(Lack of) Genetic Diversity in Immune Genes Predates Glacial Isolation in the North American Mountain Goat (*Oreamnos americanus*)

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**Abstract**

The major histocompatibility complex (MHC) plays an important role in an organism’s ability to respond to pathogens. Immunogenetic diversity is advantageous as it permits the recognition of more external antigens. For this reason, MHC and immune gene variation are considered a barometer for the genetic health of wild populations. Mountain goats (*Oreamnos americanus*) were previously shown to have little variation at the MHC Class II *Oram-DRB* locus, which was attributed to population bottlenecks during the last glacial maximum (LGM). In this paper, we extended the analysis of immunogenetic variability in mountain goats to 5 genes representing the 3 classes of MHC gene (Class I *OLA*, Class II *DRA* and *DRB*, and Class III *TNF-a*) and the natural resistance–associated macrophage protein. We sequenced approximately 3000 bp from 31 individuals sampled across the range of mountain goats and found very low levels of diversity (1–3 polymorphic sites per gene) with the exception of the Class I *Oram-OLA* gene. *Oram-OLA* was nearly 30 times more diverse than the other immune genes and appears to represent a source of increased immunogenetic diversity. This diversity may be attributed to multiple loci, mediated by pathogen exposure, or potentially influenced by social factors. The distribution of SNPs was not associated with refugial history, suggesting that the current distribution of immunogenetic diversity was present prior to the LGM. These data suggest that although they have low levels of diversity at the 4 of 5 immune loci, mountain goats may be better equipped for future climate oscillations and pathogen exposure than previously thought.

**Key words:** Caprinae, immunogenetic diversity, major histocompatibility complex, mountain goat

The capacity to adapt to environmental perturbations through evolutionary change is dependent on the presence of adaptive genetic variation (Reed and Frankham 2003). The major histocompatibility complex (MHC) family of genes harbors the greatest number of polymorphic loci in the vertebrate genome (Hedrick 1994), with high variability conferring increased pathogen resistance. Monitoring such diversity is considered an important component to evaluating population-level and species-wide health (Schwartz et al. 2007) and has become an important element in the genetic health assessment of wild populations. Moreover, variation in the MHC of wild populations has been shown to be associated with mate choice (Schwensow et al. 2008), longevity (Huchard et al. 2010), survival (Paterson et al. 1998), parasite load (Oliver et al. 2009), and individual quality (Ditchkoff et al. 2001). For all these reasons, the interest in MHC studies has grown considerably in ecology and evolution over the past 2 decades (see Sommer 2005; Milinski 2006; Piertney and Oliver 2006).

Part of the increased interest also stems from an increasing number of species becoming “genome enabled” (Kohn et al. 2006), which permits the transfer and screening of a wider array of molecular markers in wild and nonmodel organisms. One recently genome-enabled group is the Caprinae, or wild sheep and goats, which have benefited from the genomics analysis of domestic sheep, *Ovis* (e.g., Archibald et al. 2010), and cattle, *Bos* (e.g., Zimin et al. 2009). For example, Poissant et al. (2009) screened over 300 domestic sheep markers in 2 wild caprids with over 50% success. Miller et al. (2011) genotyped over 45 000 SNP loci in 2 wild sheep species using an array of markers developed for domestic sheep. Not surprisingly, nearly all MHC and immune gene
et al. 2004; Alvarez-Busto et al. 2007). This notion has been suggested that their northerly and alpine habitats have reduced parasite diversity, which in turn has relaxed the selection pressures that promote MHC variability (Schaschl et al. 2004; Alvarez-Busto et al. 2007). This notion has been used to explain the trend of decreasing MHC diversity with latitude (Mainguy et al. 2007). However, evidence for a latitudinal gradient in terrestrial mammal parasite diversity is equivocal (Bordes et al. 2010), and only a handful of studies have robustly assessed parasite load relative to MHC diversity in wild populations (e.g., Oliver et al. 2009). Alternatively, if alleles are shared between lineages, reduced diversity may be attributed to balancing selection, where pathogen-mediated selection favors only a few alleles (Takahata 1990; Klein et al. 1993; Mona et al. 2008). Yet another possible cause for the low diversity in some Caprinae species could have been the occurrence of historical bottlenecks (Mikko et al. 1999; Hedrick et al. 2001; Amills et al. 2004; Mainguy et al. 2007). Regardless of the mechanism, the limited immunogenetic diversity may be a concern (Radwan et al. 2010), especially under the auspice of climate change (Mainguy et al. 2007). With two-thirds of the Caprinae species considered threatened (Shackleton 1997), species-level assessment of genetic and immunogenetic diversity may prove important to conservation and management (Sommer 2005; Schwarz et al. 2007).

