistic, and the haplotypes show less divergence from one another. It is likely that each BoLA IIb haplotype arose from a series of stepwise point mutations, unlike the BoLA IIA-III-I region which has maintained divergent ancestral haplotypes.

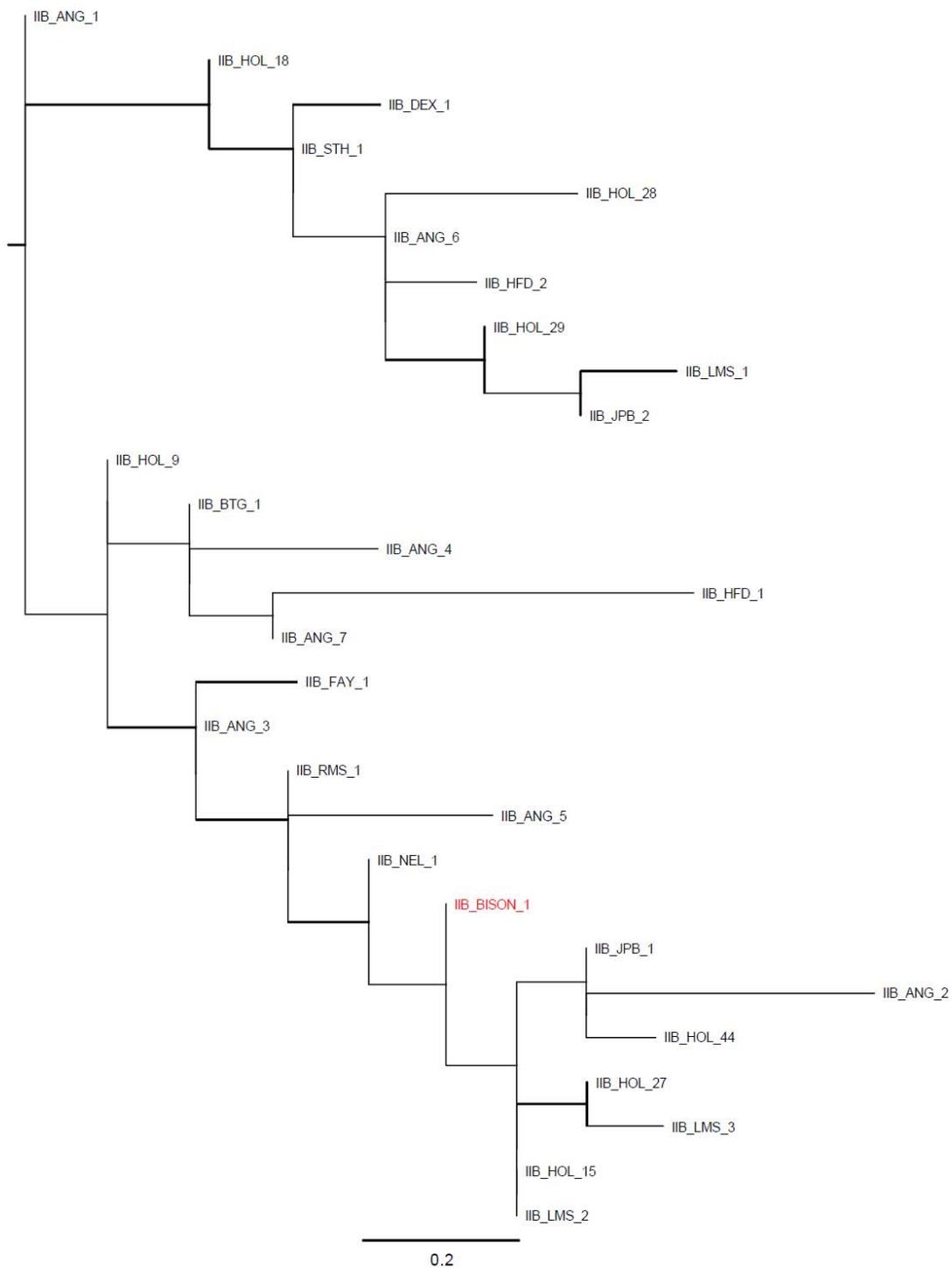


Figure 11. Phylogenetic Tree of BoLA IIB Haplotypes. Bison haplotypes are red in font color and cattle breeds are black. This tree was constructed by using FigTree software (<http://tree.bio.ed.ac.uk/software/figtree/>).

The largest numbers of homozygous BoLA IIa-III-I haplotypes are represented in the Angus (Figure 12) and Holstein (Figure 13) breeds with 598 and 698 total homozygous haplotypes, respectively. This makes the Angus and Holstein haplotypes ideal for estimating haplotype frequencies within breeds.

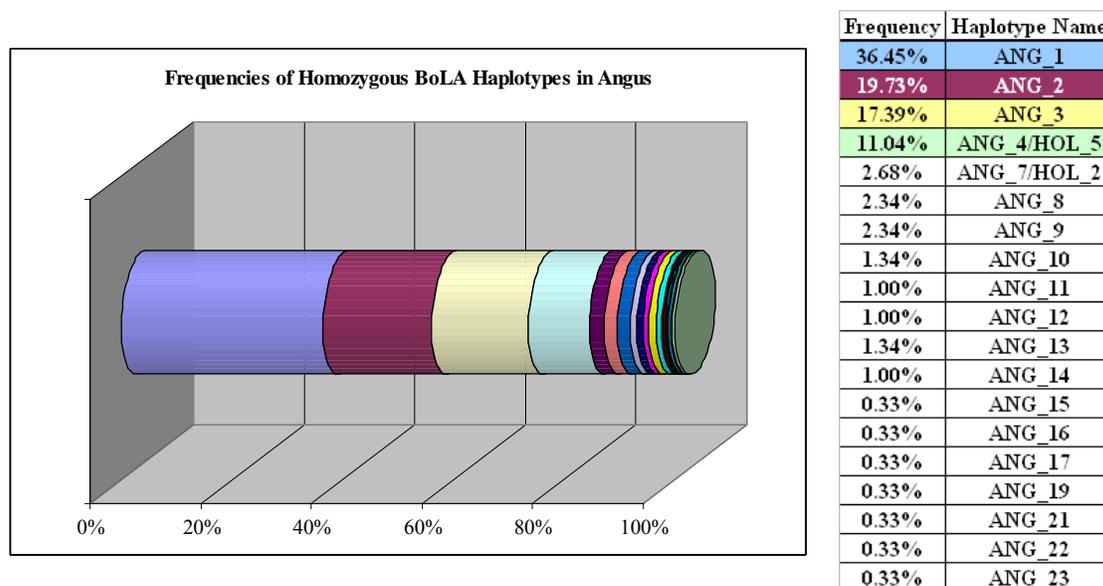


Figure 12. Angus Homozygous BoLA Haplotype Frequencies. A total of 598 homozygous Angus BoLA haplotypes were used to calculate BoLA haplotype frequencies within the Angus breed. Each colored segment of the cylinder graph represents one Angus BoLA haplotype, and each haplotype frequency is listed to the right of the graph.

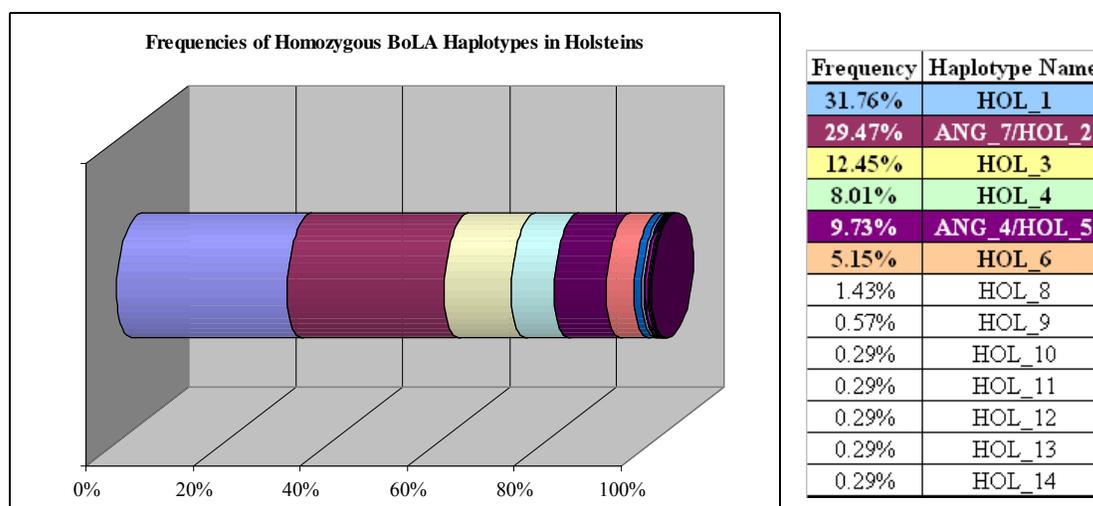


Figure 13. Holstein Homozygous BoLA Haplotype Frequencies. A total of 698 homozygous Holstein BoLA haplotypes were used to calculate BoLA haplotype frequencies within the Holstein breed. As in the Angus graph, each colored segment represents one haplotype and the frequencies are listed in a table to the right.

The four most common Angus BoLA IIa-III-I homozygous haplotypes represented 84.61% of all Angus homozygous haplotypes, and the fourth and fifth most common Angus BoLA homozygous haplotypes are also shared with the Holstein breed. The six most common Holstein BoLA homozygous haplotypes represented 96.57 % of the total haplotype number, and two of the six most common Holstein homozygous haplotypes are shared with the Angus breed. Angus and Holstein cattle have been selected for different traits (beef vs. dairy) for at least 200 years and are not interbred, so the prevalence of ANG_7/HOL_2 and ANG_4/HOL_5 haplotypes in both Angus and Holstein breeds suggests that the origin of the haplotypes predates the divergence of Angus and Holstein breeds more than 2,000 years ago (OSU 1995). The retention of these shared BoLA haplotypes over such a long period of time and the appearance of the shared haplotypes at high frequencies in modern Angus and Holstein populations suggests that the haplotypes are being selectively maintained. To more rigorously test the homozygosity indicated by the 50K SNPchip, we typed additional polymorphic markers over the homozygous BoLA haplotypes to determine if the identity of SNP-derived haplotypes across breeds would be further supported by the additional markers.

The alleles of the DRB3 gene were typed in all homozygous haplotypes with available DNA samples (Table 18). The DRB3 gene is extremely polymorphic, which makes DRB3 alleles more predictive of BoLA haplotypes than other less polymorphic BoLA genes. The DRB3 gene is also attractive for this study because it exists as a single copy on all BoLA haplotypes, and PCR amplification of DRB3 exon 2 is reliably consistent across divergent BoLA haplotypes. DRB3 alleles were always identical within a SNP-defined BoLA haplotype, and a considerable number of DRB3 alleles were shared across different BoLA haplotypes. BoLA haplotypes with the same DRB3 allele also shared SNP alleles within the class IIa region. The DRB3 alleles of bison have not been identified in cattle, but the DRB3 allele identified in gaur was identical to the DRB3*2201 allele of cattle. One divergent novel DRB3 allele was discovered within a Limousin animal containing the LMS_3 BoLA haplotype (Figure 14), but all other DRB3 alleles had been previously described.

Table 18. DRB3 Alleles of BoLA Homozygotes. This table shows the distribution of 23 different DRB3 alleles among 46 different cattle haplotypes of BoLA IIa-III-I homozygous animals. DRB3 sequences are also included for bison and gaur homozygotes. Rows are divided into sections by bold lines to show shared DRB3 alleles among animals of different breeds and haplotypes. As many as six different haplotypes share the same DRB3 allele, a state consistent with diversification of BoLA by recombination.

Haplotype	Animal Breed	Animal ID	DRB3 Allele	Haplotype	Animal Breed	Animal ID	DRB3 Allele
ANG_4/HOL_5	Angus	ANG_21990	DRB3*0101	SIM_1	Simmental	SIM_171210	DRB3*1301
ANG_4/HOL_5	Angus	ANG_22240	DRB3*0101	HOL_1	Holstein	HOL_11390	DRB3*1501
ANG_4/HOL_5	Angus	ANG_22560	DRB3*0101	HOL_1	Holstein	HOL_11910	DRB3*1501
ANG_4/HOL_5	Angus	ANG_23050	DRB3*0101	HOL_1	Holstein	HOL_12040	DRB3*1501
ANG_4/HOL_5	Angus	ANG_30950	DRB3*0101	HOL_1	Holstein	HOL_12310	DRB3*1501
ANG_4/HOL_5	Holstein	HOL_13500	DRB3*0101	HOL_1	Holstein	HOL_9950	DRB3*1501
ANG_4/HOL_5	Holstein	HOL_16830	DRB3*0101	LMS_8	Limousin	LMS_76800	DRB3*1501
ANG_4/HOL_5	Holstein	HOL_67370	DRB3*0101	RMS_1	Romossinuano	RMS_321690	DRB3*1501
ANG_4/HOL_5	Simmental	SIM_325720	DRB3*0101	ANG_21	Angus	ANG_22730	DRB3*1501
HOL_6	Holstein	HOL_10840	DRB3*0101	SIM_4	Simmental	SIM_175070	DRB3*1501
HOL_6	Holstein	HOL_45020	DRB3*0101	LMS_10	Limousin	LMS_103950	DRB3*1501
HOL_6	Holstein	HOL_9270	DRB3*0101	LMS_2	Limousin	LMS_78400	DRB3*1601
HOL_6	Limousin	LMS_73930	DRB3*0101	LMS_2	Limousin	LMS_143050	DRB3*1601
ANG_11	Angus	ANG_161120	DRB3*0101	LMS_5	Limousin	LMS_76130	DRB3*1601
ANG_3	Angus	ANG_22780	DRB3*0201	LMS_5	Limousin	LMS_143920	DRB3*1601
ANG_3	Angus	ANG_37400	DRB3*0201	HFD_3	Hereford	HFD_208520	DRB3*1601
ANG_3	Angus	ANG_5070	DRB3*0201	LMS_7	Limousin	LMS_73950	DRB3*1601
ANG_3	Angus	ANG_6810	DRB3*0201	LMS_13	Limousin	LMS_141890	DRB3*1602
ANG_3	Angus	ANG_7830	DRB3*0201	ANG_1	Angus	ANG_3100	DRB3*1801
ANG_3	Angus	ANG_320930	DRB3*0201	ANG_1	Angus	ANG_31040	DRB3*1801
ANG_3	Simmental	SIM_173690	DRB3*0201	ANG_1	Angus	ANG_321210	DRB3*1801
ANG_10	Angus	ANG_26350	DRB3*0201	ANG_1	Angus	ANG_4250	DRB3*1801
ANG_12	Angus	ANG_321440	DRB3*0201	ANG_1	Angus	ANG_44410	DRB3*1801
ANG_15	Angus	ANG_181560	DRB3*0201	ANG_1	Simmental	SIM_324070	DRB3*1801
ANG_22	Angus	ANG_167000	DRB3*0201	ANG_8	Angus	ANG_22080	DRB3*1801
ANG_23	Angus	ANG_179490	DRB3*0201	ANG_19	Angus	ANG_165060	DRB3*2502
LMS_4	Limousin	LMS_80130	DRB3*0301	ANG_2	Angus	ANG_183110	DRB3*2601
FAY_6	Finnish Ayrshire	AYR_207930	DRB3*0501	ANG_2	Angus	ANG_24940	DRB3*2601
SHN_1	Shorthorn	SHN_274230	DRB3*0501	ANG_2	Angus	ANG_31370	DRB3*2601
LMS_12	Limousin	LMS_123660	DRB3*0701	ANG_2	Angus	ANG_47790	DRB3*2601
SIM_2	Simmental	SIM_171090	DRB3*0701	ANG_2	Angus	ANG_5800	DRB3*2601
LMS_9	Limousin	LMS_96590	DRB3*0801	HOL_4	Holstein	HOL_21530	DRB3*2703
LMS_1	Limousin	LMS_74171	DRB3*1002	HOL_4	Holstein	HOL_45000	DRB3*2703
ANG_7/HOL_2	Angus	ANG_1620	DRB3*1101	HOL_4	Holstein	HOL_65860	DRB3*2703
ANG_7/HOL_2	Angus	ANG_163540	DRB3*1101	ANG_17	Angus	ANG_28720	DRB3*2707
ANG_7/HOL_2	Angus	ANG_163760	DRB3*1101	BRM_1	Brahman	BRM_29840	DRB3*3001
ANG_7/HOL_2	Angus	ANG_166550	DRB3*1101	ANG_9	Angus	ANG_28370	DRB3*3201
ANG_7/HOL_2	Holstein	HOL_16820	DRB3*1101	ANG_16	Angus	ANG_319920	DRB3*3201
ANG_7/HOL_2	Holstein	HOL_16840	DRB3*1101	HFD_4	Hereford	HFD_208490	DRB3*3201
ANG_13	Angus	ANG_22420	DRB3*1101	ANG_14	Angus	ANG_25250	DRB3*3202
ANG_13	Angus	ANG_23200	DRB3*1101	SHN_2	Shorthorn	SHN_274940	DRB3*20012
ANG_13	Angus	ANG_23220	DRB3*1101	LMS_3	Limousin	LMS_85040	NOVEL ALLELE
ANG_13	Angus	ANG_23400	DRB3*1101	BISON_3	Bison	CSP_BISON_51910	Bibi-DRB3*0502
LMS_6	Limousin	LMS_102640	DRB3*1101	BISON_4	Bison	CSP_BISON_52510	Bibi-DRB3*0701
LMS_3	Limousin	LMS_126000	DRB3*1101	GAUR_2	Gaur	GAUR_81740	BoLA-DRB3*2201
HOL_3	Holstein	HOL_11630	DRB3*1201	GAUR_1	Gaur	GAUR_81360	BoLA-DRB3*0601
HOL_3	Holstein	HOL_11830	DRB3*1201				
HOL_3	Holstein	HOL_11840	DRB3*1201				
HOL_3	Holstein	HOL_12080	DRB3*1201				
HOL_3	Holstein	HOL_9420	DRB3*1201				
HOL_3	Holstein	HOL_70430	DRB3*1201				
LMS_11	Limousin	LMS_105360	DRB3*1201				
HOL_3	Holstein	HOL_14570	DRB3*1201				

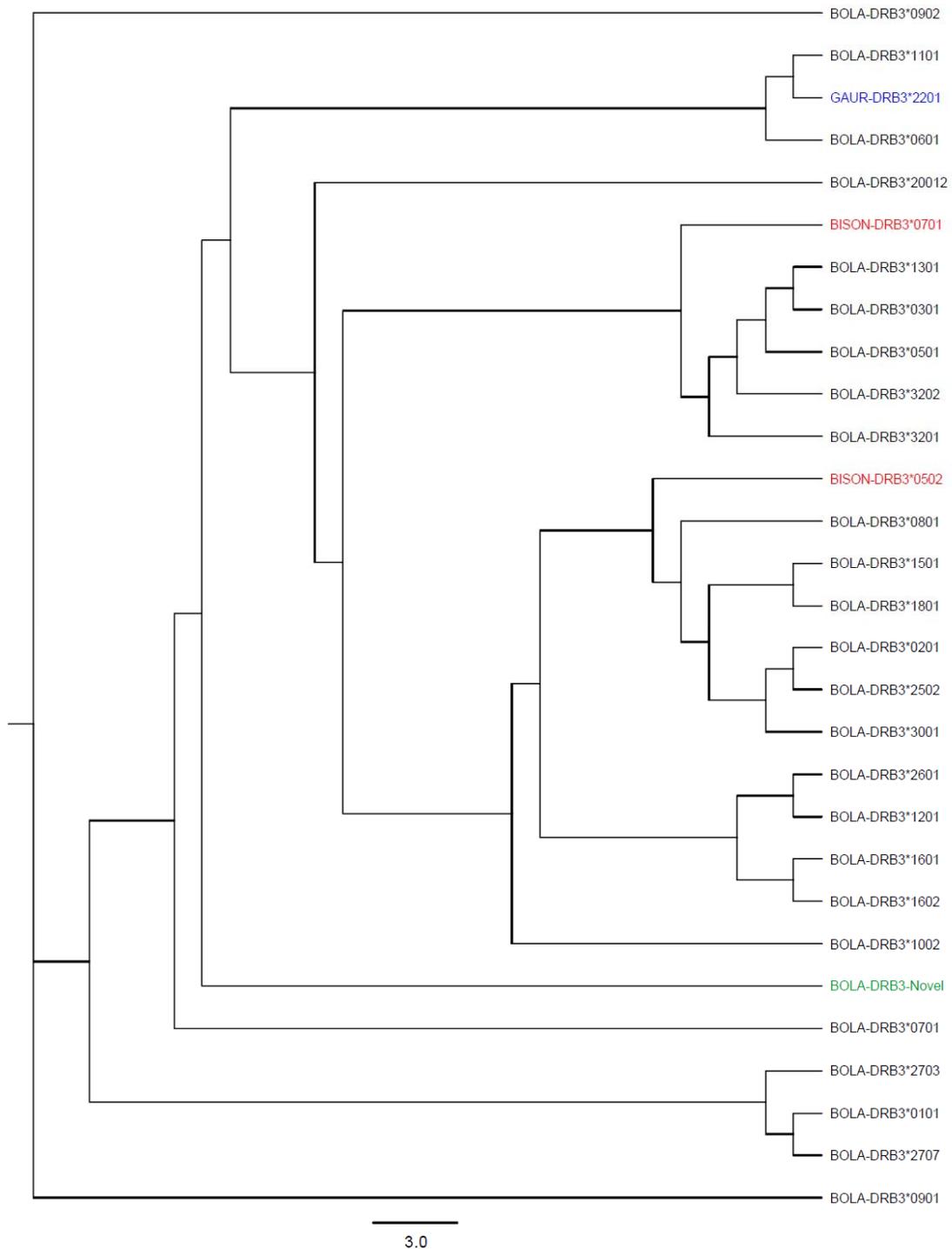


Figure 14. Phylogenetic Tree of DRB3 Exon 2 Sequences. DRB3 alleles of bison are red in font color, the DRB3 allele of gaur is blue, and the novel DRB3 allele from a Limousin animal is green. The novel DRB3 allele is highly divergent from the previously described alleles, and the DRB3 alleles of bison and gaur are not clustered together. This tree was constructed with FigTree software (<http://tree.bio.ed.ac.uk/software/figtree/>).

The BoLA IIa-III-I haplotypes defined by SNPs and DRB3 alleles were analyzed with a set of previously characterized BoLA microsatellites (Table 8) and SNPSTRs (Table 12) to further characterize BoLA haplotype structure (Table 19). The second exons of the DRA gene and DQB gene(s) were sequenced within BoLA haplotypes to investigate the correlation of SNP-defined haplotypes with alleles of BoLA genes. The DQB genes may be present in one or two copies on different BoLA haplotypes, but the DRA and DRB3 genes are always present in one copy. Many alleles are possible at DRB3 and DQB loci, but the second exon of the DRA gene has only four possible alleles generated from three synonymous SNP mutations, so different selection pressures may operate on the DRA gene than on the DRB3 and DQB genes.

Characterizing the points of divergence within haplotypes that share the same DRB3 allele may provide insight into the phylogeny and evolutionary history of BoLA haplotypes. In the following tables, haplotypes sharing the same DRB3 allele are listed together and the breakpoints in haplotype identity are highlighted in yellow. Breakpoints in identity within the same SNP-defined haplotype are highlighted in blue. If SNPs are truly predictive of BoLA IIa-III-I haplotypes, blue will only be seen in the terminal end of the extended class I region. BoLA SNPs were not selected to define haplotype structure in the region typed by terminal extended class I microsatellites.

Table 19. Additional Markers on BoLA Haplotypes with Defined DRB3 Alleles. Additional markers, some developed in this project, were typed in DNA from BoLA homozygotes with specific DRB3 alleles to further assess homozygosity and test for diversity within homozygotes. Homozygous BoLA haplotypes are grouped according to shared DRB3 alleles, and the name of the shared DRB3 allele is listed at the top of each group. The marker name, type (gene, microsatellite, or SNP), and BoLA class are listed at the top of each table. Each table represents BoLA IIa-III-I haplotypes sharing the same DRB3 allele. The BoLA IIa-III-I haplotype designation and animal ID are listed in the first two columns. Yellow indicates a polymorphism between different BoLA haplotypes, blue indicates a polymorphism within the same BoLA haplotype, and grey means the marker was not typed on a particular animal.

Table 19 Continued. BoLA Haplotypes with DRB3*0101.

Haplotype	Animal ID	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa
		Gene	Gene	Gene	Microsat	Microsat	SNP													
		DQB2	DQB1	DRB3	LA54	DRBP1	171	171	171	171	171	171	171	171	171	171	171	171	171	171
ANG_4/HOL_5	ANG_21990	none	DQB*0101 or DQB*0102	DRB3*0101	185	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A
ANG_4/HOL_5	ANG_22240	none	DQB*0101 or DQB*0102	DRB3*0101	185	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A
ANG_4/HOL_5	ANG_22560	none	DQB*0101 or DQB*0102	DRB3*0101	185	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A
ANG_4/HOL_5	ANG_23050	none	DQB*0101 or DQB*0102	DRB3*0101	185	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A
ANG_4/HOL_5	ANG_30950	none	DQB*0101 or DQB*0102	DRB3*0101	185	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A
ANG_4/HOL_5	HOL_13500	none	DQB*0101 or DQB*0102	DRB3*0101	185	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A
ANG_4/HOL_5	HOL_16830	none	DQB*0101 or DQB*0102	DRB3*0101	185	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A
ANG_4/HOL_5	HapMap - ANG 23	none	DQB*0101 or DQB*0102	DRB3*0101	185	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A
ANG_4/HOL_5	HOL_67370	none	DQB*0101 or DQB*0102	DRB3*0101	185	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A
ANG_4/HOL_5	SIM_325720	none	DQB*0101 or DQB*0102	DRB3*0101	185	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A
HOL_6	HOL_10840	none	DQB*0101 or DQB*0102	DRB3*0101	185	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A
HOL_6	HOL_45020	none	DQB*0101 or DQB*0102	DRB3*0101	185	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A
HOL_6	HOL_9270	none	DQB*0101 or DQB*0102	DRB3*0101	185	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A
HOL_6	LMS_73930	none	DQB*0101 or DQB*0102	DRB3*0101	185	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A
ANG_11	ANG_161120	none	DQB*0101 or DQB*0102	DRB3*0101	185	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A

Haplotype	Animal ID	IIa	IIa	IIa	IIa	IIa	IIa	III	I	I	I	I	I	I	Ext. I	Ext. I	
		SNP	SNP	SNP	Microsat	SNP	Gene	Microsat	Microsat	Microsat	SNP	Microsat	SNP	SNP	SNP	Microsat	Microsat
		171	171	171	171	171	DRA	198	AGER	312	415	415	415	415	1870	1687	
ANG_4/HOL_5	ANG_21990	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011	196 - (CA)13	408	454	T	475 - (AC)6	A	A	T	166	422
ANG_4/HOL_5	ANG_22240	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011	196 - (CA)13	408	454	T	475 - (AC)6	A	A	T	166	422
ANG_4/HOL_5	ANG_22560	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011	196 - (CA)13	408	454	T	475 - (AC)6	A	A	T	166	422
ANG_4/HOL_5	ANG_23050	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011	196 - (CA)13	408	454	T	475 - (AC)6	A	A	T	166	422
ANG_4/HOL_5	ANG_30950	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011	196 - (CA)13	408	454	T	475 - (AC)6	A	A	T	166	422
ANG_4/HOL_5	HOL_13500	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011	196 - (CA)13	408	454	T	475 - (AC)6	A	A	T	166	422
ANG_4/HOL_5	HOL_16830	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011	196 - (CA)13	408	454	T	475 - (AC)6	A	A	T	166	422
ANG_4/HOL_5	HapMap - ANG 23	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011	196	408	454	T	475 - (AC)6	A	A	T	166	422
ANG_4/HOL_5	HOL_67370	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011		408	454					170	418	
ANG_4/HOL_5	SIM_325720	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011		408	454					166	422	
HOL_6	HOL_10840	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011	196 - (CA)13	408	454	C	475 - (AC)6	G	A	C	166	422
HOL_6	HOL_45020	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011	196 - (CA)13	408	454	C	475 - (AC)6	G	A	C	166	422
HOL_6	HOL_9270	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011	196 - (CA)13	408	454	C	475 - (AC)6	G	A	C	166	422
HOL_6	LMS_73930	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011		408	454					166 / 170	414	
ANG_11	ANG_161120	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011		408	458					168	420	

Table 19 Continued. BoLA Haplotypes with DRB3*1101.

Haplotype	Animal ID	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa
		Gene	Gene	Gene	Microsat	Microsat	SNP												
		DQB2	DQB1	DRB3	LA54	DRBP1	171	171	171	171	171	171	171	171	171	171	171	171	171
ANG_7/HOL_2	ANG_1620	DQB*1001 or DQB*10021 or DQB*10022	DQB*1402	DRB3*1101	175	119	G	C	T	G	C	A	C	T	C	G	G	A	A
ANG_7/HOL_2	ANG_163540	DQB*1001 or DQB*10021 or DQB*10022	DQB*1402	DRB3*1101	175	119	G	C	T	G	C	A	C	T	C	G	G	A	A
ANG_7/HOL_2	ANG_163760	DQB*1001 or DQB*10021 or DQB*10022	DQB*1402	DRB3*1101	175	119	G	C	T	G	C	A	C	T	C	G	G	A	A
ANG_7/HOL_2	ANG_166550	DQB*1001 or DQB*10021 or DQB*10022	DQB*1402	DRB3*1101	175	119	G	C	T	G	C	A	C	T	C	G	G	A	A
ANG_7/HOL_2	HOL_16820	DQB*1001 or DQB*10021 or DQB*10022	DQB*1402	DRB3*1101	175	119	G	C	T	G	C	A	C	T	C	G	G	A	A
ANG_7/HOL_2	HOL_16840	DQB*1001 or DQB*10021 or DQB*10022	DQB*1402	DRB3*1101	175	119	G	C	T	G	C	A	C	T	C	G	G	A	A
ANG_7/HOL_2	HapMap - HOL 13	DQB*1001 or DQB*10021 or DQB*10022	DQB*1402	DRB3*1101	175	119	G	C	T	G	C	A	C	T	C	G	G	A	A
ANG_7/HOL_2	HapMap - HOL 53	DQB*1001 or DQB*10021 or DQB*10022	DQB*1402	DRB3*1101	175	119	G	C	T	G	C	A	C	T	C	G	G	A	A
ANG_13	ANG_22420	none	DQB*0401 or DQB*0402	DRB3*1101	187	119	G	C	T	G	C	A	C	T	C	G	G	A	A
ANG_13	ANG_23200	none	DQB*0401 or DQB*0402	DRB3*1101	187	119	G	C	T	G	C	A	C	T	C	G	G	A	A
ANG_13	ANG_23220	none	DQB*0401 or DQB*0402	DRB3*1101	187	119	G	C	T	G	C	A	C	T	C	G	G	A	A
ANG_13	ANG_23400	none	DQB*0401 or DQB*0402	DRB3*1101	187	119	G	C	T	G	C	A	C	T	C	G	G	A	A
LMS_6	LMS_102640			DRB3*1101	181	119	G	C	T	G	C	A	C	T	C	G	G	A	A
LMS_3	LMS_126000			DRB3*1101	183	119	G	C	T	G	C	A	C	T	C	G	G	A	A

Haplotype	Animal ID	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	III	I	I	I	I	I	I	Ext. I	Ext. I	
		SNP	SNP	SNP	SNP	SNP	Microsat	SNP	Gene	Microsat	Microsat	Microsat	SNP	Microsat	SNP	SNP	SNP	Microsat	Microsat
		171	171	171	171	171	171	DRA	198	AGER	312	415	415	415	415	415	1870	1687	
ANG_7/HOL_2	ANG_1620	C	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013	196 - (CA)13	408	472	T	475 - (AC)6	A	A	T	168	428
ANG_7/HOL_2	ANG_163540	C	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013	196 - (CA)13	408	472	T	475 - (AC)6	A	A	T	168	428
ANG_7/HOL_2	ANG_163760	C	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013	196 - (CA)13	408	472	T	475 - (AC)6	A	A	T	168	428
ANG_7/HOL_2	ANG_166550	C	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013	196 - (CA)13	408	472	T	475 - (AC)6	A	A	T	168	428
ANG_7/HOL_2	HOL_16820	C	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013	196 - (CA)13	408	472	T	475 - (AC)6	A	A	T	168	428
ANG_7/HOL_2	HOL_16840	C	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013	196 - (CA)13	408	472	T	475 - (AC)6	A	A	T	168	428
ANG_7/HOL_2	HapMap - HOL 13	C	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013	196	408	472	T	475 - (AC)6	A	A	T	168	422 / 428
ANG_7/HOL_2	HapMap - HOL 53	C	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013	196	408	472	T	475 - (AC)6	A	A	T	168	428
ANG_13	ANG_22420	C	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013	196 - (CA)13	404	458	C	475 - (AC)6	G	A	C	166	416
ANG_13	ANG_23200	C	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013	196 - (CA)13	404	458	C	475 - (AC)6	G	A	C	166	416
ANG_13	ANG_23220	C	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013	196 - (CA)13	404	458	C	475 - (AC)6	G	A	C	166	416
ANG_13	ANG_23400	C	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013	196 - (CA)13	404	458	C	475 - (AC)6	G	A	C	166	416
LMS_6	LMS_102640	C	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013		406	458						168	414
LMS_3	LMS_126000	C	A	G	C	C	347 - (CA)6(TG)1(CA)1	T	DRA*01014		406	456						170	434

Table 19 Continued. BoLA Haplotypes with DRB3*0201.

		IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa
		Gene	Gene	Gene	Microsat	Microsat	SNP																
Haplotype	Animal ID	DQB2	DQB1	DRB3	LA54	DRBP1	171	171	171	171	171	171	171	171	171	171	171	171	171	171	171	171	171
ANG_3	ANG_22780	none	DQB*020	DRB3*0201	193	129	G	C	T	G	C	A	C	T	C	A	G	G	C	A	G	C	C
ANG_3	ANG_37400	none	DQB*020	DRB3*0201	193	129	G	C	T	G	C	A	C	T	C	A	G	G	C	A	G	C	C
ANG_3	ANG_5070	none	DQB*020	DRB3*0201	193	129	G	C	T	G	C	A	C	T	C	A	G	G	C	A	G	C	C
ANG_3	ANG_6810	none	DQB*020	DRB3*0201	193	129	G	C	T	G	C	A	C	T	C	A	G	G	C	A	G	C	C
ANG_3	ANG_7830	none	DQB*020	DRB3*0201	193	129	G	C	T	G	C	A	C	T	C	A	G	G	C	A	G	C	C
ANG_3	ANG_320930	none	DQB*020	DRB3*0201	193	129	G	C	T	G	C	A	C	T	C	A	G	G	C	A	G	C	C
ANG_3	SIM_173690	none	DQB*020	DRB3*0201	193	129	G	C	T	G	C	A	C	T	C	A	G	G	C	A	G	C	C
ANG_10	ANG_26350	none	DQB*020	DRB3*0201	193	129	G	C	T	G	C	A	C	T	C	A	G	G	C	A	G	C	C
ANG_12	ANG_321440	none	DQB*020	DRB3*0201	193	129	G	C	T	G	C	A	C	T	C	A	G	G	C	A	G	C	C
ANG_15	ANG_181560	none	DQB*020	DRB3*0201	193	129	G	C	T	G	C	A	C	T	C	A	G	G	C	A	G	C	C
ANG_22	ANG_167000			DRB3*0201	193	129	G	C	T	G	C	A	C	T	C	A	G	G	C	A	G	C	C
ANG_23	ANG_179490	none	DQB*020	DRB3*0201	193	129	G	C	T	G	C	A	C	T	C	A	G	G	C	A	G	C	C

		IIa	IIa	IIa	IIa	III	I	I	I	I	I	I	I	Ext. I	Ext. I
		Microsat	SNP	Gene	Microsat	Microsat	Microsat	SNP	Microsat	SNP	SNP	SNP	Microsat	Microsat	
Haplotype	Animal ID	171	171	DRA	198	AGER	312	415	415	415	415	415	1870	1687	
ANG_3	ANG_22780	347 - (CA)8	T	DRA*01011	204 - (CA)2(TA)1(CA)14	406	458	C	473 - (AC)3(GC)1(AC)1	A	G	C	168	420	
ANG_3	ANG_37400	347 - (CA)8	T	DRA*01011	204 - (CA)2(TA)1(CA)14	406	458	C	473 - (AC)3(GC)1(AC)1	A	G	C	168	420	
ANG_3	ANG_5070	347 - (CA)8	T	DRA*01011	204 - (CA)2(TA)1(CA)14	406	458	C	473 - (AC)3(GC)1(AC)1	A	G	C	168	420	
ANG_3	ANG_6810	347 - (CA)8	T	DRA*01011	204 - (CA)2(TA)1(CA)14	406	458	C	473 - (AC)3(GC)1(AC)1	A	G	C	168	420	
ANG_3	ANG_7830	347 - (CA)8	T	DRA*01011	204 - (CA)2(TA)1(CA)14	406	458	C	473 - (AC)3(GC)1(AC)1	A	G	C	168	420	
ANG_3	ANG_320930	347 - (CA)8	T	DRA*01011		406	458						168	420	
ANG_3	SIM_173690	347 - (CA)8	T	DRA*01011			458						168	420	
ANG_10	ANG_26350	347 - (CA)8	T	DRA*01011		406	456						168	418	
ANG_12	ANG_321440	347 - (CA)8	T	DRA*01011		406	456						168	394	
ANG_15	ANG_181560	347 - (CA)8	T	DRA*01011		406	456						172	434	
ANG_22	ANG_167000	347 - (CA)8	T	DRA*01011		406	454						166	422	
ANG_23	ANG_179490	347 - (CA)8	T	DRA*01011		406	458						168	416	

Table 19 Continued. BoLA Haplotypes with DRB3*1201.

		IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	
		Gene	Gene	Gene	Microsat	Microsat	SNP															
Haplotype	Animal ID	DQB2	DQB1	DRB3	LA54	DRBP1	171	171	171	171	171	171	171	171	171	171	171	171	171	171	171	
HOL_3	HOL_11630	DQB*1005	DQB*1201	DRB3*1201	205	125	A	C	T	G	C	A	C	C	C	C	G	A	A	T	A	G
HOL_3	HOL_11830	DQB*1005	DQB*1201	DRB3*1201	205	125	A	C	T	G	C	A	C	C	C	C	G	A	A	T	A	G
HOL_3	HOL_11840	DQB*1005	DQB*1201	DRB3*1201	205	125	A	C	T	G	C	A	C	C	C	C	G	A	A	T	A	G
HOL_3	HOL_12080	DQB*1005	DQB*1201	DRB3*1201	205	125	A	C	T	G	C	A	C	C	C	C	G	A	A	T	A	G
HOL_3	HOL_9420	DQB*1005	DQB*1201	DRB3*1201	205	125	A	C	T	G	C	A	C	C	C	C	G	A	A	T	A	G
HOL_3	HOL_70430			DRB3*1201	205	125	A	C	T	G	C	A	C	C	C	C	G	A	A	T	A	G
HOL_3	HOL_14570			DRB3*1201	205	125	A	C	T	G	C	A	C	C	C	C	G	A	A	T	A	G
LMS_11	LMS_105360			DRB3*1201	205	125	A	C	T	G	C	A	C	C	C	C	G	A	A	T	A	G

		IIa	IIa	IIa	IIa	IIa	III	I	I	I	I	I	I	I	Ext. I	Ext. I
		SNP	SNP	Microsat	SNP	Gene	Microsat	Microsat	Microsat	SNP	Microsat	SNP	SNP	SNP	Microsat	Microsat
Haplotype	Animal ID	171	171	171	171	DRA	198	AGER	312	415	415	415	415	415	1870	1687
HOL_3	HOL_11630	C	T	345 - (CA)7	T	DRA*01011	198 - (CA)14	408	482	T	475 - (AC)6	A	A	T	168	436
HOL_3	HOL_11830	C	T	345 - (CA)7	T	DRA*01011	198 - (CA)14	408	482	T	475 - (AC)6	A	A	T	168	436
HOL_3	HOL_11840	C	T	345 - (CA)7	T	DRA*01011	198 - (CA)14	408	482	T	475 - (AC)6	A	A	T	168	436
HOL_3	HOL_12080	C	T	345 - (CA)7	T	DRA*01011	198 - (CA)14	408	482	T	475 - (AC)6	A	A	T	168	436
HOL_3	HOL_9420	C	T	345 - (CA)7	T	DRA*01011	198 - (CA)14	408	482	T	475 - (AC)6	A	A	T	168	436
HOL_3	HOL_70430	C	T	345 - (CA)7	T	DRA*01011		408	482						168	428 / 436
HOL_3	HOL_14570	C	T	345 - (CA)7	T	DRA*01011		408	482						168	414 / 436
LMS_11	LMS_105360	C	T	345 - (CA)7	T	DRA*01011		410	456						168	422

Table 19 Continued. BoLA Haplotypes with DRB3*1501.

Haplotype	Animal ID	IIa				IIa		IIa		IIa		IIa		IIa		IIa		IIa		IIa	
		Gene	Gene	Gene	Gene	Microsat	Microsat	SNP													
		DQB2	DQB1	DRB3	LA54	DRBP1	171	171	171	171	171	171	171	171	171	171	171	171	171	171	
HOL_1	HOL_11390	DQB*1001 or DQB*10021 or DQB*10022	DQB*1301	DRB3*1501	193	119	G	C	T	G	C	A	C	T	C	G	G	A	C		
HOL_1	HOL_11910	DQB*1001 or DQB*10021 or DQB*10022	DQB*1301	DRB3*1501	193	119	G	C	T	G	C	A	C	T	C	G	G	A	C		
HOL_1	HOL_12040	DQB*1001 or DQB*10021 or DQB*10022	DQB*1301	DRB3*1501	193	119	G	C	T	G	C	A	C	T	C	G	G	A	C		
HOL_1	HOL_12310	DQB*1001 or DQB*10021 or DQB*10022	DQB*1301	DRB3*1501	193	119	G	C	T	G	C	A	C	T	C	G	G	A	C		
HOL_1	HOL_9950	DQB*1001 or DQB*10021 or DQB*10022	DQB*1301	DRB3*1501	193	119	G	C	T	G	C	A	C	T	C	G	G	A	C		
HOL_1	HapMap - HOL 23	DQB*1001 or DQB*10021 or DQB*10022	DQB*1301	DRB3*1501		119	G	C	T	G	C	A	C	T	C	G	G	A	C		
LMS_8	LMS_76800			DRB3*1501	193	119	G	C	T	G	C	A	C	T	C	G	G	A	C		
LMS_10	LMS_103950			DRB3*1501	193	119	G	C	T	G	C	A	C	T	C	G	G	A	C		
RMS_1	RMS_321690			DRB3*1501			G	C	T	G	C	A	C	T	C	G	G	A	C		
ANG_21	ANG_22730			DRB3*1501	193	127	A	C	T	G	C	A	C	C	G	G	G	A	C		
SIM_4	SIM_175070			DRB3*1501	193	127	A	C	T	G	C	A	C	C	G	G	G	A	C		

Haplotype	Animal ID	IIa				IIa		IIa		III	I	I	I	I	I	I	Ext. I	Ext. I
		SNP	SNP	SNP	SNP	Microsat	SNP	Gene	Microsat	Microsat	Microsat	SNP	Microsat	SNP	SNP	SNP	Microsat	Microsat
		171	171	171	171	171	DRA	198	AGER	312	415	415	415	415	415	1870	1687	
HOL_1	HOL_11390	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013	196 - (CA)13	406	456	C	475 - (AC)6	A	G	T	168	428
HOL_1	HOL_11910	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013	196 - (CA)13	406	456	C	475 - (AC)6	A	G	T	168	428
HOL_1	HOL_12040	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013	196 - (CA)13	406	456	C	475 - (AC)6	A	G	T	168	428
HOL_1	HOL_12310	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013	196 - (CA)13	406	456	C	475 - (AC)6	A	G	T	168	428
HOL_1	HOL_9950	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013	196 - (CA)13	406	456	C	475 - (AC)6	A	G	T	168	428
HOL_1	HapMap - HOL 23	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013	196	406		C	475 - (AC)6	A	G	T	168	428
LMS_8	LMS_76800	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013		406	456						168	436
LMS_10	LMS_103950	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013		406	458						168	422
RMS_1	RMS_321690	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01013										
ANG_21	ANG_22730	A	G	C	T	345 - (CA)7	T	DRA*01011		404	458						168	422
SIM_4	SIM_175070	A	G	C	T	345 - (CA)7	T	DRA*01011			458						168	

Table 19 Continued. BoLA Haplotypes with DRB3*1601.

		IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa
		Gene	Microsat	Microsat	SNP												
Haplotype	Animal ID	DRB3	LA54	DRBP1	171	171	171	171	171	171	171	171	171	171	171	171	171
LMS_2	LMS_78400	DRB3*1601	193	119	G	C	T	G	C	A	C	T	C	G	G	A	C
LMS_2	LMS_143050	DRB3*1601	193	119	G	C	T	G	C	A	C	T	C	G	G	A	C
LMS_5	LMS_76130	DRB3*1601	195	119	G	C	T	G	C	A	C	T	C	G	G	A	C
LMS_5	LMS_143920	DRB3*1601	195	119	G	C	T	G	C	A	C	T	C	G	G	A	C
LMS_7	LMS_73950	DRB3*1601	193	119	G	C	T	G	C	A	C	T	C	G	G	A	C
HFD_3	HFD_208520	DRB3*1601	193	119	G	C	T	G	C	A	C	T	C	G	G	A	C

		IIa	IIa	IIa	IIa	IIa	IIa	IIa	III	I	Ext. I	Ext. I
		SNP	SNP	SNP	SNP	Microsat	SNP	Gene	Microsat	Microsat	Microsat	Microsat
Haplotype	Animal ID	171	171	171	171	171	171	DRA	AGER	312	1870	1687
LMS_2	LMS_78400	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011	408	476	168	394
LMS_2	LMS_143050	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011	408	476	168	394
LMS_5	LMS_76130	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011	406	458	170	428
LMS_5	LMS_143920	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011	406	458	170	428
LMS_7	LMS_73950	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011	410	456	168	394
HFD_3	HFD_208520	A	G	C	C	347 - (CA)6(CG)1(CA)1	T	DRA*01011	406	482	168	398

Table 19 Continued. BoLA Haplotypes with DRB3*1801.

		IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa
		Gene	Microsat	Microsat	SNP																
Haplotype	Animal ID	DRB3	LA54	DRBP1	171	171	171	171	171	171	171	171	171	171	171	171	171	171	171	171	171
ANG_1	ANG_3100	DRB3*1801	183	123	G	C	T	G	C	A	C	T	C	G	G	A	C	A	G	C	C
ANG_1	ANG_31040	DRB3*1801	183	123	G	C	T	G	C	A	C	T	C	G	G	A	C	A	G	C	C
ANG_1	ANG_321210	DRB3*1801	183	123	G	C	T	G	C	A	C	T	C	G	G	A	C	A	G	C	C
ANG_1	ANG_4250	DRB3*1801	183	123	G	C	T	G	C	A	C	T	C	G	G	A	C	A	G	C	C
ANG_1	ANG_44410	DRB3*1801	183	123	G	C	T	G	C	A	C	T	C	G	G	A	C	A	G	C	C
ANG_1	SIM_324070	DRB3*1801	183	123	G	C	T	G	C	A	C	T	C	G	G	A	C	A	G	C	C
ANG_8	ANG_22080	DRB3*1801	183	123	G	C	T	G	C	A	C	T	C	G	G	A	C	A	G	C	C

		IIa	IIa	IIa	IIa	III	I	I	I	I	I	I	Ext. I	Ext. I
		Microsat	SNP	Gene	Microsat	Microsat	Microsat	SNP	Microsat	SNP	SNP	SNP	Microsat	Microsat
Haplotype	Animal ID	171	171	DRA	198	AGER	312	415	415	415	415	415	1870	1687
ANG_1	ANG_3100	347 - (CA)6(CG)1(CA)1	T	DRA*01013	194 - (CA)12	408	458	C	475 - (AC)6	A	A	T	170	412
ANG_1	ANG_31040	347 - (CA)6(CG)1(CA)1	T	DRA*01013	194 - (CA)12	408	458	C	475 - (AC)6	A	A	T	170	412
ANG_1	ANG_321210	347 - (CA)6(CG)1(CA)1	T	DRA*01013	194 - (CA)12	408	458	C	475 - (AC)6	A	A	T	170	412
ANG_1	ANG_4250	347 - (CA)6(CG)1(CA)1	T	DRA*01013	194 - (CA)12	408	458	C	475 - (AC)6	A	A	T	170	412
ANG_1	ANG_44410	347 - (CA)6(CG)1(CA)1	T	DRA*01013	194 - (CA)12	408	458	C	475 - (AC)6	A	A	T	170	412
ANG_1	SIM_324070	347 - (CA)6(CG)1(CA)1	T	DRA*01013		408	458						170	412
ANG_8	ANG_22080	347 - (CA)6(CG)1(CA)1	T	DRA*01011		406	456						168	390

Table 19 Continued. BoLA Haplotypes with DRB3*2601.

		IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa
		Gene	Gene	Gene	Microsat	Microsat	SNP																
Haplotype	Animal ID	DQB2	DQB1	DRB3	LA54	DRBP1	171	171	171	171	171	171	171	171	171	171	171	171	171	171	171	171	171
ANG_2	ANG_183110	DQB*1006	DQB*0901	DRB3*2601	183	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A	G	C	C
ANG_2	ANG_24940	DQB*1006	DQB*0901	DRB3*2601	183	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A	G	C	C
ANG_2	ANG_31370	DQB*1006	DQB*0901	DRB3*2601	183	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A	G	C	C
ANG_2	ANG_47790	DQB*1006	DQB*0901	DRB3*2601	183	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A	G	C	C
ANG_2	ANG_5800	DQB*1006	DQB*0901	DRB3*2601	183	119	G	C	T	G	C	A	C	T	C	G	G	A	C	A	G	C	C

		IIa	IIa	IIa	IIa	III	I	I	I	I	I	I	Ext. I	Ext. I
		Microsat	SNP	Gene	Microsat	Microsat	Microsat	SNP	Microsat	SNP	SNP	SNP	Microsat	Microsat
Haplotype	Animal ID	171	171	DRA	198	AGER	312	415	415	415	415	415	1870	1687
ANG_2	ANG_183110	347 - (CA)6(CG)1(CA)1	C	DRA*01011	210 - (CA)20	406	476	T	475 - (AC)6	A	A	T	166	422
ANG_2	ANG_24940	347 - (CA)6(CG)1(CA)1	C	DRA*01011	210 - (CA)20	406	476	T	475 - (AC)6	A	A	T	166	422
ANG_2	ANG_31370	347 - (CA)6(CG)1(CA)1	C	DRA*01011	210 - (CA)20	406	476	T	475 - (AC)6	A	A	T	166	422
ANG_2	ANG_47790	347 - (CA)6(CG)1(CA)1	C	DRA*01011	210 - (CA)20	406	476	T	475 - (AC)6	A	A	T	166	422
ANG_2	ANG_5800	347 - (CA)6(CG)1(CA)1	C	DRA*01011	210 - (CA)20	406	476	T	475 - (AC)6	A	A	T	166	422

Table 19 Continued. BoLA Haplotypes with DRB3*2703.

		IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa	IIa
		Gene	Microsat	Microsat	SNP																
Haplotype	Animal ID	DRB3	LA54	DRBP1	171	171	171	171	171	171	171	171	171	171	171	171	171	171	171	171	171
HOL_4	HOL_21530	DRB3*2703	157	121	A	C	C	C	C	A	C	C	C	G	G	A	T	A	G	C	T
HOL_4	HOL_45000	DRB3*2703	157	121	A	C	C	C	C	A	C	C	C	G	G	A	T	A	G	C	T
HOL_4	HOL_65860	DRB3*2703	157	121	A	C	C	C	C	A	C	C	C	G	G	A	T	A	G	C	T
HOL_4	HapMap - HOL 30	DRB3*2703	157	121	A	C	C	C	C	A	C	C	C	G	G	A	T	A	G	C	T

		IIa	IIa	IIa	IIa	III	I	I	I	I	I	I	Ext. I	Ext. I
		Microsat	SNP	Gene	Microsat	Microsat	Microsat	SNP	Microsat	SNP	SNP	SNP	Microsat	Microsat
Haplotype	Animal ID	171	171	DRA	198	AGER	312	415	415	415	415	415	1870	1687
HOL_4	HOL_21530	343 - (CA)6	T	DRA*01014	194 - (CA)12	408	456	T	475 - (AC)6	A	A	T	166	436
HOL_4	HOL_45000	343 - (CA)6	T	DRA*01014	194 - (CA)12	408	456	T	475 - (AC)6	A	A	T	166	436
HOL_4	HOL_65860	343 - (CA)6	T	DRA*01014	194 - (CA)12	408	456	T	475 - (AC)6	A	A	T	166	436
HOL_4	HapMap - HOL 30	343 - (CA)6	T	DRA*01014	194	408	456	T	475 - (AC)6	A	A	T	166	436

All haplotypes that were defined as homozygous by SNPs across BoLA IIa-III-I region were also homozygous for all additional markers genotyped in this region. The only exceptions to this were microsatellites located in the terminal portion of the extended class I region. Three BoLA haplotypes containing DRB3*0101 differed in the class I region but were identical in the class IIa and III regions. Variation was identified within the extended class I region of the ANG_4/HOL_5 haplotype, but all alleles of the BoLA IIa-III-I region remained identical within the ANG_4/HOL_5 haplotype. Four different haplotypes containing the DRB3*1101 allele exhibited differences in the BoLA IIa, III, and I regions, but no variation was observed within each haplotype. All alleles were homozygous and identical within DRB3*1101 haplotypes, with the exception of one heterozygous microsatellite in the extended class I region of an ANG_7/HOL_2 haplotype. Two haplotypes with the DRB3*1201 allele showed allelic differences at the class I and extended class I regions, and a microsatellite in the extended class I region was heterozygous between two HOL_3 haplotypes. Six haplotypes containing the DRB3*1501 allele exhibited variations from each other throughout the BoLA IIa-III-I-Extended I region. All of the alleles from HOL_1 haplotypes were identical and homozygous. Four haplotypes containing the DRB3*1601 allele were identical in the class IIa region and divergent from each other in the class III, class I, and extended class I regions. All markers were homozygous and conserved within each DRB3*1601 haplotype. Two haplotypes containing the DRB3*1801 allele began to diverge from each other at the DRA allele, but all alleles of the ANG_1 haplotype were homozygous and identical. Only one haplotype containing DRB3*2601 was evaluated, but all markers on this haplotype were homozygous and identical within all individuals genotyped. There was also only one haplotype was analyzed that contained DRB3*2703, and all markers were homozygous and identical within this haplotype. SNP-defined BoLA haplotypes seem to sufficiently account for all variation in the BoLA IIa-III-I region. The accuracy in defining the class IIa region was impressive considering only five class IIa SNPs were analyzed, and two of those five had very low minimum allele frequencies. The accuracy in defining the class IIa region is probably attributed to the linkage disequilibrium of class IIa with SNPs located in other regions of BoLA.

Haplotypes Inferred from BoLA Heterozygotes

While the homozygous BoLA haplotypes were extremely useful for the initial characterization of BoLA haplotype structure, it is necessary to define haplotype structure in animals that have heterozygous BoLA haplotypes. Many of the cattle used in the Bovine HapMap Project were heterozygous for BoLA SNPs. In collaboration with Dr. John Huber, School of Rural Public Health, at Texas A&M University, we analyzed a total of 15 SNPs in BoLA Iib, four SNPs in class Iia, 18 SNPs in class III, 43 SNPs in class I, and 34 SNPs in extended class I within animals of the Bovine HapMap Project. PHASE was used to identify BoLA haplotypes within BoLA heterozygotes by using the default PHASE parameters (Stephens et al. 2001). Animals were dropped out of the PHASE analysis if they were missing too much data in a particular BoLA region, which resulted in some breeds having different numbers of animals analyzed for BoLA Iib and BoLA Iia-III-I. Trios (sire, dam, and offspring) within the Bovine HapMap Project were used to assess the accuracy of PHASE predicted BoLA haplotypes in heterozygous animals.

We began PHASE analysis by grouping all breeds together, but this was analytically impossible because the diversity of haplotypes among different cattle breeds confounded the PHASE algorithms. Cattle breeds were then categorized by *Bos indicus*, European *Bos taurus*, and African *Bos taurus*, but PHASE did not properly analyze these categories either. Ultimately, none of the breeds were combined for PHASE analysis and SNPs were phased within individual breed groups. This approach dramatically decreased the sample sizes and raised concern about the relatedness of samples within breeds, but did improve the accuracy of haplotype prediction. BoLA Iib SNPs were phased in a group separately from the BoLA Iia-III-I SNPs. A separate analysis of the Bovine HapMap data was performed with fastPHASE in collaboration with Dr. Clare Gill, Department of Animal Science, Texas A&M University using a different subset of BoLA SNPs than those in PHASE, but the results between PHASE and fastPHASE analyses were similar.

The Haploview program was used with the Haps format, the r^2 linkage disequilibrium values, and r^2 color scheme to graphically display linkage disequilibrium (LD) among haplotypes derived from PHASE (Barrett et al. 2005). Strong LD

relationships are depicted in Haploview by black boxes (value of 100), no LD relationship is indicated by white boxes (value of 0), and varying shades of grey represent intermediate LD relationships (dark grey is stronger LD, and light grey is weaker LD). The approximate distance between SNPs is displayed at the top of the Haploview figures, and rows without boxes indicate that the SNPs were monomorphic within the breed. A total of 15 SNPs in the BoLA IIb region (Figure 15) and 99 SNPs in the BoLA IIA-III-I region (Figure 16) were analyzed by PHASE and viewed in Haploview for 19 breeds of cattle used in the Bovine HapMap Project. See the appendix for a complete list of haplotypes derived from PHASE and fastPHASE, as well as Haploview figures for all breeds of the Bovine HapMap Project.

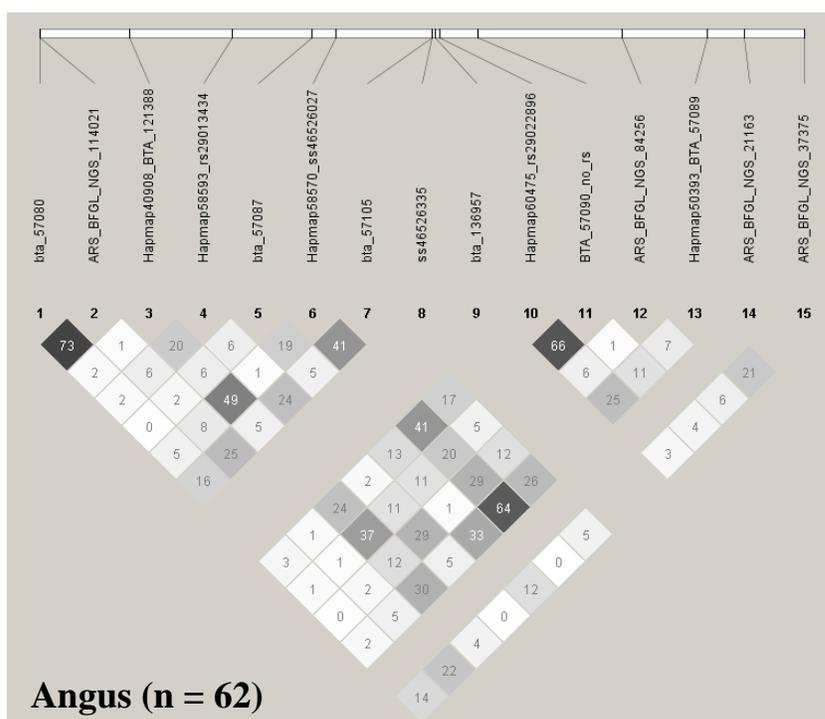


Figure 15. BoLA IIb Haploview Output for Angus HapMap Animals. BoLA IIb haplotypes were determined by PHASE within each HapMap breed and graphically displayed in Haploview. Fifteen markers and their relative positions are shown across the top. The breed and total number of animals analyzed (n) is listed in the left hand corner of the figure. Low minimum allele frequencies of several SNPs in BoLA IIb rendered the alleles monomorphic in many breeds, and monomorphic alleles are seen as blank diagonals in the Haploview output. The average distance between the 15 BoLA IIb SNPs was 31.245 kb, although they were not positioned evenly from each other. The r^2 color scheme displayed pairs of SNPs in highest linkage disequilibrium (maximum number = 100) as black boxes, low linkage disequilibrium (minimum number = 0) as white boxes, and shades of gray represent intervening levels of linkage disequilibrium.



Figure 16. BoLA IIa-III-I Haploview Output for Angus HapMap Animals. BoLA IIa-III-I haplotypes were determined by PHASE within each breed and graphically displayed in Haploview. A total of 114 SNPs and their relative positions are shown across the top. The total number of animals analyzed (n) is listed in the left bottom corner of the figure. SNPs that were monomorphic in the Angus animals are displayed as blank diagonals in the Haploview output, and SNPs with the highest levels linkage disequilibrium (maximum number = 100) are represented by black boxes, SNPs with lowest linkage disequilibrium (minimum number = 0) are white boxes, and shades of gray represent SNPs with intervening levels of linkage disequilibrium. Though the SNPs were not evenly distributed, the average distance between them was 65.653 kb. Overall, these figures show that SNPs positioned close together exhibited a higher level of linkage disequilibrium. Given the small number of animals used in each breed analysis, linkage disequilibrium may have been influenced by the total number of animals analyzed (n) and their pedigree relationships.

In general, the Haploview figures showed that markers positioned closest together had the highest levels of linkage disequilibrium. This is consistent with the findings of the Bovine HapMap Project where linkage disequilibrium was detected at distances of 100 – 250 kb in breeds that shared a recent common ancestor, 10 kb within *Bos taurus* and *Bos indicus* breeds, and never between *Bos taurus* and *Bos indicus* breeds (Gibbs et al. 2009). Linkage disequilibrium maps of bovine chromosome 14 revealed that Angus and Holstein breeds do not share haplotypes at distances greater than 10 kb, and the strongest haplotypes within each breed are below 100 kb (Marques et al. 2008).

Summary of Results

The goal of this project was to identify and characterize polymorphic markers spanning the BoLA IIb and BoLA IIa-III-I regions to provide sufficient markers to better analyze patterns of genetic variation and haplotype structure across diverse cattle breeds with different breed histories and selection pressures. The haplotype structure of the BoLA IIb region was analyzed separately from the haplotype structure of the BoLA IIa-III-I region, as the two regions are located on separate parts of chromosome 23. Four different types of markers were evaluated for their suitability to define haplotype structure including 76 microsatellites, nine SNPSTRs, 90 Bov-A2 retroposons, and 62 SNPs. Markers that showed sufficient levels of polymorphism, locus specificity, Mendelian inheritance, and accurate typing of alleles across different haplotypes were chosen to define the haplotype structure of the BoLA IIb and BoLA IIa-III-I regions and to determine where breakpoints in linkage disequilibrium may occur in the regions surrounding BoLA IIa-III-I. Out of all the markers evaluated for use in analyzing haplotype structure across the BoLA IIb and BoLA IIa-III-I regions, a total of 23 microsatellites, two SNPSTRs, 62 SNPs, and the alleles of three class IIa genes were selected. Polymorphisms of Bov-A2 retroposons were not chosen to be evaluated in BoLA haplotype structure because null alleles were prevalent and difficult to overcome, but this study presented the first report of polymorphisms in Bov-A unit number among wild and feral bovids belonging to the *Bos* and *Bison* genera.

Analysis of microsatellites in pedigreed families revealed two recombination events in BoLA IIb (~ 450kb), four in the region just centromeric to class IIa (~ 2 Mb) region, three in the BoLA IIa-III-I region (~ 4 Mb), and two in the extended class I region (~ 2Mb). The recombination rate calculated for the BoLA IIa-III-I region (0.18 cM / 1 Mb) was lower than the average recombination rate estimated for the bovine genome (1.25 cM / 1 Mb), but the recombination rate of the BoLA IIb region (1.21 cM / 1 Mb) was consistent with the genome average. It was necessary to genotype markers across the entire BoLA IIa-III-I region to define BoLA IIa-III-I haplotypes, as the DRB3 allele alone was not always predictive of BoLA IIa-III-I haplotypes. A total of 796 animals representing 26 breeds of cattle and three additional bovids (gaur, bison, Cape buffalo) were typed as homozygous for 52 SNPs spanning the BoLA IIa-III-I region, and analysis

of the boundaries of SNP-defined BoLA IIA-III-I homozygosity demonstrated a sharp break in homozygosity centromeric to class IIA and prolonged homozygosity through extended class I. A small number of BoLA IIA-III-I homozygous haplotypes appeared at high frequencies and represented the majority of all BoLA IIA-III-I homozygous haplotypes within a breed; for example, four Angus haplotypes accounted for 84.61% and six Holstein haplotypes accounted for 96.57% of the BoLA IIA-III-I homozygous haplotype breed totals. Seven SNP-defined BoLA IIA-III-I homozygous haplotypes were shared across different breeds of cattle, suggesting these haplotypes have been created and maintained since the divergence of the cattle breeds. Additional markers with different mutation rates and selection pressures, including intergenic and intronic microsatellites and SNPSTRs and protein-coding class IIA genes, all appeared homozygous and identical within the SNP-defined BoLA IIA-III-I homozygous haplotypes, with the exception of microsatellites located in the terminal portion of the extended class I region.

The phase of markers in homozygous BoLA haplotypes was inherently known, but the phase of alleles in heterozygous haplotypes was unknown and required a method of haplotype inference. PHASE, fastPHASE, and a novel approach based on known homozygote haplotype frequencies were used to infer BoLA heterozygous haplotypes within each cattle breed. The PHASE results visualized with the Haploview program did not show strong linkage disequilibrium throughout the BoLA IIA-III-I region; however, consistent with other regions of the bovine genome, SNPs located close together showed the highest levels of linkage disequilibrium. The major finding of this project was that BoLA IIA-III-I haplotypes are highly conserved and 52 SNPs from the Illumina 50K SNPchip were sufficient to predict BoLA IIA-III-I haplotypes. This should offer a cost-effective means for screening large sample sizes for bovine haplotype/disease association studies in the future.

CHAPTER IV

DISCUSSION AND CONCLUSIONS

Genotyping and Selection of BoLA Microsatellites

Characterization of Microsatellites

Microsatellites, also known as short tandem repeats (STRs) or short sequence repeats (SSRs), were chosen as genetic markers for this study because of their highly polymorphic nature and prevalence throughout the BoLA IIa-III-I and BoLA IIb regions. Microsatellites are widely distributed throughout the nuclear genomes of eukaryotes and consist of 2-5 base pairs of tandemly repeated DNA sequences that can vary in repeat unit number (Schlotterer and Tautz 1992; Tautz 1994). Slippage during DNA replication is considered to be the primary mechanism of microsatellite mutation, and a correlation has been demonstrated between defective DNA repair systems and an increased rate of microsatellite slippage (Lindahl et al. 1997; Strand et al. 1993). The abundance of microsatellites in the BoLA regions is consistent with the observations that larger eukaryotic nuclear genomes generally have a higher percentage of microsatellites, with the exception of plants where the density of microsatellites is inversely related to genome size (Hancock 1996; Morgante et al. 2002), and that mammalian genomes have the highest density of microsatellites when compared to all available eukaryotic genome sequences (Tóth et al. 2000). Over one million microsatellite loci have been identified in the human genome (Sachidanandam et al. 2001), and dinucleotide (AC)_n repeats are the most common microsatellite motif in the human genome, followed by (AT)_n, (GA)_n, and (GC)_n (Rockman and Wray 2002; Sachidanandam et al. 2001). Microsatellite markers were also appealing because they carried more information than SNPs, as a larger number of possible alleles may occur at each microsatellite locus, and microsatellites have a faster mutation rate than SNPs. Dinucleotide repeat polymorphisms have a mutation rate of approximately 1.5×10^{-3} per microsatellite per generation (Zhivotovsky et al. 2001), which is higher than the average human nucleotide mutation rate at about 2.5×10^{-8} (Nachman and Crowell 2000).

The most polymorphic microsatellites identified in this study contained (AC) motifs with more than five perfect repeats, which are repeated units without any point

mutations. This is consistent with findings in the human genome, where microsatellites with more than ten perfect (AC)_n repeat units, or motifs uninterrupted by point mutations, exhibited the highest levels of polymorphism with frequencies estimated to exceed 90% (Macaubas et al. 1997; Rockman and Wray 2002). The distribution of microsatellite lengths within a genome may represent a balance between the expansionary tendencies of slippage mutations with contractions caused by point mutations (Bell and Jurka 1997; Kruglyak et al. 1998). The reason most polymorphic microsatellites in the BoLA regions had more than five repeated units is that the most important factor affecting microsatellite mutation rate is the number of repeated units, with mutation rates rising as the number of repeats increases (Goldstein and Clark 1995; Wierdl et al. 1997). Other factors that have been reported to influence the extent of microsatellite polymorphism include the length and motif of the repeat unit (Chakraborty et al. 1997; Rockman and Wray 2002), the flanking sequence of the microsatellite (Glenn et al. 1996), nucleotide interruptions within the microsatellite (Kruglyak et al. 1998), mismatch-repair efficiency (Strand et al. 1993), recombination rate (Begun and Aquadro 1992), transcription rate (Mellon et al. 1996), and the age and gender of the organism (Brinkmann et al. 1998). It would also stand to reason that the polymorphism of some microsatellites may be under functional constraints, such as microsatellites that are located within protein-coding sequences. Microsatellites have been reported to play additional functional roles that include altering nearby chromatin structure (Otten and Tapscott 1995), regulating gene expression and transcription factor binding (Martin et al. 2005), and stimulating homologous recombination and gene conversion (Wahls et al. 1990).

This study sought to characterize polymorphic microsatellites to analyze BoLA haplotype structure and identify markers that could potentially be in linkage disequilibrium with important BoLA genes. The first polymorphic microsatellite shown to be in linkage disequilibrium with genes of the MHC was located near the tumor necrosis factor (TNF) genes of the mouse (Jongeneel et al. 1990), and the first description of linkage disequilibrium between microsatellites and HLA genes was also identified between microsatellites and the TNF genes of HLA (Jongeneel et al. 1991). A total of 389 microsatellite primer pairs targeting 281 regions of the extended HLA have been developed since the first discovery that microsatellites were in linkage disequilibrium

with HLA genes (Gourraud et al. 2006). Out of a total 76 microsatellites investigated in the BoLA regions, 23 of the microsatellites were selected for use in this study, including the two previously characterized microsatellites LA54 (Ellegren et al. 1993) and DRBP1 (Creighton et al. 1992). The 23 chosen microsatellites were used to identify recombination events in BoLA IIb and BoLA IIA-III-I within pedigreed families, to characterize linkage disequilibrium across the BoLA regions, and to validate SNP-defined haplotype block structure in individuals homozygous for BoLA IIA-III-I.

The availability of the bovine genome sequence allowed microsatellites to be identified and correctly positioned within BoLA IIb and BoLA IIA-III-I regions. Correct marker order was critical for analyzing BoLA haplotype structure, so different BoLA assemblies were evaluated to choose the most accurate assembly for positioning BoLA markers. Ultimately the Btau4.0 assembly was chosen to position all BoLA IIA-III-I markers, and BoLA IIb markers were positioned with an independent BoLA IIb sequence assembly (Childers et al. 2006). The Btau4.0 assembly was chosen over the Btau3.1 and UMD assemblies because the gene order and content of the BoLA IIA-III-I region showed more homology to HLA in the Btau4.0 assembly, and a high resolution radiation hybrid map of BoLA independently validated the improved accuracy of the Btau4.0 assembly over the Btau3.1 assembly (Brinkmeyer-Langford et al. 2009). After the BoLA microsatellites had been identified and positioned in the Btau4.0 assembly, microsatellite primers were designed and initially genotyped across pedigreed families to evaluate polymorphism, locus specificity, and normal Mendelian inheritance. This approach identified problematic markers and helped to avoid complicating factors, such as null alleles, which inhibited accurate microsatellite allele scoring.

Null alleles were commonly observed in our study, and each BoLA microsatellite primer pair was typically redesigned at least one time to eliminate the null alleles. Analyses of human (AC)_n repeats revealed that null alleles were generated by nucleotide substitutions and deletions underneath the microsatellite primer binding sites (Callen et al. 1993; Koorey et al. 1993). The null alleles resulted in preferential amplification of specific microsatellite alleles and the non-Mendelian inheritance of alleles at a single locus; however, the null alleles were successfully eliminated after the microsatellite primers were redesigned in the Koorey et al. 1993 study. The polymorphic complexity

that is characteristic of the MHC may present a greater challenge in developing robust primers to amplify microsatellite alleles across many different MHC haplotypes because the increased level of polymorphism leads to a higher probability of designing a primer over a polymorphic site that will generate null alleles. Null alleles are problematic because they create misleading data that could obstruct the accuracy of linkage disequilibrium and disease association studies. The power to identify null alleles is dependent on the number of haplotypes that are analyzed with microsatellite markers in pedigreed individuals.

This study analyzed microsatellites across pedigreed International Reference Families representing nine different breeds of *Bos taurus* and *Bos indicus* cattle with 33 different BoLA IIA-III-I haplotypes and 43 different BoLA IIB haplotypes. Microsatellites were not retained for use in the analysis of BoLA haplotype structure if the null alleles persisted after the primers were redesigned multiple times, with the exception of two previously published microsatellites in the class IIA region - LA54 (Ellegren et al. 1993) and DRBP1 (Creighton et al. 1992). Null alleles were found in LA54 and DRBP1, but they were retained for use in this study to connect these results to previous studies of BoLA. Null alleles were prevalent in the microsatellites evaluated for this study, and they also appear to be common in human microsatellite studies. Seven of the twenty-three (AC)_n repeats analyzed by Callen et al. 1993 in the parents of forty Centre d'Etude du Polymorphisme Humain (CEPH) families were found to exhibit null alleles.

