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# Selection and Demography Drive Range-Wide Patterns of Mhc Variation in Mule Deer (odocoileus Hemionus)

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# SELECTION AND DEMOGRAPHY DRIVE RANGE-WIDE PATTERNS OF MHC VARIATION IN MULE DEER (*Odocoileus hemionus*)

by

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ABSTRACT

SELECTION AND DEMOGRAPHY DRIVE RANGE-WIDE PATTERNS OF MHC VARIATION IN MULE DEER (*Odocoileus hemionus*)

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Under the Supervision of Professor Emily Latch

Variation at functional genes involved in immune response is of increasing concern as wildlife diseases continue to emerge and threaten populations. The amount of standing genetic variation in a population is directly associated with its potential for rapid adaptation to novel environments. For genes in the major histocompatibility complex (MHC), which are crucial in activating the immune response and which have extremely high levels of polymorphism, the genetic variation has been shown to be influenced by both parasite-mediated selection and historical population demography. To better understand the relative roles of parasite-mediated selection and demography on MHC evolution in large populations, I analyzed geographic patterns of variation at the MHC DRB class II locus in mule deer (*Odocoileus hemionus*). I identified 31 new MHC-DRB alleles which were phylogenetically similar to other cervid MHC alleles, and I found 1 allele that was shared with white-tailed deer (*Odocoileus virginianus*). I found evidence for selection on the MHC based on high dN/dS ratios, positive neutrality tests, deviations from Hardy-Weinberg Equilibrium (HWE) and greater isolation-by-distance (IBD) than expected under neutrality. However, I also saw evidence that historical demography is important in shaping variation at the MHC, in the similar variation structures between MHC and microsatellites and the lack of significant environmental drivers of variation at either locus. These results show that both natural selection and historical demography are important drivers in

the evolution of the MHC in mule deer and may aid in predicting how future adaptation is shaped when this species is confronted with environmental challenges.

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| | |
|---|---|
| ABS | Antigen-Binding Site |
| BEB | Bayes Empirical Bayes |
| DAPC | Discriminant Analysis of Principal Components |
| DOC | Degree of Change |
| GEA | Genotype-Environment Associations |
| HLA | Human Leukocyte Antigen |
| HWE | Hardy-Weinberg Equilibrium |
| IBD | Isolation by Distance |
| MHC | Major Histocompatibility Complex |
| PCR | Polymerase Chain Reaction |
| RDA | Redundancy Analysis |

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## Introduction

Organisms are exposed to a wide range of environmental changes. Broad-scale environmental transformations have been catalyzed by global climate change and other human-mediated habitat alterations such as fragmentation, increasing pollution, introduction of invasive species, and the establishment of novel pathogens. Natural populations can persist in changing environments by shifting their geographic distributions to track favorable habitats, or by adapting to new conditions (Pease et al. 1989, Janik et al. 2021, Thompson and Fronhofer 2019, Cai et al. 2015). The fate of a population ultimately depends on its adaptive capacity. Populations capable of rapid adaptive responses can maintain biodiversity in rapidly changing environments, whereas those slower to respond are vulnerable to population declines or extinction. Understanding how populations respond to environmental change and the conditions that promote adaptation is important for understanding how biodiversity persists in changing environments.

The amount of standing genetic variation in a population is directly associated with its potential to adapt or evolve in new environmental conditions (Barrett and Schluter 2008; Lai et al. 2019). Populations with high levels of standing genetic variation are predicted to harbor alleles that may not confer a fitness advantage in the current environment but become beneficial as conditions change (Przeworski et al. 2005). By acting on available variation, natural selection can promote rapid evolution through directional (positive) selection and, even with highly polygenic traits, through soft selective sweeps where multiple adaptive alleles sweep to high frequency simultaneously (Messer and Petrov 2013, Bernatchez 2016, Hermisson and Pennings 2005). Fixation of any one allele is delayed under a soft sweep, maintaining genetic variation within and across populations (Orr and Betancourt 2001, Innan and Kim 2004). Soft selective

sweeps are expected to be more important in large populations with abundant standing genetic variation (Karasov et al. 2010, Barton 2010), whereas signatures of directional selection are often stronger in small populations (Ochoa et al. 2020).

The adaptive capacity of a population or species is also affected by its demographic history. Across species, different ecologies and life histories are expected to influence genetic diversity and differentiation. At the population level, different demographic histories yield heterogeneous population dynamics over space and time. Highly variable environments can create fluctuations in population size that impact genetic variation across the genome. If populations become small or isolated, genetic drift will erode genetic variation and drive differentiation among populations, potentially swamping natural selection (Cai et al. 2015, Bollmer et al. 2011, Taylor et al. 2012, Peng et al. 2021, Elbers et al. 2017, Eimes et al. 2011). Gene flow can mitigate the effects of genetic drift if populations are well connected and contributes to a population's ability to adapt to changing environments. These demographically based neutral evolutionary processes impact variation at all loci in the genome, including loci that impact fitness. Thus, standing variation at adaptive loci is shaped by both natural selection and demographic history, driven by the neutral process of mutation, gene flow, and genetic drift. Though it is challenging to disentangle these mechanisms in natural systems, it is critical to understanding the evolution of standing genetic variation at functional loci and their adaptive capacity to respond to changing environments.