The mountain goat (Oreamnos americanus) is 1 of only 4 caprids found in North America. Range-wide census data are sporadic, but mountain goats likely number close to 100,000 and are under no immediate threat (Festa-Bianchet and Côté 2008). Initial screening of MHC and neutral genetic markers showed minimal diversity (Mainguy et al. 2005, 2007; Poissant et al. 2009), with the patterns of neutral genetic variation echoed in an extensive range-wide analysis (Shafer et al. 2011). Shafer et al. (2011) also found evidence of 2 major refugia during the last glacial maximum (LGM); the 2 refugial clades diverged over 200,000 years ago and are separated latitudinally by more than 1500 km. The ranges of 2 other caprids, bighorn sheep (Ovis canadensis) and thinhorn sheep (Ovis dalli), cover these refugial sites along with the northern and southern limits of mountain goats. Interestingly, MHC allelic diversity of bighorn sheep (Gutiérrez-Espeleta et al. 2001) and thinhorn sheep (Worley et al. 2006) are among the highest and lowest in wild ungulates, respectively. This is consistent with a clinal pattern (Mainguy et al. 2007—but see Qutob et al. 2011 for another scenario), as bighorn sheep live south of their thinhorn relatives. Based on this observed latitudinal cline (Mainguy et al. 2007), we hypothesized that immunogenetic diversity would be lower in the mountain goat lineage from the northern refugium than the south. We also screened additional immunogenetic markers to better characterize MHC diversity (Spurgin and Richardson 2010) and to assess whether the paucity of MHC diversity observed by Mainguy et al. (2007) was representative of the species range.

### Materials and Methods

#### Immune Gene Background and Nomenclature

The MHC is an immunologically important gene cluster with the primary function of coding antigen-presenting proteins. The MHC gene structure is conserved among ungulates and is divided into 3 classes all with different functions (Amills et al. 1998). Class I genes primarily respond to intracellular parasites, viruses, and proteins by presenting antigens to cytotoxic T cells. The Class I protein is a heterodimer consisting of a heavy and light chain, with the α-I and α-II domains of the heavy chain being the peptide-binding region (PBR; Hughes and Yeager 1998). Class II genes code for peptides that present antigens from extracellular parasites and proteins to helper T cells. Similarly, Class II proteins are a heterodimer, but the PBR is found on the α-I and β-I domains (Hughes and Yeager 1998). Class III genes are considered highly conserved and are not well characterized for caprids (Dukkipati et al. 2006; Qin et al. 2008). These genes code for cytokines and proteins involved in the complement cascade (Dukkipati et al. 2006). For the MHC structure, we followed the nomenclature of Ovis (Dukkipati et al. 2006; Gao et al. 2010) and used the prefix Oram to be consistent with Mainguy et al. (2007). We also analyzed the natural resistance–associated macrophage protein (NRAMP), another immunologically relevant gene involved in responding to intracellular pathogens through controlling cation concentrations (Canonne-Hergaux et al. 1999). The NRAMP protein is located at the endosomal/lysosomal layer of macrophages and is induced by inflammatory agents (Gruenheid et al. 1997).

#### Tissue Sampling and DNA Preparation

We obtained tissue samples from across the mountain goat’s native range, representing the Canadian provinces of British Columbia and Alberta, Yukon and Northwest Territories, and the American states of Idaho, Alaska, Montana, and South Dakota (Supplementary Appendix I). Most tissue samples were collected from hunted goats during inspection or registration. Some tissues from Alberta, British Columbia, and Alaska were from ear punches taken during field studies. Handling of live animals was in accordance with animal care guidelines (Gannon et al. 2007). Most samples were stored in 95% ethanol at either −20 or −80°C, but some tissues from Montana were directly frozen at −20°C.