Another challenge in characterizing microsatellites in BoLA was identifying size homoplasy, where microsatellite alleles were identical by size but not by descent (Estoup et al. 2002). Microsatellites showed size homoplasy when they exhibited the same allele size in a genotyping reaction but had different internal sequence compositions. This was most frequently seen in PCR amplicons that contained two different microsatellite motifs, although it was occasionally observed with polymorphic microsatellites amplified along with polymorphic stretches of mononucleotide (A)_n or with larger insertion/deletion polymorphisms. A commonly observed compound microsatellite, wherein two microsatellite motifs were present within three base pairs of each other, was (AC)_n(AT)_n. If both the (AC)_n and (AT)_n repeats were polymorphic, the size of one elongating motif

would be cancelled out by the contraction of the second motif. For example, $(AC)_{10}(AT)_6$ would be genotyped to have the same allele as $(AC)_5(AT)_{11}$ because they both have the same total size of $(AN)_{16}$. Size homoplasy is unfavorable because it reduces the number of observed alleles, heterozygosity, and gene diversity, and it inaccurately infers population divergence times by making them appear more recent than they actually are (Ramakrishnan and Mountain 2004). Size homoplasy was identified by sequencing a subset of microsatellite PCR products in parents of the International Reference Families representing diverse breeds of cattle with divergent BoLA haplotypes. Amplicons demonstrating homoplasy were eliminated from this study, or if possible, the primers were redesigned to amplify only one polymorphic microsatellite.

Characterization of SNPSTRs

SNPSTRs were developed to reduce the analytical problems posed by size homoplasy in microsatellite markers. One SNPSTR consisted of a single polymorphic microsatellite located within 500 bp of one or more SNPs (Mountain et al. 2002). The differing mutation rates of the microsatellite and SNPs within a SNPSTR generated a small, independently evolving compound haplotype that was unlikely to be broken up by recombination. SNPSTRs were identified in this study by designing microsatellite primers to amplify the largest possible segment that could be resolved in a genotyping reaction (max size standard = 500 bp) to increase the probability of finding SNPs in the sequence surrounding the microsatellite. There may be a higher probability of identifying SNPs in sequences surrounding microsatellites, as some studies have suggested that there is an elevated nucleotide substitution rate in regions immediately flanking microsatellites (Brohede and Ellegren 1999; Vowles and Amos 2004).

SNPSTRs were characterized in the BoLA IIA-III-I region by sequencing microsatellite amplicons from parents of the International Reference Families, and priority was given to SNPSTRs containing the largest amount of SNPs with a minimum allele frequency greater than 0.05 because they were considered to be the most informative. At least one SNP was identified in the flanking sequences of all nine BoLA IIA-III-I microsatellites investigated for SNPSTRs, eight out of the nine SNPSTRs contained SNPs with minimum allele frequencies greater than 0.05, and two of the SNPs

were tri-allelic. The two SNPSTRs containing the largest number of SNPs were the 171 microsatellite in the class IIa region (17 SNPs with MAF > 5%) and the 415 microsatellite in the class I region (7 SNPs with MAF > 5%). Analysis of SNPSTRs in different BoLA IIa-III-I haplotypes is likely to reveal additional SNPs and increased minimum allele frequencies of pre-defined SNPs. About 25% of human microsatellites were found to have SNPs in the flanking sequences (Mountain et al. 2002), but 100% of the BoLA IIa-III-I microsatellites were found to have SNPs in the surrounding sequences. The high frequencies of SNPs identified around BoLA microsatellites may be reflective of highly divergent BoLA IIa-III-I haplotype sequences.

Determining the phase of a heterozygous microsatellite with heterozygous SNPs in a SNPSTR required the cloning and sequencing of PCR products. SNPs identified within cloned PCR sequences were validated by their presence in the direct PCR sequencing product to eliminate any errors caused by Taq polymerase during the PCR reaction as well as errors caused by in vitro recombinant PCR products (Grimaldi and Crouau-Roy 1997). It has been estimated that conventional Taq polymerase creates one error, usually a nucleotide substitution, for every 1421 nucleotides amplified in a PCR reaction (Ennis et al. 1990). Taq errors did not appear in the direct PCR product sequences because they were present at very low frequencies among many copies of alleles sequenced together, but the errors readily appeared in the single alleles of cloned PCR product sequences. Previously published methods of typing SNPSTRs have utilized different colored dyes to bind to specific SNP alleles in the microsatellite genotyping reactions (Mountain et al. 2002). The alternative approach of sequencing the entire SNPSTR amplicon was more conducive to the high density of SNPs present within the SNPSTRs selected for use in this study.

Association of Microsatellites with Repeat Elements

The association of microsatellites within or near retroposons created a challenge in designing BoLA microsatellite genotyping primers, because primers that were designed within a retroposon would often show weak amplification, high background, and non-locus specific amplification. Pedigreed samples were used to verify the Mendelian inheritance of alleles, and whenever possible, placing PCR primers within

highly repeated elements was avoided to ensure locus-specific amplification. It has been proposed that microsatellites arise from the expansion of pre-existing tandem repeats or from the conversion of sequences with high cryptic simplicity (López-Giráldez et al. 2006). Retroposons are an abundant source of such sequences in mammalian genomes, and a close association has been observed between retroposons and microsatellites in sheep (Buchanan et al. 1993), pigs (Alexander et al. 1995), cattle (Band and Ron 1996), primates (Arcot et al. 1995), humans (Nadir et al. 1996), horses (Gallagher et al. 1999b), and canines (López-Giráldez et al. 2006). The 3' polyadenylation of retroposons occurs before their incorporation into the genome, and their poly-A tails are thought to become the source of many A-rich microsatellite motifs (Arcot et al. 1995; Nadir et al. 1996). In support of this, the most common tetranucleotide microsatellite motif in mammalian genomes is (AAAN)_n (Katti et al. 2001), and human Alu sequences often generate microsatellites from point mutations and slippage events within the Alu poly-A tails (Arcot et al. 1995; Nadir et al. 1996). The authors of López-Giráldez et al. 2006 suggested that (AG)_n and A-rich microsatellites coevolved in carnivore genomes with the pyrimidine rich “Poly-Y” region and the poly-A tail of tRNA^{Lys}-derived SINES. The coevolution of microsatellite motifs with retroposons may explain why particular microsatellite motifs are often associated with specific types of repeat elements.

This study found that the majority of the A-rich tetranucleotide repeats in the BoLA IIB region were located within 40bp of the 3' end of a SINE or LINE, and over 92% (25/27) of microsatellites with (AGTTC)_n and (AACTG)_n motifs in the BoLA IIB region were associated with SINE-ART2 repeat elements, which was a previously described microsatellite-retroposon association (Malik et al. 1998). The bovid-specific Bov-A2 repeat element was usually found to be tailed with (AGC)_n or (ACG)_n trinucleotide repeats (Kaukinen et al. 1992), but none of the polymorphic (AGC)_n microsatellites associated with Bov-A2 repeat elements were retained for use in this study because of non-specific primer amplification and too much size variation. The association of the (ACG)_n microsatellite motif with the ruminant-specific Bov-A2 retroposon is an excellent example of how the coevolution of microsatellites with particular retroposons can create variable distributions of microsatellite motifs in different mammalian species. The (ACG)_n microsatellite motif occurs at a 90-fold higher

rate in cattle compared to humans and a 142-fold higher rate compared to dogs (The Bovine Genome Sequencing and Analysis Consortium et al. 2009).

Over 94% (33/35) of the (AGC)_n or (ACG)_n microsatellites in the BoLA IIb were associated with Bov-A2 repeat elements, and over 273 Bov-A2 repeat elements with (AGC)_n or (ACG)_n microsatellite tails were located in the BoLA IIa/III/I region. Ruminant-specific Bov-A2 retroposons are widely distributed throughout the bovine genome (The Bovine Genome Sequencing and Analysis Consortium et al. 2009), accounting for approximately 1.8% of the entire cattle genome (Lenstra et al. 1993). The Bov-A2 retroposon consists of one to six Bov-A units connected by a linker sequences of (CACTTT)_n (Onami et al. 2007). Polymorphisms in Bov-A unit number have been identified among domestic cattle breeds (Onami et al. 2007) and were identified in this study within wild and feral bovids belonging to the *Bos* and *Bison* genera. The high mutation frequency of Bov-A2 retroposons (Damiani et al. 2000) made Bov-A polymorphisms poorly suited for use in this study because of the prevalence of null alleles; however, 11 of the 90 Bov-A2 retroposons evaluated within the BoLA regions were found to be polymorphic in Bov-A unit number among nine breeds of cattle and two North American Bison from Yellowstone National Park. This was the first description of Bov-A unit polymorphism in ruminants outside of *Bos taurus* domestic cattle breeds.

Microsatellite-Derived Haplotypes and DRB3 Alleles

The exon 2 sequence of the BoLA class IIa DRB3 gene was used as an additional marker in this study (Baxter et al. 2008) because DRB3 alleles have been associated with several diseases of cattle including mastitis (Park et al. 2004), dermatophilosis (Maillard et al. 2003), persistent lymphocytosis (Juliarena et al. 2008), host resistance to the Lone Star tick (Untalan et al. 2007), and vaccination response to epitopes of infectious diseases (Ballingall et al. 2004b; Garcia-Briones et al. 2000). The exon 2 sequence of the BoLA class IIa DRB3 gene is highly polymorphic and encodes the peptides lining the antigen binding site, which determine what exogenous antigens are bound and presented to helper T cells to illicit an effective humoral immune response (Doherty and Zinkernagel 1975b; Stern et al. 1994). The large number of possible alleles at the DRB3 gene (at least 104 alleles have been reported) makes it a stronger candidate than most genes to predict

BoLA IIA-III-I haplotypes, but this study identified same DRB3 allele on different microsatellite-derived haplotype backgrounds; therefore, DRB3 alleles were not found to be predictive of BoLA IIA-III-I haplotypes. This finding has been previously alluded to in studies of the LA54 microsatellite, located within an intron of the DRB3 gene (Ellegren et al. 1993), where microsatellite alleles were predictive of some, but not all, DRB3 alleles. The same DRB3 allele appearing on different BoLA IIA-III-I haplotype backgrounds is evidence for diversification by recombination at the time of BoLA IIA-III-I haplotype generation. Similar findings have been reported in the HLA class II region, as deeply divergent HLA class II haplotypes have demonstrated high levels of linkage disequilibrium (Raymond et al. 2005) and otherwise different HLA haplotype sequences shared 158 kb of class II sequence containing the DQ and DR genes (Traherne et al. 2006b). Recombination hotspots have been identified in the HLA class II region (Cullen et al. 1997; Jeffreys et al. 2001; Jeffreys et al. 2000), but there seems to be an evolutionary propensity to maintain ancestral class II haplotypes in human populations (Raymond et al. 2005).

Since DRB3 alleles were not predictive of all BoLA IIA-III-I haplotypes, animals with the same DRB3 allele may have had stable but divergent alleles throughout the remainder of BoLA IIA-III-I, even though the region had repressed recombination. This is a very important factor to consider in disease association studies, especially since many BoLA disease association studies have been done with haplotype identification based solely on alleles of the DRB3 gene. Additional variation, present as hitchhiking genes, could confound disease association studies. Considering the context of an allele on the background of the larger BoLA haplotype gives a clearer picture of the factors influencing a particular BoLA-associated phenotype. This study used BoLA microsatellites to identify BoLA IIA-III-I haplotype identity in 21 Holstein animals selected for a study in vaccine response because they shared at least one copy of the DRB3*1101 allele (Mwangi et al., unpublished results), and BoLA IIA-III-I haplotype identity among the offspring of three sires implicated in differential response to the Lone Star tick (Untalan et al. 2007).

The 21 Holstein animals with at least one copy of the DRB3*1101 allele also shared at least one copy of identical BoLA IIA-III-I microsatellite-derived haplotypes that

genotyped with the SNP-derived ANG_7/HOL_2 haplotype, which is completely conserved across Angus and Holstein breeds. Given that the 21 Holstein animals had the same BoLA IIA-III-I haplotype; it is very likely that they all shared at least one copy of all of their BoLA genes. This includes any number of genes that may have also played an important role in vaccine response including the alleles and copy number polymorphisms of DQB, DQA, and BoLA class I genes. It is possible that one of the other shared BoLA genes was influencing vaccine response, or that additive effects of multiple alleles at BoLA genes were producing a specific phenotype. Although all 21 of the Holstein animals shared identical DRB3*1101 alleles and entire BoLA IIA-III-I haplotypes, four homozygous BoLA IIA-III-I haplotypes in samples from the University of Missouri shared the same DRB3*1101 allele and showed differences in markers of the class IIA, class III, class I, and extended class I regions. Therefore, the DRB3*1101 allele is not always predictive of the entire BoLA IIA-III-I haplotype, as it happened to be in the 21 Holstein animals. This indicates that markers spanning the entire BoLA IIA-III-I region are needed to accurately define BoLA haplotypes.

Resistance to the Lone Star Tick was associated with the DRB3*4401 allele that calves inherited from their sire, Bull 12 or 13 (Untalan et al. 2007). The DRB3*4401 allele was also associated with alleles of microsatellites LA54 and DRBP1. After genotyping additional microsatellites of BoLA IIA-III-I in the tick study samples, we found that the offspring inherited intact parental BoLA IIA-III-I haplotypes from their sires. This was true for offspring that were the most susceptible and the most resistant to the Lone Star tick, but resistant animals received a statistically greater number of haplotypes containing DRB3*4401 alleles. It seems counterintuitive that the BoLA IIA-III-I region would be the sole factor implicated in Lone Star tick resistance if both susceptible and resistant animals had the same BoLA IIA-III-I haplotype. The BoLA region may be a contributing factor in tick resistance (Regitano et al. 2008), but it seems unlikely that BoLA alleles or haplotypes are the only factor conferring tick resistance. The authors of Regitano et al. 2008 mapped quantitative trait loci (QTL) for *Rhipicephalus (Boophilus) microplus* tick load to bovine chromosomes 4, 5, 7, 10, 14, 18, and 23, out of a total of 20 chromosomes analyzed. Tick load was also found to be influenced by coat color and hair type, as well as environmental factors, as different

QTLs were identified in the rainy and dry seasons. The two QTLs explained 13.07% of the total phenotypic variation during the rainy season and 11.28% during the dry season. The complex multiloci nature of tick resistance substantiates our finding that BoLA IIa-III-I haplotypes are not the only determining factor of tick resistance. Future studies should take the entire BoLA haplotype into account when investigating contributing factors to tick resistance, as many genes within the BoLA region have potential to contribute to this phenotype.

Observed Recombination Events

No recombination was observed from analysis of BoLA IIa-III-I microsatellites within the sires and offspring of the Lone Star tick resistance study or within the DRB3*1101 haplotypes of the 21 Holstein animals used in a vaccine response study, but two recombination events were observed within the Bovine International Reference Families. One male and one female recombination event were observed in BoLA IIb, the first within the region spanning *DYA* to *PSMB9* and the second near the *DMB* gene. Two recombination events observed within 368 meioses over 450 kb of the BoLA IIb region is consistent with the average rate of recombination for the bovine genome, 1.25cM / 1Mb (Arias et al. 2009). Although recombination hotspots are not often conserved across species, it is interesting to note that the *TAP2* gene – a recognized recombination hotspot in humans – is located within the BoLA IIb region. The BoLA IIb region may have been unstable prior to its transposition ~20 Mb away from the remainder of BoLA. The BoLA IIb region may have developed a recombination rate different than that of the larger BoLA IIa-III-I region because it retained a portion of the instability that led to its transposition or because higher selection pressures operate to keep the BoLA IIa-III-I region intact than those to keep the BoLA IIb region intact. Unpublished results from Schnabel et al. show the BoLA IIb region has the same rate of recombination as the rest of BTA 23 (Figure 4).

Nine recombination events out of 406 meioses were observed within the BoLA IIa-III-I region in samples from the Lone Star tick study and the International Bovine Reference Family Panel. Of these nine recombination events, three occurred in male meioses and six occurred during female meioses. The largest number of recombination

events (four) took place in the ~2 Mb region centromeric to BoLA class IIa (~2 Mb), three in the BoLA IIa-III-I region (~4 Mb), and two within the extended class I region (~2 Mb). The recombination rate of 0.18cM / 1Mb in the BoLA IIa-III-I region was much lower than the genome wide average of 1.25cM / 1Mb (Arias et al. 2009) and is consistent with a lowered recombination rate in the BoLA IIa-III-I region observed in the bovine chromosome 23 linkage study results by Schnabel et al. (unpublished results). Similar results have been reported in the HLA after excluding the extended HLA regions. The HLA was found to have a 0.7852 cM / Mb recombination rate, which is lower than the average human genome recombination rate of 1.1 cM / Mb (Kong et al. 2002). Evolutionarily successful HLA recombination events that span multiple pedigrees occur infrequently, so there seems to be an evolutionary advantage to maintaining high levels of non-recombinant HLA haplotypes at the population level.

Various hypotheses have been offered to explain the lowered recombination rates in the MHC. Recombination may be suppressed due to selection against offspring with recombinant haplotypes that would disrupt combinations of functionally well-coordinated alleles. Van Oosterhout (2009) proposed that recessive deleterious mutations within MHC haplotypes reinforce linkage disequilibrium and reduce the effective rate of recombination because recombinants combine the deleterious mutations of both parental haplotypes. Divergent blocks of DNA subsequently evolve with elevated levels of linkage disequilibrium. Van Oosterhout's model also showed that novel haplotypes did not coexist with parental haplotypes for very long before the novel haplotypes became eliminated, so there was clear evidence for selection against derived MHC haplotypes. Additionally, the higher rate of inbreeding in cattle may increase the frequencies of a small subset of BoLA haplotypes within a breed, and this reduction in heterozygosity may limit the production of novel haplotypes by recombination. This would explain a reduced level of observed recombination events, but it would not explain the strong conservation of BoLA IIa-III-I haplotypes across long-diverged populations of cattle. Such strong conservation of BoLA IIa-III-I haplotypes supports the idea of strong linkage disequilibrium and reduced rates of recombination throughout the BoLA IIa-III-I region.

Additional supporting evidence for an increased level of linkage disequilibrium across the BoLA IIa-III-I region is found in the region centromeric to class IIa. Blocks of

linkage disequilibrium are usually marked by boundaries of recombination hotspots, and microsatellite analysis in this study revealed that largest number of recombination events occurred in the region centromeric to class IIa. Analysis of heterozygosity surrounding SNP-defined homozygous BoLA haplotypes revealed an abrupt breakpoint in homozygosity centromeric to class IIa, and this may indicate a possible recombination hotspot directly centromeric to the class IIa region. Markers in the extended class I region showed a moderate and gradual increase in heterozygosity and recombination compared to the centromeric to class IIa region. However, there may be a recombination hotspot distal to the class I region that is located outside of the scope of marker placement for this study.

While this work added supporting evidence to the idea of a reduced rate of recombination at BoLA IIa-III-I, the major finding of this study is the strong linkage disequilibrium and conservation of BoLA haplotypes within breeds of cattle. Similar ancestral MHC haplotype conservation has been observed in humans (Degli-Esposti et al. 1992b), and the knowledge of ancestral HLA haplotypes has been used to identify a region between HLA-B and BAT3 that contains genes involved in conferring susceptibility to insulin dependent diabetes mellitus (IDDM) (Degli-Esposti et al. 1992a). Understanding the content of BoLA haplotypes can be used in a similar way to identify haplotypes conferring susceptibility to specific diseases of cattle. This study found that the conservation of BoLA class IIa-III-I haplotypes extended beyond the class I region, which is consistent with the finding that the human extended class I region is in strong linkage disequilibrium with the HLA (Malfroy et al. 1997). Many of the genes present in the HLA extended class I region are also present in cattle, including large families of olfactory receptor genes, zinc-finger genes, tRNA genes, and histone genes (Horton et al. 2004). The HLA extended class I region is significant portion of the human genome because the extended class I olfactory-receptor gene cluster represents one of the largest blocks of linkage disequilibrium in the human genome (Miretti et al. 2005), and the largest cluster of histone and tRNA genes in the human genome is found in the extended HLA (Horton et al. 2004). The clustering of histone and tRNA genes in both human and cattle extended class I regions may promote elevated levels of transcription, and genes of

the MHC may be hitchhiking with histone and tRNA gene clusters in the extended class I region to increase their own rates of transcription.

Additional Genetic Markers

This study generated additional genetic markers to further characterize linkage disequilibrium within the BoLA IIA-III-I region. These markers included SNPSTRs, exon 2 sequences of BoLA class IIA genes DRB3, DRA, and DQB, and Bov-A2 retroposons. We expected that BoLA IIA-III-I haplotypes would subdivide into smaller blocks of linkage disequilibrium as the density of BoLA markers increased. Surprisingly, subdivisions were never observed as more markers were added to BoLA IIA-III-I haplotypes that were defined by 52 SNPs in the BoLA IIA-III-I region. The markers that were added to the haplotypes had different mutation rates, with the microsatellites of SNPSTRs mutating more rapidly than SNPs and Bov-A2 repeat elements, and the class IIA genes mutating under different selection pressures than the other marker types because of their location in protein-coding regions. The class IIA genes themselves were under different selection pressures, as the DRB3 gene is present in one copy with many alleles that alter the DRB3 peptide composition (Baxter et al. 2008), the DQB gene shows copy number polymorphism and allelic polymorphism at each DQB copy (Glass et al. 2000), and the DRA gene is relatively monomorphic with only three possible synonymous SNPs reported in the second exon (Zhou et al. 2007). None of the different marker mutation rates were reflected in the BoLA haplotypes because all of the different marker types had identical alleles on the same BoLA IIA-III-I haplotype. This suggests that all of the different marker types have been fixed and maintained within BoLA IIA-III-I haplotypes for a long period of time.

The SNPSTR markers 171 and 415 were typed over animals homozygous for 52 SNPs spanning the BoLA IIA-III-I region, and in all instances the SNPSTRs were homozygous and had identical alleles within the same BoLA IIA-III-I SNP-defined haplotypes. The independently evolving microsatellite and SNP(s) of SNPSTRs have been used in population genetics to estimate the age of phylogenetic relationships, such as the time of the first human migration out of Africa (Mountain et al. 2002). SNPSTRs were particularly compelling markers within this experiment because the sequence of

SNPSTRs 171 and 415 in BoLA IIA-III-I homozygotes revealed complete identity of all SNPs and each microsatellite within haplotypes defined by 52 SNPs of the Illumina 50K SNPchip. This suggested that the SNPs and microsatellites have not undergone mutations since the establishment and divergence of breeds sharing the same BoLA IIA-III-I haplotypes and implies that the haplotype structures observed in this study are relatively old.

The alleles of three BoLA class II genes were investigated for their identity within BoLA IIA-III-I haplotypes defined by SNPs, microsatellites, and SNPSTRs. The second exon of class IIA genes, encoding the polymorphic antigen binding site, was sequenced with published primers for DRB3 (Baxter et al. 2008; Miltiadou et al. 2003), DRA (Zhou et al. 2007), and DQB (Russell 2000). Although each of these genes was likely to have different selection pressures based on their varying rates of polymorphism, all of the sequenced class IIA alleles were found to be identical within BoLA IIA-III-I haplotypes. This suggests that the gene content of a BoLA IIA-III-I haplotype may be predicted by SNPs typed throughout the BoLA IIA-III-I, which would be particularly beneficial in the development of a SNPchip for the BoLA region.

This study also examined the variability in the ruminant Bov-A2 repetitive elements as a possible source of markers for BoLA analysis. Bov-A2 retroposons may be polymorphic in Bov-A unit number, with each Bov-A monomer being about 120 bp in length, and retroposons with two to six Bov-A monomers have been reported in domestic cattle (Onami et al. 2007). The Bov-A2 retroposon appeared throughout the BoLA regions because it is one of the most common repeat elements in the bovine genome, and a total of 90 Bov-A2 retroposons located within the BoLA regions were analyzed for polymorphism in Bov-A unit number across nine breeds of cattle and two North American bison from Yellowstone National Park. Eleven of the 90 Bov-A2 elements analyzed in this study showed polymorphism in Bov-A unit number, including one in class IIb, two centromeric to class IIA, three in class IIA, and five in class I. Null alleles were common throughout the typing of Bov-A2 repeat elements, and it was difficult to design one primer pair to successfully amplify all BoLA haplotypes. The increased nucleotide mutation rate in Bov-A2 retroposons (Damiani et al. 2000) and unequal crossing over between Bov-A units (Onami et al. 2007) may have inhibited the binding of

many Bov-A2 primers among different BoLA haplotypes, which did not make Bov-A2 retroposons sufficiently robust markers for typing BoLA haplotypes.

However, the phylogeny of Bov-A2 repeat elements was investigated after polymorphism in Bov-A unit number was observed in domestic cattle and Yellowstone National Park bison. Onami et al. 2007 proposed that Bov-A unit number polymorphism was unique to domestic cattle breeds, and the authors interpreted this to indicate that domestication and selective breeding of cattle have destabilized the bovine genome. Onami et al. did not investigate any members of the *Bos* or *Bison* genera, with the exception of several *Bos taurus* domestic cattle breeds. Onami et al. 2007 analyzed five polymorphic Bov-A2 repeat elements in *Bos taurus* breeds of cattle, one bongo (*Tragelaphus euryceros*), one Arabian Oryx (*Oryx leucoryx*), one Axis deer (*Axis axis*), one Reticulated Giraffe (*Giraffa camelopardalis*), one Pronghorn (*Antilocapra Americana*), and one Lesser mouse deer (*Tragulus javanicus*). The experimental design of Onami et al. 2007 did not have the power to detect the evolution of Bov-A unit number polymorphism within the Bovini tribe.

This study investigated Bov-A unit number polymorphism within members of the Bovini tribe, and the Bov-A2 elements studied by Onami et al. 2007 were found to be polymorphic within domestic cattle, feral cattle, gaur, banteng, and bison. Clearly Bov-A unit number polymorphism is not a consequence of domestication and selective breeding because it was observed in wild and domestic members of the *Bos* and *Bison* genera. Work in this study supports the idea that domestic cattle have maintained substantial levels of genetic diversity because they were derived from a large and genetically diverse ancestral population (Gibbs et al. 2009), and that the high levels of genetic diversity observed in cattle are not indicative of genome instability resulting from domestication. Bov-A2 markers seem useful for phylogenetic studies, and they may also be useful for studies of gene expression. The CACT_n (n = 3, 4, 3) Bov-A2 linker sequence has been suggested to play a role in post-transcriptionally regulating genes to increase their expression in response to environmental stresses or activation signals (Damiani et al. 2008), so the polymorphism of Bov-A unit number may have evolved in *Bos* and *Bison* genera as a way of diversifying their capability to respond to stress.

Although Bov-A2 retroposons were not used as markers in the characterization of BoLA haplotype structure, other markers including microsatellites, SNPSTRs, and the exon 2 sequences of class IIa genes were available for use in this study. These markers demonstrated that all homozygous haplotypes defined by 52 SNPs spanning the BfoLA IIa-III-I region were continually typed as homozygous, regardless of how many additional markers were added to the region. Not only were the haplotypes consistently homozygous, but every marker would exhibit the identical allele on the SNP-defined BoLA IIa-III-I haplotype. This was even true for haplotypes shared across different breeds of cattle. Seven entire BoLA IIa-III-I haplotypes were conserved among different *Bos taurus* breeds of cattle, including shared haplotypes between Angus and Simmental; Angus, Simmental, and Brown Swiss; Angus, Holstein, and Simmental; Angus and Holstein; Holstein and Finnish Ayrshire; Limousin, Kerry, and Shorthorn; and Maine Anjou and Santa Gertrudis.

Although all of these shared haplotypes are considered to be *Bos taurus* in origin, these breeds have undergone divergent population histories and selection pressures. Angus cattle are bred for quality beef and originated in Scotland around the eighteenth century, while the Simmental breed came from Switzerland and is one of the oldest breeds in the world (OSU 1995). The Brown Swiss breed also came from Switzerland, but the Brown Swiss breed is younger than Simmental and only became a prominent dairy breed in the last one hundred years (OSU 1995). The Limousin originated in France and is another one of the oldest breeds of cattle (OSU 1995). The Ayrshire breed was founded in Scotland before 1800 and probably has genetic relationships with the Shorthorn and Holstein breeds, the Holstein breed was founded in what is now the Netherlands, and the Shorthorn breed originated on the northeastern coast of England as early as 1580 (OSU 1995). Santa Gertrudis cattle are crossbred *Bos taurus* x *Bos indicus* with approximately 5/8 Shorthorn and 3/8 Brahman breeding (OSU 1995). The Kerry breed probably descended from the Celtic Shorthorn, which was imported to Ireland as early as 2000 B.C (OSU 1995), and the Maine-Anjou breed began in southern France early in the 18th century (OSU 1995). The shared BoLA haplotypes must have predated the divergence of these breeds and been retained in tact over hundreds of years.

Some cattle shared haplotypes across the entire BoLA IIA-III-I region, while others showed strong haplotype blocks surrounding the DRB3 allele and extending through the class IIA region. It is not surprising that strong linkage disequilibrium would exist within class II haplotypes. Deeply divergent haplotypes of the HLA class II region have been preserved by linkage disequilibrium over tens of millions of years (Raymond et al. 2005), and class I genes generally undergo a more rapid birth-and-death process than class II genes (Wan et al. 2009). BoLA haplotypes that are identical at alleles of the class II region but divergent at the alleles of their class III or I regions may have descended from an ancient recombination event between the class II and class I regions prior to domestication of cattle. The identification of haplotype structure in additional breeds of cattle, within and among domestication clusters, will provide a test of this hypothesis.

Most of the homozygous BoLA IIA-III-I haplotypes analyzed in this study were from *Bos taurus* breeds of cattle. The few *Bos indicus* samples that were analyzed showed strong linkage disequilibrium across the BoLA IIA-III-I region, as observed in the *Bos taurus* samples, but future analysis of additional *Bos indicus* BoLA IIA-III-I haplotypes will provide a more complete understanding of BoLA haplotype structure. It is expected that *Bos indicus* BoLA haplotypes will be different than those of *Bos taurus* cattle because *Bos taurus* and *Bos indicus* cattle descended from two different subspecies of aurochs (Loftus et al. 1994), diverging at least 100,000 ago and possibly as long as one million years ago (Bradley et al. 1996; Loftus et al. 1994; MacHugh et al. 1998). While their BoLA haplotypes may be different from each other, it is likely that the same mechanism of BoLA IIA-III-I haplotype conservation is operating on *Bos taurus* and *Bos indicus* cattle, so strong conservation of BoLA IIA-III-I haplotypes is likely to be found after analyzing more animals of *Bos indicus* breeds.

Analysis of BoLA Single Nucleotide Polymorphisms

This project was able to utilize data from a whole genome SNP project that used the Illumina 50K SNPchip to type 13,914 animals from twenty six breeds of cattle and three additional bovid species including gaur, bison, and Cape buffalo. Out of all of the animals typed on the 50K SNPchip, 796 of them had haplotypes that were homozygous

for 52 SNPs spanning BoLA IIa-III-I (Table 20). Five of the 52 total SNPs were located in class IIa, 12 in class III, 26 in class I, and nine in the extended class I region. Many of the SNPs typed as homozygous on non-cattle DNA samples, such as gaur, bison, and Cape buffalo. It is likely that most of the SNPs in cattle were not maintained across different bovid species, so the cattle SNPs typed on other species often revealed fixed monomorphic nucleotides. Many wild and feral bovid populations are endangered and have gone through genetics bottlenecks (Nguyen et al. 2007), so the overall homozygosity of their genomes could also be contributing to the monomorphism observed in SNP genotypes. There were also many SNPs that did not successfully genotype at all on other species, which was probably due to surrounding nucleotide divergence.

Seven of the BoLA IIa-III-I homozygous haplotypes were found to be shared across breeds, but the majority of haplotypes analyzed were breed-specific. The Angus and Holstein breeds were represented by the most individuals, and it is probable that additional shared BoLA IIa-III-I haplotypes would have been identified if there were larger numbers of animals analyzed within other breeds. A total of eleven shared BoLA IIa-III-I haplotypes were identified, but four of these haplotypes were not counted because they were only shared between one specific breed and MARC animals. Many different breeds comprise the MARC animal data set, but the breed of each MARC animal is unknown so it seemed likely that a MARC haplotype shared with one particular breed was actually an animal belonging to that breed. Haplotypes of MARC animals that were not shared with other breeds were counted as breed specific haplotypes and retained for use in the phylogenetic tree, although the breed of the MARC haplotype remained unknown.

Table 20. Summary of BoLA IIA-III-I Homozygous Haplotypes. This table summarizes the BoLA IIA-III-I homozygous haplotypes that were identified in 796 individuals among a total of 13,914 animals representing 26 breeds of cattle and three wild bovids - gaur, bison, and Cape buffalo. A total of 108 haplotypes, consisting of 97 breed-specific haplotypes and 11 haplotypes shared among breeds, were identified within individuals homozygous for BoLA IIA-III-I. Data is presented according to the frequencies within each breed.

Breed	Total Number of Individuals	Percent Homozygotes	Number of Homozygous Individuals	Number of Homozygous Haplotypes	Number of Breed-Specific Haplotypes	Number of Individuals with Shared Haplotypes											Percent Shared Haplotypes
						ANG_1	ANG_3	ANG_4/HOL_5	ANG_7/HOL_2	ANG_15	HOL_12	HFD_2	HFD_4	LMS_14	SIM_1	MAO_2	
Angus	5213	5.74%	299	19	14	109	52	33	8	1							68.89%
Holstein	4343	7.52%	349	13	10			34	103		1						39.54%
MARC (multiple breeds)	1496	1.67%	25	16	10		2	1		2		1	4			4	56.00%
Limousin	1271	2.44%	31	14	13									2			6.45%
Simmental	442	1.81%	8	6	2	1	1	1								3	75.00%
Finnish Ayrshire	390	2.31%	9	6	5						1						11.11%
Hereford	124	8.87%	11	5	3							2	2				36.36%
Bralunan	81	9.88%	8	2	2												0
Shorthorn	79	5.06%	4	3	2									1			25.00%
Jersey	77	6.50%	5	4	4												0
Brown Swiss	66	6.06%	4	3	2		1										0
Nelore	51	7.84%	4	3	3												0
Bison	50	16.00%	8	6	6												0
Gaur	40	10.00%	4	3	3												0
Japanese Black	30	10.00%	3	2	2												0
Romagnola	29	13.79%	4	2	2												0
Gir	22	4.55%	1	1	1												0
Guernsey	21	4.76%	1	1	1												0
Norwegian Red	21	4.76%	1	1	1												0
Santa Gertrudis	21	9.52%	2	2	1											1	50.00%
Cape Buffalo	8	50.00%	4	1	1												0
Romosinuano	6	16.67%	1	1	1												0
Scottish Highland	6	16.67%	1	1	1												0
Maine Anjou	5	40.00%	2	2	1											1	50.00%
Salers	5	20.00%	1	1	1												0
White Park	5	40.00%	2	2	2												0
Belted Galloway	4	25.00%	1	1	1												0
Red Poll	4	25.00%	1	1	1												0
Dexter	2	50.00%	1	1	1												0
Kerry	2	50.00%	1	1	0								1				100.00%
Totals	13914		796		97												

There were 97 breed-specific BoLA homozygous haplotypes, giving a total of 108 SNP-defined BoLA IIA-III-I homozygous haplotypes. Holstein animals exhibited about 29% more breed-specific haplotypes than Angus, as approximately 68% of all Angus BoLA IIA-III-I homozygous haplotypes were shared with other breeds. The prevalence of ANG_7/HOL_2 and ANG_4/HOL_5 haplotypes in both Angus and Holstein breeds suggested that the origin of the haplotypes predated the divergence of Angus and Holstein breeds more than 2,000 years ago (OSU 1995).

Of the breeds with more than 100 homozygous BoLA IIA-III-I haplotypes, the average percent of homozygotes was 4.34%. The Angus and Holstein breeds had the largest sample sizes (5,213 and 4,343 animals, respectively), so they were ideal for calculating homozygous haplotype frequencies. The four most common Angus BoLA IIA-III-I homozygous haplotypes represented 84.61% of all Angus homozygous haplotypes, and the six most common Holstein BoLA homozygous haplotypes represented 96.57% of the total Holstein homozygous haplotype number. Although there is not a large percentage of BoLA IIA-III-I homozygotes in the Angus and Holstein populations (5.74% and 7.52%, respectively), it is interesting that such a small number of haplotypes accounts for virtually all of the BoLA IIA-III-I homozygous haplotypes in the Angus and Holstein breeds. According to the Hardy-Weinberg principle, the most frequent homozygous haplotypes will also be the most frequent haplotypes in heterozygotes, and this principle was used to infer haplotypes in animals heterozygous for BoLA IIA-III-I.

If the Holstein and Angus breeds are representative of all other cattle breeds, we project that about 16 common BoLA IIA-III-I haplotypes exist in every breed and four to six of these represent the majority of haplotypes for that breed. The high frequencies of small number of BoLA IIA-III-I haplotypes within the Angus and Holstein breeds may be attributed to genetic drift and bottlenecks during the domestication and development of the breeds, selection against BoLA haplotypes that were susceptible to disease, or the artificial selection pressures imposed on domestic breeds such as inbreeding, extensive use of artificial insemination, husbandry practices that eliminate natural selection pressures like veterinary care for sick animals, large quantities of readily available food and water, reduced lifespans, and antibiotic administration for disease prevention.