Variation at functional genes involved in immune response is of increasing concern as wildlife diseases emerge as serious threats to populations, seen in examples like amphibian chytridiomycosis (Fisher et al. 2009, Van Rooij et al. 2015), white-nose syndrome in bats (Zukal et al. 2014, Foley et al. 2011, Cryan et al. 2013, Yi et al. 2020), and chronic wasting disease in

ungulates (Carlson et al. 2018, Mawdsley 2020, Cullingham et al. 2020, Monello et al. 2017). Genes in the major histocompatibility complex (MHC) are some of the most polymorphic in vertebrate genomes, maintained by natural selection in response to an ever-changing environment of pathogens (Borghans et al. 2004, Piertney and Oliver 2006, Spurgin and Richardson 2010, Winternitz et al. 2013, Radwan et al. 2020). Pathogen-mediated selection at the MHC can occur by way of directional selection in response to the presence of specific parasites or pathogens (Teacher et al. 2009), or balancing selection through non-mutually exclusive mechanisms including heterozygote advantage, negative frequency dependence, and fluctuating selection from heterogenous host-pathogen dynamics (Niskanen et al. 2013, Hedrick 2012, Ejsmond et al. 2010, Eizaguirre et al. 2012, Ashby and Boots 2017). High MHC variation in host populations is expected to be maintained where pathogen populations are diverse and dynamic (Bernatchez and Landry 2003, Sommer 2003, Fraser and Neff 2010, Strand et al. 2012, Quemere et al. 2018). Sexual selection may also play a role as MHC allelic richness has been linked with sexually selected traits (Ivy-Israel et al. 2021, Moore 2019, Reusch et al. 2001, Penn 2002, Winternitz et al. 2013, Dunn et al. 2012, Whittingham et al. 2015).

Historical population demography also influences MHC variation and can be challenging to disentangle from the effects of natural selection. Strong genetic drift in small and isolated populations reduces variation at all loci, including the MHC (Cai et al. 2015, Bollmer et al. 2011, Taylor et al. 2012, Peng et al. 2021, Elbers et al. 2017, Eimes et al. 2011). Comparing patterns of genetic variation at functional and neutral loci can help isolate the role of selection in maintaining MHC variation in natural populations. MHC variation driven by neutral evolutionary mechanisms like genetic drift and demography would be expected to show patterns of variation similar to neutral loci (Miller et al. 2010, Zeisset et al. 2014, Cortazar-Chinarro et al.

2017), whereas parasite-mediated balancing selection would yield divergent patterns of variation (Ekblom et al. 2007, Loiseau et al. 2009, Kyle et al. 2014). Previous studies have used this comparative approach to learn more about adaptation at the MHC (Bateson et al. 2015, Kennedy et al. 2011, Bollmer et al. 2011, Taylor et al. 2012, Peng et al. 2021, Elbers et al. 2017). However, many of these studies have considered populations that are small, isolated, or have experienced recent bottlenecks. Large, highly connected populations may show drastically different results, because genetic drift is not as prominent, and natural selection can drive adaptation of complex traits (Barton 2010).

To better understand the relative roles of parasite-mediated selection and demography on MHC evolution in large populations, I analyzed geographic patterns of variation at the MHC DRB class II locus in mule deer, (*Odocoileus hemionus*). Mule deer are distributed in diverse environments across a broad geographical range, spanning over forty degrees of latitude. Populations are generally large and well connected, lacking strong signatures of genetic drift (Latch et al. 2014), facilitating direct comparison of functional and neutral diversity at a broad scale. In the absence of strong genetic drift, I expect that the pattern of MHC variation among populations will be driven by pathogen-mediated selection. Comparing patterns of adaptive and neutral variation across the mule deer range will not only help disentangle the relative influence of evolutionary mechanisms impacting MHC evolution but may also aid in predicting how these processes might shape future adaptation in this ecologically important and widespread species in the face of changing environments. As the first characterization of MHC in mule deer, these data are also useful as a foundation for future work on MHC evolution in mule deer and other ungulates.

## Materials and Methods

### *Sample collection*

*Odocoileus hemionus* tissue samples were collected across the species' latitudinal range, from animals harvested by hunters between 1995 and 2005. I sampled 24 individuals from each of 16 populations (Figure 1). Populations were located in the core mule deer range (the 'MD' genetic cluster identified in Latch et al. 2014) and outside the known hybrid zone with black-tailed deer in the Pacific Northwest (Latch et al. 2011, Haines et al. 2019). Sampled animals were at least 1 year old and 63.8% of individuals were male.

### *Genotyping at microsatellite and MHC loci*

DNA was extracted from all 384 samples using a modified ammonium acetate protein precipitation protocol (Latch et al. 2008). DNA was assessed by agarose gel electrophoresis and standardized to ~10 ng/µL in TLE buffer (10mm Tris-HCl, pH 8.0, 0.1mm EDTA). All samples were previously genotyped at a panel of 9 microsatellite loci (Latch et al. 2014), which I used in this study for comparative purposes. The microsatellite dataset had 0.4% missing data and no loci that exhibited null alleles (Latch et al. 2014).

I amplified a 390bp product containing the entire MHC class II DRB exon 2 in 20 µL Polymerase Chain Reactions (PCRs) containing 10 ng genomic DNA, 0.5 µM each primer (LA31 and LA32; Sigurdardottir et al. 1991), 200 µM dNTPs, 1 U Phusion DNA polymerase (New England Biolabs), and 1X Phusion buffer. Primers were modified with adapter sequences on both primers and a 10bp individual barcode on the forward primer. PCRs were performed with an initial denaturation at 98 $^{o}$C for 30 s, followed by 25 cycles of 98 $^{o}$C for 15 s, 50 $^{o}$C for

30 s, and 98 °C for 15 s, and a final extension at 72 °C for 10 min. PCR products were cleaned with a Qiagen PCR Purification Kit followed by Agencourt AMPure XP magnetic beads, and DNA concentration was quantified on a Qubit 2.0 fluorometer. PCR products were pooled into eight pools, each with 48 samples, and bead cleaned twice more. DNA concentration was quantified and checked on an Agilent Bioanalyzer chip for each PCR pool. Sequencing of cleaned PCR pools was performed on a Roche 454 FLX Genome Sequencer using Titanium chemistry at the University of Illinois.