A DNeasy Blood and Tissue kit (QIAGEN) was used to extract DNA from mountain goat tissue samples. We followed the manufacturer’s protocol with the following exceptions: 1) An extra 1-min spin at 20,000 × g was performed before elution to ensure the removal of all excess proteins involved in the complement cascade (Dukkipati et al. 2006; Gao et al. 2010) and thinhorn sheep (Worley et al. 2006). For the MHC structure, we followed the nomenclature of Ovis (Dukkipati et al. 2006; Gao et al. 2010) and used the prefix Oram to be consistent with Mainguy et al. (2007). We also analyzed the natural resistance–associated macrophage protein (NRAMP), another immunologically relevant gene involved in responding to intracellular pathogens through controlling cation concentrations (Canonne-Hergaux et al. 1999). The NRAMP protein is located at the endosomal/lysosomal layer of macrophages and is induced by inflammatory agents (Gruenheid et al. 1997).

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ethanol and 2) the column was incubated in elution buffer AE for at least 5 min before centrifugation. Two hundred microliters of eluant was recovered per sample and stored at −20 °C. All samples were quantified using a Nanodrop 2000 (Thermo Scientific).

PCR of Immune Genes

We first screened an array of immune gene primers taken from Bovid and Caprinae species. Those markers that consistently produced a PCR product were analyzed in this study (details on other markers available from corresponding author). We amplified portions of exons 2 and 3 (encoding the α-1 domain) along with intron 2 of the Class I MHC gene using the primers B5 (5’-GCT ACG TGG ACG ACA CGC-3’) and B6 (5’-AGC GGA GGT CCT CGT TC-3’) from Miltiadou et al. (2005). Two Class II genes were amplified:

- The first domain (exon 2) of the DRB1 gene was amplified using primers DRB-For (5’-CCC CCTT TTC TGCT TTT CAG AG-3’) and DRB-Rev (5’-CAA TTC CCA AGT CTA GGA GGA CTG-3’) from Sena et al. (2003) and exon 2 of the DRB3 gene using the LA31-K (5’-ATC CTC TCT CTG CAG CAC ATT TCC T-3’) and LA32-K (5’-TCA CCT CGC CGC TGC ACA-3’) primers modified by Worley et al. (2006). The fourth exon and the 3’ untranslated region (UTR) of Class III MHC gene, tumor necrosis factor alpha (TNF-α), were amplified using primers ovTNF-C1 (5’-CTG CCG GAA TAC CTG GAC TA-3’) and ovTNF-C2 (5’-TCC AGT AGT CCT TGG TGA TGG TT-3’) from Alvarez-Busto et al. (2004). All PCR ingredients and cycling conditions are provided in Supplementary Appendices II and III. Because Mainuy et al. (2007) only analyzed a small portion of the mountain goat range, we first screened the Class II Orm-DRB gene in individuals from across the entire range representing all known subpopulations detected by Shafer et al. (2011).

We amplified exons 5–7 and the intervening introns of the NRAMP gene using 2 primer sets from Worley et al. (2006): NRAMP-2F (5’-CTC TCC TGG GGA TCA CCA TC-3’) and NRAMP-2R (5’-CAC GAT GGT GAT GAG GAC AC-3’) and NRAMP-3F (5’-GTG GTA GAT GCA CAC TCC TG-3’) and NRAMP-3R (5’-CCG AAG GTC AAA GCC ATT AT-3’). The PCR conditions are detailed in the Supplementary Appendices II and III.

Sequencing and Cloning of PCR Products

An aliquot of all PCR products was first visualized on an agarose gel (1–2%) to confirm amplification. Ten microliters of PCR product were treated with 0.25 U each of exonuclease I (ExoI; USB) and shrimp alkaline phosphatase (SAP; USB) resulting in a 15 µl reaction. Exo-SAP treated samples were placed in a 2-step incubation of 15 min at 30 °C followed by 15 min at 80 °C. All samples were then directly sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), ethanol precipitated, and resuspended in 10 µl of water. Two microliters of the products were mixed in 8 µl Hi-Di (Applied Biosystems) and run on ABI 3730 DNA Analyzer (Applied Biosystems).

The Class I gene amplification produced multiple products, thus requiring cloning before sequencing. For this gene, PCRs were conducted using the high-fidelity Phusion Taq (New England Biolabs). PCR fragments were inserted into a pJET/blunt vector (Fermentas) following the manufacturer’s sticky-end protocol. Vectors were transformed into competent DH5α cells (Invitrogen) and grown on Luria-Broth (LB) agar plates with ampicillin. Colonies were plucked and resuspended in 100 µl of water used for colony PCR (Supplementary Appendices II and III). Sequencing followed the above protocol.