Phylogenetic trees of SNP-defined homozygous haplotypes showed different relationships among BoLA IIb haplotypes than those among BoLA IIA-III-I haplotypes. BoLA IIA-III-I haplotypes are more divergent from each other, whereas the BoLA IIb haplotypes show more simplistic relationships that would be expected from a normally recombining region of the bovine genome. Divergent BoLA IIA-III-I haplotypes are found within and between breeds and do not show clear clustering of BoLA IIA-III-I haplotypes by breed. This may be attributed to the sharing of BoLA IIA-III-I haplotypes across divergent breeds. Three *Bos indicus* BoLA IIA-III-I haplotypes, two from the Brahman breed and one from the Nelore breed, cluster together with haplotypes of two French *Bos taurus* breeds, Maine Anjou and Limousin. The Maine Anjou haplotype, MAO_2, was also found to be shared with a crossbred Santa Gertrudis animal. It was assumed that MAO_2 was a shared *Bos taurus* haplotype between Maine Anjou and Santa Gertrudis, but its clustering with three *Bos indicus* haplotypes may indicate that it is actually a BoLA IIA-III-I haplotype shared between *Bos indicus* and *Bos taurus* breeds. Increasing the number of samples typed from breeds other than Angus and Holstein will improve the conclusions that can be drawn about the phylogenetic relationships of BoLA haplotypes among domestic breeds of cattle. Very few *Bos indicus* breeds were used in the evaluation of SNP-defined BoLA haplotypes, so the power of this study to detect shared haplotypes between *Bos taurus* and *Bos indicus* is limited. There is a clear separation of cattle BoLA IIA-III-I haplotypes from African buffalo, bison, and to some extent gaur in the phylogenetic tree, and this most likely represents the ancestral SNPs BoLA IIA-III-I haplotypes.

The limited number of haplotypes observed within breeds and the sharing of haplotypes among divergent breeds suggests that the haplotypes of BoLA are genetically stable and have likely been fixed by a combination of reduced recombination, selection and inbreeding. Modern domestic cattle breeds have originated from at least two centers of domestication, wherein *Bos indicus* and *Bos taurus* cattle were domesticated from two different auroch subspecies (Loftus et al. 1994; Troy et al. 2001). Additional domestication events may have occurred in Africa (Bradley et al. 1996) and East Asia (Lai et al. 2006; Mannen et al. 2004). We propose that the multiple cattle domestication events from a large and genetically diverse ancestral population spanning large

geographical areas of the world captured a small portion of the wide variety of BoLA IIA-III-I haplotypes in ancestral wild cattle and these haplotypes have since been passed down, some largely in tact, in the lineages of various cattle breeds. Some haplotypes may have been lost over time, but this study has shown that at least seven BoLA haplotypes have remained completely conserved over hundreds of years since the divergence of certain domestic breeds of cattle that currently share entire BoLA IIA-III-I haplotypes. As more animals of various breeds, especially *Bos indicus* breeds, are defined for BoLA IIA-III-I haplotypes, the phylogenetic history of BoLA haplotypes will tell a more complete story.

Haplotypes Inferred from BoLA Heterozygotes

Haplotypes were not observed directly unless the individual was homozygous, so haplotypes present in heterozygotes were inferred statistically from their genotyping data. The SNP allele phases of animals with heterozygous BoLA haplotypes were determined with PHASE, fastPHASE, and a novel approach based on known homozygous haplotype frequencies. The diverse breed structure and lineage diversity of cattle complicated PHASE analysis, so PHASE was used to analyze each breed individually. PHASE performed best within breeds and among individuals that shared common haplotypes, but PHASE still made some mistakes in predicting the BoLA haplotypes of heterozygotes when evaluated in pedigreed samples (Table 21). We have developed a method to identify known BoLA haplotypes, which reduced the need for PHASE in Angus and Holstein heterozygotes. The success of this method was attributed to the high frequency of common BoLA homozygous haplotypes, the strong conservation of haplotypes across the entire BoLA IIA-III-I region, and the divergence of BoLA IIA-III-I haplotypes from each other. The divergence of BoLA IIA-III-I haplotypes gives them unique combinations of alleles that may be easily ascertained and distinguished from one another. Our method of inferring unknown haplotypes with known homozygous haplotypes was also useful for imputing missing data because the linkage of multiple alleles across the region was already known.

Table 21. Errors in Haplotype Inference. The SNPs of BoLA in a trio of individuals including HOL000001 (sire), HOL000007 (dam), and HOL000010 (offspring) is shown as incorrectly phased in the first set of haplotypes and correctly phased in the second set. Gray cells indicate that the SNP was not included in the analysis. The haplotype the offspring inherited from its sire is highlighted blue and the haplotype inherited from its dam is highlighted pink in each of the analysis methods. All three of the methods - fastPHASE, PHASE, and known haplotype based inference - correctly determined the BoLA IIB haplotypes for this particular trio, but mistakes were inserted to show examples of the different kinds of errors that were encountered throughout the course of this analysis. The mistakes in the second set include errors in genotyping (green), a double recombinant haplotype (yellow), and the incorrect phasing of alleles (red). There is clearly a genotyping error (green) because the SNP at position 7384619 appears homozygous T/T in both parents and heterozygous G/T in the offspring. The double recombination (yellow) event required to produce the inferred HOL000007 haplotypes is very rare, so it is more likely that these haplotypes are the result of an error in the phasing of the SNP alleles at position 7280210. A clear example of the incorrect phasing of alleles (red) is given at SNP position 7398492 in HOL000010 because the G allele had to come from the sire and the A allele had to come from the dam. All of these errors were detected because they were evaluated in pedigreed individuals.

		IIB														
		7228956	7229152	7280210	7339117	7384619	7398492	7453429	7453668	7455564	7458121	7479927	7562289	7611266	7632049	7666392
fastPHASE	HOL000001			A	A	T	G	G		T	A			A		
fastPHASE	HOL000001			A	G	T	G	G		T	A			A		
PHASE	HOL000001	G	G	A	A	T	G	G	C	T	A	A	A	A	G	G
PHASE	HOL000001	G	G	A	G	T	G	G	C	T	A	A	A	A	G	G
Known Haplotype	HOL000001	G	G	A	A	T	G	G	C	T	A	A	A	A	G	G
Known Haplotype	HOL000001	G	G	A	G	T	G	G	C	T	A	A	A	A	G	G
fastPHASE	HOL000007			A	A	T	A	G		T	C			A		
fastPHASE	HOL000007			G	A	T	A	G		T	A			G		
PHASE	HOL000007	G	G	G	A	T	A	G	C	T	C	G	G	A	G	G
PHASE	HOL000007	G	G	A	A	T	A	G	C	T	A	A	G	G	G	G
Known Haplotype	HOL000007	G	G	A	A	T	A	G	C	T	C	G	G	A	G	G
Known Haplotype	HOL000007	G	G	G	A	T	A	G	C	T	A	A	G	G	G	G
fastPHASE	HOL000010			A	A	T	G	G		T	C			A		
fastPHASE	HOL000010			A	A	G	A	G		T	A			A		
PHASE	HOL000010	G	G	A	A	T	A	G	C	T	C	G	G	A	G	G
PHASE	HOL000010	G	G	A	A	G	G	G	C	T	A	A	A	A	G	G
Known Haplotype	HOL000010	G	G	A	A	T	A	G	C	T	C	G	G	A	G	G
Known Haplotype	HOL000010	G	G	A	A	G	G	G	C	T	A	A	A	A	G	G

Table 21 Continued.

		IIB														
		7228956	7229152	7280210	7339117	7384619	7398492	7453429	7453668	7455564	7458121	7479927	7562289	7611266	7632049	7666392
fastPHASE	HOL000001			A	A	G	G	G		T	A			A		
fastPHASE	HOL000001			A	G	T	G	G		T	A			A		
PHASE	HOL000001	G	G	A	A	G	G	G	C	T	A	A	A	A	G	G
PHASE	HOL000001	G	G	A	G	T	G	G	C	T	A	A	A	A	G	G
Known Haplotype	HOL000001	G	G	A	A	G	G	G	C	T	A	A	A	A	G	G
Known Haplotype	HOL000001	G	G	A	G	T	G	G	C	T	A	A	A	A	G	G
fastPHASE	HOL000007			A	A	T	A	G		T	C			A		
fastPHASE	HOL000007			G	A	T	A	G		T	A			G		
PHASE	HOL000007	G	G	A	A	T	A	G	C	T	C	G	G	A	G	G
PHASE	HOL000007	G	G	G	A	T	A	G	C	T	A	A	G	G	G	G
Known Haplotype	HOL000007	G	G	A	A	T	A	G	C	T	C	G	G	A	G	G
Known Haplotype	HOL000007	G	G	G	A	T	A	G	C	T	A	A	G	G	G	G
fastPHASE	HOL000010			A	A	T	A	G		T	C			A		
fastPHASE	HOL000010			A	A	G	G	G		T	A			A		
PHASE	HOL000010	G	G	A	A	T	A	G	C	T	C	G	G	A	G	G
PHASE	HOL000010	G	G	A	A	G	G	G	C	T	A	A	A	A	G	G
Known Haplotype	HOL000010	G	G	A	A	T	A	G	C	T	C	G	G	A	G	G
Known Haplotype	HOL000010	G	G	A	A	G	G	G	C	T	A	A	A	A	G	G

Haploview is a program that was used to calculate and visualize the linkage disequilibrium statistic r^2 among SNPs across the BoLA IIB and BoLA IIA-III-I regions. If stronger linkage disequilibrium had been observed in Haploview, this program could have also identified BoLA haplotype blocks and tagSNPs. It was surprising that the strong level of linkage disequilibrium noted across the BoLA IIA-III-I region in our previous analyses was not reflected in the Haploview figures (Figure 16). If conclusions were to be drawn from the Haploview figures alone, the BoLA IIA-III-I region would not be considered unique, as it appears to have blocks of linkage disequilibrium similar to the rest of the bovine genome. It seems that these samples may be violating some of the assumptions underlying the algorithms of PHASE or Haploview such that they are not recognizing the high levels of BoLA linkage disequilibrium within breeds.

Improving methods of heterozygous BoLA haplotype inference did not drastically change the outcome of the Haploview figures. Haploview figures were generated with a much larger data set ($n > 350$) derived from Angus BoLA homozygous haplotypes and haplotypes inferred from Angus heterozygotes by comparison to known BoLA homozygous haplotypes (Figure 17), but the low levels of linkage disequilibrium observed in the Haploview outputs were not resolved by this approach. Slightly higher linkage disequilibrium and an elimination of monomorphic SNPs are observed in Figure 17 when compared to the haplotypes generated by PHASE in Figure 16, but overall Figures 16 and 17 look quite similar with markers located close together showing the highest levels of linkage disequilibrium. Therefore, errors in PHASE and small sample sizes are not responsible for the low levels of linkage disequilibrium observed in the Haploview figures.

Improving the accuracy of heterozygote haplotype inference and increasing the sample size within a breed did not increase the amount of linkage disequilibrium observed in the Haploview figures. Perhaps the highly divergent combinations of alleles within BoLA haplotypes are negating the calculations of linkage disequilibrium. PHASE detected linkage disequilibrium when markers were located close together, so increasing the SNP density of the BoLA region may allow PHASE and Haploview to detect higher levels of linkage disequilibrium. Although PHASE and Haploview did not detect high levels of linkage disequilibrium in BoLA IIA-III-I, the results of this study have demonstrated that high levels of linkage disequilibrium exist within the BoLA IIA-III-I region. The information provided by this study demonstrates that a tagSNP approach to defining BoLA IIA-III-I haplotypes would offer more information and improve the efficiency of future disease association studies of cattle.

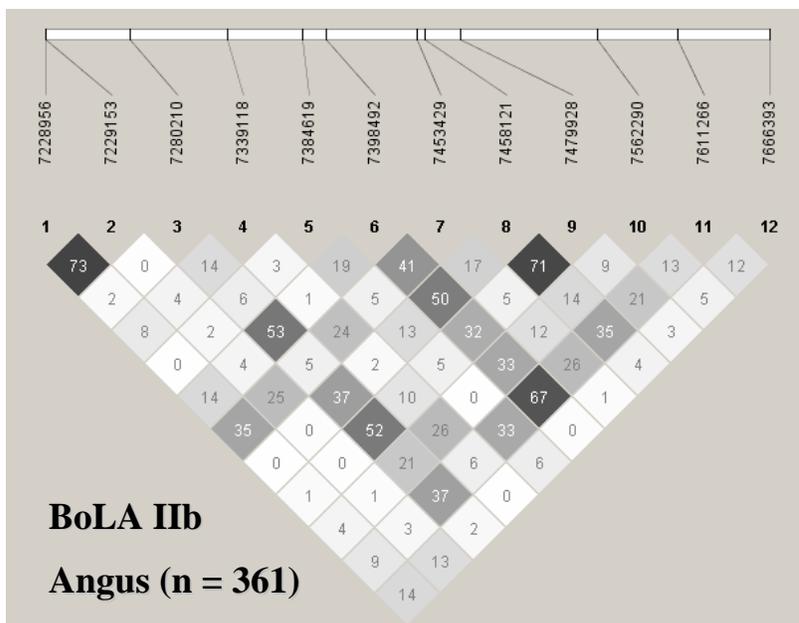


Figure 17. Angus BoLA IIA-III-I and BoLA IIb Haploview Outputs. This figure was generated with an increased sample size and more accurate haplotype predictions than Figure 16, as most of the animals were homozygous for BoLA IIA-III-I. The total number of animals analyzed (n) is listed in the left bottom corner of the figure. SNPs with the highest levels linkage disequilibrium (maximum number = 100) are represented by black boxes, SNPs with lowest linkage disequilibrium (minimum number = 0) are white boxes, and shades of gray represent SNPs with intervening levels of linkage disequilibrium. This figure demonstrates that larger amounts of linkage disequilibrium were not observed in Haploview after improving the accuracy of haplotype inference and increasing sample size of BoLA IIb and BoLA IIA-III-I.

Addressing Criticisms of tagSNPs and Haplotypes

Concerns have been raised about the utility of tagSNPs, including tagSNP selection bias towards high frequency SNPs that may not detect rare variants and the transferability of tagSNPs across different populations. The vast diversity among cattle breeds (Gibbs et al. 2009) may be a hinderance to utilizing tagSNPs in other regions of the bovine genome, but the high degree of conservation, divergence, and linkage disequilibrium in the BoLA IIa-III-I region is ideal for utilizing tagSNPs. This work has demonstrated that haplotypes of the BoLA IIa-III-I region can be predicted with a subset of 52 SNPs from the Illumina 50K SNPchip because overlaying different types of markers with varying mutation rates across the SNP-defined BoLA IIa-III-I haplotypes did not identify any further subdivisions within the haplotypes.

Various criticisms have been made about utilizing BoLA IIa-III-I haplotypes in disease association studies. A general criticism of haplotype disease associations is that the causal genetic element(s) behind susceptible and resistant phenotypes is not identified. However, the power and robustness of mapping the causal genetic elements of diseases has been shown to be significantly improved by analyzing haplotypes rather than a putative single locus (Akey et al. 2001; De Bakker et al. 2005). A disease association study is strengthened by analyzing haplotypes over alleles of a single locus because more information is provided by the linkage disequilibrium of multiple markers within the haplotype, which allows for the evolution of otherwise unmeasured causal variants and the interaction of multiple variants within the haplotype. After a BoLA haplotype has been associated with a disease phenotype, it may be further characterized to identify the specific causal genetic element(s) within the haplotype. However, identifying the causal element(s) is not required to select against the haplotypes that are most susceptible to a particular disease. The susceptible haplotype only needs to be correctly identified within a disease association study, and then recognized and selected against at the population level. Culling animals with a BoLA class II haplotype (DRB3*0301-0302 / DRB3*3401-3402 and DQB*1804) strongly associated with susceptibility to dermatophilosis reduced the disease prevalence from 0.76 to 0.02 over a five year period within a Brahman cattle population (Maillard et al. 2003). Although in this example only the BoLA class IIa haplotype was selected against, this demonstrates how knowledge of BoLA haplotypes

can be successfully used to reduce the prevalence of specific diseases affecting cattle populations.

BoLA haplotypes will be useful for identifying disease associations with genes positioned in the BoLA region; however, this approach will not detect causal associations with genes outside of the BoLA region, and many genes located in other regions of the genome play important roles in the immune response pathway. Over 1500 genes have been implicated in the human immune response, representing 7% of the human genome (Kelley et al. 2005). It is presently unclear which of these genes contribute in significant detectable ways to individual differences in disease resistance or susceptibility. Furthermore, one can expect that the contribution of each gene will be determined to some extent by the genomic context in which it functions.

This study provides genetic resources for associating BoLA haplotypes with diseases, but the complex nature of many diseases should be considered when drawing conclusions from BoLA disease association studies because the additive effects of many loci may be contributing to a disease phenotype. Evidence from this project supported the conclusions of Regitano et al. 2008 that host resistance to ticks is a complex multi-factorial trait, as some calves that were classified as most susceptible or most resistant to the Lone Star tick had identical BoLA IIA-III-I haplotypes in our study and Regitano et al. 2008 found quantitative trait loci (QTL) for *Rhipicephalus (Boophilus) microplus* tick load on BTA 23 and six other chromosomes (BTA 4, 5, 7, 10, 14, and 18) in cattle. Genes within BoLA are frequently associated with mastitis resistance, but genes of the innate immune system that are located on other chromosomes also appear to play a significant role in defending against mastitis. Putative mastitis QTLs have been identified on cattle chromosomes 3, 4, 6, 14, and 27 (Klungland et al. 2001). Bovine β -defensin genes, clustered together on BTA 27 (Gallagher et al. 1995), are expressed in mammary gland tissue and may be candidates for resistance to mastitis (Roosen et al. 2004). Other studies have demonstrated that cattle infected with mastitis showed associations with β -defensin 5 located on chromosome 27, toll-like receptor 2 (TLR2) located on chromosome 17, and TLR4 located on chromosome 8 (Goldammer et al. 2004; Wang et al. 2002; White et al. 2003). Mastitis is a multi-factorial disease that is influenced by many environmental factors like proper management and hygienic

milking practices, and different environmental and genetic factors may be implicated in susceptibility to different strains of bacteria causing the disease (Heringstad et al. 2000).

Disease associations with BoLA haplotypes do not have the power to detect causal genetic variants located outside of the BoLA regions; therefore, whole-genome or whole-chromosome association studies may be more appropriate to understand complex diseases that are influenced by multiple genetic variants positioned throughout the genome, such as cattle susceptibility to ticks and mastitis (Klungland et al. 2001; Regitano et al. 2008). Whole genome studies present some challenges including problems with defining intermediate phenotypes and understanding the interactions among many polymorphisms. The complex nature of the immune response imposes limitations on the capacity of any disease-association study to identify all causative agents involved in differential immune responses, as even an experiment designed to represent all genes involved the immune response would still not account for environmental factors, such as management practices and stress levels (Webster et al. 2002). The data provided by this study will increase the amount of information obtained from disease association studies in cattle by incorporating loci across the entire BoLA IIa-III-I region rather than the limited amount of information gained from single BoLA markers appearing on the background of multiple BoLA IIa-III-I haplotypes. The SNPs defining BoLA IIa-III-I haplotypes could easily be incorporated into a genome-wide immune response SNPchip that integrates markers of other important immune system genes to identify disease QTLs positioned outside of BoLA.

Implications for Future Research

Information from this project should be considered in future BoLA disease association studies. The potentially high frequencies of a small number of BoLA IIa-III-I haplotypes within each cattle breed should be taken into account during the design and analysis of case-control disease association studies. A haplotype present at high frequency within a particular disease phenotype may simply be due to the high frequency of that haplotype within a particular breed or population, and not due to a true statistical association with the disease. Ideally, all disease association studies with BoLA IIa-III-I haplotypes should be performed and evaluated within a single breed of cattle to minimize statistical errors in disease associations. The phasing of all BoLA heterozygous haplotypes should be performed within a breed, as this study found many inaccuracies when BoLA haplotypes were inferred with different breeds grouped together. Although seven BoLA IIa-III-I haplotypes were discovered to be entirely shared across breeds in this study, most BoLA haplotypes were breed specific and little haplotype sharing has been observed in the bovine genome across different cattle breeds (Gibbs et al. 2009). If the same BoLA haplotype appears within the most resistant and the most susceptible animals of a study, it is unlikely that the BoLA haplotype is the only factor contributing to the disease phenotype. Complex diseases of cattle may involve the interactions of multiple loci throughout the genome, as well as many different environmental factors. Integrating the BoLA haplotype with variants of other important immune system genes will give a more complete understanding for the genetic basis of susceptibility and resistance to a particular disease of cattle.

We found no evidence that adding additional markers to the BoLA IIA/III/I region would identify any additional haplotypes and conclude that the 52 SNPs of the Illumina 50K SNPchip are sufficient to define BoLA IIA-III-I haplotypes among the cattle breeds used in this study. However, the data set was heavily biased towards taurine breeds and more *Bos indicus* animals should be investigated to ensure that these SNPs have the capacity to define additional haplotypes that are specific to *Bos indicus* breeds. Haplotypes sharing alleles for 52 SNPs of the Illumina 50K SNPchip distributed across the BoLA IIA-III-I region had identical alleles for known class IIA genes DRB3, DQB, and DRA, and identical alleles for microsatellites and SNPSTRs in the class III and class I regions. This would be especially useful in typing certain genes that have been challenging to define in the past, such as those in the class I region. Defining BoLA classical class I genes has been laborious and time consuming because the number of class I loci differs between BoLA haplotypes (Birch et al. 2006), so a new set of locus-specific class I primers must be redesigned for every breed. Copy number polymorphisms (Glass et al. 2000) also create challenges in defining the gene content of different BoLA haplotypes. As BoLA IIA-III-I haplotypes are defined, members of BoLA research community could undertake the task of identifying alleles of BoLA genes on the background of these SNP-defined haplotypes. Different labs have previously determined the alleles of various genes within BoLA, and many labs have DNA samples with pre-defined BoLA alleles that could easily be integrated with haplotypes defined by the BoLA tagSNPs. Such a collaborative effort would be cost-effective for determining the gene content of BoLA IIA-III-I SNP-defined haplotypes, and provide a very valuable resource for future disease association studies in cattle.

REFERENCES

1. Aarestrup F, Jensen N, Ostergard H (1995) Analysis of associations between major histocompatibility complex (BoLA) class I haplotypes and subclinical mastitis of dairy cows. *J Dairy Sci* 78, 1684-1692
2. Abi-Rached L, Gilles A, Shiina T, Pontarotti P, Inoko H (2002) Evidence of en bloc duplication in vertebrate genomes. *Nat Genet* 31, 100-105
3. Achilli A, Olivieri A, Pellecchia M, Ubaldi C, Colli L, Al-Zahery N, Accetturo M, Pala M, Kashani B, Perego U (2008) Mitochondrial genomes of extinct aurochs survive in domestic cattle. *Curr Biol* 18, 157-158
4. Agrafioti I, Stumpf M (2007) SNPSTR: a database of compound microsatellite-SNP markers. *Nucleic Acids Res* 35, D71-D75
5. Aguilar A, Roemer G, Debenham S, Binns M, Garcelon D, Wayne R (2004) High MHC diversity maintained by balancing selection in an otherwise genetically monomorphic mammal. *Proc Natl Acad Sci USA* 101, 3490-3494
6. Akey J, Jin L, Xiong M (2001) Haplotypes vs single marker linkage disequilibrium tests: what do we gain? *Eur J Hum Genet* 9, 291-300
7. Alexander L, Rohrer G, Stone R, Beattie C (1995) Porcine SINE-associated microsatellite markers: evidence for new artiodactyl SINEs. *Mamm Genome* 6, 464-468
8. Allard M, Miyamoto M, Jarecki L, Kraus F, Tennant M (1992) DNA systematics and evolution of the artiodactyl family Bovidae. *Proc Natl Acad Sci USA* 89, 3972-3976
9. Altschul S, Gish W, Miller W, Myers E, Lipman D (1990) Basic local alignment search tool. *J Mol Biol* 215, 403-410
10. Amadou C (1999) Evolution of the MHC class I region: the framework hypothesis. *Immunogenetics* 49, 362-367
11. Amiel J (1967) Study of the human leukocyte phenotypes in Hodgkin's disease. *Histocompatibility Testing* 79-81
12. Anziani O, Zimmermann G, Guglielmone A, Vazquez R, Suarez V (2001) Avermectin resistance in *Cooperia pectinata* in cattle in Argentina. *Vet Rec* 149, 58-59

13. Arcot S, Wang Z, Weber J, Deininger P, Batzer M (1995) Alu repeats: a source for the genesis of primate microsatellites. *Genomics* 29, 136-144
14. Arias J, Keehan M, Fisher P, Coppieters W, Spelman R (2009) A high density linkage map of the bovine genome. *BMC Genet* 10, 18
15. Arnold M, Hodges S (1995) Are natural hybrids fit or unfit relative to their parents? *Trends Ecol Evol* 10, 67-71
16. Bahram S, Bresnahan M, Geraghty D, Spies T (1994) A second lineage of mammalian major histocompatibility complex class I genes. *Proc Natl Acad Sci USA* 91, 6259-6263
17. Baker C, Vant M, Dalebout M, Lento G, O'Brien S, Yuhki N (2006) Diversity and duplication of DQB and DRB-like genes of the MHC in baleen whales (suborder: Mysticeti). *Immunogenetics* 58, 283-296
18. Ballingall K, Ellis S, MacHugh N, Archibald S, McKeever D (2004a) The DY genes of the cattle MHC: expression and comparative analysis of an unusual class II MHC gene pair. *Immunogenetics* 55, 748-755
19. Ballingall K, Luyai A, Rowlands G, Sales J, Musoke A, Morzaria S, McKeever D (2004b) Bovine leukocyte antigen major histocompatibility complex class II DRB3* 2703 and DRB3* 1501 alleles are associated with variation in levels of protection against *Theileria parva* challenge following immunization with the sporozoite p67 antigen. *Infect Immun* 72, 2738-2741
20. Band M, Ron M (1996) Creation of a SINE enriched library for the isolation of polymorphic (AGC)_n microsatellite markers in the bovine genome. *Anim Genet* 27, 243-248
21. Barendse W, Vaiman D, Kemp S, Sugimoto Y, Armitage S, Williams J, Sun H, Eggen A, Agaba M, Aleyasin S (1997) A medium-density genetic linkage map of the bovine genome. *Mamm Genome* 8, 21-28
22. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21, 263-265
23. Baschal E, Aly T, Jasinski J, Steck A, Johnson K, Noble J, Erlich H, Eisenbarth G (2009) The frequent and conserved DR3-B8-A1 extended haplotype confers less diabetes risk than other DR3 haplotypes. *Diabetes Obes Metab* 11, 25-30
24. Baxter R, Hastings N, Law A, Glass E (2008) A rapid and robust sequence-based genotyping method for BoLA-DRB3 alleles in large numbers of heterozygous cattle. *Anim Genet* 39, 561-563

25. Begun D, Aquadro C (1992) Levels of naturally occurring DNA polymorphism correlate with recombination rates in *D. melanogaster*. *Nature* 356, 519-520
26. Beja-Pereira A, Luikart G, England PR, Bradley DG, Jann OC, Bertorelle G, Chamberlain AT, Nunes TP, Metodiev S, Ferrand N, Erhardt G (2003) Gene-culture coevolution between cattle milk protein genes and human lactase genes. *Nat Genet* 35, 311-313
27. Bell G, Jurka J (1997) The length distribution of perfect dimer repetitive DNA is consistent with its evolution by an unbiased single-step mutation process. *J Mol Evol* 44, 414-421
28. Belov K, Deakin JE, Papenfuss AT, Baker ML, Melman SD, Siddle HV, Gouin N, Goode DL, Sargeant TJ, Robinson MD, Wakefield MJ, Mahony S, Cross JG, Benos PV, Samollow PB, Speed TP, Graves JA, Miller RD (2006) Reconstructing an ancestral mammalian immune supercomplex from a marsupial major histocompatibility complex. *PLoS Biol* 4, e46
29. Benacerraf B (1981) Role of MHC gene products in immune regulation. *Science* 212, 1229-1238
30. Birch J, Codner G, Guzman E, Ellis S (2008a) Genomic location and characterisation of nonclassical MHC class I genes in cattle. *Immunogenetics* 60, 267-273
31. Birch J, De Juan Sanjuan C, Guzman E, Ellis S (2008b) Genomic location and characterisation of MIC genes in cattle. *Immunogenetics* 60, 477-483
32. Birch J, Ellis S (2007) Complexity in the cattle CD94/NKG2 gene families. *Immunogenetics* 59, 273-280
33. Birch J, Murphy L, MacHugh N, Ellis S (2006) Generation and maintenance of diversity in the cattle MHC class I region. *Immunogenetics* 58, 670-679
34. Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC (1987a) The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature* 329, 512-518
35. Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC (1987b) Structure of the human class I histocompatibility antigen, HLA-A2. *Nature* 329, 506-512
36. Bos D, Waldman B (2006) Evolution by recombination and transspecies polymorphism in the MHC class I gene of *Xenopus laevis*. *Mol Biol Evol* 23, 137-143

37. Bover KH (1927) Homoisotransplantation van epidermis bei eineigen zwillingen. *Beitr Klin Chir* 141, 442-447
38. The Bovine Genome Sequencing and Analysis Consortium, Elsik C, Tellam R, Worley K (2009) The genome sequence of taurine cattle: a window to ruminant biology and evolution. *Science* 324, 522-528
39. Bradley DG, MacHugh DE, Cunningham P, Loftus RT (1996) Mitochondrial diversity and the origins of African and European cattle. *Proc Natl Acad Sci USA* 93, 5131-5135
40. Bradshaw C, Brook B (2007) Ecological-economic models of sustainable harvest for an endangered but exotic megaherbivore in northern Australia. *Natural Res Modeling* 20, 129-156
41. Bradshaw C, Isagi Y, Kaneko S, Brook B, Bowman D, Frankham R (2007) Low genetic diversity in the bottlenecked population of endangered non-native banteng in northern Australia. *Mol Ecol* 16, 2998-3008
42. Braud V, Allan D, O'Callaghan C, Söderström K, D'Andrea A, Ogg G, Lazetic S, Young N, Bell J, Phillips J (1998) HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* 391, 795-799
43. Brinkmann B, Klintschar M, Neuhuber F, Hühne J, Rolf B (1998) Mutation rate in human microsatellites: influence of the structure and length of the tandem repeat. *Am J Hum Genet* 62, 1408-1415
44. Brinkmeyer-Langford C, Childers C, Fritz K, Gustafson-Seabury A, Cothran M, Raudsepp T, Womack J, Skow L (2009) A high resolution RH map of the bovine major histocompatibility complex. *BMC Genomics* 10, 182
45. Brohede J, Ellegren H (1999) Microsatellite evolution: polarity of substitutions within repeats and neutrality of flanking sequences. *Proc Biol Sci* 266, 825-833
46. Brown J, Jardetzky T, Gorga J, Stern L, Urban R, Strominger J, Wiley D (1993) Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature* 364, 33-39
47. Buchanan F, Littlejohn R, Galloway S, Crawford A (1993) Microsatellites and associated repetitive elements in the sheep genome. *Mamm Genome* 4, 258-264
48. Caesar JG (1869) *Commentaries on the Gallic war, book VI*. Harper & Brothers, New York

49. Callen D, Thompson A, Shen Y, Phillips H, Richards R, Mulley J, Sutherland G (1993) Incidence and origin of "null" alleles in the (AC)_n microsatellite markers. *Am J Hum Genet* 52, 922-927
50. Campbell RD, Trowsdale J (1993) Map of the human MHC. *Immunol Today* 14, 349-352
51. Cao K, Hollenbach J, Shi X, Shi W, Chopek M, Fernández-Viña M (2001) Analysis of the frequencies of HLA-A, B, and C alleles and haplotypes in the five major ethnic groups of the United States reveals high levels of diversity in these loci and contrasting distribution patterns in these populations. *Hum Immunol* 62, 1009-1030
52. Carroll M, Campbell R, Bentley D, Porter R (1984) A molecular map of the human major histocompatibility complex class III region linking complement genes C4, C2 and factor B. *Nature* 307, 237-241
53. Castro LF, Furlong RF, Holland PW (2004) An antecedent of the MHC-linked genomic region in amphioxus. *Immunogenetics* 55, 782-784
54. CattleNetwork (July, 2009) CattleNetwork world report: cattle population by country. <http://www.cattlenetwork.com/Content.asp?contentid=226025>
55. Chakraborty R, Kimmel M, Stivers D, Davison L, Deka R (1997) Relative mutation rates at di-, tri-, and tetranucleotide microsatellite loci. *Proc Natl Acad Sci USA* 94, 1041-1046
56. Chalmers DG, Coombs RR, Gurner BW, Dausset J (1959) The mixed antiglobulin reaction in the detection of human isoantibodies against leucocytes, platelets and HeLa cells. *Br J Haematol* 5, 225-231
57. Chaves R, Santos S, Guedes-Pinto H (2004) Comparative analysis (Hippotragini versus Caprini, Bovidae) of X-chromosome's constitutive heterochromatin by in situ restriction endonuclease digestion: X-chromosome constitutive heterochromatin evolution. *Genetica* 121, 315-325
58. Chen Y, Cicciarelli J, Pravica V, Hutchinson I (2009) Long-range linkage on chromosome 6p of VEGF, FKBP5, HLA and TNF alleles associated with transplant rejection. *Mol Immunol* Epub ahead of print
59. Childers CP, Newkirk HL, Honeycutt DA, Ramlachan N, Muzney DM, Sodergren E, Gibbs RA, Weinstock GM, Womack JE, Skow LC (2006) Comparative analysis of the bovine MHC class IIb sequence identifies inversion breakpoints and three unexpected genes. *Anim Genet* 37, 121-129

60. Choudhury A (2002) Distribution and conservation of the gaur *Bos gaurus* in the Indian subcontinent. *Mammal Rev* 32, 199-226
61. Clark MS, Pontarotti P, Gilles A, Kelly A, Elgar G (2000) Identification and characterization of a beta proteasome subunit cluster in the Japanese pufferfish (*Fugu rubripes*). *J Immunol* 165, 4446-4452
62. Clark MS, Shaw L, Kelly A, Snell P, Elgar G (2001) Characterization of the MHC class I region of the Japanese pufferfish (*Fugu rubripes*). *Immunogenetics* 52, 174-185
63. Clarke B, Kirby D (1966) Maintenance of histocompatibility polymorphisms. *Nature* 211, 999-1000
64. Coder G (1975) The national movement to preserve the American buffalo in the United States and Canada between 1880 and 1920. PhD. dissertation, Ohio State University, Columbus, OH
65. Crawford D, Bhangale T, Li N, Hellenthal G, Rieder M, Nickerson D, Stephens M (2004) Evidence for substantial fine-scale variation in recombination rates across the human genome. *Nat Genet* 36, 700-706
66. Creighton P, Eggen A, Fries R, Jordan S, Hetzel J, Cunningham E, Humphries P (1992) Mapping of bovine markers CYP21, PRL, and BoLA DRBP1 by genetic linkage analysis in reference pedigrees. *Genomics* 14, 526-528
67. Cullen M, Erlich H, Klitz W, Carrington M (1995) Molecular mapping of a recombination hotspot located in the second intron of the human TAP2 locus. *Am J Hum Genet* 56, 1350-1358
68. Cullen M, Noble J, Erlich H, Thorpe K, Beck S, Klitz W, Trowsdale J, Carrington M (1997) Characterization of recombination in the HLA class II region. *Am J Hum Genet* 60, 397-407
69. Cullen M, Perfetto S, Klitz W, Nelson G, Carrington M (2002) High-resolution patterns of meiotic recombination across the human major histocompatibility complex. *Am J Hum Genet* 71, 759-776
70. Daar A, Fuggle S, Fabre J, Ting A, Morris P (1984) The detailed distribution of HLA-A, B, C antigens in normal human organs. *Transplantation* 38, 287-292
71. Dadi H, Tibbo M, Takahashi Y, Nomura K, Hanada H, Amano T (2008) Microsatellite analysis reveals high genetic diversity but low genetic structure in Ethiopian indigenous cattle populations. *Anim Genet* 39, 425-431

72. Damiani G, Florio S, Budelli E, Bolla P, Caroli A (2000) Single nucleotide polymorphisms (SNPs) within Bov-A2 SINE in the second intron of bovine and buffalo k-casein (CSN3) gene. *Anim Genet* 31, 277-279
73. Damiani G, Florio S, Panelli S, Capelli E, Cuccia M (2008) The Bov-A2 retroelement played a crucial role in the evolution of ruminants. *Riv Biol* 101, 375-404
74. Danchin E, Abi-Rached L, Gilles A, Pontarotti P (2003) Conservation of the MHC-like region throughout evolution. *Immunogenetics* 55, 141-148
75. Danchin E, Vitiello V, Vienne A, Richard O, Gouret P, McDermott MF, Pontarotti P (2004) The major histocompatibility complex origin. *Immunol Rev* 198, 216-232
76. Dary D (1974) *The buffalo book*. Avon Books, New York
77. Dausset J (1958) Iso-leuko-antibodies. *Acta Haematol* 20, 156-166
78. Dausset J, Nenna A, Brecy H (1954) Leukoagglutinins. V. Leukoagglutinins in chronic idiopathic or symptomatic pancytopenia and in paroxysmal nocturnal hemoglobinuria. *Blood* 9, 696-720
79. De Bakker P, Yelensky R, Pe'er I, Gabriel S, Daly M, Altshuler D (2005) Efficiency and power in genetic association studies. *Nat Genet* 37, 1217-1223
80. De Bakker PI, McVean G, Sabeti PC, Miretti MM, Green T, Marchini J, Ke X, Monsuur AJ, Whittaker P, Delgado M, Morrison J, Richardson A, Walsh EC, Gao X, Galver L, Hart J, Hafler DA, Pericak-Vance M, Todd JA, Daly MJ, Trowsdale J, Wijmenga C, Vyse TJ, Beck S, Murray SS, Carrington M, Gregory S, Deloukas P, Rioux JD (2006) A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. *Nat Genet* 38, 1166-1172
81. Deakin J, Siddle H, Cross J, Belov K, Graves J (2007) Class I genes have split from the MHC in the tammar wallaby. *Cytogenet Genome Res* 116, 205-211
82. Degli-Esposti M, Abraham L, McCann V, Spies T, Christiansen F, Dawkins R (1992a) Ancestral haplotypes reveal the role of the central MHC in the immunogenetics of IDDM. *Immunogenetics* 36, 345-356
83. Degli-Esposti M, Leaver A, Christiansen F, Witt C, Abraham L, Dawkins R (1992b) Ancestral haplotypes: conserved population MHC haplotypes. *Hum Immunol* 34, 242-252
84. Diamond J (2002) Evolution, consequences and future of plant and animal domestication. *Nature* 418, 700-707