To determine the number of MHC alleles per individual, I used the web server AmpliSAT (Sebastian et al. 2016) to de-multiplex samples within pools (AmpliSAS), cluster amplicon sequences within individuals, and filter individual sequences. Although the MHC has not been characterized in mule deer, I predicted a single expressed copy of the MHC-DRB gene based on findings in other New World deer (*Odocoileinae*; Van den Bussche et al. 1999, Mikko and Andersson 1995, Mikko et al. 1999, Kennedy et al. 2011, Ivy-Israel et al. 2020). However, to accommodate possible gene duplication in mule deer, I set the maximum number of alleles per individual to four. Minimum amplicon depth was set to 100 to remove potential artefacts and samples with low reads. I selected the degree of change (DOC) criterion filtering parameter to estimate the number of true alleles based on sequencing depth (Lighten et al. 2014). All other parameters were set to the default settings. Data from individuals was merged into a single file using AmpliCOMBINE within AmpliSAT. I aligned and translated sequences into amino acids in MEGA7 (Kumar et al. 2016) and no stop codons were observed in any samples.

*Population Genetic Data Analysis*

For each of the 16 populations, I calculated measures of genetic diversity separately for MHC and microsatellites. I used GenAlEx 6.5 (Peakall and Smouse 2012) to estimate observed ($H_O$) and expected ($H_E$) heterozygosity, total number of alleles (A), and number of private alleles ($A_P$), and HP-Rare (Kalinowski 2005) to calculate allelic richness ($A_R$) and private allelic richness ($A_{PR}$). I tested for deviation from Hardy-Weinberg Equilibrium (HWE) in GenAlEx, employing a false discovery rate correction for multiple tests (Benjamini and Hochberg 1995). To explore the genetic structure and connectivity of populations at both locus types, I used Discriminant Analysis of Principal Components (DAPC; Jombart et al. 2010) using the adegenet package v2.1.0 in R (Jombart 2008). I also calculated genetic differentiation (Jost's *D*; Jost 2008) between pairs of populations using GenAlEx.

I used several selection and neutrality tests to investigate the influence of natural selection on MHC variation in our mule deer samples. I calculated relative frequencies of nonsynonymous (dN) and synonymous (dS) substitutions for dN/dS (ω) ratios using *codeml* in PAML 4.9j (Yang 2007) which follows the Nei and Gojobori (1986) method. Ratios near 1 indicate selective neutrality whereas values of $\omega > 1$ and $\omega < 1$ suggest positive selection and balancing selection, respectively. I calculated ω values for the entire MHC-DRB exon 2, antigen-binding sites (ABS; inferred from human HLA-DRB (Reche & Reinherz 2003)), and non-ABS. I tested for site-specific positive selection by comparing two pairs of codon substitution models: model M1a (nearly neutral) to M2a (positive selection) and model M7 (beta) to M8 (beta plus ω) (Yang et al. 2000). Pairwise likelihood ratio tests were used to identify codons under selection and the Bayes Empirical Bayes (BEB) method was used to calculate the posterior probability and determine significance of the identified codons (Yang et al. 2005). Posterior probabilities of

>0.95 were considered supported, and codons were considered to be under positive selection if they were identified by either models M2a or M8. I also performed neutrality tests in each population, including Tajima's D (Tajima 1989), Fu's F, Fu and Li's F* and Fu and Li's D* using DnaSP v6.12 (Rozas et al. 2017) to further investigate possible selection at the MHC locus.

To compare patterns of diversity between the putatively adaptive MHC locus and the neutral microsatellite loci, I tested for correlations between MHC- and microsatellite-based genetic diversity (allelic richness and observed heterozygosity) and differentiation (Jost's *D*) using Pearson's correlation coefficients (r) and Mantel (1967) tests with 999 permutations in R. I tested for isolation-by-distance (IBD) in MHC and microsatellites by conducting a Mantel test with 999 permutations between a geographic distance matrix and pairwise genetic distance matrices of Jost's *D*. An absent or weak IBD correlation suggests high gene flow or selection that favors similar alleles across locations, whereas strong correlations suggest population isolation or divergent selection. I compared IBD patterns between markers to gain additional insight into the relative importance of connectivity and selection in this system.

*Environmental Analysis*

I examined the relationship between population-level genetic diversity and latitude, elevation, and environmental variables for MHC and microsatellites. To account for variation in rates of polymorphism between marker types (average 12 alleles per locus in MHC and 5 in microsatellites) I used mean-centered allelic richness (Bateson et al. 2015). I used a linear regression to quantify the relationship between mean-centered allelic richness and latitude. To quantify environmental influences on genetic diversity, I used redundancy analysis (RDA; Forester et al. 2018). RDA is a multivariate ordination method which can help detect genotype-

environment associations (GEAs) and loci under selection. I used latitude, longitude, elevation, and 15 bioclimatic variables for each population obtained from the ClimateWNA database (www.climateWNA.com; Wang et al. 2012) from the years 1981 – 2010. The bioclimatic variables included mean annual temperature, mean warmest month temperature, mean coldest month temperature, temperature difference between warmest month and coldest month (or seasonality), mean annual precipitation, annual heat-moisture index ((temperature+10)/(precipitation/1000)), mean annual relative humidity, and mean temperature and precipitation for each season (winter, spring, summer, and fall). I also obtained elevation for each population from www.mapcoordinates.net/en. I tested for multicollinearity between variables and removed correlated variables (> 0.8). I ran RDA analyses for microsatellites using allelic richness at each locus, and for MHC using allele frequencies per population. I plotted the RDA axes using the vegan package v2.5-7 in R (Oksanen et al. 2020) to visualize how environmental predictors explained the genetic variation at each locus. F statistics were used to test the significance of the full RDA models and each constrained axis.

*MHC phylogeny*

I constructed a maximum likelihood phylogenetic tree using IQ-TREE (v2.1.2, Nguyen et al. 2015) to visualize evolutionary relationships between our mule deer MHC alleles and other cervid MHC alleles. I used ModelFinder (Kalyaanamoorthy et al. 2017) as implemented within IQ-TREE to determine the best substitution model based on Bayesian Information Criteria. Branch support was assessed using 1000 ultrafast bootstrap replicates (UFBoot2; Hoang et al. 2018) using the F81+F+I+G4 model in IQ-TREE. I visualized the phylogeny with FigTree

(v1.4.4). The phylogeny contains the 250 bp MHC-DRB exon 2 sequences from the 31 new mule deer alleles generated in this study, the 30 MHC DRB alleles previously described in white-tailed deer (*Odocoileus virginianus*), and MHC DRB sequences from five outgroup species within Cervidae [moose (*Alces alces*); roe deer (*Capreolus capreolus*); elk (red deer, *Cervus elaphus*); sika deer (*Cervus nippon*); and caribou (reindeer, *Rangier tarandus*); Table 3]. The tree was rooted with cattle DQB sequences (*Bos taurus*).