Sequence and Statistical Analysis

The chromatograms and sequence data were analyzed using Bioedit 7.0.9 (Hall 1999). All sequences were screened on GenBank to find similar sequences and ensure that the appropriate gene was amplified. Sequences were then aligned and converted to amino acids using the software MEGA5 (Tamura et al. 2011). The numbers of SNPs, haplotypes, along with haplotype (b) and nucleotide diversity (π), were calculated using the software DnaSP 5.0.0 (Rozas and Labrador 2009). Deviation from Hardy–Weinberg equilibrium (HWE) was assessed in GenAlEx 6.2 (Peakall and Smouse 2006) when applicable. We calculated average rate of non-synonymous (dN) and synonymous (dS) substitutions for each coding region according to the Nei–Gojobori method (Nei and Gojobori 1986) implemented in MEGA5. A Z-test of neutrality was conducted between dN and dS using 1000 bootstrapped replicates. Because of the multiple Class I products, we conducted a phylogenetic analysis on the exonic sequence data to identify gene clusters (sensu Miltiadou et al. 2005) and any nonclassical genes (sensu Zidi et al. 2008). A neighbor joining tree was constructed from the K2P nucleotide distances in MEGA5. Sequence data from Ovis, Bos, Capra, and Sus were also included. For Ovis, we included representatives of all 4 Class I gene clusters observed in Miltiadou et al. (2005). Confidence in topology was assessed using 1000 bootstrapped replicates.

We tested for an association between parsimony informative SNPs and amino acid changes with northern and southern refugial origins designated by Shafer et al. (2011). Because of the high levels of differentiation and vicariant refugial history in mountain goats (Shafer et al. 2011), we interpreted a lack of association to mean that the SNPs did not arise from, or post, the LGM. This was done using a binomial logistic regression with bias reduction (Firth 1993) implemented by the brglm package (Kosmidis 2011) in R2.12.1 (http://www.rproject.org/). Parameter estimates along with the z statistic and P value are given.

Results

MHC and NRAMP Sequence Data

A total of 212 individuals were sequenced at 249 bp of the Orm-DRB exon 2. The only SNP was observed a G → T transversion at position 233 resulting an amino acid change from glycine to valine. Thirty-two individuals were
heterozygous, and only 6 were homozygous for the T allele. These genotypic frequencies deviated from what is expected under HWE in the direction of heterozygote deficiency across the range (P < 0.01) and in the southern refugium (P < 0.01) but not in the northern refugium (P = 0.08). Both alleles were previously deposited in GenBank (DQ648492-3) and are most similar to Bos grunniens (AY374126) and Ovis moschatus (AF162657).

Due to the limited variability in Oram-DRB, we opted to only sequence 31 individuals representing all DRB haplotypes and the entire mountain goat range for remainder immune genes. We sequenced 254 bp of the Class II Oram-DRA exon 2, which was found to be monomorphic (GenBank Accession numbers: JN861547–77) and 98% identical to Capra falconeri (FM986346) and Ovis aries (Z116000). The Class III Oram-TNF-α exon 4 and 3′ UTR were also monomorphic at the 232 bp sequence (GenBank Accession numbers: JN861578–608), and 98% similar to Ovis aries (EF446377). The majority of the Oram-TNF-α sequence (189 bp) was the noncoding 3′ UTR.

For the Class I gene, we sequenced 298 clones from the 31 individuals. Based on GenBank searches and amino acid conversion, 37 were the Class I Oram-OLA gene, 68 were a nonclassical Class I gene, one was intermediate, and 192 were pseudogenes (GenBank Accession numbers: JN861250–546). On average, each individual had only one detectable Class I allele, with the highest number being 4 (Supplementary Appendix IV). Of the 192 pseudogenes, 73 unique haplotypes were observed. Product sizes of the Class I alleles ranged from 445 to 505 bp with the differences in length almost entirely attributable to intron indels. Since we detected high variability and could not get genotypes for all individuals, we did not test for deviation of HWE. The neighbor joining tree with introns 5 and 6. We detected 3 SNPs, 2 of which producing a 929 bp fragment spanning exons 5, 6, and 7, along with introns 5 and 6. We detected 3 SNPs, 2 of which were intronic and 1 synonymous mutation in exon 7; these were at site 170 (C → G), site 186 (A → C), and site 896 (G → T), respectively (GenBank Accession numbers: JN861609–39). The lone exonic SNP was in HWE (P = 0.28), whereas both intronic SNPs were not (P < 0.05). When accounting for refugial origin, all loci were in HWE except for the exonic SNP in the southern refugium. These sequences shared their highest similarity (98%) to Ovis dalli (AJ920417). With the exception of the DRA gene which used primers from the water buffalo (Bubalus bubalis), all sequenced genes used primers from sheep studies.