85. Dietz A, Detilleux J, Freeman A, Kelley D, Stabel J, Kehrli M (1997) Genetic association of bovine lymphocyte antigen DRB3 alleles with immunological traits of Holstein cattle. *J Dairy Sci* 80, 400-405
86. Dobromylskyj M, Ellis S (2007) Complexity in cattle KIR genes: transcription and genome analysis. *Immunogenetics* 59, 463-472
87. Doherty P, Zinkernagel R (1975a) Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex. *Nature* 256, 50-52
88. Doherty PC, Zinkernagel RM (1975b) H-2 compatibility is required for T-cell-mediated lysis of target cells infected with lymphocytic choriomeningitis virus. *J Exp Med* 141, 502-507
89. Dohm J, Tsend-Ayush E, Reinhardt R, Grützner F, Himmelbauer H (2007) Disruption and pseudoautosomal localization of the major histocompatibility complex in monotremes. *Genome Biol* 8, R175
90. Drummond A, Rambaut A, Shapiro B, Pybus O (2005) Bayesian coalescent inference of past population dynamics from molecular sequences. *Mol Biol Evol* 22, 1185-1192
91. Dunn D, Romphruk A, Leelayuwat C, Bellgard M, Kulski J (2005) Polymorphic Alu insertions and their associations with MHC class I alleles and haplotypes in the northeastern Thais. *Ann Hum Genet* 69, 364-372
92. Edwards CJ, Bollongino R, Scheu A, Chamberlain A, Tresset A, Vigne JD, Baird JF, Larson G, Ho SY, Heupink TH, Shapiro B, Freeman AR, Thomas MG, Arbogast RM, Arndt B, Bartosiewicz L, Benecke N, Budja M, Chaix L, Choyke AM, Coqueugniot E, Dohle HJ, Goldner H, Hartz S, Helmer D, Herzig B, Hongo H, Mashkour M, Ozdogan M, Pucher E, Roth G, Schade-Lindig S, Schmolcke U, Schulting RJ, Stephan E, Uerpmann HP, Voros I, Voytek B, Bradley DG, Burger J (2007) Mitochondrial DNA analysis shows a Near Eastern Neolithic origin for domestic cattle and no indication of domestication of European aurochs. *Proc Biol Sci* 274, 1377-1385
93. Ehlers A, Beck S, Forbes SA, Trowsdale J, Volz A, Younger R, Ziegler A (2000) MHC-linked olfactory receptor loci exhibit polymorphism and contribute to extended HLA/OR-haplotypes. *Genome Res* 10, 1968-1978
94. Ellegren H, Davies C, Andersson L (1993) Strong association between polymorphisms in an intronic microsatellite and in the coding sequence of the BoLA-DRB3 gene: implications for microsatellite stability and PCR-based DRB3 typing. *Anim Genet* 24, 269-275

95. Ellis SA, Bontrop RE, Antczak DF, Ballingall K, Davies CJ, Kaufman J, Kennedy LJ, Robinson J, Smith DM, Stear MJ, Stet RJ, Waller MJ, Walter L, Marsh SG (2006) ISAG/IUIS-VIC comparative MHC nomenclature committee report, 2005. *Immunogenetics* 57, 953-958
96. Ennis P, Zemmour J, Salter R, Parham P (1990) Rapid cloning of HLA-A, B cDNA by using the polymerase chain reaction: frequency and nature of errors produced in amplification. *Proc Natl Acad Sci USA* 87, 2833-2837
97. Estoup A, Jarne P, Cornuet J (2002) Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. *Mol Ecol* 11, 1591-1604
98. Excoffier L, Yang Z (1999) Substitution rate variation among sites in 9 mitochondrial hypervariable region I of humans and chimpanzees. *Mol Biol Evol* 16, 1357-1368
99. Feder J, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy D, Basava A, Dormishian F, Domingo R, Ellis M, Fullan A (1996) A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 13, 399-408
100. Fernando MM, Stevens CR, Walsh EC, De Jager PL, Goyette P, Plenge RM, Vyse TJ, Rioux JD (2008) Defining the role of the MHC in autoimmunity: a review and pooled analysis. *PLoS Genet* 4, e1000024
101. Figueroa F, Günther E, Klein J (1988) MHC polymorphism pre-dating speciation. *Nature* 335, 265-267
102. Flamand J, Vankan D, Gairhe K, Duong H, Barker J (2003) Genetic identification of wild Asian water buffalo in Nepal. *Anim Conserv* 6, 265-270
103. Frankham R, Loebel D (1992) Modeling problems in conservation genetics using captive *Drosophila* populations: rapid genetic adaptation to captivity. *Zoo Biol* 11, 343-351
104. Fraser D, Bailey E (1998) Polymorphism and multiple loci for the horse DQA gene. *Immunogenetics* 47, 487-490
105. Frazer K, Ballinger D, Cox D, Hinds D, Stuve L, Gibbs R, Belmont J, Boudreau A, Hardenbol P, Leal S (2007) A second generation human haplotype map of over 3.1 million SNPs. *Nature* 449, 851-861
106. Freeman A, Meghen C, Machugh D, Loftus R, Achukwi M, Bado A, Sauveroche B, Bradley D (2004) Admixture and diversity in West African cattle populations. *Mol Ecol* 13, 3477-3487

107. Frisch J (1981) Changes occurring in cattle as a consequence of selection for growth rate in a stressful environment. *J Agric Sci* 96, 23-38
108. Gaddum R, Cook R, Furze J, Ellis S, Taylor G (2003) Recognition of bovine respiratory syncytial virus proteins by bovine CD8 β T lymphocytes. *Immunology* 108, 220-229
109. Galbreath G, Mordacq J, Weiler F (2006) Genetically solving a zoological mystery: was the kouprey (*Bos sauveli*) a feral hybrid? *J Zool* 270, 561-564
110. Gallagher D, Davis S, De Donato M, Burzlaff J, Womack J, Taylor J, Kumamoto A (1999a) A molecular cytogenetic analysis of the tribe bovini (Artiodactyla: Bovidae: Bovinae) with an emphasis on sex chromosome morphology and NOR distribution. *Chromosome Res* 7, 481-492
111. Gallagher D, Ryan A, Diamond G, Bevins C, Womack J (1995) Somatic cell mapping of α -defensin genes to cattle syntenic group U25 and fluorescence in situ localization to chromosome 27. *Mamm Genome* 6, 554-556
112. Gallagher DS, Jr., Womack JE (1992) Chromosome conservation in the Bovidae. *J Hered* 83, 287-298
113. Gallagher P, Lear T, Coogle L, Bailey E (1999b) Two SINE families associated with equine microsatellite loci. *Mamm Genome* 10, 140-144
114. Gao H, Williamson S, Bustamante C (2007) A Markov chain Monte Carlo approach for joint inference of population structure and inbreeding rates from multilocus genotype data. *Genetics* 176, 1635-1651
115. Garcia-Briones M, Russell G, Oliver R, Tami C, Taboga O, Carrillo E, Palma E, Sobrino F, Glass E (2000) Association of bovine DRB3 alleles with immune response to FMDV peptides and protection against viral challenge. *Vaccine* 19, 1167-1171
116. Geraghty D, Koller B, Hansen J, Orr H (1992) The HLA class I gene family includes at least six genes and twelve pseudogenes and gene fragments. *J Immunol* 149, 1934-1946
117. Geraghty D, Wei X, Orr H, Koller B (1990) Human leukocyte antigen F (HLA-F). An expressed HLA gene composed of a class I coding sequence linked to a novel transcribed repetitive element. *J Exp Med* 171, 1-18
118. Gerner W, Hammer S, Wiesmuller K, Saalmuller A (2009) Identification of major histocompatibility complex restriction and anchor residues of foot-and-mouth disease virus-derived bovine T-cell epitopes. *J Virol* 83, 4039-4050

119. Gibbs RA, Taylor JF, Van Tassell CP, Barendse W, Eversole KA, Gill CA, Green RD, Hamernik DL, Kappes SM, Lien S, Matukumalli LK, McEwan JC, Nazareth LV, Schnabel RD, Weinstock GM, Wheeler DA, Ajmone-Marsan P, Boettcher PJ, Caetano AR, Garcia JF, Hanotte O, Mariani P, Skow LC, Sonstegard TS, Williams JL, Diallo B, Hailemariam L, Martinez ML, Morris CA, Silva LO, Spelman RJ, Mulatu W, Zhao K, Abbey CA, Agaba M, Araujo FR, Bunch RJ, Burton J, Gorni C, Olivier H, Harrison BE, Luff B, Machado MA, Mwakaya J, Plastow G, Sim W, Smith T, Thomas MB, Valentini A, Williams P, Womack J, Woolliams JA, Liu Y, Qin X, Worley KC, Gao C, Jiang H, Moore SS, Ren Y, Song XZ, Bustamante CD, Hernandez RD, Muzny DM, Patil S, San Lucas A, Fu Q, Kent MP, Vega R, Matukumalli A, McWilliam S, Sclep G, Bryc K, Choi J, Gao H, Grefenstette JJ, Murdoch B, Stella A, Villa-Angulo R, Wright M, Aerts J, Jann O, Negrini R, Goddard ME, Hayes BJ, Bradley DG, Barbosa da Silva M, Lau LP, Liu GE, Lynn DJ, Panzitta F, Dodds KG (2009) Genome-wide survey of SNP variation uncovers the genetic structure of cattle breeds. *Science* 324, 528-532
120. Glass E (2007) Genetic variation and responses to vaccines. *Anim Health Res Rev* 5, 197-208
121. Glass EJ, Oliver RA, Russell GC (2000) Duplicated DQ haplotypes increase the complexity of restriction element usage in cattle. *J Immunol* 165, 134-138
122. Glenn T, Stephan W, Dessauer H, Braun M (1996) Allelic diversity in alligator microsatellite loci is negatively correlated with GC content of flanking sequences and evolutionary conservation of PCR amplifiability. *Mol Biol Evol* 13, 1151-1154
123. Goldammer T, Brunner R, Schwerin M (1997) Comparative analysis of Y chromosome structure in *Bos taurus* and *B. indicus* by FISH using region-specific, microdissected, and locus-specific DNA probes. *Cytogenet Cell Genet* 77, 238-241
124. Goldammer T, Zerbe H, Molenaar A, Schuberth H, Brunner R, Kata S, Seyfert H (2004) Mastitis increases mammary mRNA abundance of -defensin 5, toll-like-receptor 2 (TLR2), and TLR4 but not TLR9 in cattle. *Clin Vaccine Immunol* 11, 174-185
125. Goldstein D, Clark A (1995) Microsatellite variation in North American populations of *Drosophila melanogaster*. *Nucleic Acids Res* 23, 3882-3886
126. Goodfellow P, Jones E, Van Heyningen V, Solomon E, Bobrow M, Miggiano V, Bodmer W (1975) The beta2-microglobulin gene is on chromosome 15 and not in the HL-A region. *Nature* 254, 267-269

127. Gorer P, Lyman S, Snell G (1948) Studies on the genetic and antigenic basis of tumour transplantation. Linkage between a histocompatibility gene and 'fused' in mice. *Proc R Soc Lond B Biol Sci*, 499-505
128. Gorer PA (1936) The detection of a hereditary antigenic difference in the blood of mice by means of human group A serum. *J Genet* 32, 17-31
129. Gotherstrom A, Anderung C, Hellborg L, Elburg R, Smith C, Bradley DG, Ellegren H (2005) Cattle domestication in the Near East was followed by hybridization with aurochs bulls in Europe. *Proc Biol Sci* 272, 2345-2350
130. Gourraud P, Feolo M, Hoffman D, Helmberg W, Cambon-Thomsen A (2006) The dbMHC microsatellite portal: a public resource for the storage and display of MHC microsatellite information. *Tissue Antigens* 67, 395-401
131. Graham R, Ortmann W, Rodine P, Espe K, Langefeld C, Lange E, Williams A, Beck S, Kyogoku C, Moser K (2007) Specific combinations of HLA-DR2 and DR3 class II haplotypes contribute graded risk for disease susceptibility and autoantibodies in human SLE. *Eur J Hum Genet* 15, 823-830
132. Graser R, O'hUigin C, Vincek V, Meyer A, Klein J (1996) Trans-species polymorphism of class II Mhc loci in danio fishes. *Immunogenetics* 44, 36-48
133. Gray A (1972) Mammalian hybrids: a check-list with bibliography, revised ed. Technical Communication of the Commonwealth Bureau of Animal Breeding and Genetics, Edinburgh
134. Gregersen J, Kranc K, Ke X, Svendsen P, Madsen L, Thomsen A, Cardon L, Bell J, Fugger L (2006) Functional epistasis on a common MHC haplotype associated with multiple sclerosis. *Nature* 443, 574-577
135. Grimaldi M, Crouau-Roy B (1997) Microsatellite allelic homoplasy due to variable flanking sequences. *J Mol Evol* 44, 336-340
136. Gu Z, Zhao X, Li N, Wu C (2007) Complete sequence of the yak (*Bos grunniens*) mitochondrial genome and its evolutionary relationship with other ruminants. *Mol Phylogenet Evol* 42, 248-255
137. Guo S, Savolainen P, Su J, Zhang Q, Qi D, Zhou J, Zhong Y, Zhao X, Liu J (2006) Origin of mitochondrial DNA diversity of domestic yaks. *BMC Evol Biol* 6, 73
138. Halbert N, Derr J (2007) A comprehensive evaluation of cattle introgression into US federal bison herds. *J Hered* 98, 1-12

139. Halbert N, Raudsepp T, Chowdhary B, Derr J (2004) Conservation genetic analysis of the Texas state bison herd. *J Mammal* 85, 924-931
140. Halbert N, Ward T, Schnabel R, Taylor J, Derr J, USDA (2005) Conservation genomics: disequilibrium mapping of domestic cattle chromosomal segments in North American bison populations. *Mol Ecol* 14, 2343-2362
141. Haldane J (1922) Sex ratio and unisexual sterility in animal hybrids. *J Genet* 12, 101-109
142. Hameed K, Sender G, Korwin-Kossakowska A (2008) An association of BoLA alleles DRB3. 2* 16 and DRB3. 2* 23 with occurrence of mastitis caused by different bacterial species in two herds of dairy cows. *Anim Sci Pap Rep* 26, 37-48
143. Hancock J (1996) Simple sequences in a 'minimal' genome. *Nat Genet* 14, 14-15
144. Hassanin A, Douzery E (2003) Molecular and morphological phylogenies of ruminantia and the alternative position of the moschidae. *Syst Biol* 52, 206-228
145. Hassanin A, Douzery EJ (1999) The tribal radiation of the family Bovidae (Artiodactyla) and the evolution of the mitochondrial cytochrome b gene. *Mol Phylogenet Evol* 13, 227-243
146. Hassanin A, Ropiquet A (2004) Molecular phylogeny of the tribe Bovini (Bovidae, Bovinae) and the taxonomic status of the Kouprey, *Bos sauveli* Urbain 1937. *Mol Phylogenet Evol* 33, 896-907
147. Hassanin A, Ropiquet A (2007) Resolving a zoological mystery: the kouprey is a real species. *Proc R Soc B* 274, 2849-2855
148. Hassanin A, Ropiquet A, Cornette R, Tranier M, Pfeffer P, Candegabe P, Lemaire M (2006) Has the kouprey (*Bos sauveli* Urbain, 1937) been domesticated in Cambodia? *C R Biol* 329, 124-135
149. Hedrick P (2002) Pathogen resistance and genetic variation at MHC loci. *Evolution* 56, 1902-1908
150. Hedrick P, Parker K, Gutierrez-Espeleta G, Rattink A, Lievers K (2000) Major histocompatibility complex variation in the Arabian oryx. *Evolution* 54, 2145-2151
151. Heringstad B, Klemetsdal G, Ruane J (2000) Selection for mastitis resistance in dairy cattle: a review with focus on the situation in the Nordic countries. *Livest Prod Sci* 64, 95-106

152. Horin P, Matiasovic J (2002) A second locus and new alleles in the major histocompatibility complex class II (ELA-DQB) region in the horse. *Anim Genet* 33, 196-200
153. Horton R, Gibson R, Coghill P, Miretti M, Allcock R, Almeida J, Forbes S, Gilbert J, Halls K, Harrow J (2008) Variation analysis and gene annotation of eight MHC haplotypes: the MHC haplotype project. *Immunogenetics* 60, 1-18
154. Horton R, Wilming L, Rand V, Lovering RC, Bruford EA, Khodiyar VK, Lush MJ, Povey S, Talbot CC, Jr., Wright MW, Wain HM, Trowsdale J, Ziegler A, Beck S (2004) Gene map of the extended human MHC. *Nat Rev Genet* 5, 889-899
155. Hughes A, Hughes M, Howell C, Nei M (1994) Natural selection at the class II major histocompatibility complex loci of mammals. *Philos Trans R Soc Lond B Biol Sci* 346, 359-366
156. Hughes A, Nei M (1990) Evolutionary relationships of class II major-histocompatibility-complex genes in mammals. *Mol Biol Evol* 7, 491-514
157. Hughes A, Ota T, Nei M (1990) Positive Darwinian selection promotes charge profile diversity in the antigen-binding cleft of class I major-histocompatibility-complex molecules. *Mol Biol Evol* 7, 515-524
158. Hume D, Merrill J, Miller B, Thorn G (1955) Experiences with renal homotransplantation in the human: report of nine cases. *J Clin Invest* 34, 327-382
159. Hurt P, Walter L, Sudbrak R, Klages S, Müller I, Shiina T, Inoko H, Lehrach H, Günther E, Reinhardt R (2004) The genomic sequence and comparative analysis of the rat major histocompatibility complex. *Genome Res* 14, 631-639
160. Iannuzzi L, Di Meo G (2009) *Genome Mapping and Genomics in Animals*. Heidelberg: Springer-Verlag.
161. International HapMap Consortium (2005) A haplotype map of the human genome. *Nature* 437, 1299-1320
162. Jeffreys A, Kauppi L, Neumann R (2001) Intensely punctate meiotic recombination in the class II region of the major histocompatibility complex. *Nat Genet* 29, 217-222
163. Jeffreys A, Ritchie A, Neumann R (2000) High resolution analysis of haplotype diversity and meiotic crossover in the human TAP2 recombination hotspot. *Hum Mol Genet* 9, 725-733

164. Jongeneel C, Acha-Orbea H, Blankenstein T (1990) A polymorphic microsatellite in the tumor necrosis factor alpha promoter identifies an allele unique to the NZW mouse strain. *J Exp Med* 171, 2141-2146
165. Jongeneel C, Briant L, Udalova I, Sevin A, Nedospasov S, Cambon-Thomsen A (1991) Extensive genetic polymorphism in the human tumor necrosis factor region and relation to extended HLA haplotypes. *Proc Natl Acad Sci USA* 88, 9717-9721
166. Juliarena M, Poli M, Ceriani C, Sala L, Rodriguez E, Gutierrez S, Dolcini G, Odeon A, Esteban E (2009) Antibody response against three widespread bovine viruses is not impaired in Holstein cattle carrying bovine leukocyte antigen DRB3. 2 alleles associated with bovine leukemia virus resistance. *J Dairy Sci* 92, 375-381
167. Juliarena M, Poli M, Sala L, Ceriani C, Gutierrez S, Dolcini G, Rodriguez E, Marino B, Rodriguez-Dubra C, Esteban E (2008) Association of BLV infection profiles with alleles of the BoLA-DRB3.2 gene. *Anim Genet* 39, 432-438
168. Katti M, Ranjekar P, Gupta V (2001) Differential distribution of simple sequence repeats in eukaryotic genome sequences. *Mol Biol Evol* 18, 1161-1167
169. Kaufman J, Milne S, Göbel T, Walker B, Jacob J, Auffray C, Zoorob R, Beck S (1999) The chicken B locus is a minimal essential major histocompatibility complex. *Nature* 401, 923-925
170. Kaukinen J, Varvio S (1992) Artiodactyl retroposons: association with microsatellites and use in SINEmorph detection by PCR. *Nucleic Acids Res* 20, 2955-2958
171. Kelley J, de Bono B, Trowsdale J (2005) IRIS: A database surveying known human immune system genes. *Genomics* 85, 503-511
172. Kelm S, Dettilleux J, Freeman A, Kehrli M, Dietz A, Fox L, Butler J, Kasckovics I, Kelley D (1997) Genetic association between parameters of innate immunity and measures of mastitis in periparturient Holstein cattle. *J Dairy Sci* 80, 1767-1775
173. Kent W (2002) BLAT-the BLAST-like alignment tool. *Genome Res* 12, 656-664
174. Klungland H, Sabry A, Heringstad B, Olsen H, Gomez-Raya L, Våge D, Olsaker I, Ødegård J, Klemetsdal G, Schulman N (2001) Quantitative trait loci affecting clinical mastitis and somatic cell count in dairy cattle. *Mamm Genome* 12, 837-842

175. Koller B, Geraghty D, Shimizu Y, DeMars R, Orr H (1988) HLA-E. A novel HLA class I gene expressed in resting T lymphocytes. *Journal of immunology* (Baltimore, Md.: 1950) 141, 897
176. Kong A, Gudbjartsson D, Sainz J, Jonsdottir G, Gudjonsson S, Richardsson B, Sigurdardottir S, Barnard J, Hallbeck B, Masson G (2002) A high-resolution recombination map of the human genome. *Nat Genet* 31, 241-247
177. Konnai S, Usui T, Ikeda M, Kohara J, Hirata T, Okada K, Ohashi K, Onuma M (2006) Tumor necrosis factor-alpha genetic polymorphism may contribute to progression of bovine leukemia virus-infection. *Microbes Infect* 8, 2163-2171
178. Koorey D, Bishop G, McCaughan G (1993) Allele non-amplification: a source of confusion in linkage studies employing microsatellite polymorphisms. *Hum Mol Genet* 2, 289-291
179. Královicová J, Houngninou-Molango S, Krämer A, Vorechovsky I (2004) Branch site haplotypes that control alternative splicing. *Hum Mol Genet* 13, 3189-3202
180. Kruglyak S, Durrett R, Schug M, Aquadro C (1998) Equilibrium distributions of microsatellite repeat length resulting from a balance between slippage events and point mutations. *Proc Natl Acad Sci USA* 95, 10774-10778
181. Kuehn D (1986) Population and social characteristics of the Tamarao (*Bubalus mindorensis*). *Biotropica* 18, 263-266
182. Kumanovics A, Takada T, Lindahl KF (2003) Genomic organization of the mammalian MHC. *Annu Rev Immunol* 21, 629-657
183. Kumar S, Nagarajan M, Sandhu J, Kumar N, Behl V, Nishanth G (2007) Mitochondrial DNA analyses of Indian water buffalo support a distinct genetic origin of river and swamp buffalo. *Anim Genet* 38, 227-232
184. Lai SJ, Liu YP, Liu YX, Li XW, Yao YG (2006) Genetic diversity and origin of Chinese cattle revealed by mtDNA D-loop sequence variation. *Mol Phylogenet Evol* 38, 146-154
185. Lander E, Linton L, Birren B, Nusbaum C, Zody M, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W (2001) Initial sequencing and analysis of the human genome. *Nature* 409, 860-921
186. Lawlor D, Ward F, Ennis P, Jackson A, Parham P (1988) HLA-A and B polymorphisms predate the divergence of humans and chimpanzees. *Nature* 335, 268-271

187. Lei C, Zhang W, Chen H, Lu F, Liu R, Yang X, Zhang H, Liu Z, Yao L, Lu Z (2007) Independent maternal origin of Chinese swamp buffalo (*Bubalus bubalis*). *Anim Genet* 38, 97-102
188. Lemecha H, Mulatu W, Hussein I, Rege E, Tekle T, Abdicho S, Ayalew W (2006) Response of four indigenous cattle breeds to natural tsetse and trypanosomosis challenge in the Ghibe Valley of Ethiopia. *Vet Parasitol* 141, 165-176
189. Lenstra J, Boxtel J, Zwaagstra K, Schwerin M (1993) Short interspersed nuclear element (SINE) sequences of the Bovidae. *Anim Genet* 24, 33-39
190. Lenstra J, Bradley D (1999) Systematics and phylogeny of cattle. UK CABI Publishing, Oxfordshire
191. Li A, Davey R, Miller R, George J (2005) Mode of inheritance of amitraz resistance in a Brazilian strain of the southern cattle tick, *Boophilus microplus* (Acari: Ixodidae)*. *Exp Appl Acarol* 37, 183-198
192. Lindahl T, Karran P, Wood R (1997) DNA excision repair pathways. *Curr Opin Genet Dev* 7, 158-169
193. Lindblad-Toh K, Wade C, Mikkelsen T, Karlsson E, Jaffe D, Kamal M, Clamp M, Chang J, Kulbokas E, Zody M (2005) Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature* 438, 803-819
194. Little CC, Tyzzer EE (1916) Further experimental studies on the inheritance of susceptibility to a transplantable tumor, carcinoma (JWA) of the Japanese waltzing mouse. *J Med Res* 33, 393-453
195. Loftus RT, MacHugh DE, Bradley DG, Sharp PM, Cunningham P (1994) Evidence for two independent domestications of cattle. *Proc Natl Acad Sci USA* 91, 2757-2761
196. López-Giráldez F, Andrés O, Domingo-Roura X, Bosch M (2006) Analyses of carnivore microsatellites and their intimate association with tRNA-derived SINEs. *BMC Genomics* 7, 269
197. Macaubas C, Jin L, Hallmayer J, Kimura A, Mignot E (1997) The complex mutation pattern of a microsatellite. *Genome Res* 7, 635-641
198. MacEachern S, McEwan J, Goddard M (2009) Phylogenetic reconstruction and the identification of ancient polymorphism in the Bovini tribe (Bovidae, Bovinae). *BMC Genomics* 10, 177

199. MacHugh DE, Loftus RT, Cunningham P, Bradley DG (1998) Genetic structure of seven European cattle breeds assessed using 20 microsatellite markers. *Anim Genet* 29, 333-340
200. Maillard J, Berthier D, Chantal I, Thevenon S, Sidibé I, Stachurski F, Belemsaga D, Razafindraïbé H, Elsen J (2003) Selection assisted by a BoLA-DR/DQ haplotype against susceptibility to bovine dermatophilosis. *Genet Sel Evol* 35, 193-200
201. Malfroy L, Roth MP, Carrington M, Borot N, Volz A, Ziegler A, Coppin H (1997) Heterogeneity in rates of recombination in the 6-Mb region telomeric to the human major histocompatibility complex. *Genomics* 43, 226-231
202. Malik H, Eickbush T (1998) The RTE class of non-LTR retrotransposons is widely distributed in animals and is the origin of many SINES. *Mol Biol Evol* 15, 1123-1134
203. Mannen H, Kohno M, Nagata Y, Tsuji S, Bradley DG, Yeo JS, Nyamsamba D, Zagdsuren Y, Yokohama M, Nomura K, Amano T (2004) Independent mitochondrial origin and historical genetic differentiation in North Eastern Asian cattle. *Mol Phylogenet Evol* 32, 539-544
204. Marques E, Schnabel R, Stothard P, Kolbehdari D, Wang Z, Taylor J, Moore S (2008) High density linkage disequilibrium maps of chromosome 14 in Holstein and Angus cattle. *BMC Genet* 9, 45
205. Martin P, Makepeace K, Hill S, Hood D, Moxon E (2005) Microsatellite instability regulates transcription factor binding and gene expression. *Proc Natl Acad Sci USA* 102, 3800-3804
206. Matsuo MY, Asakawa S, Shimizu N, Kimura H, Nonaka M (2002) Nucleotide sequence of the MHC class I genomic region of a teleost, the medaka (*Oryzias latipes*). *Immunogenetics* 53, 930-940
207. Matukumalli L, Lawley C, Schnabel R, Taylor J, Allan M, Heaton M, O'Connell J, Moore S, Smith T, Sonstegard T (2009) Development and characterization of a high density SNP genotyping assay for cattle. *PLoS ONE* 4, e5350
208. Mayer W, Jonker M, Klein D, Ivanyi P, van Seventer G, Klein J (1988) Nucleotide sequences of chimpanzee MHC class I alleles: evidence for trans-species mode of evolution. *EMBO J* 7, 2765-2774
209. McConnell T, Talbot W, McIndoe R, Wakeland E (1988) The origin of MHC class II gene polymorphism within the genus *Mus*. *Nature* 332, 651-654

210. McKeever D, Taracha E, Morrison W, Musoke A, Morzaria S (1999) Protective immune mechanisms against *Theileria parva*: evolution of vaccine development strategies. *Parasitol Today* 15, 263-267
211. McMaster M, Librach C, Zhou Y, Lim K, Janatpour M, DeMars R, Kovats S, Damsky C, Fisher S (1995) Human placental HLA-G expression is restricted to differentiated cytotrophoblasts. *J Immunol* 154, 3771-3778
212. Mellon I, Rajpal D, Koi M, Boland C, Champe G (1996) Transcription-coupled repair deficiency and mutations in human mismatch repair genes. *Science* 272, 557-560
213. Meo G, Perucatti A, Floriot S, Incarnato D, Rullo R, Jambrenghi A, Ferretti L, Vonghia G, Cribiu E, Eggen A (2005) Chromosome evolution and improved cytogenetic maps of the Y chromosome in cattle, zebu, river buffalo, sheep and goat. *Chromosome Res* 13, 349-355
214. The MHC Sequencing Consortium (1999) Complete sequence and gene map of a human major histocompatibility complex. *Nature* 401, 921-923
215. Mikko S, Spencer M, Morris B, Stabile S, Basu T, Stormont C, Andersson L (1997) A comparative analysis of Mhc DRB3 polymorphism in the American bison (*Bison bison*). *J Hered* 88, 499-503
216. Miltiadou D, Law A, Russell G (2003) Establishment of a sequence-based typing system for BoLA-DRB3 exon 2. *Tissue Antigens* 62, 55-65
217. Miretti M, Walsh E, Ke X, Delgado M, Griffiths M, Hunt S, Morrison J, Whittaker P, Lander E, Cardon L (2005) A high-resolution linkage-disequilibrium map of the human major histocompatibility complex and first generation of tag single-nucleotide polymorphisms. *Am J Hum Genet* 76, 634-646
218. Montpetit A, Nelis M, Laflamme P, Magi R, Ke X, Remm M, Cardon L, Hudson T, Metspalu A (2006) An evaluation of the performance of tag SNPs derived from HapMap in a Caucasian population. *PLoS Genet* 2, e27
219. Morgante M, Hanafey M, Powell W (2002) Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. *Nat Genet* 30, 194-200
220. Mountain J, Knight A, Jobin M, Gignoux C, Miller A, Lin A, Underhill P (2002) SNPSTRs: empirically derived, rapidly typed, autosomal haplotypes for inference of population history and mutational processes. *Genome Res* 12, 1766-1772

221. Mungall AJ, Palmer SA, Sims SK, Edwards CA, Ashurst JL, Wilming L, Jones MC, Horton R, Hunt SE, Scott CE, Gilbert JG, Clamp ME, Bethel G, Milne S, Ainscough R, Almeida JP, Ambrose KD, Andrews TD, Ashwell RI, Babbage AK, Bagguley CL, Bailey J, Banerjee R, Barker DJ, Barlow KF, Bates K, Beare DM, Beasley H, Beasley O, Bird CP, Blakey S, Bray-Allen S, Brook J, Brown AJ, Brown JY, Burford DC, Burrill W, Burton J, Carder C, Carter NP, Chapman JC, Clark SY, Clark G, Clee CM, Clegg S, Cobley V, Collier RE, Collins JE, Colman LK, Corby NR, Coville GJ, Culley KM, Dhami P, Davies J, Dunn M, Earthrowl ME, Ellington AE, Evans KA, Faulkner L, Francis MD, Frankish A, Frankland J, French L, Garner P, Garnett J, Ghorri MJ, Gilby LM, Gillson CJ, Glithero RJ, Grafham DV, Grant M, Gribble S, Griffiths C, Griffiths M, Hall R, Halls KS, Hammond S, Harley JL, Hart EA, Heath PD, Heathcott R, Holmes SJ, Howden PJ, Howe KL, Howell GR, Huckle E, Humphray SJ, Humphries MD, Hunt AR, Johnson CM, Joy AA, Kay M, Keenan SJ, Kimberley AM, King A, Laird GK, Langford C, Lawlor S, Leongamornlert DA, Leversha M, Lloyd CR, Lloyd DM, Loveland JE, Lovell J, Martin S, Mashreghi-Mohammadi M, Maslen GL, Matthews L, McCann OT, McLaren SJ, McLay K, McMurray A, Moore MJ, Mullikin JC, Niblett D, Nickerson T, Novik KL, Oliver K, Overton-Larty EK, Parker A, Patel R, Pearce AV, Peck AI, Phillimore B, Phillips S, Plumb RW, Porter KM, Ramsey Y, Ranby SA, Rice CM, Ross MT, Searle SM, Sehra HK, Sheridan E, Skuce CD, Smith S, Smith M, Spraggon L, Squares SL, Steward CA, Sycamore N, Tamlyn-Hall G, Tester J, Theaker AJ, Thomas DW, Thorpe A, Tracey A, Tromans A, Tubby B, Wall M, Wallis JM, West AP, White SS, Whitehead SL, Whittaker H, Wild A, Willey DJ, Wilmer TE, Wood JM, Wray PW, Wyatt JC, Young L, Younger RM, Bentley DR, Coulson A, Durbin R, Hubbard T, Sulston JE, Dunham I, Rogers J, Beck S (2003) The DNA sequence and analysis of human chromosome 6. *Nature* 425, 805-811
222. Myers S, Bottolo L, Freeman C, McVean G, Donnelly P (2005) A fine-scale map of recombination rates and hotspots across the human genome. *Science* 310, 321-324
223. Nachman M, Crowell S (2000) Estimate of the mutation rate per nucleotide in humans. *Genetics* 156, 297-304
224. Nadir E, Margalit H, Gallily T, Ben-Sasson S (1996) Microsatellite spreading in the human genome: evolutionary mechanisms and structural implications. *Proc Natl Acad Sci USA* 93, 6470-6475
225. National Center for Biotechnology Information (July, 2009) NCBI database SNP summary. http://www.ncbi.nlm.nih.gov/SNP/snp_summary.cgi
226. Naughton JM, O'Dea K, Sinclair AJ (1986) Animal foods in traditional Australian aboriginal diets: polyunsaturated and low in fat. *Lipids* 21, 684-690

227. Newman M, Truax R, French D, Dietrich M, Franke D, Stear M (1996) Evidence for genetic control of vaccine-induced antibody responses in cattle. *Vet Immunol Immunopathol* 50, 43-54
228. Nguyen T, Aniskin V, Gerbault-Seureau M, Planton H, Renard J, Nguyen B, Hassanin A, Volobouev V (2008) Phylogenetic position of the saola (*Pseudoryx nghetinhensis*) inferred from cytogenetic analysis of eleven species of Bovidae. *Cytogenet Genome Res* 122, 41-54
229. Nguyen T, Genini S, Bui L, Voegeli P, Stranzinger G, Renard J, Maillard J, Nguyen B (2007) Genomic conservation of cattle microsatellite loci in wild gaur (*Bos gaurus*) and current genetic status of this species in Vietnam. *BMC Genet* 8, 77
230. Niedermann G, Grimm R, Geier E, Maurer M, Realini C, Gartmann C, Soll J, Omura S, Rechsteiner MC, Baumeister W, Eichmann K (1997) Potential immunocompetence of proteolytic fragments produced by proteasomes before evolution of the vertebrate immune system. *J Exp Med* 186, 209-220
231. Nijman I, Otsen M, Verkaar E, De Ruijter C, Hanekamp E, Ochieng J, Shamshad S, Rege J, Hanotte O, Barwegen M (2003) Hybridization of banteng (*Bos javanicus*) and zebu (*Bos indicus*) revealed by mitochondrial DNA, satellite DNA, AFLP and microsatellites. *Heredity* 90, 10-16
232. Nijman I, van Boxtel D, van Cann L, Marnoch Y, Cuppen E, Lenstra J (2008) Phylogeny of Y chromosomes from bovine species. *Cladistics* 24, 723-726
233. Norimine J, Brown W (2005) Intrahaplotype and interhaplotype pairing of bovine leukocyte antigen DQA and DQB molecules generate functional DQ molecules important for priming CD4+ T-lymphocyte responses. *Immunogenetics* 57, 750-762
234. Oetting W, Lee H, Flanders D, Wiesner G, Sellers T, King R (1995) Linkage analysis with multiplexed short tandem repeat polymorphisms using infrared fluorescence and M13 tailed primers. *Genomics* 30, 450-458
235. Ohno S (1970) *Evolution by gene duplication*. Springer-Verlag, New York
236. Ohta Y, McKinney EC, Criscitiello MF, Flajnik MF (2002) Proteasome, transporter associated with antigen processing, and class I genes in the nurse shark *Ginglymostoma cirratum*: evidence for a stable class I region and MHC haplotype lineages. *J Immunol* 168, 771-781
237. Onami J, Nikaido M, Mannen H, Okada N (2007) Genomic expansion of the Bov-A2 retroposon relating to phylogeny and breed management. *Mamm Genome* 18, 187-196