## Results

### *Sequencing Results*

I successfully sequenced the entire 250 bp MHC class II DRB2 in 384 individuals across 16 populations of mule deer. After filtering, I obtained an average of 1452 amplicons per individual for 380 individuals (Figure S1). Four individuals, each from a different population, yielded few reads (< 100) and were removed from further analysis. There were 69 variable sites, corresponding to amino acid changes at 37 of the 83 codons. All alleles were unique at the amino acid level. The number of amino acid differences between alleles ranged from 1 to 26. Each individual had a maximum of two alleles, consistent with the presence of a single MHC-DRB locus (Figure S1). One individual showed three alleles, but one of the called alleles had low reads and could be ruled out (Figure S1). I observed 31 new alleles and one allele (Odvi-DRB*09) previously recorded in white-tailed deer (Van Den Bussche et al. 2002). The new mule deer alleles (Odhe-DRB*01-31) were deposited in GenBank. Although four alleles (Odhe-DRB*23, Odhe-DRB*25, Odhe-DRB*27, and Odhe-DRB*31) were each observed only once, each had over 500 reads when present and thus were considered to be true alleles and included in downstream analyses (Figure S1).

### *Population Genetic Data Analysis*

MHC allelic richness varied among populations while microsatellite allelic richness remained relatively constant (Tables 1 and 2). The number of observed MHC alleles in each population ranged from 6 in the northernmost population (YK-SE) to 17 at middle latitudes (NV-PC and WY-SH), with only two populations having private alleles (MT-RV and TX-AL; Table

2). The most common MHC allele (Odhe-DRB*01) was the only allele observed in all populations (Figure 1). Although white-tailed deer are primarily found in the eastern half of North America, the shared allele between mule deer and white-tailed deer (Odvi-DRB*09) was not restricted to populations along the contact zone (Figure 1). As with MHC, microsatellite allelic richness was lowest at the most northerly site YK-SE (4.19), but in contrast to MHC diversity, was highest at other northern sites AB-OR and MT-RV (5.74 and 5.71, respectively; Table 1). Private alleles in microsatellite loci were observed in most populations, with the southwestern site AZ-KF having the highest private allelic richness (0.28; Table 1). Overall, allelic richness was positively correlated between MHC and microsatellites ($r = 0.697$; $p = 0.003$). Observed heterozygosity ranged more widely for MHC than microsatellites and was not significantly correlated between genetic markers ($r = 0.092$; $p = 0.73$). The microsatellite loci were at HWE in most populations (with 4/144 tests deviating from HWE across the 9 microsatellite loci), whereas deviations from HWE were evident at the MHC for 8 of 16 populations (Tables 1 and 2). Neutral and demographic processes affect all loci at expectedly the same level, thus the presence of heterozygote deficiencies specifically in the MHC suggest selection at that locus. DAPC analysis of both MHC and microsatellites revealed little overall population structure, with most population clusters overlapping (Figure 2). I saw a slight latitudinal trend in the microsatellite DAPC scatterplot along axis 1 that was not present in the MHC data (Figure 2). TX-AL and YK-SE populations were slightly more distinct from other populations in both analyses (Figure 2).

I observed evidence for selection on MHC class II DRB2 in mule deer. dN/dS ratios ($\omega$) above 1 for the full exon ($\omega = 1.18$) and the ABS sites ($\omega = 2.05$) indicate positive selection, whereas non-ABS sites showed evidence of balancing selection ($\omega = 0.59$). Further support for

positive selection comes from comparing codon substitution models; models M2a and M8 provided a better fit to the MHC sequence data than M1a and M7 ($p < 0.01$). Nine sites were identified as under positive selection in model M2a (posterior probability > 0.95). Model M8 identified the same positively selected sites as M2a, as well as an additional 3 (Figure 4). Ten of the twelve sites identified as being under positive selection using these codon substitution models corresponded to human HLA antigen binding sites (Figure 4). Neutrality tests showed positive values across all populations (Table 2). Fu and Li's D* and F* tests showed 6 and 5 populations with statistically significant values, respectively (Fu and Li's D*: AZ-30, CO-SJ, KS-BD, NV-PC, SK-14, and YK-SE; Fu and Li's F* significant values included the same as D* except for NV-PC). The YK-SE population showed statistically significant values across all neutrality analyses. Significant values suggest that selection may be driving MHC variation in these populations, and positive values point to balancing selection, recent population bottlenecks, or the presence of intermediate frequency alleles.

Genetic differentiation between populations was not driven by the same evolutionary processes in both marker types (Mantel $r = 0.10$, $p = 0.28$). Whereas both MHC and microsatellite differentiation could be explained using a simple IBD pattern of gene flow, the slope was significantly steeper for MHC (0.180) than microsatellites (0.022) ($p < 0.0001$; Figure 3). Both marker types experience identical gene dispersal patterns, thus stronger IBD (steeper slope) suggests the presence of evolutionary factors that act exclusively on MHC variation.