**Distribution of Diversity**

Diversity statistics for all the genes and refugial lineages are presented in Tables 1 and 2. For the Class I genes, all pseudogenes and duplicated sequences within individuals were removed from the analyses in Tables 1–3. With the exception of the Class I gene, all genes showed very low levels of diversity, and there was no difference between dN and dS. Both Class I genes showed a marked increase in diversity metrics, with the nonclassical gene showing evidence of purifying selection (dN < dS). Diversity statistics by refugial origin showed nearly equivalent levels of diversity, except for the Oram-DRB, which was elevated in the south. Within the Class II Oram-DRB gene, the G haplotype (glycine) was not associated with refugial origin (β = −0.63 ± 0.95, z = −0.67, degrees of freedom [df] = 211, P = 0.50), but the T haplotype (valine) was found predominantly in individuals from the southern refugium (β = −1.02 ± 0.45, z = −2.27, df = 211, P = 0.02). Heterozygotes shared the same association for the southern refugium (β = −1.00 ± 0.45, z = −2.24, df = 211, P = 0.03). Within the Class I genes, no SNP or amino acid change was associated with refugial origin nor were any of the NRAMP SNPs (all Ps > 0.05). The remaining markers were not suitable for such analysis.

**Discussion**

Patterns of immunodiversity in mountain goats from across their range were relatively low with the exception of the Class I Oram-OLA gene. No sequence diversity was observed at MHC Class II Oram-DRA and Class III Oram-TNF-α. Low levels of variation were observed at the Class II Oram-DRB and NRAMP genes with 1 and 3 SNPs, respectively. The Class I Oram-OLA gene showed an increase in diversity (Table 1) and appears to offer a novel source of variation in the immune genes of mountain goats. Compared with studies on related ungulates examining the same genes, mountain goats had lower levels of polymorphism (e.g., Class II DRA and DRB—Schaschl et al. 2006; Worley et al. 2006; Ballingall et al. 2010; Class III TNF-α—Alvarez-Busto et al. 2004; and NRAMP—Worley et al. 2006). With the exception of 1 Oram-DRB allele, we did not find an association or difference between immunodiversity and refugial origin, refuting our hypothesis of a within-species latitudinal cline. Additional screening of noncoding MHC regions will be required to fully substantiate the absence of acline, but a study by Shafer et al. (2011) showed a similar nonexistent clinal pattern in neutral loci. Given the high degree of population differentiation and long-term separation of refugial lineages (Shafer et al. 2011), this lack of an association would suggest that the current distribution of immunodiversity is not a direct result of the LGM and that the apparent dearth of diversity was present well before the start of the holocene.

The relative paucity of immunodiversity is somewhat difficult to assess, as few studies of wild organisms have assessed genes other than Class II DRA gene. For example, the Class II DRA region is generally considered to have relatively low diversity (e.g., Yuhki et al. 2003), but recent studies have shown increased variation in domestic sheep (Ballingall et al. 2010) as well as in wild cetaceans (Xu et al. 2012:103(3)).
2007, 2008) and caribou, *Rangifer tarandus* (Kennedy et al. 2011). The upregulation of the Class II *DRA* gene is associated with parasite resistance (Diez-Tascón et al. 2005), but the functional relevance of haplotype variation is unknown. The same goes for the Class III *TNF-a* gene, with preliminary studies finding variation in sheep (Alvarez-Busto et al. 2004), with the importance of this variation remaining unclear. In these genes, we found complete monomorphism across the range of mountain goats, which may be explained by either an ancient bottleneck (pre-LGM).