238. University of Sydney and Australian National Genomic Information Service (July, 2009) Online mendelian inheritance in animals. <http://omia.angis.org.au/>
239. OSU (July, 2009) Oklahoma State University breeds of livestock. <http://www.ansi.okstate.edu/breeds/cattle/>
240. Otten A, Tapscott S (1995) Triple repeat expansion in myotonic dystrophy alters the adjacent chromatin structure. *Proc Natl Acad Sci USA* 92, 5465- 5469
241. Ovsyannikova I, Pankratz V, Vierkant R, Jacobson R, Poland G (2006) Human leukocyte antigen haplotypes in the genetic control of immune response to measles-mumps-rubella vaccine. *J Infect Dis* 193, 655-663
242. Park Y, Joo Y, Park J, Moon J, Kim S, Kwon N, Ahn J, Davis W, Davies C (2004) Characterization of lymphocyte subpopulations and major histocompatibility complex haplotypes of mastitis-resistant and susceptible cows. *J Vet Sci* 5, 29-40
243. Penn D, Potts W (1999) The evolution of mating preferences and major histocompatibility complex genes. *Am Nat* 153, 145-164
244. Perkins D, Jr. (1969) Fauna of catal huyuk: evidence for early cattle domestication in Anatolia. *Science* 164, 177-179
245. Perzanowski K, Olech W (2007) A future for European Bison *bison bonasus* in the Carpathian ecoregion? *Wildlife Biol* 13, 108-112
246. Pilgrim G (1939) The fossil bovidae of India, memoirs of the geological survey of India. *Palaeontol Indica* 26, 1-356.
247. Pilgrim G, Hopwood A (1947) The evolution of the buffaloes, oxen, sheep and goats. *Zool J Linn Soc* 41, 272-286
248. Preston P, Hall F, Glass E, Campbell J, Darghouth M, Ahmed J, Shiels B, Spooner R, Jongejan F, Brown C (1999) Innate and adaptive immune responses co-operate to protect cattle against *Theileria annulata*. *Parasitol Today* 15, 268-274
249. Ptak S, Roeder A, Stephens M, Gilad Y, Paabo S, Przeworski M (2004) Absence of the TAP2 human recombination hotspot in chimpanzees. *PLoS Biol* 2, 849-855
250. Pushkina D, Raia P (2008) Human influence on distribution and extinctions of the late Pleistocene Eurasian megafauna. *J Hum Evol* 54, 769-782

251. Rakyant V, Hildmann T, Novik K, Lewin J, Tost J, Cox A, Andrews T, Howe K, Otto T, Olek A (2004) DNA methylation profiling of the human major histocompatibility complex: a pilot study for the human epigenome project. *PLoS Biol* 2, e405
252. Ramakrishnan U, Mountain J (2004) Precision and accuracy of divergence time estimates from STR and SNPSTR variation. *Mol Biol Evol* 21, 1960-1971
253. Rambaut A (July, 2009) FigTree. <http://tree.bio.ed.ac.uk/software/figtree/>
254. Raymond C, Kas A, Paddock M, Qiu R, Zhou Y, Subramanian S, Chang J, Palmieri A, Haugen E, Kaul R (2005) Ancient haplotypes of the HLA Class II region. *Genome Res* 15, 1250-1257
255. Regitano L, Ibelli A, Gasparin G, Miyata M, Azevedo A, Coutinho L, Teodoro R, Machado M, Silva M, Nakata L (2008) On the search for markers of tick resistance in bovines. *Dev Biol (Basel)* 132, 225-230
256. Rhodes D, Stammers M, Malcherek G, Beck S, Trowsdale J (2001) The cluster of BTN genes in the extended major histocompatibility complex. *Genomics* 71, 351-362
257. Ritz L, Glowatzki-Mullis M, MacHugh D, Gaillard C (2000) Phylogenetic analysis of the tribe Bovini using microsatellites. *Anim Genet* 31, 178-185
258. Robin ED, Wong R (1988) Mitochondrial DNA molecules and virtual number of mitochondria per cell in mammalian cells. *J Cell Physiol* 136, 507-513
259. Robinson J, Waller MJ, Parham P, de Groot N, Bontrop R, Kennedy LJ, Stoehr P, Marsh SG (2003) IMGT/HLA and IMGT/MHC: sequence databases for the study of the major histocompatibility complex. *Nucleic Acids Res* 31, 311-314
260. Rockman M, Wray G (2002) Abundant raw material for cis-regulatory evolution in humans. *Mol Biol Evol* 19, 1991-2004
261. Roosen S, Exner K, Paul S, Schröder J, Kalm E, Looft C (2004) Bovine β -defensins: identification and characterization of novel bovine β -defensin genes and their expression in mammary gland tissue. *Mamm Genome* 15, 834-842
262. Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol* 132, 365-386
263. Russell G (2000) Sequence duplication at the 3' end of BoLA-DQB genes suggests multiple allelic lineages. *Immunogenetics* 52, 101-106

264. Sabatti C, Risch N (2002) Homozygosity and linkage disequilibrium. *Genetics* 160, 1707-1719
265. Sachdev M, Sankaranarayanan R, Reddanna P, Thangaraj K, Singh L (2005) Major histocompatibility complex class I polymorphism in Asiatic lions. *Tissue Antigens* 66, 9-18
266. Sachidanandam R, Weissman D, Schmidt S, Kakol J, Stein L, Marth G, Sherry S, Mullikin J, Mortimore B, Willey D (2001) A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* 409, 928-933
267. Sanderson M, Donoghue M (1989) Patterns of variation in levels of homoplasmy. *Evolution* 43, 1781-1795
268. Santos P, Füst G, Prohászka Z, Volz A, Horton R, Miretti M, Yu C, Beck S, Uchanska-Ziegler B, Ziegler A (2008) Association of smoking behavior with an odorant receptor allele telomeric to the human major histocompatibility complex. *Genet Test* 12, 481-486
269. Sato A, Figueroa F, Murray BW, Malaga-Trillo E, Zaleska-Rutczynska Z, Sultmann H, Toyosawa S, Wedekind C, Steck N, Klein J (2000) Nonlinkage of major histocompatibility complex class I and class II loci in bony fishes. *Immunogenetics* 51, 108-116
270. Savolainen P, Zhang Y, Luo J, Lundeberg J, Leitner T (2002) Genetic evidence for an East Asian origin of domestic dogs. *Science* 298, 1610-1613
271. Scheet P, Stephens M (2006) A fast and flexible statistical model for large-scale population genotype data: applications to inferring missing genotypes and haplotypic phase. *Am J Hum Genet* 78, 629-644
272. Schlotterer C, Tautz D (1992) Slippage synthesis of simple sequence DNA. *Nucleic Acids Res* 20, 211-215
273. Schneider P, Carroll M, Alper C, Rittner C, Whitehead A, Yunis E, Colten H (1986) Polymorphism of the human complement C4 and steroid 21-hydroxylase genes. Restriction fragment length polymorphisms revealing structural deletions, homoduplications, and size variants. *J Clin Invest* 78, 650-657
274. Schreiber A, Seibold I, Notzold G, Wink M (1999) Cytochrome b gene haplotypes characterize chromosomal lineages of anoa, the Sulawesi dwarf buffalo. *J Hered* 90, 165-176

275. Shapiro B, Drummond A, Rambaut A, Wilson M, Matheus P, Sher A, Pybus O, Gilbert M, Barnes I, Binladen J (2004) Rise and fall of the Beringian steppe bison. *Science* 306, 1561-1565
276. Sharif S, Mallard B, Sargeant J (2000) Presence of glutamine at position 74 of pocket 4 in the BoLA-DR antigen binding groove is associated with occurrence of clinical mastitis caused by *Staphylococcus* species. *Vet Immunol Immunopathol* 76, 231-238
277. Sharif S, Mallard B, Wilkie B, Sargeant J, Scott H, Dekkers J, Leslie K (1998) Associations of the bovine major histocompatibility complex DRB3 (BoLA-DRB3) alleles with occurrence of disease and milk somatic cell score in Canadian dairy cattle. *Anim Genet* 29, 185-193
278. Shiina T, Inoko H, Kulski J (2004) An update of the HLA genomic region, locus information and disease associations. *Tissue Antigens* 64, 631-649
279. Shiina T, Ota M, Shimizu S, Katsuyama Y, Hashimoto N, Takasu M, Anzai T, Kulski J, Kikkawa E, Naruse T (2006) Rapid evolution of major histocompatibility complex class I genes in primates generates new disease alleles in humans via hitchhiking diversity. *Genetics* 173, 1555-1570
280. Shiina T, Tamiya G, Oka A, Takishima N, Yamagata T, Kikkawa E, Iwata K, Tomizawa M, Okuaki N, Kuwano Y (1999) Molecular dynamics of MHC genesis unraveled by sequence analysis of the 1,796,938-bp HLA class I region. *Proc Natl Acad Sci USA* 96, 13282-13287
281. Sibly R, Meade A, Boxall N, Wilkinson M, Corne D, Whittaker J (2003) The structure of interrupted human AC microsatellites. *Mol Biol Evol* 20, 453-459
282. Sigurdardóttir S, Borsch C, Gustafsson K, Andersson L (1992) Gene duplications and sequence polymorphism of bovine class II DQB genes. *Immunogenetics* 35, 205-213
283. Sigurdardóttir S, Lundén A, Andersson L (1988) Restriction fragment length polymorphism of DQ and DR class II genes of the bovine major histocompatibility complex. *Anim Genet* 19, 133-150
284. Sigurdsson M, Smith A, Bjornsson H, Jonsson J (2009) HapMap methylation-associated SNPs, markers of germline DNA methylation, positively correlate with regional levels of human meiotic recombination. *Genome Res* 19, 581-589
285. Sitte K, Brinkworth R, East I, Jazwinska E (2002) A single amino acid deletion in the antigen binding site of BoLA-DRB3 is predicted to affect peptide binding. *Vet Immunol Immunopathol* 85, 129-135

286. Sivori S, Vitale M, Bottino C, Marcenaro E, Sanseverino L, Parolini S, Moretta L, Moretta A (1996) CD94 functions as a natural killer cell inhibitory receptor for different HLA class I alleles: identification of the inhibitory form of CD94 by the use of novel monoclonal antibodies. *Eur J Immunol* 26, 2487-2492
287. Smit A, Hubley R, Green P, Institute for Systems Biology (July 2009) Repeatmasker open-3.0. <http://www.repeatmasker.org>
288. Smith LC, Shih CS, Dachenhausen SG (1998) Coelomocytes express SpBf, a homologue of factor B, the second component in the sea urchin complement system. *J Immunol* 161, 6784-6793
289. Smith T, Rohrer G, Alexander L, Troyer D, Kirby-Dobbels K, Janzen M, Cornwell D, Louis C, Schook L, Beattie C (1995) Directed integration of the physical and genetic linkage maps of swine chromosome 7 reveals that the SLA spans the centromere. *Genome Res* 5, 259-271
290. Snell GD (1948) Methods for the study of histocompatibility genes. *J Genet* 49, 87-108
291. Snell GD, Higgins GF (1951) Alleles at the histocompatibility-2 locus in the mouse as determined by tumor transplantation. *Genetics* 36, 306-310
292. Spencer C, Deloukas P, Hunt S, Mullikin J, Myers S, Silverman B, Donnelly P, Bentley D, McVean G (2006) The influence of recombination on human genetic diversity. *PLoS Genetics* 2, e148
293. Starckenburg R, Hansen L, Kehrli M, Chester-Jones H (1997) Frequencies and effects of alternative DRB3.2 alleles of bovine lymphocyte antigen for Holsteins in milk selection and control lines. *J Dairy Sci* 80, 3411-3419
294. Stephens M, Smith N, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 68, 978-989
295. Stephens R, Horton R, Humphray S, Rowen L, Trowsdale J, Beck S (1999) Gene organisation, sequence variation and isochore structure at the centromeric boundary of the human MHC. *J Mol Biol* 291, 789-799
296. Stern L, Brown J, Jardetzky T, Gorga J, Urban R, Strominger J, Wiley D (1994) Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. *Nature* 368, 215-221

297. Stewart CA, Horton R, Allcock RJ, Ashurst JL, Atrazhev AM, Coggill P, Dunham I, Forbes S, Halls K, Howson JM, Humphray SJ, Hunt S, Mungall AJ, Osoegawa K, Palmer S, Roberts AN, Rogers J, Sims S, Wang Y, Wilming LG, Elliott JF, de Jong PJ, Sawcer S, Todd JA, Trowsdale J, Beck S (2004) Complete MHC haplotype sequencing for common disease gene mapping. *Genome Res* 14, 1176-1187
298. Stone J (2004) Sheltered load associated with S-alleles in *Solanum carolinense*. *Heredity* 92, 335-342
299. Strand M, Prolla T, Liskay R, Petes T (1993) Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. *Nature* 365, 274-276
300. Swierkosz JE, Rock K, Marrack P, Kappler JW (1978) The role of H-2 linked genes in helper T-cell function. II. Isolation on antigen-pulsed macrophages of two separate populations of F1 helper T cells each specific for antigen and one set of parental H-2 products. *J Exp Med* 147, 554-570
301. Takahashi K, Rooney AP, Nei M (2000) Origins and divergence times of mammalian class II MHC gene clusters. *J Hered* 91, 198-204
302. Takahata N, Satta Y, Klein J (1992) Polymorphism and balancing selection at major histocompatibility complex loci. *Genetics* 130, 925
303. Takeda K, Satoh M, Neopane S, Kuwar B, Joshi H, Shrestha N, Fujise H, Tasai M, Tagami T, Hanada H (2004) Mitochondrial DNA analysis of Nepalese domestic dwarf cattle Lulu*. *Anim Sci J* 75, 103-110
304. Takeshima S, Matsumoto Y, Chen J, Yoshida T, Mukoyama H, Aida Y (2008) Evidence for cattle major histocompatibility complex (BoLA) class II DQA1 gene heterozygote advantage against clinical mastitis caused by *Streptococci* and *Escherichia* species. *Tissue Antigens* 72, 525-531
305. Tautz D (1994) Simple sequences. *Curr Opin Genet Dev* 4, 832-837
306. Tellam R, Lemay D, Van Tassell C, Lewin H, Worley K, Elsik C (2009) Unlocking the bovine genome. *BMC Genomics* 10, 193
307. Terasaki P (1991) *History of Transplantation: Thirty-Five Recollections*. Los Angeles: UCLA Tissue Typing Laboratory.
308. Teuber M (2001) Veterinary use and antibiotic resistance. *Curr Opin Microbiol* 4, 493-499

309. Thompson D, Muriel P, Russell D, Osborne P, Bromley A, Rowland M, Creigh-Tyte S, Brown C (2002) Economic costs of the foot and mouth disease outbreak in the United Kingdom in 2001. *Rev Sci Tech* 21, 675-685
310. Tishkoff S, Pakstis A, Stoneking M, Kidd J, Destro-Bisol G, Sanjantila A, Lu R, Deinard A, Sirugo G, Jenkins T (2000) Short tandem-repeat polymorphism/Alu haplotype variation at the PLAT locus: implications for modern human origins. *Am J Hum Genet* 67, 901-925
311. Tishkoff SA, Reed FA, Ranciaro A, Voight BF, Babbitt CC, Silverman JS, Powell K, Mortensen HM, Hirbo JB, Osman M, Ibrahim M, Omar SA, Lema G, Nyambo TB, Ghorri J, Bumpstead S, Pritchard JK, Wray GA, Deloukas P (2007) Convergent adaptation of human lactase persistence in Africa and Europe. *Nat Genet* 39, 31-40
312. Tóth G, Gáspári Z, Jurka J (2000) Microsatellites in different eukaryotic genomes: survey and analysis. *Genome Res* 10, 967-981
313. Trachtulec Z, Forejt J (2001) Synteny of orthologous genes conserved in mammals, snake, fly, nematode, and fission yeast. *Mamm Genome* 12, 227-231
314. Traherne J, Barcellos L, Sawcer S, Compston A, Ramsay P, Hauser S, Oksenberg J, Trowsdale J (2006a) Association of the truncating splice site mutation in BTNL2 with multiple sclerosis is secondary to HLA-DRB1* 15. *Hum Mol Genet* 15, 155-161
315. Traherne J, Horton R, Roberts A, Miretti M, Hurles M, Stewart C, Ashurst J, Atrazhev A, Coggill P, Palmer S (2006b) Genetic analysis of completely sequenced disease-associated MHC haplotypes identifies shuffling of segments in recent human history. *PLoS Genet* 2, 81-91
316. Traherne JA (2008) Human MHC architecture and evolution: implications for disease association studies. *Int J Immunogenet* 35, 179-192
317. Traul D, Bhushan B, Eldridge J, Crawford T, Li H, Davies C (2005) Characterization of Bison bison major histocompatibility complex class IIa haplotypes. *Immunogenetics* 57, 845-854
318. Troy CS, MacHugh DE, Bailey JF, Magee DA, Loftus RT, Cunningham P, Chamberlain AT, Sykes BC, Bradley DG (2001) Genetic evidence for Near-Eastern origins of European cattle. *Nature* 410, 1088-1091
319. Udina I, Karamysheva E, Turkova S, Orlova A, Sulimova G (2003) Genetic mechanisms of resistance and susceptibility to leukemia in Ayrshire and black pied cattle breeds determined by allelic distribution of gene Bola-DRB3. *Russ J Genet* 39, 306-317

320. Uinuk-ool TS, Mayer WE, Sato A, Takezaki N, Benyon L, Cooper MD, Klein J (2003) Identification and characterization of a TAP-family gene in the lamprey. *Immunogenetics* 55, 38-48
321. Untalan P, Pruett J, Steelman C (2007) Association of the bovine leukocyte antigen major histocompatibility complex class II DRB3* 4401 allele with host resistance to the Lone Star tick, *Amblyomma americanum*. *Vet Parasitol* 145, 190-195
322. USDA Economic Research Service (July, 2009) US beef and cattle industry: background statistics and information. <http://www.ers.usda.gov/news/BSECoverage.htm>
323. Uyenoyama M (1997) Genealogical structure among alleles regulating self-incompatibility in natural populations of flowering plants. *Genetics* 147, 1389-1400
324. Van Den Bussche R, Ross T, Hooper S, Blake B (2002) Genetic variation at a major histocompatibility locus within and among populations of white-tailed deer (*Odocoileus virginianus*). *J Mammal* 83, 31-39
325. Van Hooft W, Groen A, Prins H (2000) Microsatellite analysis of genetic diversity in African buffalo (*Syncerus caffer*) populations throughout Africa. *Mol Ecol* 9, 2017-2025
326. Van Hooft W, Groen A, Prins H (2002) Phylogeography of the African buffalo based on mitochondrial and Y-chromosomal loci: Pleistocene origin and population expansion of the Cape buffalo subspecies. *Mol Ecol* 11, 267-279
327. Van Oosterhout C (2009) A new theory of MHC evolution: beyond selection on the immune genes. *Proc R Soc B* 276, 657-665
328. Van Rood J, Van Leeuwen A, Schippers A, Balner H (1968) Human histocompatibility antigens in normal and neoplastic tissues. *Cancer Res* 28, 1415-1422
329. Verkaar E, Nijman I, Beeke M, Hanekamp E, Lenstra J (2004) Maternal and paternal lineages in cross-breeding bovine species. Has wisent a hybrid origin? *Mol Biol Evol* 21, 1165-1170
330. Vienne A, Shiina T, Abi-Rached L, Danchin E, Vitiello V, Cartault F, Inoko H, Pontarotti P (2003) Evolution of the proto-MHC ancestral region: more evidence for the plesiomorphic organisation of human chromosome 9q34 region. *Immunogenetics* 55, 429-436
331. Vila C, Seddon J, Ellegren H (2005) Genes of domestic mammals augmented by backcrossing with wild ancestors. *Trends Genet* 21, 214-218

332. Vogel A, Tenggardjaja K, Edmands S, Halbert N, Derr J, Hedgecock D (2007) Detection of mitochondrial DNA from domestic cattle in bison on Santa Catalina Island. *Anim Genet* 38, 410
333. Vowles E, Amos W (2004) Evidence for widespread convergent evolution around human microsatellites. *PLoS Biol* 2, e199
334. Vyse T, Todd J (1996) Genetic analysis of autoimmune disease. *Cell* 85, 311-318
335. Wahls W, Wallace L, Moore P (1990) The Z-DNA motif d (TG) 30 promotes reception of information during gene conversion events while stimulating homologous recombination in human cells in culture. *Mol Cell Biol* 10, 785-793
336. Wan Q, Zeng C, Ni X, Pan H, Fang S (2009) Giant panda genomic data provide insight into the birth-and-death process of mammalian major histocompatibility complex class II genes. *PLoS One* 4, e4147
337. Wang N, Akey J, Zhang K, Chakraborty R, Jin L (2002) Distribution of recombination crossovers and the origin of haplotype blocks: the interplay of population history, recombination, and mutation. *Am J Hum Genet* 71, 1227-1234
338. Ward T, Bielawski J, Davis S, Templeton J, Derr J (1999) Identification of domestic cattle hybrids in wild cattle and bison species: a general approach using mtDNA markers and the parametric bootstrap. *Anim Conserv* 2, 51-57
339. Ward T, Skow L, Gallagher D, Schnabel R, Nall C, Kolenda C, Davis S, Taylor J, Derr J (2001) Differential introgression of uniparentally inherited markers in bison populations with hybrid ancestries. *Anim Genet* 32, 89-91
340. Weber J, May P (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am J Hum Genet* 44, 388-396
341. Webster J, Tonelli L, Sternberg E (2002) Neuroendocrine regulation of immunity. *Annu Rev Immunol* 20, 125-163
342. Wedekind C, Seebeck T, Bettens F, Paepke A (1995) MHC-dependent mate preferences in humans. *Proc Biol Sci* 260, 245-249
343. Weimann C, Kraus M, Gauly M, Erhardt G (2003) Differences in recombination rates on chromosome 23 between German Angus and German Simmental and breed specific linkage mapping. *Anim Genet* 34, 229-231
344. Wenink P, Groen A, Roelke-Parker M, Prins H (1998) African buffalo maintain high genetic diversity in the major histocompatibility complex in spite of historically known population bottlenecks. *Mol Ecol* 7, 1315-1322

345. White S, Kata S, Womack J (2003) Comparative fine maps of bovine toll-like receptor 4 and toll-like receptor 2 regions. *Mamm Genome* 14, 149-155
346. Wierdl M, Dominska M, Petes T (1997) Microsatellite instability in yeast: dependence on the length of the microsatellite. *Genetics* 146, 769-779
347. Wilson A, Cann R, Carr S, George M, Gyllensten U, Helm-Bychowski K, Higuch R, Palumbi S, Prager E, Sage R, Stoneking M (1985) Mitochondrial DNA and two perspectives on evolutionary genetics. *Biol J Linn Soc Lond* 26, 375-400
348. Wilson M, Hills L, Shapiro B (2008) Late Pleistocene northward-dispersing *Bison antiquus* from the Bighill Creek Formation, Gallelli Gravel Pit, Alberta, Canada, and the fate of *Bison occidentalis*. *Can J Earth Sci* 45, 827-859
349. Wilson M, Kenady S, Schalk R (2009) Late Pleistocene *Bison antiquus* from Orcas Island, Washington, and the biogeographic importance of an early postglacial land mammal dispersal corridor from the mainland to Vancouver Island. *Quat Int* 71, 49-61
350. Winckler W, Myers S, Richter D, Onofrio R, McDonald G, Bontrop R, McVean G, Gabriel S, Reich D, Donnelly P (2005) Comparison of fine-scale recombination rates in humans and chimpanzees. *Science* 308, 107-111
351. Wojcik J, Kawalko A, Tokarska M, Jaarola M, Vallenback P, Pertoldi C (2009) Post-bottleneck mtDNA diversity in a free-living population of European bison: implications for conservation. *J Zool* 277, 81-87
352. Woolhouse M, Webster J, Domingo E, Charlesworth B, Levin B (2002) Biological and biomedical implications of the co-evolution of pathogens and their hosts. *Nat Genet* 32, 569-577
353. Xie T, Rowen L, Aguado B, Ahearn ME, Madan A, Qin S, Campbell RD, Hood L (2003) Analysis of the gene-dense major histocompatibility complex class III region and its comparison to mouse. *Genome Res* 13, 2621-2636
354. Xu S, Sun P, Zhou K, Yang G (2007) Sequence variability at three MHC loci of finless porpoises (*Neophocaena phocaenoides*). *Immunogenetics* 59, 581-592
355. Yang G, Yan J, Zhou K, Wei F (2005) Sequence variation and gene duplication at MHC DQB loci of baiji (*Lipotes vexillifer*), a Chinese river dolphin. *J Hered* 96, 310-317

356. Yang Y, Chung E, Wu Y, Savelli S, Nagaraja H, Zhou B, Hebert M, Jones K, Shu Y, Kitzmiller K (2007) Gene copy-number variation and associated polymorphisms of complement component C4 in human systemic lupus erythematosus (SLE): low copy number is a risk factor for and high copy number is a protective factor against SLE susceptibility in European Americans. *Am J Hum Genet* 80, 1037-1054
357. Yoshino M, Xiao H, Jones EP, Kumanovics A, Amadou C, Fischer Lindahl K (1997) Genomic evolution of the distal Mhc class I region on mouse Chr 17. *Hereditas* 127, 141-148
358. Yu Y, Nie L, He Z, Wen J, Jian C, Zhang Y (1999) Mitochondrial DNA variation in cattle of south China: origin and introgression. *Anim Genet* 30, 245-250
359. Yuhki N, Beck T, Stephens R, Neelam B, O'Brien S (2007) Comparative genomic structure of human, dog, and cat MHC: HLA, DLA, and FLA. *J Hered* 98, 390-399
360. Yuhki N, Mullikin J, Beck T, Stephens R, O'Brien S (2008) Sequences, annotation and single nucleotide polymorphism of the major histocompatibility complex in the domestic cat. *PLoS ONE* 3, e2674
361. Zai G, Bezchlibnyk Y, Richter M, Arnold P, Burroughs E, Barr C, Kennedy J (2004) Myelin oligodendrocyte glycoprotein (MOG) gene is associated with obsessive-compulsive disorder Gwyneth Zai and Yarema B. Bezchlibnyk contributed equally to this work. *Am J Med Genet B Neuropsychiatr Genet* 129, 64-68
362. Zanotti M, Poli G, Ponti W, Polli M, Rocchi M, Bolzani E, Longeri M, Russo S, Lewin H, van Eijk M (1996) Association of BoLA class II haplotypes with subclinical progression of bovine leukaemia virus infection in Holstein-Friesian cattle. *Anim Genet* 27, 337-341
363. Zhivotovsky L, Goldstein D, Feldman M (2001) Genetic sampling error of distance ($(\Delta \mu)^2$) and variation in mutation rate among microsatellite loci. *Mol Biol Evol* 18, 2141-2145
364. Zhou H, Hickford J, Fang Q, Byun S (2007) Short communication: Identification of allelic variation at the bovine DRA locus by polymerase chain reaction-single strand conformational polymorphism. *J Dairy Sci* 90, 1943-1946
365. Zimin A, Delcher A, Florea L, Kelley D, Schatz M, Puiu D, Hanrahan F, Pertea G, Van Tassell C, Sonstegard T (2009) A whole-genome assembly of the domestic cow, *Bos taurus*. *Genome Biol* 10, R42

366. Zinkernagel R, Doherty P (1975) H-2 compatability requirement for T-cell-mediated lysis of target cells infected with lymphocytic choriomeningitis virus. Different cytotoxic T-cell specificities are associated with structures coded for in H-2K or H-2D. *J Exp Med* 141, 1427-1436

Results of PHASE on Angus HapMap Animals for BoLA I1b

BoLA	I1b	BoLA	I1b																														
Animal ID SNP Position	7228956	7229152	7280210	7339117	7384619	7398492	7453429	7453668	7455564	7458121	7479927	7562289	7611266	7632049	7666392	Animal ID SNP Position	7228956	7229152	7280210	7339117	7384619	7398492	7453429	7453668	7455564	7458121	7479927	7562289	7611266	7632049	7666392		
ANG000001	G	G	A	G	T	G	G	C	T	C	G	G	A	G	G	ANG001778	?	G	A	G	?	G	?	?	?	C	G	G	A	G	G		
ANG000001	G	G	A	G	T	A	A	C	T	A	C	G	G	G	G	ANG001778	?	G	G	A	?	A	?	?	?	A	A	G	G	G	G		
ANG000002	G	G	A	G	G	G	G	C	T	C	G	G	A	G	G	ANG001779	?	G	G	A	?	A	?	?	?	A	A	G	G	G	G		
ANG000002	A	A	A	G	T	A	A	C	T	C	G	G	G	G	A	ANG001779	?	G	G	A	?	A	?	?	?	C	G	G	G	G	G		
ANG000003	G	G	A	A	G	G	G	C	T	C	G	A	A	G	G	ANG001780	?	G	A	A	?	G	?	?	?	C	G	A	G	G	A		
ANG000003	G	G	A	A	G	G	G	C	T	C	G	A	A	G	G	ANG001780	?	G	G	A	?	A	?	?	?	A	A	G	G	G	G		
ANG000004	G	G	A	G	T	A	A	C	T	A	G	G	G	G	G	ANG001781	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G		
ANG000004	G	G	A	T	A	G	C	T	A	A	G	G	G	G	ANG001781	?	G	G	A	?	A	?	?	?	A	A	G	G	G	G	G		
ANG000005	G	G	A	A	T	G	G	C	T	C	G	A	G	G	A	ANG001782	?	G	G	A	?	A	?	?	?	A	A	G	G	G	G		
ANG000005	G	G	A	G	T	A	A	C	T	A	G	G	G	G	G	ANG001782	?	G	G	A	?	A	?	?	?	C	G	G	G	G	G		
ANG000006	G	G	A	A	T	G	G	C	T	C	G	A	A	G	G	ANG001783	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G		
ANG000006	G	G	A	T	A	G	C	T	A	A	G	G	G	G	ANG001783	?	G	G	A	?	A	?	?	?	C	G	G	G	G	G	G		
ANG000007	G	G	A	A	G	G	G	C	T	C	G	G	A	G	G	ANG001784	?	G	A	A	?	G	?	?	?	A	A	A	A	G	G		
ANG000007	G	G	A	T	A	G	C	T	A	A	G	G	G	G	ANG001784	?	G	G	A	?	A	?	?	?	A	A	G	G	G	G	G		
ANG000008	G	G	A	G	G	G	C	T	C	G	G	A	G	G	ANG001785	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	A		
ANG000008	G	G	A	T	A	G	C	T	A	A	G	G	G	G	ANG001785	?	G	A	G	?	G	?	?	?	C	G	G	A	G	G	G		
ANG000009	G	G	A	G	T	A	A	C	T	A	G	G	G	G	A	ANG001786	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	A	
ANG000009	G	G	A	G	G	G	C	T	C	G	A	A	G	G	ANG001786	?	G	A	G	?	A	?	?	?	A	G	G	G	G	G	G		
ANG000010	G	G	A	G	G	G	C	T	C	G	G	A	A	G	G	ANG001787	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	A	
ANG000010	G	G	A	G	T	G	G	C	T	C	A	A	A	G	G	ANG001787	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	A	
ANG000011	G	G	A	G	T	A	A	C	T	A	G	G	G	G	ANG001788	?	G	A	A	?	G	?	?	?	A	A	A	A	G	G	G		
ANG000011	G	G	A	T	A	A	C	T	A	A	G	G	G	G	ANG001788	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	G		
ANG000012	A	A	A	G	T	A	A	C	T	C	G	G	G	G	A	ANG001789	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	A	
ANG000012	G	G	A	G	G	G	C	T	C	G	G	A	G	G	ANG001789	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	A		
ANG000013	A	A	A	G	T	A	A	C	T	C	G	G	A	G	A	ANG001790	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	A	
ANG000013	G	G	A	G	T	A	A	C	T	A	G	G	G	G	ANG001790	?	G	G	A	?	A	?	?	?	C	G	G	G	G	G	G		
ANG000014	G	G	A	A	T	G	G	C	T	C	A	A	G	G	A	ANG001791	?	G	A	G	?	G	?	?	?	C	G	G	A	G	G	A	
ANG000014	G	G	A	T	A	G	C	T	A	A	G	G	G	G	ANG001791	?	G	A	G	?	G	?	?	?	C	G	G	A	G	G	A		
ANG000015	G	G	A	A	T	G	G	C	T	A	A	A	A	G	G	ANG001792	?	G	A	G	?	G	?	?	?	C	G	G	A	G	G	A	
ANG000015	G	G	A	T	A	G	C	T	A	A	G	G	G	G	ANG001792	?	G	A	G	?	G	?	?	?	C	G	G	A	G	G	A		
ANG000016	A	G	A	A	G	G	C	T	C	G	A	A	G	G	ANG001793	?	G	A	A	?	G	?	?	?	C	G	G	A	?	G	G	A	
ANG000016	G	G	A	G	G	A	A	C	T	A	G	G	G	G	ANG001793	?	G	G	A	?	A	?	?	?	A	A	G	G	?	G	G	A	
ANG000017	G	G	A	G	G	G	C	T	C	G	G	A	G	G	ANG001794	?	G	A	G	?	G	?	?	?	C	G	G	A	A	G	G	A	
ANG000017	G	G	A	T	G	G	C	T	C	G	A	A	G	G	ANG001794	?	G	G	A	?	A	?	?	?	A	A	G	G	G	G	G	A	
ANG000018	G	G	A	G	G	G	C	T	C	G	G	A	A	G	G	ANG001795	?	G	A	A	?	?	?	?	?	A	A	A	A	G	G	A	
ANG000018	G	G	A	G	G	G	C	T	C	G	G	A	A	G	G	ANG001795	?	G	G	A	?	?	?	?	?	A	A	G	G	G	G	A	
ANG000019	G	G	A	A	T	G	G	C	T	C	G	A	A	G	A	ANG001796	?	G	G	A	?	A	?	?	?	C	G	G	G	G	G	A	
ANG000019	G	G	A	G	G	G	C	T	C	G	G	A	A	G	G	ANG001796	?	G	G	A	?	A	?	?	?	A	A	G	G	G	G	A	
ANG000020	G	G	A	G	T	G	G	C	T	C	G	G	A	G	G	ANG001797	?	G	A	G	?	G	?	?	?	C	G	G	A	A	G	G	
ANG000020	G	G	A	G	G	G	C	T	C	G	G	A	G	G	ANG001797	?	G	A	G	?	G	?	?	?	C	G	G	A	A	G	G	A	
ANG000021	G	G	A	A	T	G	G	C	T	C	G	A	A	G	G	ANG001798	?	G	A	G	?	G	?	?	?	C	G	G	A	A	G	G	A
ANG000021	G	G	A	T	A	G	C	T	C	G	G	G	G	G	ANG001798	?	G	A	G	?	G	?	?	?	C	G	G	A	A	G	G	A	
ANG000022	G	G	A	A	T	G	G	C	T	C	G	A	A	G	A	ANG001799	?	G	A	G	?	G	?	?	?	C	G	G	A	A	G	G	A
ANG000022	G	G	A	A	T	G	G	C	T	C	G	A	A	G	A	ANG001799	?	A	A	G	?	A	?	?	?	C	G	G	G	G	G	A	?
ANG000023	G	G	A	A	T	G	G	C	T	C	G	A	A	G	G	ANG001800	?	G	A	G	?	G	?	?	?	C	G	G	A	A	G	G	A
ANG000023	G	G	A	A	T	G	G	C	T	C	G	A	A	G	A	ANG001800	?	G	G	A	?	A	?	?	?	A	A	G	G	G	G	A	?
ANG000024	G	G	A	A	T	G	G	C	T	C	G	A	A	G	A	ANG001801	?	G	A	G	?	G	?	?	?	C	G	G	A	A	G	G	A
ANG000024	G	G	A	G	T	G	G	C	T	C	G	G	A	G	G	ANG001801	?	G	A	G	?	G	?	?	?	C	G	G	A	A	G	G	A
ANG000025	G	G	A	A	T	G	G	C	T	C	G	G	A	G	G	ANG001802	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	A	?
ANG000025	G	G	A	T	A	G	C	T	C	G	G	A	G	G	ANG001802	?	G	G	A	?	A	?	?	?	A	A	G	G	G	G	A	?	
ANG000026	G	G	A	A	G	G	C	T	C	G	A	A	G	G	ANG001803	?	G	A	A	?	G	?	?	?	A	A	A	A	G	G	A	?	?
ANG000026	G	G	A	G	G	G	C	T	C	G	G	A	A	G	G	ANG001803	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	A	?
ANG000027	G	G	A	G	T	G	G	C	T	A	A	A	A	G	G	ANG001804	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	A	?
ANG000027	G	G	A	G	G	G	C	T	C	G	G	A	A	G	G	ANG001804	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	A	?
ANG001400	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	ANG001805	?	G	A	G	?	G	?	?	?	C	G	G	A	A	G	G	A
ANG001400	?	G	A	G	?	G	?	?	?	C	G	A	A	G	G	ANG001805	?	G	G	A	?	A	?	?	?	A	A	G	G	G	G	A	?
ANG001775	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	ANG001806	?	G	A	A	?	A	?	?	?	C	G	G	G	G	G	A	?
ANG001775	?	G	A	G	?	G	?	?	?	C	G	G	A	A	G	ANG001806	?	G	G	A	?	A	?	?	?	C	G	G	G	G	G	A	?
ANG001776	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	ANG001807	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	A	?
ANG001776	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	ANG001807	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	A	?
ANG001777	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	ANG001808	?	G	A	A	?	G	?	?	?	C	G	A	A	G	G	A	?
ANG001777	?	G	A	G	?	G	?	?	?	C	G	G	A	A	G	ANG001808	?	A	A	G	?	A	?	?	?	C	G	G	G	G	A	?	?