*Environmental analysis*

MHC and microsatellite diversity were not significantly affected by latitude, elevation, or any bioclimatic variables. Mean-centered allelic richness values were similar across latitudes

(Figure 5) and a low coefficient of determination ($R^2$ = 0.0028 for MHC and 0.0007 for microsatellites) suggests that latitude is not an important driver of among-population variation in allelic richness in our system. Investigation of environmental variables revealed many that were correlated with each other; 11 predictors were removed from analysis leaving latitude, longitude, elevation, mean annual temperature, temperature difference between warmest and coldest month, mean annual precipitation, and relative humidity variables in the RDAs. The first two axes in the microsatellite RDA explained 46% and 23% of the variance in allelic richness across populations and both the full RDA model and axis 1 were significant (p = 0.05 and 0.03; Figure 6a). Populations tended to follow logical patterns in terms of relation to environmental predictors; populations located in southwestern North America tended to be positively related to mean annual temperature and negatively related to mean annual precipitation and seasonality, while northern populations showed the opposite relationships (Figure 6a). The MHC-based RDA showed similar spatial patterns of populations; sites which had higher latitudes also were negatively related to mean annual temperature, but positively related to temperature difference between warmest and coldest month (Figure 6b). Axes 1 and 2 explained 27% and 21% of the variance, respectively, in allele frequencies for MHC across the 16 populations (Figure 6b). However, the full RDA model and all axes within the MHC RDA were not significant (all p > 0.1), thus these results do not provide evidence for any specific environmental conditions that may be acting as a selective force on the MHC locus in mule deer populations.

*MHC phylogeny*

The maximum likelihood phylogenetic tree showed little evidence for distinct MHC lineages among cervid species (Figure 7). The bootstrap support values were low (< 0.92)

throughout the tree.  I observed some clustering of the 3 moose sequences and a cluster containing all 3 caribou sequences. Mule deer, white-tailed deer, sika deer, elk, and roe deer were all polyphyletic, though most *Odocoileus* sequences were in a clade separate from other cervids, albeit with weak support (0.53; Figure 7).

## Discussion

Understanding the relative effects of natural selection and demography at functionally important immune genes in wildlife is increasingly important as emerging epizootic and zoonotic diseases threaten animal and human health (Daszak et al. 2000, Russell et al. 2020). Our comparative analysis of broad-scale variation at the MHC and neutral microsatellite loci in a widespread and mobile species adds much to the developing picture of how evolutionary mechanisms work collectively to shape functional variation at a broad scale. In this study, I found that while selection plays an inherent role in the evolution of the MHC in mule deer, demography is also a significant predictor of broad scale patterns of MHC variation. Population differentiation was stronger at the MHC than at neutral microsatellite loci, resulting from selection for locally adapted MHC variation driving differentiation beyond that arising from evolutionary processes with genome-wide impact (i.e., drift and gene flow). Overall, our results are consistent with the hypothesis that local pathogens play a role in shaping variation at immune genes. But they also reveal that demography is a major contributor to functional diversity at a broad scale, even in large and mobile populations where drift is expected to be weak. Our data also provide the first assessment of MHC polymorphism in mule deer, adding to our understanding of cervid evolution and selection pressures on MHC polymorphism across ruminants; MHC alleles I sequenced were evolutionarily similar to other cervid MHC alleles with similar patterns of variation.

Patterns of polymorphism in the mule deer MHC revealed classic signatures of natural selection. I found high dN/dS ratios and positive neutrality test values in the MHC sequences, on the entire locus and in peptide-binding regions. Tests of codon substitution models also revealed

that the codon sites that were under selection closely matched the inferred antigen-binding sites from the human HLA molecule. Our results showing that selection acts on functional codons within the MHC are consistent with previous studies in ungulates and other species (Ivy-Israel et al. 2020, Van Den Bussche et al. 1999, Cai et al. 2015, Perez-Espona et al. 2019, Gao et al. 2018, Mikko and Andersson 1995, Mikko et al. 1997 and 1999, Swarbrick et al. 1995, Wu et al. 2004, Eimes et al. 2010).

Signatures of selection that I observed at the MHC were not present in the neutral microsatellite loci. Heterozygote deficiencies at the MHC locus were found in 50% of the mule deer populations surveyed, whereas microsatellites were consistently in Hardy-Weinberg equilibrium (6 loci exhibited no deviations, and the other 3 loci exhibited heterozygote deficiencies in 1 or 2 populations). Deviations from expected heterozygosity and HWE can typically be attributed to non-random mating, population mixing, genetic drift, or selection (Zhai et al. 2017). Because these deviations were present at functional but not neutral loci, the most likely cause for the observed MHC heterozygote deficiencies is selection. The MHC also experienced greater genetic distance over the same geographic space compared to the microsatellites, seen by the steeper slope in the IBD analysis. This supports the idea that while neutral processes may establish a baseline of genetic diversity across all loci, natural selection is driving additional differentiation at the MHC locus. The stronger population differentiation at the MHC than at neutral loci has also been seen in other vertebrates (Ekblom et al. 2007, Kyle et al. 2014, Loiseau et al 2009, Oliver et al. 2009). Many previous studies looked at variation in small, isolated, or recently bottlenecked populations (Arguello-Sanchez et al. 2018, Bollmer et al. 2011, Campos et al. 2006, Elbers et al. 2017, Miller et al. 2010, Taylor et al. 2012, Strand et al. 2012), where drift can have an outsized impact on adaptive variation (Ejsmond and Radwan 2011,

Eimes et al. 2011, Bateson et al. 2015). In our large and well-connected populations, drift is likely to be negligible and differentiation patterns at adaptive loci are likely to be driven by differences in local selection pressures (Alcaide et al. 2008). MHC variation is expected to coincide with differences in pathogen prevalence, but pathogen communities are dynamic over both space and time (Salkeld et al. 2008). Examining how MHC variation changes with spatially or temporally varying parasite pressure would yield additional insight into the relative roles of drift and selection (e.g., Dionne et al. 2007, Eizaguirre et al. 2012, Charbonnel and Pemberton 2005, Schwensow et al. 2007).