Figure 1. Neighbour joining tree of the mountain goat Class I gene (exons 2 and 3). The nonclassical gene is identified, along with 4 clusters within the *Oram-OLA* gene. N and S in parentheses denote the northern and southern haplotype origin of the individual, respectively, in accordance with Shafer et al. (2011). For *Ovis*, Class I cluster origin is provided from Miltiadou et al. (2005). Support values >50% are shown and are from 1000 bootstrapped replicates.
that wiped out all the diversity or by ongoing purifying selection. Similarly, the NRAMP gene had only 3 SNPs, all of which were noninformative. Both sheep and cattle have double this number of SNPs (Ables et al. 2002; Worley et al. 2006), and genetic variants have been associated to disease resistance in bovids (Barthel et al. 2001; Borriello et al. 2006) and mice (Vidal et al. 1995; Roy and Malo 2002). We suggest additional screening within these genes coupled with disease assays may be warranted to confirm the observed low levels of diversity.

The Class II Oram-DRB gene is considered among the most diverse in mammals, with evidence of positive selection in wild ungulates (Schaschl et al. 2006). MHC diversity is inversely correlated to latitude in ungulates, with mountain goats containing among the lowest DRB allelic variation (Mainguy et al. 2007). Our expanded sampling suggests that the DRB paucity is indeed spread across the range of mountain goats. Mainguy et al. (2007) attributed this limited variation to a population bottleneck, which the range-wide analysis would support in the form of 2 major ice-age refugia (Shafer et al. 2011). However, given the larger sampling scheme presented here and the significant population structure of mountain goats (Shafer et al. 2011), it seems reasonable to suggest that a bottleneck during the LGM is not the only culprit of low diversity, and perhaps either an ancient pre-LGM bottleneck or some degree of purifying selection is acting on the Class II Oram-DRB locus in mountain goats—the latter conclusion was put forth in great crested newts (Triturus cristatus), which like mountain goats had only 2 DRB alleles across a geographically expansive postglacial range (Babik et al. 2009). Interestingly, in mountain goats, the T allele (the majority found in heterozygous state) is largely restricted to the southern refugial lineage and deviates from HWE. This suggests that there may be a selective pressure favoring the T allele in the southern part of the mountain goat's range, perhaps conferring a heterozygote advantage (e.g., Huchard et al. 2010). Currently, data on pathogen exposure in mountain goats are limited to a handful of historic observations (reviewed by Coˆte´ and Festa-Bianchet 2003), but the remote high altitudinal distribution likely limits pathogen exposure to some extent.

We also identified a largely monomorphic Class I nondiagnostic gene that had evidence of purifying selection (Table 2), likely due to a functional constraint. Similar nondiagnostic genes and phylogenetic structure have been identified in domestic goats (Capra hircus) and horses (Equus caballus) (Zidi et al. 2008; Tallmadge et al. 2010) and generally have low levels of polymorphism (Tallmadge et al. 2010). We also found 1 intermediate gene (British Columbia 09 in Figure 1) that is nested between nonclassical and Oram-DRA clades. Similar sequences were detected in horses (Tallmadge et al. 2010) with their exact function unknown. Unlike the other MHC and NRAMP genes that showed minimal sequence variability, the Class I Oram-OLA gene showed a high degree of sequence variation. The Class I gene codes for glycoproteins involved in immune surveillance of intracellular pathogens and viruses and in regulating innate immunity. Our phylogenetic structure is largely in accordance with sheep (Miltiadou et al. 2005) and horses (Tallmadge et al. 2010). Although both Miltiadou et al. (2005) and Tallmadge et al. (2010) examined transcribed sequences compared with our genomic phylogenetic tree (Figure 1), they shared the common structure of 4 major clades. These clusters may represent differential levels of diversity.

### Table 1 Genetic diversity statistics for immunogenes in mountain goats (Oreamnos americanus)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Class I—Oram-OLA</th>
<th>Class I—Nonclassical</th>
<th>Class II—Oram-DRA</th>
<th>Class II—Oram-DRB</th>
<th>Class III—Oram-TNF-α</th>
<th>NRAMP</th>
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<tbody>
<tr>
<td>n</td>
<td>31(24)</td>
<td>31(19)</td>
<td>31</td>
<td>212</td>
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<td>31</td>
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<td>1</td>
<td>0</td>
<td>3</td>
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<tr>
<td>Haplotypes</td>
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<td>5</td>
<td>1</td>
<td>2</td>
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<td>6</td>
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<tr>
<td>b</td>
<td>0.92</td>
<td>0.31</td>
<td>—</td>
<td>0.18</td>
<td>—</td>
<td>0.65</td>
</tr>
<tr>
<td>π</td>
<td>0.08</td>
<td>0.02</td>
<td>—</td>
<td>7.3 × 10⁻⁰⁴</td>
<td>—</td>
<td>1.1 × 10⁻⁰⁵</td>
</tr>
</tbody>
</table>

n, number of individuals analyzed; bp, number of base pairs sequenced; SNPs, total number of single nucleotide polymorphisms; Haplotypes, number of unique haplotypes; b, haplotype diversity; π, nucleotide diversity. NRAMP data include introns, with the MHC statistics only in reference to exonic regions. Parentheses in the Class I markers denote the number of analyzed sequences as not all individuals had the functional copy amplified.