Results of PHASE on Beefmaster and Brahman HapMap Animals for BoLA IIB

BoLA	IIB	BoLA	IIB	IIB	IIB	IIB	IIB	IIB	IIB	IIB	IIB	IIB	IIB	IIB																		
Animal ID SNP Position	7228956	7229152	7280210	7339117	7384619	7398492	7453429	7453668	7455564	7458121	7479927	7562289	7611266	7632049	7666392	Animal ID SNP Position	7228956	7229152	7280210	7339117	7384619	7398492	7453429	7453668	7455564	7458121	7479927	7562289	7611266	7632049	7666392	
BMA000001	G	G	A	A	G	G	G	C	T	C	G	G	G	G	G	BRM000001	G	G	A	A	T	G	G	C	C	A	G	G	A	G	G	
BMA000001	G	G	A	A	T	G	G	C	T	A	G	G	A	G	G	BRM000001	G	G	A	A	T	G	G	C	C	A	G	G	A	G	G	
BMA000002	G	G	A	G	T	G	G	C	T	C	G	A	A	G	G	BRM000002	G	G	A	A	T	G	G	C	T	C	G	G	A	G	G	
BMA000002	G	G	G	A	G	A	A	C	T	C	G	A	G	G	A	BRM000002	G	G	A	A	T	A	G	C	T	A	G	G	A	G	A	
BMA000003	G	G	A	A	T	G	G	C	C	A	G	G	A	G	G	BRM000003	G	G	A	A	T	G	G	C	C	A	G	G	A	G	G	
BMA000003	G	G	G	A	T	G	G	C	C	A	G	G	A	G	G	BRM000003	G	G	A	A	T	G	G	C	C	A	G	G	A	G	G	
BMA000004	G	G	A	G	T	G	G	C	T	C	G	A	A	G	A	BRM000004	G	G	A	A	?	G	G	C	C	A	?	G	A	G	G	
BMA000004	G	G	G	A	G	A	A	C	T	C	G	A	G	G	A	BRM000004	G	G	A	A	?	G	G	C	C	A	?	G	A	G	G	
BMA000005	G	G	A	A	G	G	G	C	? A	? G	A	G	G	G	A	BRM000005	G	G	A	A	T	G	G	C	C	A	G	G	A	G	G	
BMA000005	G	G	A	A	T	G	G	C	? A	? G	A	G	G	A	BRM000005	G	G	A	A	T	G	G	C	C	A	G	G	A	G	G		
BMA000006	G	G	A	A	T	G	G	C	T	A	G	G	A	G	G	BRM000006	G	G	A	A	T	G	G	C	C	A	G	G	A	G	G	
BMA000006	G	G	G	A	G	A	A	C	T	C	G	A	G	G	A	BRM000006	G	G	A	A	T	A	G	C	T	C	G	G	A	G	A	
BMA000007	G	G	A	A	T	G	G	C	T	A	A	A	A	G	G	BRM000007	G	G	A	A	T	G	G	C	T	A	G	G	A	G	G	
BMA000007	G	G	G	A	T	A	G	C	C	A	A	G	A	G	A	BRM000007	G	G	A	A	T	G	G	C	C	A	G	G	A	G	G	
BMA000008	G	G	G	A	G	A	G	C	T	C	G	G	G	G	A	BRM000008	G	G	A	A	T	G	G	C	? A	G	G	A	G	G	G	
BMA000008	G	G	G	A	T	A	A	C	T	C	G	A	G	G	A	BRM000008	G	G	A	A	T	G	G	C	? A	G	G	A	G	G	G	
BMA000009	G	G	A	A	G	G	G	C	T	C	G	A	G	G	A	BRM000014	G	G	A	A	T	G	G	C	T	A	G	G	A	G	G	
BMA000009	G	G	G	A	T	A	A	C	T	C	G	A	G	G	A	BRM000014	G	G	A	A	T	G	G	C	T	A	G	G	A	G	G	
BMA000010	G	G	A	A	T	G	G	C	T	A	A	A	A	G	A	BRM000017	G	G	A	A	T	G	G	C	C	A	? G	A	G	G	G	
BMA000010	G	G	G	A	G	A	A	C	T	C	G	A	G	G	A	BRM000017	G	G	A	A	T	G	G	C	C	A	? G	A	G	G	G	
BMA000011	G	G	A	A	T	G	G	C	? A	G	G	G	G	G	A	BRM000020	G	G	A	A	T	G	G	C	T	C	G	G	A	G	A	
BMA000011	G	G	A	A	G	G	G	C	? C	G	G	G	G	G	A	BRM000020	G	G	A	A	T	G	G	C	T	A	G	G	G	G	G	
BMA000012	G	G	A	A	T	G	G	C	? A	? G	A	G	G	A	BRM000021	G	G	A	A	T	G	G	C	? A	? G	A	G	G	G	G	G	
BMA000012	G	G	A	A	T	G	G	C	? A	? G	A	G	G	A	BRM000021	G	G	A	A	T	G	G	C	? A	? G	A	G	G	G	G	G	
BMA000013	G	G	A	A	T	G	G	C	? A	? G	A	G	G	A	BRM000022	G	G	A	A	T	G	G	C	? A	? G	A	G	G	G	G	G	
BMA000013	G	G	G	A	G	A	G	C	T	C	G	G	G	G	A	BRM000022	G	G	A	G	G	G	G	C	? C	G	G	A	G	A	G	A
BMA000014	G	G	A	A	G	G	G	C	? A	? G	A	G	G	A	BRM000025	G	G	A	A	T	G	G	C	T	A	G	G	A	G	A	G	
BMA000014	G	G	G	A	T	G	G	C	? A	? G	A	G	G	A	BRM000025	G	G	A	A	T	G	G	C	T	A	G	G	A	G	A	G	
BMA000015	G	G	A	A	G	G	G	C	T	A	G	G	A	G	G	BRM000026	G	G	A	A	T	G	G	C	T	A	G	G	A	G	A	G
BMA000015	G	G	A	G	G	G	G	C	T	C	G	G	A	G	G	BRM000026	G	G	A	A	T	G	G	C	T	A	G	G	A	G	A	G
BMA000016	G	G	A	A	T	G	G	C	T	A	G	G	A	G	G	BRM000027	G	G	A	A	T	G	G	C	T	A	A	A	A	G	G	G
BMA000016	G	G	G	A	G	A	A	C	T	C	G	A	G	A	A	BRM000027	A	G	A	G	T	G	G	C	C	A	A	G	A	G	A	G
BMA000017	G	G	A	A	T	G	G	C	C	A	G	G	A	G	G	BRM000028	G	G	A	A	T	G	G	C	C	A	G	G	G	G	G	G
BMA000017	G	G	A	A	T	G	G	C	C	A	G	G	A	G	G	BRM000028	G	G	A	A	T	G	G	C	C	A	G	G	A	G	A	G
BMA000018	G	G	A	A	T	G	G	C	T	A	G	G	A	G	G	BRM000029	G	G	A	A	T	G	G	C	C	A	? G	A	G	A	G	G
BMA000018	G	G	G	A	G	A	A	C	T	C	G	A	G	G	A	BRM000029	G	G	A	A	T	G	G	C	C	A	? G	A	G	A	G	G
BMA000019	G	G	A	A	T	G	G	C	T	A	G	G	A	G	G	BRM000030	G	G	A	A	T	G	G	C	? C	G	G	A	G	A	G	G
BMA000019	G	G	A	G	T	G	G	C	T	C	G	A	A	G	G	BRM000030	G	G	A	A	T	G	G	C	? A	G	G	A	G	A	G	G
BMA000020	A	A	A	G	T	A	A	C	T	C	G	G	G	G	G	BRM000031	G	G	A	A	T	G	G	C	C	A	? G	A	G	A	G	G
BMA000020	G	G	G	A	G	A	A	C	T	C	G	A	G	G	A	BRM000031	G	G	A	A	T	G	G	C	C	A	? G	A	G	A	G	G
BMA000021	G	G	G	A	G	A	A	C	T	C	G	A	G	G	A	BRM000032	G	G	A	A	T	G	G	C	C	A	G	G	A	G	A	G
BMA000021	G	G	A	G	A	G	A	C	T	C	G	A	G	A	A	BRM000032	G	G	A	A	T	G	G	C	C	A	G	G	A	G	A	G
BMA000022	G	G	A	A	T	G	G	C	C	A	G	G	G	G	A	BRM000033	G	G	A	A	T	G	G	C	C	A	G	G	G	G	G	G
BMA000022	G	G	G	A	G	A	G	C	T	C	G	G	G	A	A	BRM000033	G	G	A	A	T	G	G	C	C	A	G	G	A	G	A	G
BMA000023	G	G	A	A	T	G	G	C	C	A	G	G	A	G	A	BRM000034	G	G	A	A	T	G	G	C	C	A	G	G	G	G	G	G
BMA000023	G	G	A	A	G	G	G	C	C	A	G	G	A	G	A	BRM000034	G	G	A	A	T	G	G	C	C	A	G	G	A	G	A	G
BMA000024	G	G	A	A	G	G	G	C	T	A	G	G	A	G	A	BRM000035	G	G	A	A	T	G	G	C	C	A	G	G	A	G	A	G
BMA000024	G	G	G	A	G	A	G	C	T	C	G	G	G	G	A	BRM000035	G	G	A	A	T	G	G	C	C	A	G	G	A	G	A	G
																BRM000038	G	G	A	A	T	G	G	C	C	A	G	G	A	G	A	G
																BRM000038	G	G	A	A	T	G	G	C	C	A	G	G	A	G	A	G

Results of PHASE on Limousin and N'Dama HapMap Animals for BoLA IIB

BoLA	IIB	BoLA	IIB	IIB	IIB	IIB	IIB	IIB	IIB	IIB	IIB	IIB	IIB	IIB																		
Animal ID SNP Position	7228956	7229152	7280210	7339117	7384619	7398492	7453429	7453668	7455564	7458121	7479927	7562289	7611266	7632049	7666392	Animal ID SNP Position	7228956	7229152	7280210	7339117	7384619	7398492	7453429	7453668	7455564	7458121	7479927	7562289	7611266	7632049	7666392	
LMS000001	G	G	A	G	T	A	G	C	T	A	A	A	A	G	G	NDA000001	G	G	A	A	G	G	G	C	T	C	G	G	A	G	G	
LMS000001	A	G	G	A	T	A	A	C	T	C	G	G	G	G	G	NDA000001	G	G	A	G	G	G	G	C	T	C	G	G	A	G	G	
LMS000002	G	G	A	G	T	G	G	C	T	A	A	G	A	G	G	NDA000004	A	G	A	A	T	G	G	C	T	A	A	A	A	G	G	
LMS000002	G	G	G	A	T	G	G	C	T	A	A	A	A	A	G	G	NDA000004	G	G	A	A	T	G	G	C	T	A	A	A	A	G	G
LMS000003	A	G	A	A	G	A	G	C	T	C	A	A	A	A	G	G	NDA000005	G	G	A	A	T	G	G	C	T	C	G	G	A	G	G
LMS000003	G	G	A	G	G	G	G	C	T	A	A	G	A	G	G	NDA000005	G	G	A	A	G	G	G	C	T	C	G	G	A	G	G	
LMS000004	G	G	A	G	G	G	G	C	T	A	A	G	A	G	G	NDA000006	G	G	A	A	G	G	G	C	T	C	G	G	A	G	G	
LMS000004	G	G	A	G	G	G	C	T	A	A	G	A	G	G	G	NDA000006	G	G	A	A	G	G	G	C	T	C	G	G	A	G	G	
LMS000005	G	G	G	A	T	A	G	C	T	C	G	G	G	G	G	NDA000010	G	G	A	A	G	G	G	C	T	C	G	G	A	G	G	
LMS000005	A	G	G	A	T	A	A	C	T	C	G	G	G	G	G	NDA000010	G	G	G	A	T	G	G	C	T	A	A	G	A	G	G	
LMS000006	G	G	A	G	G	G	G	C	T	C	G	G	G	G	G	NDA000011	G	G	G	A	G	G	G	C	T	C	G	G	A	G	G	
LMS000006	G	G	G	A	T	G	G	C	T	C	G	G	A	G	G	NDA000011	G	G	G	A	G	G	G	C	T	A	A	G	A	G	G	
LMS000007	G	G	G	A	T	G	G	C	T	A	A	A	A	G	G	NDA000012	G	G	A	A	G	G	G	C	T	C	G	G	A	G	G	
LMS000007	G	G	G	G	T	G	G	C	T	C	G	G	A	G	G	NDA000012	G	G	G	A	G	G	G	C	T	C	G	G	A	G	G	
LMS000008	A	G	A	A	G	A	G	C	T	C	A	A	A	G	G	NDA000013	G	G	A	A	? G	G	C	T	C	G	A	A	G	G	G	
LMS000008	G	G	G	A	T	G	G	C	T	C	G	A	A	G	A	NDA000013	G	G	A	A	? G	G	C	T	C	G	G	A	G	G	G	
LMS000009	G	G	A	G	G	G	C	T	C	G	G	A	G	G	G	NDA000014	G	G	A	A	T	A	G	C	T	A	G	G	A	G	G	
LMS000009	G	G	G	A	T	A	G	C	T	C	G	G	G	G	G	NDA000014	G	G	G	A	T	A	G	C	T	A	G	G	A	G	G	
LMS000010	G	G	A	A	T	G	G	C	T	C	G	A	A	G	A	NDA000015	G	G	A	A	T	G	G	C	? C	G	G	A	G	G	G	
LMS000010	A	G	A	A	G	A	G	C	T	C	A	A	A	G	G	NDA000015	G	G	G	A	T	A	G	C	? A	G	G	A	G	G	G	
LMS000011	A	G	G	A	T	A	A	C	T	C	G	G	G	G	G	NDA000017	G	G	A	A	G	G	G	C	T	C	G	G	A	G	G	
LMS000011	G	G	G	T	G	G	C	T	C	G	G	A	G	G	G	NDA000017	G	G	G	T	G	G	C	T	A	A	G	A	G	G	G	
LMS000012	A	G	G	A	T	A	A	C	T	C	G	G	G	G	G	NDA000019	A	G	A	A	G	G	G	C	T	C	G	G	A	G	G	G
LMS000012	G	G	G	T	G	G	C	T	C	G	A	A	G	G	G	NDA000019	A	G	G	A	G	G	G	C	T	C	G	G	A	G	G	G
LMS000013	G	G	A	G	T	G	G	C	T	A	A	G	A	G	G	NDA000021	G	G	A	A	G	G	G	C	T	C	G	G	A	G	G	G
LMS000013	G	G	A	G	T	A	G	C	T	A	A	A	A	G	G	NDA000021	G	G	G	A	T	A	G	C	T	A	G	G	A	G	G	G
LMS000014	G	G	A	G	T	A	G	C	T	A	A	A	A	G	G	NDA000022	A	G	G	A	G	A	G	C	T	A	G	G	A	G	G	G
LMS000014	G	G	A	G	T	A	G	C	T	A	A	A	A	G	G	NDA000022	G	G	G	A	G	G	G	C	T	A	A	G	A	G	G	G
LMS000015	G	G	A	G	T	A	G	C	T	A	A	G	A	G	G	NDA000024	A	G	A	A	G	G	G	C	T	C	G	G	A	G	G	G
LMS000015	G	G	G	A	T	G	G	C	T	C	G	A	A	G	A	NDA000024	G	G	A	A	T	A	G	C	T	A	G	G	A	G	G	G
LMS000016	G	G	A	G	T	A	A	C	T	C	G	G	A	A	G	NDA000025	A	G	A	A	G	G	G	C	T	C	G	G	A	G	G	G
LMS000016	G	G	G	T	G	G	C	T	C	G	G	A	G	G	G	NDA000025	G	G	A	G	T	G	G	C	T	A	A	G	A	G	G	G
LMS000017	G	G	A	G	T	A	A	C	T	A	A	G	A	G	G	NDA000027	A	G	A	A	G	G	G	C	T	A	A	G	A	G	G	G
LMS000017	G	G	G	T	G	G	C	T	C	G	G	A	G	G	G	NDA000027	G	G	A	A	G	G	G	C	T	A	G	G	A	G	G	G
LMS000018	G	G	A	A	G	G	C	T	A	A	A	A	A	G	G	NDA000029	G	G	A	A	G	G	G	C	T	A	A	G	A	G	G	G
LMS000018	G	G	A	G	G	G	C	T	A	A	G	A	G	G	G	NDA000029	G	G	A	A	T	A	G	C	T	A	G	G	A	G	G	G
LMS000019	G	G	A	G	T	A	A	C	T	A	A	G	A	G	G	NDA000030	G	G	A	A	G	G	G	C	T	A	G	G	A	G	G	G
LMS000019	G	G	G	A	T	G	G	C	T	C	G	G	A	G	G	NDA000030	G	G	A	A	T	A	G	C	T	A	G	G	A	G	G	G
LMS000020	G	G	A	G	T	A	A	C	? C	G	G	A	A	G	G	NDA000033	G	G	A	G	T	A	G	C	T	A	G	G	A	G	G	G
LMS000020	G	G	G	T	G	G	C	? C	G	G	A	G	G	G	NDA000033	G	G	G	G	G	G	G	C	T	C	G	G	A	G	G	G	
LMS000021	G	G	A	A	T	G	G	C	T	C	G	A	A	G	G	NDA000035	G	G	A	A	T	G	G	C	T	A	A	A	A	G	G	G
LMS000021	A	G	A	T	A	A	C	T	C	G	G	G	G	G	NDA000035	G	G	A	G	T	G	G	C	T	A	A	A	A	G	G	G	
LMS000022	A	G	A	G	T	A	A	C	T	C	G	G	G	G	G	NDA000036	G	G	G	A	T	G	G	C	T	A	A	A	A	G	G	G
LMS000022	G	G	A	G	T	A	A	C	T	C	G	A	A	G	G	NDA000036	G	G	G	T	G	G	C	T	C	G	G	A	G	G	G	G
LMS000023	G	G	A	T	G	G	C	T	A	A	A	A	A	G	G	NDA000041	A	G	A	A	G	G	G	C	? C	G	G	A	G	G	G	G
LMS000023	G	G	G	T	G	G	C	T	C	G	G	A	G	G	NDA000041	G	G	G	A	T	A	G	C	? A	G	G	A	G	G	G	G	
LMS000024	G	G	A	G	T	A	A	C	T	C	G	G	A	A	G	NDA000043	G	G	A	G	T	G	G	C	T	C	G	G	A	G	G	G
LMS000024	G	G	A	T	G	G	C	T	A	A	A	A	A	G	G	NDA000043	G	G	G	T	G	G	C	T	C	G	G	A	G	G	G	G
LMS000025	G	G	A	A	T	G	C	T	C	G	A	A	A	G	G	NDA000044	G	G	A	A	T	A	G	C	T	A	G	G	A	G	G	G
LMS000025	G	G	A	G	T	A	A	C	T	A	A	A	A	G	G	NDA000044	G	G	A	A	T	A	G	C	T	C	G	A	A	G	G	G

Results of PHASE on Limousin and Nelore HapMap Animals for BoLA IIb

BoLA	IIb	BoLA	IIb	IIb	IIb	IIb	IIb	IIb	IIb	IIb	IIb	IIb	IIb	IIb																	
Animal ID SNP Position	7228956	7229152	7280210	7339117	7384619	7398492	7453429	7453668	7455564	7458121	7479927	7562289	7611266	7632049	7666392	Animal ID SNP Position	7228956	7229152	7280210	7339117	7384619	7398492	7453429	7453668	7455564	7458121	7479927	7562289	7611266	7632049	7666392
LMS000026	G	G	A	A	G	A	G	C	T	A	A	A	A	G	G	NEL000001	G	G	A	A	G	G	G	C	T	C	G	G	A	G	G
LMS000026	G	G	A	G	T	A	A	C	T	C	G	G	A	A	G	NEL000001	G	G	A	G	G	G	G	C	T	C	G	G	A	G	G
LMS000027	G	G	A	A	T	G	G	C	T	C	G	A	A	G	G	NEL000002	A	G	A	A	T	G	G	C	T	A	A	A	A	G	G
LMS000027	G	G	A	A	T	G	G	C	T	C	G	A	A	G	A	NEL000002	G	G	A	A	T	G	G	C	T	A	A	A	A	G	G
LMS000028	G	G	G	A	T	G	G	C	T	A	A	A	A	G	G	NEL000003	G	G	A	A	T	G	G	C	T	C	G	G	A	G	G
LMS000028	G	G	G	A	T	G	G	C	T	A	A	A	A	G	G	NEL000003	G	G	A	A	G	G	G	C	T	C	G	G	A	G	G
LMS000029	G	G	A	A	T	G	G	C	T	C	G	G	A	G	A	NEL000004	G	G	A	A	G	G	G	C	T	C	G	G	A	G	G
LMS000029	G	G	A	G	G	G	G	C	T	C	G	G	A	G	G	NEL000004	G	G	A	A	G	G	G	C	T	C	G	G	A	G	G
LMS000030	G	G	A	G	T	A	A	C	T	C	G	G	A	A	G	NEL000005	G	G	A	A	G	G	G	C	T	C	G	G	A	G	G
LMS000030	G	G	G	A	T	G	G	C	T	A	A	A	A	G	G	NEL000005	G	G	G	A	T	G	G	C	T	A	A	G	A	G	G
LMS000031	G	G	A	G	T	G	G	C	T	A	A	G	A	G	G	NEL000006	G	G	G	A	G	G	G	C	T	C	G	G	A	G	G
LMS000031	G	G	G	T	G	G	C	T	C	G	G	A	G	G	NEL000006	G	G	G	A	G	G	G	C	T	A	A	G	A	G	G	
LMS000032	G	G	G	A	G	G	G	C	T	C	G	A	A	G	A	NEL000007	G	G	A	A	G	G	G	C	T	C	G	G	A	G	G
LMS000032	G	G	G	G	A	G	C	T	C	G	G	A	G	G	NEL000007	G	G	G	A	G	G	G	C	T	C	G	G	A	G	G	
LMS000033	G	G	G	A	T	G	G	C	T	C	G	A	A	G	A	NEL000008	G	G	A	A	?	G	G	C	T	C	G	A	A	G	G
LMS000033	G	G	G	G	T	G	G	C	T	C	G	G	A	G	G	NEL000008	G	G	A	A	?	G	G	C	T	C	G	G	A	G	G
LMS000034	G	G	A	A	T	G	G	C	T	A	A	A	A	G	G	NEL000009	G	G	A	A	T	A	G	C	T	A	G	G	A	G	G
LMS000034	G	G	A	A	T	G	G	C	T	A	A	A	A	G	G	NEL000009	G	G	G	A	T	A	G	C	T	A	G	G	A	G	G
LMS000035	G	G	A	G	T	A	A	C	T	C	G	G	A	A	G	NEL000010	G	G	A	A	T	G	G	C	?	C	G	G	A	G	G
LMS000035	G	G	G	A	T	G	G	C	T	A	A	A	A	G	G	NEL000010	G	G	G	A	T	A	G	C	?	A	G	G	A	G	G
LMS000036	G	G	A	A	T	G	G	C	T	A	A	A	A	G	G	NEL000011	G	G	A	A	G	G	G	C	T	C	G	G	A	G	G
LMS000036	G	G	G	A	G	G	G	C	T	C	G	A	A	G	A	NEL000011	G	G	G	T	G	G	C	T	A	A	G	A	G	G	
LMS000037	G	G	A	A	T	G	G	C	T	A	A	A	A	G	G	NEL000012	A	G	A	A	G	G	G	C	T	C	G	G	A	G	G
LMS000037	G	G	A	G	G	G	G	C	T	A	A	A	A	G	G	NEL000012	A	G	G	A	G	G	G	C	T	C	G	G	A	G	G
LMS000038	G	G	A	A	T	G	G	C	T	C	G	A	A	A	G	NEL000013	G	G	A	A	G	G	G	C	T	C	G	G	A	G	G
LMS000038	G	G	A	A	T	G	G	C	T	C	G	A	A	A	G	NEL000013	G	G	G	A	T	A	G	C	T	A	G	G	A	G	G
LMS000039	G	G	A	G	T	A	A	C	T	C	G	G	A	A	G	NEL000014	A	G	G	A	G	A	G	C	T	A	G	G	A	G	G
LMS000039	G	G	G	A	T	G	G	C	T	A	A	A	A	G	G	NEL000014	G	G	G	A	G	G	G	C	T	A	A	G	A	G	G
LMS000040	G	G	A	G	T	A	A	C	T	C	G	G	A	A	G	NEL000015	A	G	A	A	G	G	G	C	T	C	G	G	A	G	G
LMS000040	G	G	A	G	T	A	A	C	T	C	G	G	A	A	G	NEL000015	G	G	A	A	T	A	G	C	T	A	G	G	A	G	G
LMS000041	G	G	G	G	A	G	C	T	C	G	G	A	G	G	NEL000016	A	G	A	A	G	G	G	C	T	C	G	G	A	G	G	G
LMS000041	G	G	G	G	T	G	G	C	T	C	G	G	A	G	G	NEL000016	G	G	A	G	T	G	G	C	T	A	A	G	A	G	G
LMS000042	A	G	A	A	G	A	G	C	?	C	A	A	A	G	G	NEL000017	A	G	A	A	G	G	G	C	T	A	A	G	A	G	G
LMS000042	G	G	G	G	A	G	C	?	C	G	G	A	G	G	NEL000017	G	G	A	A	G	G	G	C	T	A	G	G	A	G	G	G
LMS000609	?	G	A	A	?	G	?	?	?	C	G	A	A	A	G	NEL000018	G	G	A	A	G	G	G	C	T	A	A	G	A	G	G
LMS000609	?	G	G	A	?	G	?	?	?	C	G	A	A	G	A	NEL000018	G	G	A	A	T	A	G	C	T	A	G	G	A	G	G
LMS000610	?	G	A	G	?	A	?	?	?	C	G	G	A	A	G	NEL000019	G	G	A	A	G	G	G	C	T	A	G	G	A	G	G
LMS000610	?	G	G	A	?	A	?	?	?	C	G	G	G	G	G	NEL000019	G	G	A	A	T	A	G	C	T	A	G	G	A	G	G
LMS900042	?	G	A	A	?	A	?	?	?	C	A	A	A	G	G	NEL000020	G	G	A	G	T	A	G	C	T	A	G	G	A	G	G
LMS900042	?	G	G	G	?	A	?	?	?	C	G	G	A	G	G	NEL000020	G	G	G	G	G	G	G	C	T	C	G	G	A	G	G
															NEL000021	G	G	A	A	T	G	G	C	T	A	A	A	A	G	G	
															NEL000021	G	G	A	G	T	G	G	C	T	A	A	G	A	G	G	G
															NEL000022	G	G	G	A	T	G	G	C	T	A	A	A	A	G	G	G
															NEL000022	G	G	G	G	T	G	G	C	T	C	G	G	A	G	G	G
															NEL000023	A	G	A	A	G	G	G	C	?	C	G	G	A	G	G	G
															NEL000023	G	G	G	A	T	A	G	C	?	A	G	G	A	G	G	G
															NEL000024	G	G	A	G	G	G	G	C	T	C	G	G	A	G	G	G
															NEL000024	G	G	G	G	T	G	G	C	T	C	G	A	A	G	G	G

Results of PHASE on Norwegian Red and Piedmontese HapMap Animals for BoLA IIB

BoLA	I1b	I2b	I3b	I4b	I5b	I6b	I7b	I8b	I9b	I10b	I11b	I12b	I13b	I14b	BoLA	I1b	I2b	I3b	I4b	I5b	I6b	I7b	I8b	I9b	I10b	I11b	I12b	I13b	I14b						
Animal ID SNP Position	7228956	7229152	7280210	7339117	7384619	7398492	7453429	7453668	7455564	7458121	7479927	7562289	7611266	7632049	7666392	Animal ID SNP Position	7228956	7229152	7280210	7339117	7384619	7398492	7453429	7453668	7455564	7458121	7479927	7562289	7611266	7632049	7666392				
NRC000001	G	G	A	A	T	G	G	C	T	C	G	A	A	G	G	PMT000001	G	G	G	A	T	G	G	C	T	A	A	A	A	G	G				
NRC000001	G	G	A	A	T	G	G	C	T	C	G	A	A	G	G	PMT000001	G	G	G	A	T	G	G	C	T	A	A	A	A	G	G				
NRC000002	G	G	A	A	G	A	G	C	T	C	G	G	G	G	G	PMT000002	G	G	A	G	T	G	G	C	T	A	A	A	A	G	G				
NRC000002	G	G	A	G	T	G	G	C	T	A	G	A	A	G	A	PMT000002	G	G	A	G	T	G	G	C	T	A	A	A	A	G	A				
NRC000003	A	G	G	A	G	A	G	C	T	A	A	A	A	G	G	PMT000003	G	G	A	G	G	G	G	C	T	C	G	A	A	A	G	A			
NRC000003	G	G	G	A	T	G	G	C	T	C	G	G	G	G	G	PMT000003	G	G	G	A	G	G	G	C	T	C	G	G	A	G	A				
NRC000004	G	G	A	A	T	G	G	C	T	A	G	A	A	G	G	PMT000004	G	G	A	G	T	A	A	C	T	C	G	A	G	G	G	A			
NRC000004	G	G	A	A	T	G	G	C	T	A	G	A	A	G	A	PMT000004	A	G	G	A	G	G	G	C	T	C	G	G	A	G	A	A			
NRC000005	G	G	G	A	T	A	G	C	T	C	G	G	G	G	G	PMT000005	G	G	G	A	T	G	G	C	T	A	A	A	A	G	G	A			
NRC000005	G	G	G	A	T	A	G	C	T	C	G	G	G	G	G	PMT000005	A	G	G	A	G	A	G	C	T	C	G	G	A	G	G	A			
NRC000006	G	G	A	A	T	G	G	C	T	A	G	A	A	G	G	PMT000006	G	G	A	G	T	A	A	C	T	C	G	A	G	G	G	A			
NRC000006	A	G	A	G	T	G	G	C	T	C	G	A	A	G	G	PMT000006	G	G	A	T	G	G	C	T	A	A	A	A	G	G	A	G	A		
NRC000007	G	G	A	A	T	A	G	C	? C	G	G	A	A	G	G	PMT000007	G	G	A	G	T	G	G	C	T	A	A	A	A	G	G	A			
NRC000007	G	G	G	A	T	A	G	C	? C	G	G	G	G	G	G	PMT000007	G	G	A	G	T	A	A	C	T	C	G	A	G	G	G	A			
NRC000009	G	G	A	A	G	A	G	C	T	A	A	A	A	G	G	PMT000008	A	G	A	G	G	A	G	C	T	C	G	G	A	G	G	A	G		
NRC000009	A	G	G	A	G	G	G	C	T	C	G	G	G	G	G	PMT000008	G	G	G	A	T	G	G	C	T	A	A	A	A	G	A	A	A		
NRC000010	G	G	A	G	T	G	G	C	T	C	G	G	A	G	G	PMT000009	G	G	A	A	G	G	G	C	T	C	G	G	A	G	G	A	A		
NRC000010	G	G	G	A	T	A	G	C	T	C	G	G	G	G	G	PMT000009	G	G	G	A	T	A	A	C	T	C	G	A	A	G	G	A	A		
NRC000011	G	G	A	G	T	A	G	C	T	C	G	G	A	G	G	PMT000010	G	G	A	A	G	A	G	C	T	A	A	A	A	G	G	A	A		
NRC000011	A	G	A	G	T	G	G	C	T	C	G	A	A	G	G	PMT000010	G	G	A	G	T	G	G	C	T	C	G	A	A	G	A	A	A		
NRC000012	G	G	A	A	G	A	G	C	T	C	G	G	G	G	G	PMT000011	A	G	A	G	G	G	G	C	T	C	G	G	A	G	G	A	A		
NRC000012	A	G	A	G	T	G	G	C	T	C	G	A	A	G	G	PMT000011	G	G	A	G	A	G	A	G	C	T	A	A	A	A	G	G	A	A	
NRC000013	G	G	A	A	T	G	G	C	T	A	G	G	A	G	G	PMT000012	G	G	G	A	T	G	G	C	T	A	A	A	A	G	A	A	A	A	
NRC000013	G	G	G	A	T	A	G	C	T	A	A	G	G	G	G	PMT000012	G	G	G	A	T	G	G	C	T	A	A	A	A	G	A	A	A	A	
NRC000014	A	G	A	G	T	G	G	C	T	C	G	A	A	G	G	PMT000013	G	G	A	A	T	G	G	C	T	C	G	A	A	G	G	A	A	A	
NRC000014	G	G	G	A	T	A	G	C	T	A	A	A	A	G	G	PMT000013	G	G	G	A	T	G	G	C	T	A	A	A	A	G	G	A	A	A	
NRC000015	G	G	A	A	T	G	G	C	? C	G	A	A	G	G	G	PMT000014	G	G	A	A	T	G	G	C	T	C	G	A	A	A	G	A	A	A	
NRC000015	G	G	G	A	T	A	G	C	? C	G	G	A	G	G	G	PMT000014	G	G	A	G	T	G	G	C	T	A	A	A	A	G	G	A	A	A	
NRC000016	G	G	A	A	G	A	G	C	T	C	G	G	G	G	G	PMT000015	G	G	A	A	G	G	G	C	T	A	A	A	A	G	G	A	A	A	
NRC000016	G	G	G	A	T	A	G	C	T	A	A	A	A	G	G	PMT000015	G	G	A	A	G	A	G	C	T	A	A	A	A	G	G	A	A	A	
NRC000017	G	G	G	A	T	A	G	C	T	A	A	A	A	G	G	PMT000016	G	G	A	A	G	A	G	C	T	A	A	A	A	G	G	A	A	A	
NRC000017	G	G	G	A	T	A	G	C	T	C	G	G	G	G	G	PMT000016	G	G	A	A	T	G	G	C	T	C	G	A	A	A	G	A	A	A	
NRC000019	G	G	A	A	G	A	G	C	T	C	G	G	G	G	G	PMT000017	A	G	A	G	G	G	G	C	T	C	G	G	A	G	G	A	A	A	
NRC000019	G	G	A	A	T	A	G	C	T	C	G	G	A	G	G	PMT000017	G	G	A	G	T	G	G	C	T	A	A	A	A	G	G	A	A	A	
NRC000020	G	G	A	A	G	A	A	C	T	A	A	A	A	G	G	PMT000018	G	G	A	G	T	G	G	C	T	C	G	A	A	G	A	A	A	A	
NRC000020	G	G	A	G	T	A	G	C	T	C	G	G	G	G	G	PMT000018	G	G	G	A	G	G	G	C	T	C	G	A	A	G	A	A	A	A	
NRC000021	G	G	A	A	G	A	A	C	T	A	A	A	A	G	G	PMT000019	G	G	A	A	G	A	G	C	T	A	A	A	A	G	A	A	A	A	
NRC000021	G	G	A	G	T	A	G	C	T	C	G	G	A	G	G	PMT000019	G	G	G	A	T	G	G	C	T	C	G	A	A	G	A	A	A	A	
NRC000023	G	G	A	A	T	G	G	C	T	C	G	A	A	G	G	PMT000020	G	G	A	A	G	G	G	C	T	C	G	G	A	A	G	A	A	A	
NRC000023	G	G	A	A	G	A	G	C	T	C	G	G	G	G	G	PMT000020	G	G	A	G	T	G	G	C	T	A	A	A	A	G	A	A	A	A	
NRC000025	G	G	G	A	T	A	G	C	T	C	G	G	G	G	G	PMT000021	G	G	A	A	G	G	G	C	T	A	A	A	A	G	G	A	A	A	
NRC000025	G	G	G	A	T	A	G	C	T	C	G	G	G	G	G	PMT000021	G	G	A	G	T	G	G	C	T	A	A	A	A	G	G	A	A	A	
																PMT000022	G	G	A	G	T	G	G	C	T	C	G	A	A	G	A	A	A	A	
																PMT000022	G	G	G	A	T	G	G	C	T	A	A	A	A	G	G	A	A	A	
																PMT000023	G	G	A	A	G	A	G	C	T	A	A	A	A	G	G	A	A	A	A
																PMT000023	G	G	A	G	G	G	G	C	T	C	G	A	A	G	A	A	A	A	A
																PMT000024	G	G	A	A	G	A	G	C	T	A	A	A	A	G	A	A	A	A	A
																PMT000024	G	G	G	A	G	G	G	C	T	A	A	A	A	G	G	A	A	A	A