Selection-based spatial genetic structure at the MHC may be mediated by one or more mechanisms including heterozygote advantage, frequency-dependent selection, or varying pathogen pressures. The difference in environmental conditions across populations at a broad scale may foster differences in pathogen abundance and community composition, creating variable selective pressure across the mule deer range (Bell 2010, Bergland et al. 2014, O'Connor et al. 2020). The true landscape of parasite-induced selection pressure is not well described in mule deer but may be associated with latitude (Awadi et al. 2018, Dionne et al. 2007, Andreani et al 2020, Guernier et al. 2004, Tonteri et al 2010), or environmental factors (Hawkins and Diniz-Filho 2004). Spatially heterogenous selection would be expected to maintain polymorphism and facilitate local adaptation (Alcaide et al 2008, Charbonnel et al. 2005, Collin et al. 2013, Ekblom et al 2007, Loiseau et al 2009, Sallaberry-Pincheira et al 2016, Quemere et al. 2018). High polymorphism across populations at MHC genes may help populations respond to a wide variety of pathogens and increase population fitness, though costs to maintain diversity may impose an upper limit (Gagnon et al. 2020). In mule deer, low rates of

gene duplication and allelic richness compared to other mammals suggest that such a trade-off may not be critical in ungulates (Winternitz et al. 2013).

Historical demography works to further shape MHC evolution in mule deer, and its effects are evident even in large and well-connected populations. DAPC analysis revealed similar patterns of genetic structure in both MHC and microsatellite datasets, and neither dataset showed strong correlations with specific environmental variables. Both the microsatellites and the MHC showed patterns of IBD, which suggests that neutral genetic processes are important in shaping genome-wide diversity at both neutral and functional loci. MHC allelic richness was overall higher than microsatellites, but when corrected for overall levels of variation was similar to microsatellites and did not show statistically significant latitudinal or environmental trends that might be expected if parasite-mediated selection was the driving force behind MHC evolution (Guernier et al. 2004, Preisser 2019). The 16 populations studied here all have historically large effective population sizes and are from the same evolutionary lineage, suggesting that they have experienced similar long-term evolutionary histories (Latch et al. 2014).

There are several reasons why I could see genomic signatures of both selection and demography in this system. One possibility is that high effective population sizes and high levels of standing genetic variation may contribute to natural selection that is widespread and sustained, yet weak. A combination of demographic influence and weak selection across many functional loci has been shown in mule deer, albeit in a different adaptive context (Haines et al. 2019). Weak pathogen-mediated selection is feasible in deer, where pathogens are often rare relative to their host or have low virulence (Myers et al. 2015). High survival and a lack of predators in their natural environments likely also contributes to weaker selective pressures in mule deer

(Bergman et al. 2015, Lendrum et al 2018). A second possible reason that signatures of both selection and demography could be maintained in MHC class II variation is if other genes are important in pathogen response. MHC genes are highly linked in mammals (Nonaka et al. 1997, Ohta et al. 2000), but genomic signatures of selection on different MHC classes have been shown to vary depending on species (Minias et al. 2016 and 2018). Along these lines, a virulent or prevalent pathogen may not trigger a class II response but may be defended against via another pathway, such as toll-like receptors and cytokines that have been associated with disease resistance and survival in wild populations (Tschirren et al. 2013, Turner et al. 2011, Grueber et al. 2013). A third possibility is that the MHC diversity I estimated using genomic DNA sequences may not reflect the expressed diversity, thus providing an incomplete picture of selection. For example, a comparison of cDNA and genomic DNA in songbirds revealed fewer expressed MHC-I alleles than predicted by sequence data (O'Connor and Westerdahl 2021). Comparing our genomic variation in MHC class II with cDNA diversity would show whether expressed diversity displays stronger signatures of selection in mule deer.

Another important step made with our data is that I was able to characterize the MHC locus in a new species and compare it to other ungulates. Our phylogenetic analysis of evolutionary relationships between MHC DRB2 alleles in mule deer and other cervids showed MHC alleles from similar species tend to cluster together. The white-tailed deer and mule deer MHC alleles tended to be intermixed but formed their own clade. Our phylogenetic tree was similar to the MHC-DRB2 tree from Ivy-Israel et al. (2020), and indeed used many of the same taxa, though our tree had higher bootstrap supports throughout. Shared alleles across species and polyphyletic grouping appears to be common for MHC (Eimes et al. 2010 and 2015, Bollmer et al. 2011, Lenz et al. 2013). I observed one shared allele between mule deer and white-tailed deer

(Odvi-DRB*09). This could be an example of a trans-species polymorphism, where similar historical biogeography between species typically leads to shared alleles or similar MHC variation patterns (Ballingall et al. 2010, Klein 1987). Because white-tailed deer and mule deer are closely related species that share many pathogens (Stephens et al. 2017), selection may have maintained the shared allele to confer resistance to a common pathogen. Alternatively, it is possible that the shared allele is an independent identical-by-state mutation. The fact that the shared allele is more recently derived according to the phylogenetic tree, and the fact that it is present in populations where white-tailed and mule deer ranges do not overlap suggests that it may not be a true trans-species polymorphism. A broader survey of *Odocoileus* and related species from North America would help determine the extent and evolutionary history of this shared polymorphism. More detailed studies that characterize the pathogen community and connect estimates of fitness for particular alleles at the MHC and other immune genes are needed to better understand how natural selection influences the evolution of the adaptive immune response in ruminants.