### Table 2 Genetic diversity statistics between north and south glacial refugia for immune genes in mountain goats (Oreamnos americanus)

<table>
<thead>
<tr>
<th>Gene</th>
<th>North (h/n)</th>
<th>South (h/n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I—Oram-OLA</td>
<td>0.90/0.09</td>
<td>0.96/0.09</td>
</tr>
<tr>
<td>Class II—Oram-DRA</td>
<td>0.12/0.001</td>
<td>0.23/0.001</td>
</tr>
<tr>
<td>NRAMP</td>
<td>0.20/0.001</td>
<td>0.21/0.001</td>
</tr>
</tbody>
</table>

h, haplotype diversity; π, nucleotide diversity.

### Table 3 Nonsynonymous (dN) and synonymous substitutions (dS) in immune-related exons of mountain goats (Oreamnos americanus) across North America

<table>
<thead>
<tr>
<th>Gene</th>
<th>dN</th>
<th>dS</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I—Oram-OLA</td>
<td>0.08</td>
<td>0.11</td>
<td>−1.30</td>
<td>0.20</td>
</tr>
<tr>
<td>Class I—Nonclassical</td>
<td>0.02</td>
<td>0.04</td>
<td>−2.56</td>
<td>0.01</td>
</tr>
<tr>
<td>Class II—Oram-DRB</td>
<td>1.0 × 10⁻⁰³</td>
<td>0.00</td>
<td>1.12</td>
<td>0.26</td>
</tr>
<tr>
<td>NRAMP</td>
<td>0.00</td>
<td>4.0 × 10⁻⁰³</td>
<td>−1.04</td>
<td>0.30</td>
</tr>
</tbody>
</table>

The Z statistic and corresponding P value are reported for the null hypothesis that dN = dS.

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mountain goats are alpine specialists with limited genetic diversity and evolution of the Mhc-DRB1 gene in the two endemic Iberian subspecies of Pyrenean chamois, Capra pyrenaica. Heredity. 93:266–272.


Oram-OLA OLA gene, suggests that the high functional gene according to the translated sequence (Supplementary Appendix IV). The second is that mountain goats may be exposed to more intracellular pathogens (e.g., MHC Class II OLA gene, in particular the Class I OLA gene, suggests that the paucity of variation seen by Mainguy et al. (2007) is not entirely reflective of the mountain goat MHC. Identifying specific transcribed Class I loci should be the next step. From such data, we could confirm whether we are amplifying single or multiple loci, verify our initial assessments of diversity, and also examine this variation relative to external factors such as mate choice and parasite exposure. This approach will help disentangle the relative contribution of these hypothesized factors in maintaining the mountain goats’ MHC Class I diversity.

In conclusion, by analyzing portions of genes from all the 3 MHC classes and the NRAMP gene in mountain goats, we garnered a better sense of the immunodiversity in this species. With many species becoming genome-enabled, future studies should attempt to screen additional immune genes to better characterize MHC diversity. A possible mechanism comes from the association between killer immunoglobulin-like receptors (KIR) found on natural killer cells and the MHC Class I ligands, where more KIR–Class I combinations confer greater disease resistance in humans (Parham 2005). Indeed, MHC Class I variability has been linked to disease resistance (Bonneaud et al. 2006; Madsen and Ujvari 2006) and longevity (Madsen and Ujvari 2006) in wild populations. Third, the mountain goat MHC Class I gene may be involved in social behavior or mate choice (see Leypold et al. 2002; Leinders-Zufall et al. 2004), which could explain the high individual variation. Overall, the additional screening of immune genes, in particular the Class I OLA gene, may be better equipped for climatic oscillations and pathogen exposure than we originally thought, as the minimal diversity appears to have persisted through historical glacial cycles.

**Supplementary Material**

Supplementary material can be found at http://www.jhered. oxfordjournals.org/.

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**References**