Results of PHASE on Red Angus and Romagnola HapMap Animals for BoLA IIB

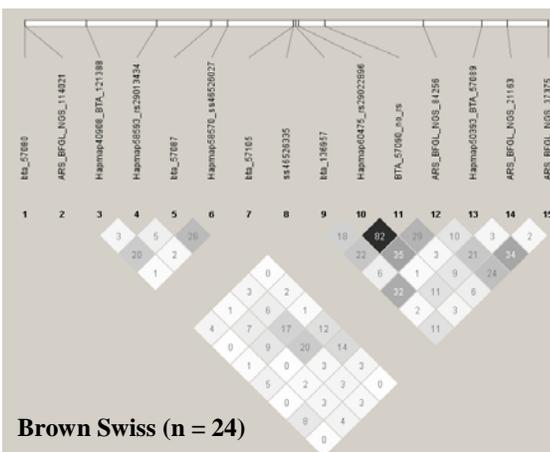
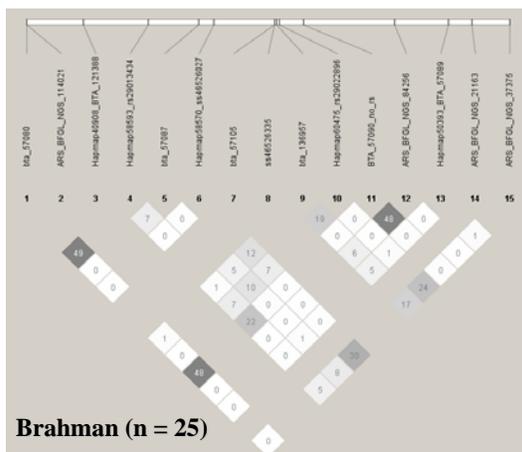
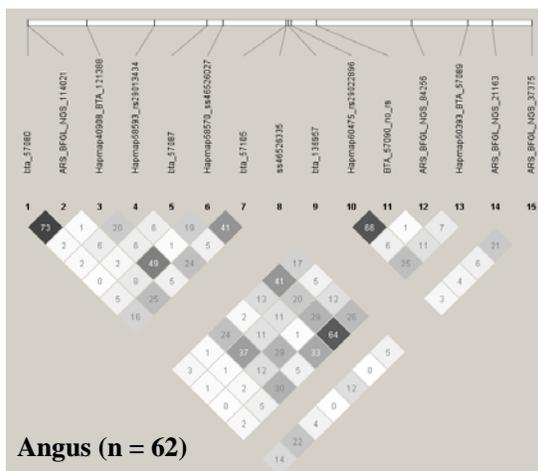
BoLA	I1b	I2b	I3b	I4b	I5b	I6b	I7b	I8b	I9b	I10b	I11b	I12b	I13b	I14b	BoLA	I1b	I2b	I3b	I4b	I5b	I6b	I7b	I8b	I9b	I10b	I11b	I12b	I13b	I14b			
Animal ID SNP Position	7228956	7229152	7280210	7339117	7384619	7398492	7453429	7453668	7455564	7458121	7479927	7562289	7611266	7632049	7666392	Animal ID SNP Position	7228956	7229152	7280210	7339117	7384619	7398492	7453429	7453668	7455564	7458121	7479927	7562289	7611266	7632049	7666392	
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RGU000001	G	G	G	A	T	A	G	C	T	A	A	G	G	G	G	RMG000001	G	G	G	A	T	A	G	C	T	A	G	G	A	G	G	
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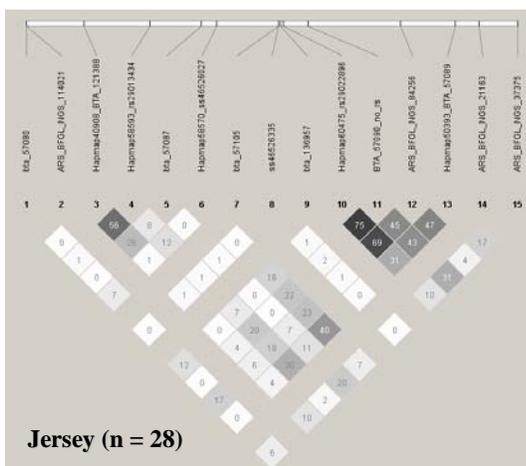
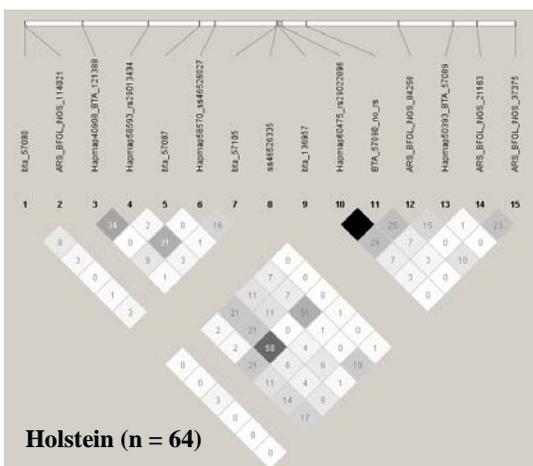
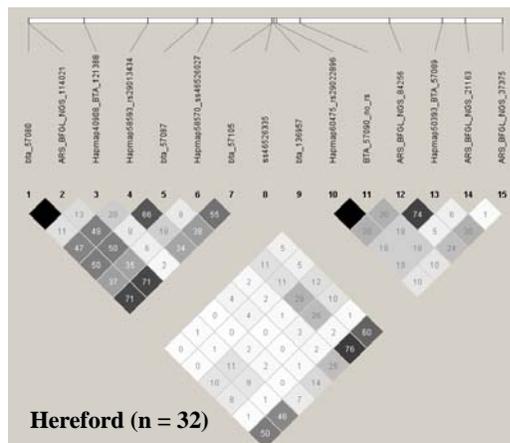
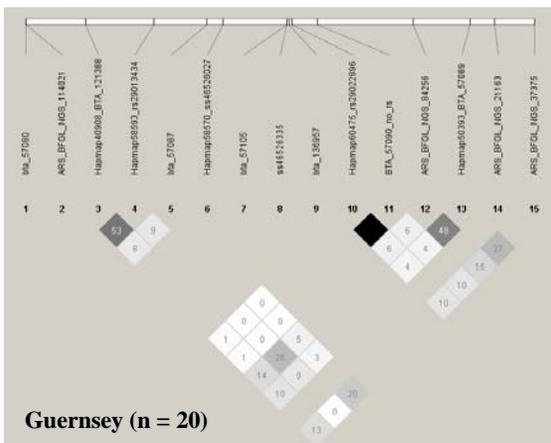
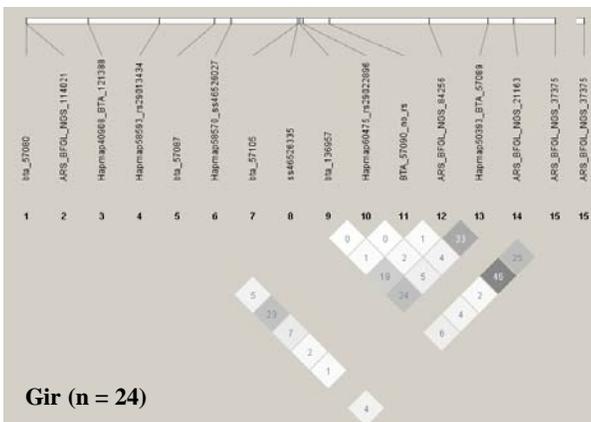
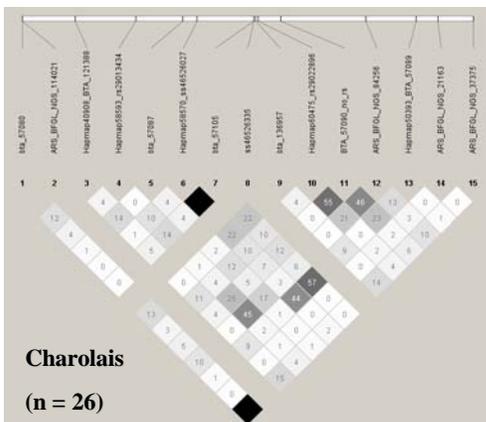
Results of PHASE on Santa Gertrudis and Sheko HapMap Animals for BoLA IIB

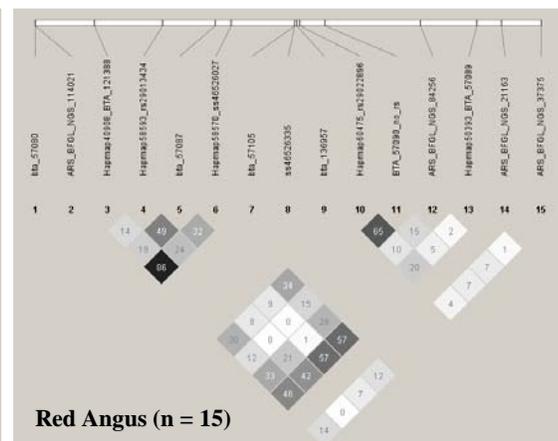
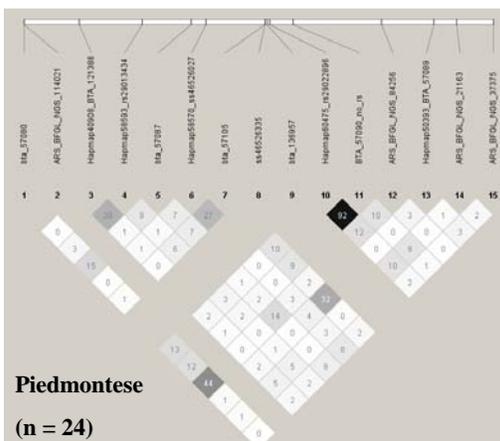
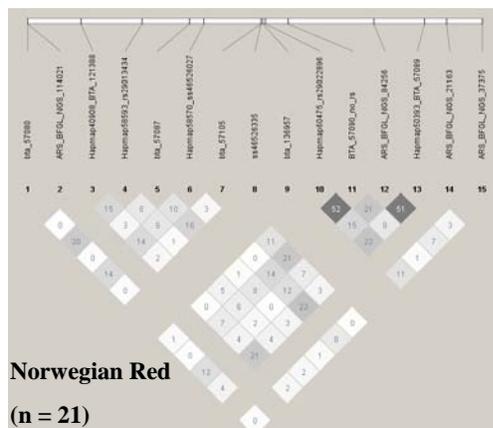
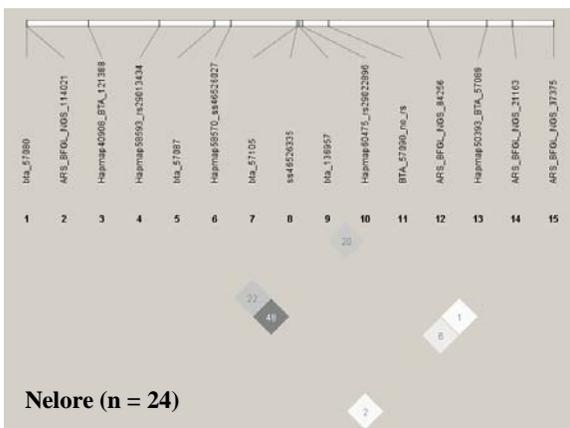
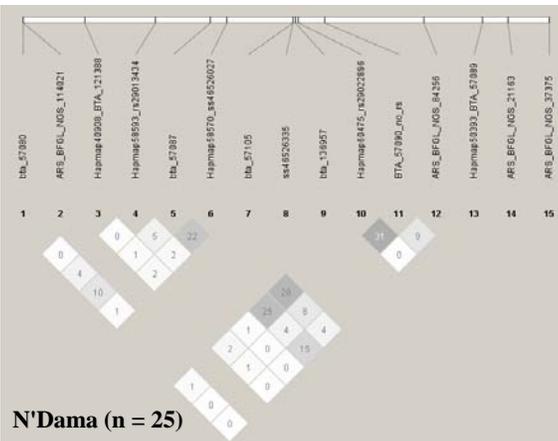
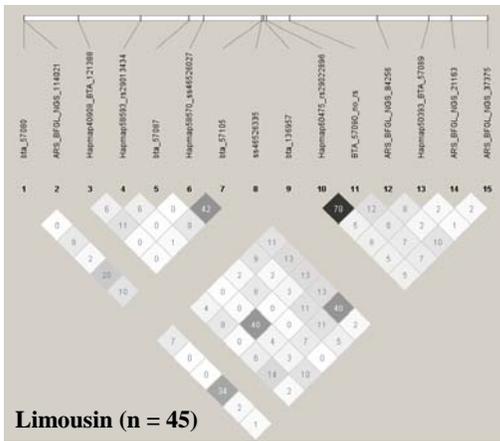
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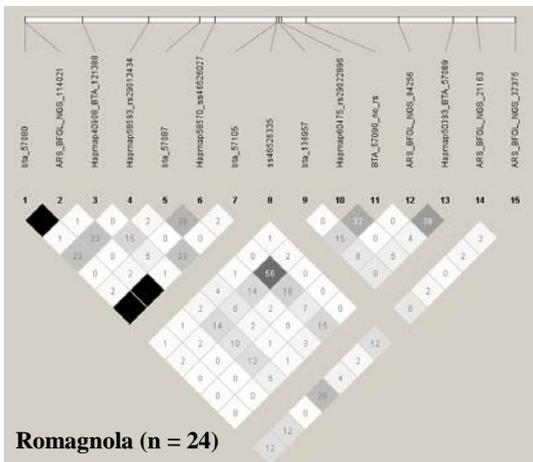
PHASE Haplotypes of BoLA IIA-III-I across HapMap Breeds. A total of 114 SNPs were analyzed by PHASE in animals from the Bovine HapMap Project. The SNP positions are listed across the top row, SNPs are color-coded according to their BoLA class, and each animal has two rows of SNPs representing two BoLA haplotypes.

BoLA IIB Haploview Output for HapMap Breeds. BoLA IIB haplotypes were determined by PHASE within each breed and graphically displayed in Haploview. The breed and total number of animals analyzed (n) is listed in the left hand corner of the figure. Low minimum allele frequencies of SNPs rendered the alleles monomorphic in many breeds, and monomorphic alleles are seen as blank diagonals in the Haploview output.

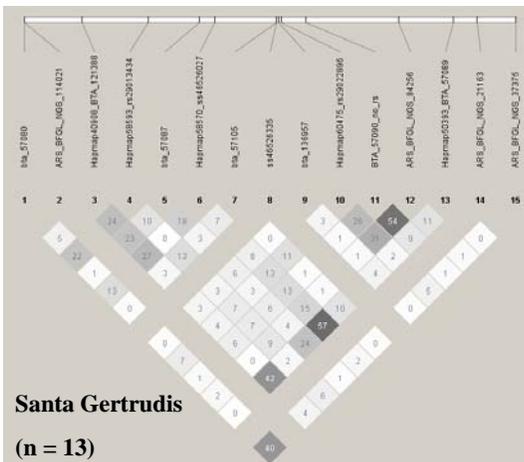






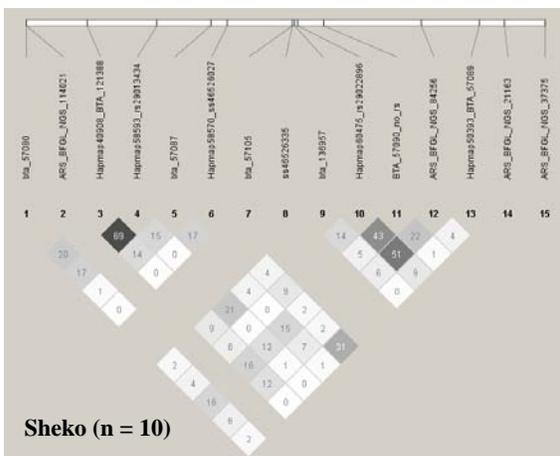


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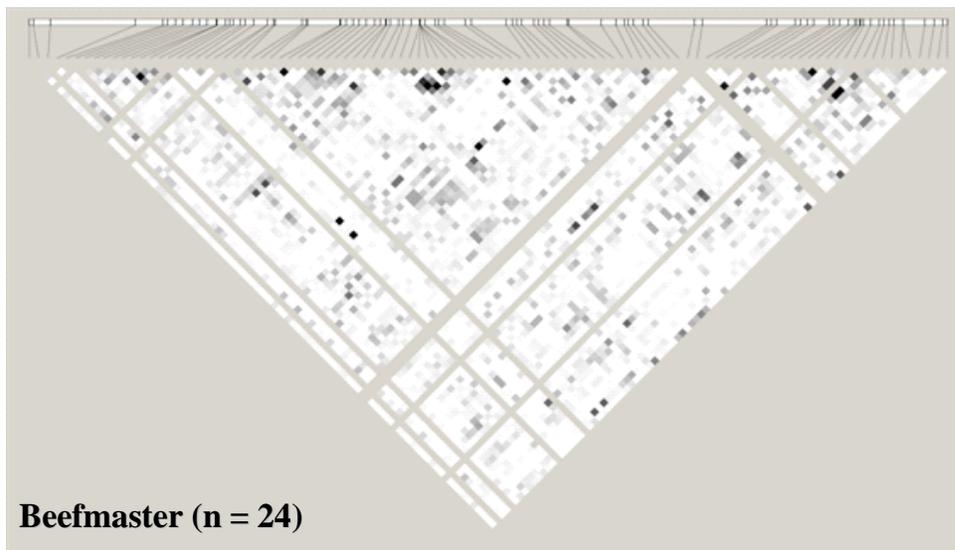
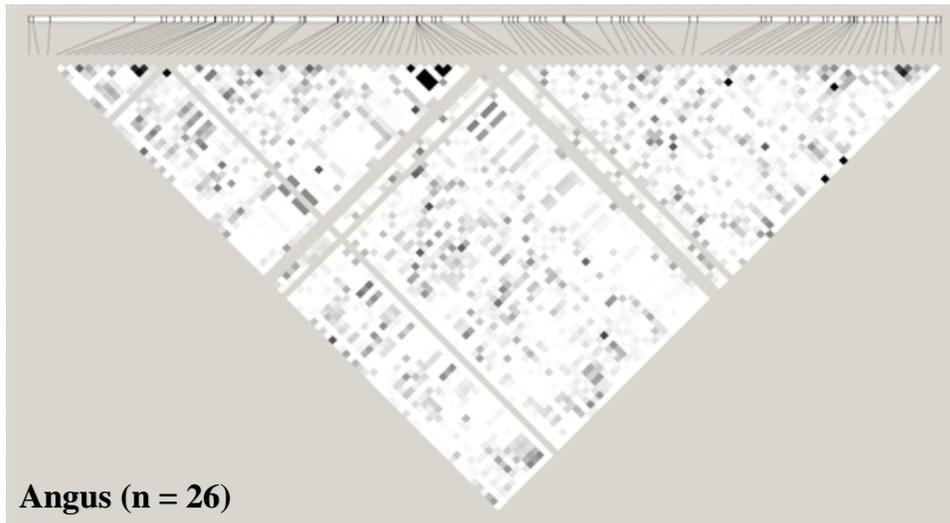
Santa Gertrudis

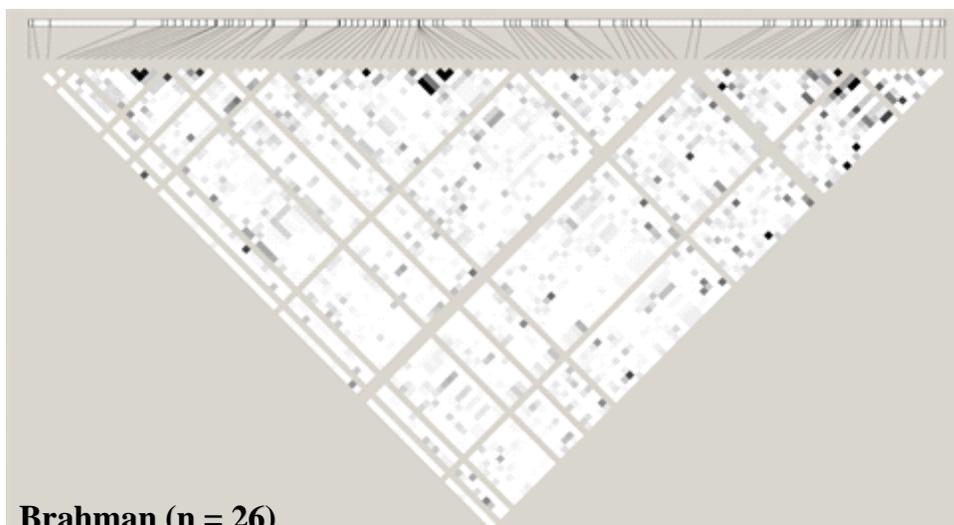
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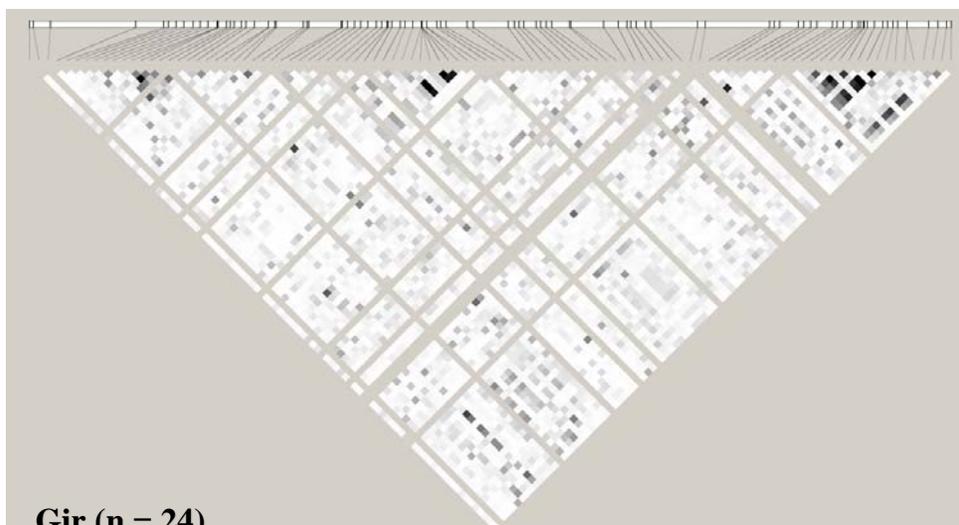


Sheko (n = 10)

BoLA IIa-III-I Haploview Outputs for HapMap Breeds. BoLA IIa-III-I haplotypes were determined by PHASE within each breed and graphically displayed in Haploview. Although some breeds showed evidence of stronger linkage disequilibrium than others, these figures show that over all breeds SNPs positioned close together exhibited a higher level of linkage disequilibrium. Linkage disequilibrium may have been influenced by the total number of animals analyzed (n) and their pedigree relationships.



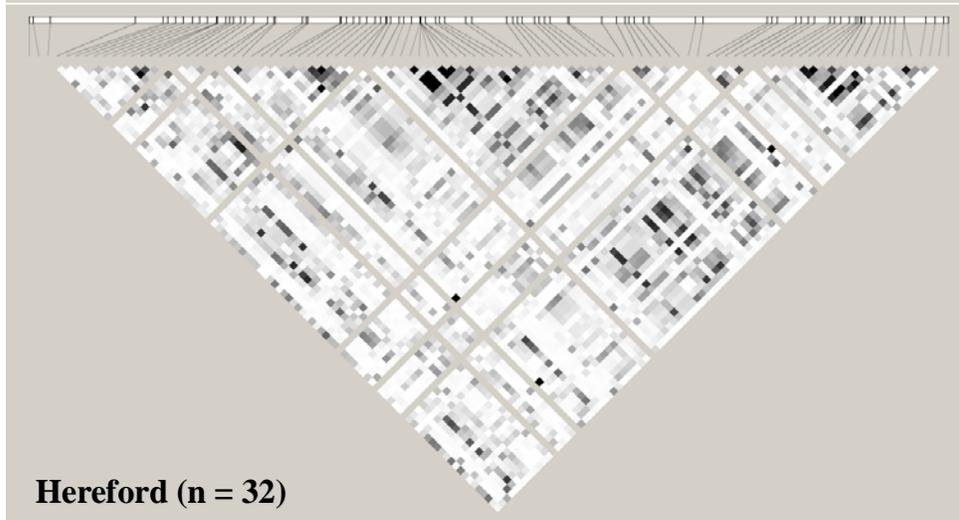




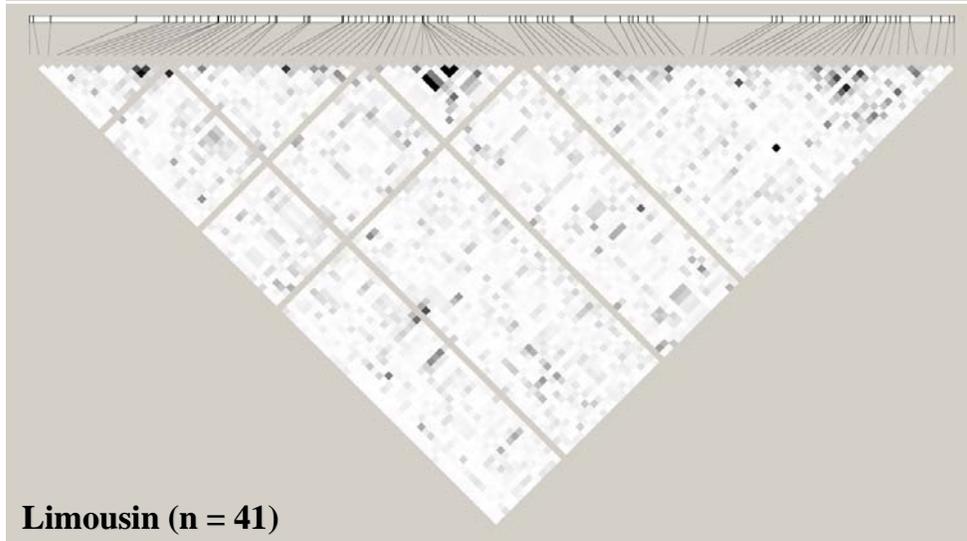
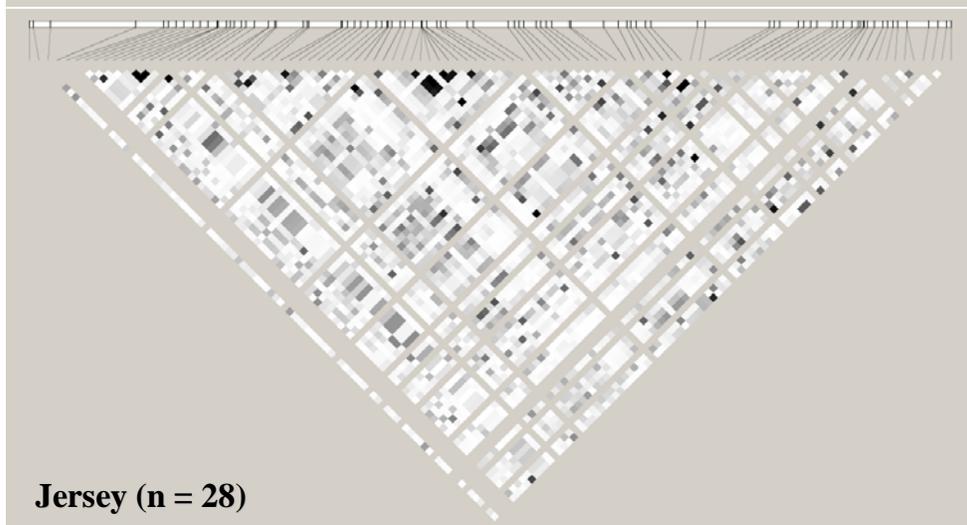
Gir (n = 24)

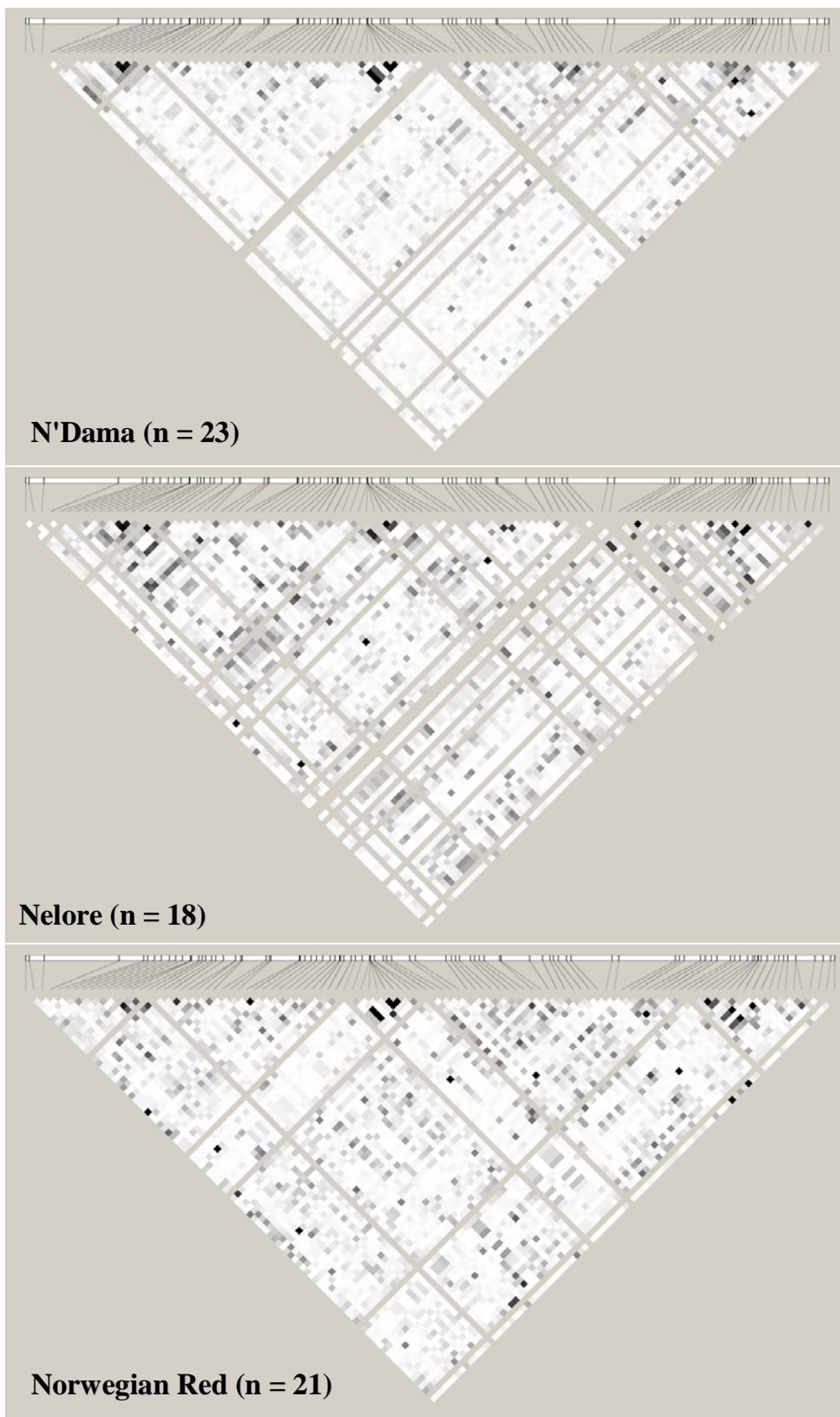


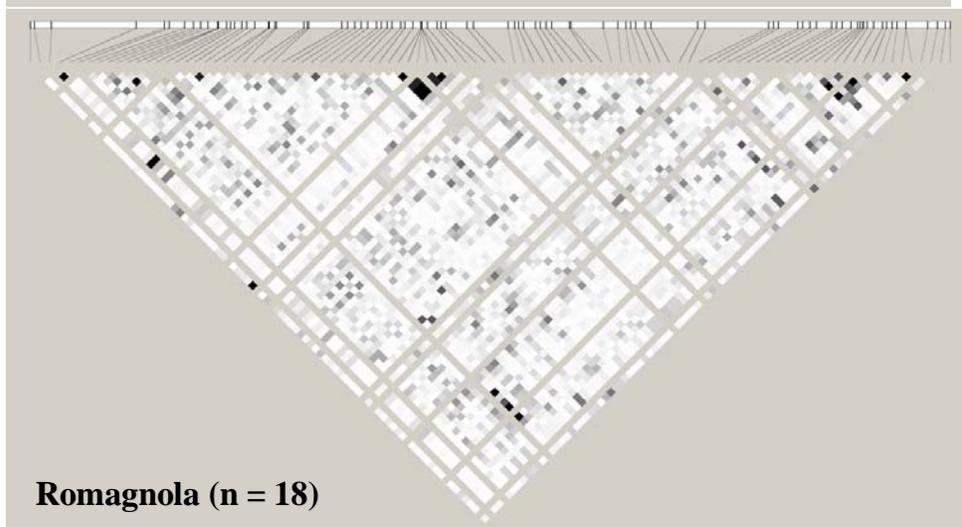
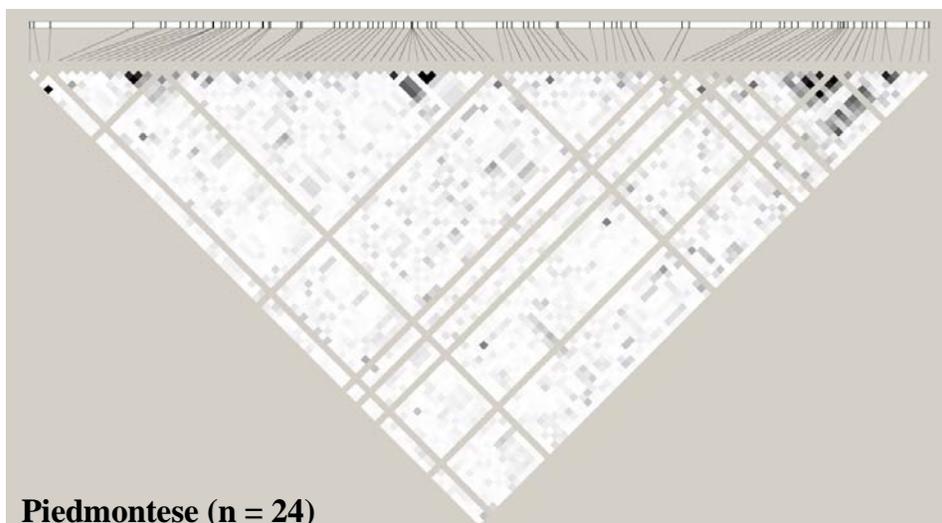
Guernsey (n = 21)

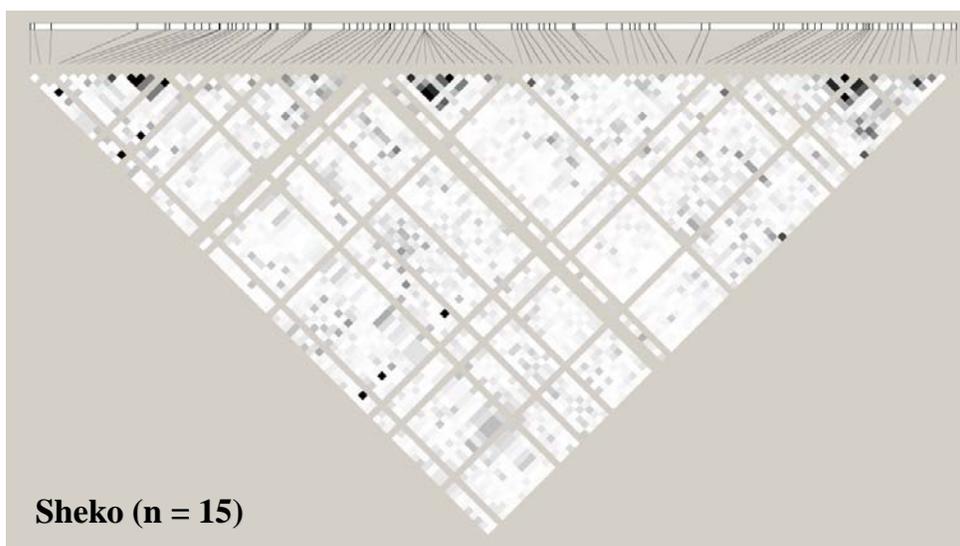


Hereford (n = 32)









VITA

Krista L. Fritz

Texas A&M University
 Department of Veterinary Integrative Biosciences
 College Station, TX 77843-4458
 kfritz@cvm.tamu.edu

Education

Texas A&M University, College Station, Texas Ph.D. in genetics	Sept. 2004 - Dec. 2009
University of Illinois, Urbana-Champaign, Illinois B.S. in animal science, minor in chemistry	Aug. 2000 - May 2004

Honors and Awards

Best Graduate Student Presentation at Texas Genetics Society Meeting	April 2009
First Place Oral Presentation at CVM GSA Research Symposium	April 2008
Awarded L.T. Jordan Fellowship for Independent Research Abroad	June 2006
Awarded "Exceptional Performance as a Genetics TA"	Feb. 2005
CVM Graduate Student Association Travel Award Recipient	Jan. 2006, 2007, 2008
Bachelor of Science Graduation with Honors	May 2004
Jonathan Baldwin Turner Scholar	Aug. 2000, 2002, 2003
ACES James Scholar Honors Program	Aug. 2000 - May 2002

Student Activities

Texas A&M College of Veterinary Medicine Graduate Student Association	
- President	Aug. 2006 - May 2007
- Member	Sept. 2005 - Dec. 2009
Texas A&M Genetics Graduate Student Association	Sept. 2005 - Dec. 2009

Publications

The Bovine Genome Sequencing and Analysis Consortium, Elvik C, Tellam R, Worley K (2009) The genome sequence of taurine cattle: a window to ruminant biology and evolution. *Science* 324, 522-528

Brinkmeyer-Langford C, Childers C, **Fritz K**, Gustafson-Seabury A, Cothran M, Raudsepp T, Womack J, Skow L (2009) A high resolution RH map of the bovine major histocompatibility complex. *BMC Genomics* 10, 182

Rogatcheva M, **Fritz K**, Rund L, Pollock C, Beever J, Counter C, Schook L (2007) Characterization of the porcine ATM gene: towards the generation of a novel non-murine animal model for Ataxia-Telangiectasia. *Gene* 405, 27-35