**Table 1. Summary of genetic variation in 9 microsatellite loci by population.**

| Population | | Microsatellites | | | | |
|---|---|---|---|---|---|---|
| | n | $A_R$ | $A_{PR}$ | $H_O \pm$ (SE) | $H_E \pm$ (SE) | HWE (loci) |
| AB-728 | 24 | 5.29 | 0 | 0.657 (0.059) | 0.659 (0.043) | ns |
| AB-OR | 23 | 5.74 | 0 | 0.652 (0.058) | 0.645 (0.050) | ns |
| AZ-30 | 24 | 4.98 | 0.10 | 0.605 (0.069) | 0.606 (0.067) | ns |
| AZ-KF | 24 | 4.42 | 0.28 | 0.625 (0.082) | 0.586 (0.072) | * (1) |
| CO-SJ | 24 | 5.32 | 0.01 | 0.648 (0.064) | 0.652 (0.048) | ns |
| CO-ST | 24 | 5.51 | 0.12 | 0.648 (0.080) | 0.617 (0.059) | ns |
| KS-BD | 24 | 5.31 | 0 | 0.616 (0.067) | 0.598 (0.064) | ns |
| MT-RV | 24 | 5.71 | 0.22 | 0.648 (0.052) | 0.648 (0.049) | ns |
| ND-SW | 24 | 5.29 | 0 | 0.653 (0.063) | 0.631 (0.060) | ns |
| NV-PC | 24 | 5.19 | 0.03 | 0.667 (0.043) | 0.663 (0.044) | ns |
| SD-CU | 24 | 5.31 | 0 | 0.704 (0.046) | 0.657 (0.043) | ns |
| SK-14 | 23 | 4.8 | 0 | 0.560 (0.081) | 0.587 (0.064) | ns |
| SO-CS | 24 | 4.69 | 0 | 0.620 (0.069) | 0.592 (0.062) | ns |
| TX-AL | 23 | 5.00 | 0.06 | 0.589 (0.074) | 0.581 (0.074) | ns |
| WY-SH | 24 | 5.43 | 0.19 | 0.611 (0.070) | 0.640 (0.055) | * (2) |
| YK-SE | 23 | 4.19 | 0.21 | 0.541 (0.067) | 0.572 (0.061) | * (1) |

Populations are listed with their sample sizes (n). Microsatellite statistics include allelic richness ($A_R$), private allelic richness ($A_{PR}$), observed ($H_O$) and expected heterozygosity ($H_E$) ± standard error (SE), and deviations from HWE (using a False Discovery Rate significance threshold value: * = $p<0.0056$) listed with the corresponding number of loci (HWE (loci)). Population codes are alphanumeric, with the prefix describing the state or province of collection and the suffix describing the specific sample location.

**Table 2. Summary of MHC class II DRB2 variation by population.**

| Population | | MHC | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | A | $A_P$ | $H_O$ | $H_E$ | HWE p value | Tajima's D | Fu's Fs | Fu and Li's D* | Fu and Li's F* |
| AB-728 | 24 | 13 | 0 | 0.667 | 0.837 | * | 1.25 | 11.69 | 1.31 | 1.54 |
| AB-OR | 23 | 14 | 0 | 0.696 | 0.868 | ** | 1.48 | 10.38 | 1.07 | 1.46 |
| AZ-30 | 24 | 10 | 0 | 0.750 | 0.867 | ns | 1.32 | 17.42 | 1.57* | 1.76* |
| AZ-KF | 24 | 11 | 0 | 0.750 | 0.814 | ns | 1.16 | 13.65 | 1.12 | 1.36 |
| CO-SJ | 24 | 10 | 0 | 0.583 | 0.839 | * | 1.65 | 18.13 | 1.67** | 1.99** |
| CO-ST | 24 | 13 | 0 | 0.583 | 0.859 | ns | 0.82 | 10.96 | 1.11 | 1.20 |
| KS-BD | 24 | 11 | 0 | 0.625 | 0.859 | * | 0.67 | 12.46 | 2.04** | 1.84* |
| MT-RV | 24 | 16 | 3 | 0.708 | 0.893 | ** | 1.42 | 6.95 | 0.96 | 1.35 |
| ND-SW | 24 | 15 | 0 | 0.583 | 0.832 | *** | 0.61 | 7.36 | 1.21 | 1.18 |
| NV-PC | 24 | 17 | 0 | 0.750 | 0.917 | ns | 0.91 | 6.46 | 1.60* | 1.61 |
| SD-CU | 24 | 10 | 0 | 0.542 | 0.824 | *** | 1.06 | 15.58 | 0.77 | 1.05 |
| SK-14 | 23 | 11 | 0 | 0.435 | 0.831 | *** | 0.98 | 12.64 | 1.76** | 1.76* |
| SO-CS | 24 | 8 | 0 | 0.583 | 0.829 | ns | 1.66 | 20.09 | 1.04 | 1.51 |
| TX-AL | 23 | 11 | 2 | 0.783 | 0.836 | ns | 1.42 | 15.52 | 1.10 | 1.45 |
| WY-SH | 24 | 17 | 0 | 0.833 | 0.880 | ns | 1.06 | 6.66 | 1.24 | 1.40 |
| YK-SE | 23 | 6 | 0 | 0.565 | 0.793 | ns | 2.24* | 26.75 | 2.00** | 2.49** |

Populations are listed with their sample sizes (n). MHC statistics include number of alleles (A), number of private alleles ($A_P$), observed ($H_O$) and expected heterozygosity ($H_E$), neutrality test values including Tajima's D, Fu's Fs, Fu and Li's D* and F*, and deviations from HWE listed as a significance value. Population codes are alphanumeric, with the prefix describing the state or province of collection and the suffix describing the specific sample location. Significance values: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

**Table 3. Taxa used in phylogenetic analysis.**

| | Sample name | GenBank ID | Reference |
|---|---|---|---|
| **Mule deer** | Odhe-DRB*01-31 | | This study |
| *Odocoileus hemionus* | | | |
| **White-tailed deer** | Odvi-DRB*01-15 | AF082161-AF082175 | Van Den Bussche et al. 1999 |
| *Odocoileus virginianus* | Odvi-DRB*16-18 | AF407169-AF407171 | Van Den Bussche et al. 2002 |
| | Odvi-DRB*19-30 | MK952679-MK952690 | Ivy-Israel et al. 2020 |
| **Moose** | Alal-DRB*01 | X82398 | Mikko & Andersson 1995 |
| *Alces alces* | Alal-DRB*02-03 | X83278-79 | |
| **Roe deer** | Caca-DRB*0101 | U90923-U90925 | Mikko et al. 1997 |
| *Capreolus capreolus* | Caca-DRB*0201 | | |
| | Caca-DRB*0301 | | |
| **Elk** | Ceel-DRB*10-11 | U11110-U11111 | Swarbrick et al. 1995 |
| *Cervus elaphus* | Ceel-DRB*19 | U11119 | |
| **Sika deer** | Ceni-DRB*02 | AY679485 | Wu et al. 2004 |
| *Cervus nippon* | Ceni-DRB*09-10 | AY679492-AY679493 | |
| **Caribou** | Rata-DRB*0102 | AF012717 | Mikko et al. 1999 |
| *Rangier tarandus* | Rata-DRB*0201 | AF012719 | |
| | Rata-DRB*0401 | AF012721 | |
| **Cattle** | Bola-DQB1*1A | S83910 | Sigurdardottier et al. 1992 |
| *Bos taurus* | Bola-DQB1*02 | U77787 | |

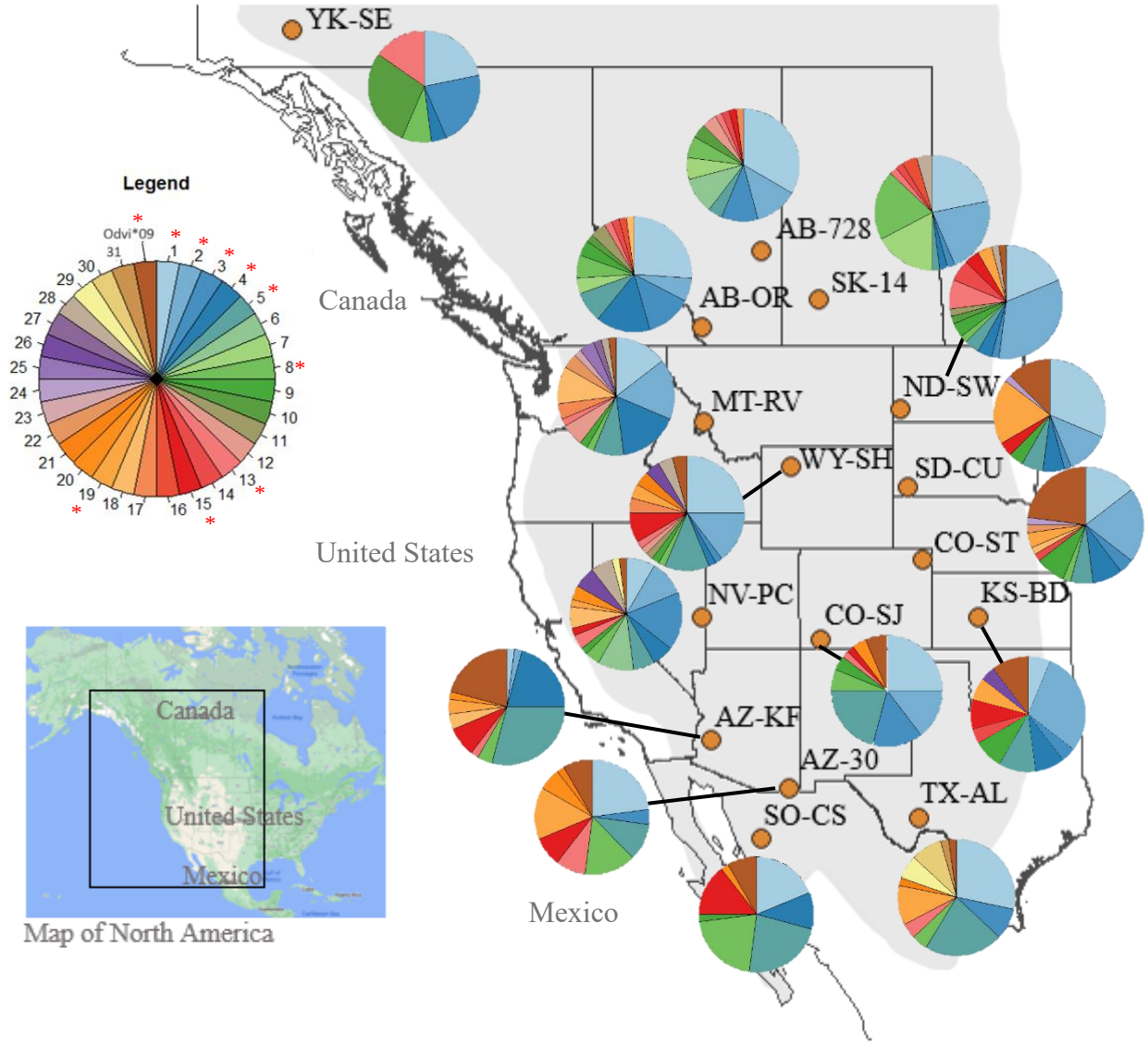


**Figure 1. Sampling sites and MHC allele frequencies.** 24 individuals were sampled from each of 16 sites spanning the range of mule deer (shown in light grey) between 1995 and 2005. The orange circles show the location of the sampling sites. The pie charts represent the proportion of different MHC alleles found in each population, with each allele's respective color shown in the legend chart (1= Odhe-DRB*01). The top 10 most common alleles are labeled in the legend with asterisks. Only the most common allele, Odhe-DRB*01, shown in the lightest blue, was found in every population. The shared allele between mule deer and white-tailed deer, Odvi-DRB*09, is shown in the darkest brown.
(*AB-728* Alberta, Wildlife Management Unit 728; *AB-OR* Alberta, Oldman River; *AZ-30* Arizona, Game Management Unit 30A; *AZ-KF* Arizona, Kofa area; *CO-SJ* Colorado, San Juan; CO-ST Colorado, Sterling; *KS-BD* Kansas, Burdett; *MT-RV* Montana, Ravali County; *ND-SW* North Dakota, southwestern; *NV-PC* Nevada, Pioche Wildlife Management Unit 231; *SD-CU* South Dakota, Custer; *SK-14* Saskatchewan, Wildlife Management Zone 14E; *SO-CS* Sonora, central; *TX-AL* Texas, Alpine/Stockton/Sanderson; *WY-SH* Wyoming, Shoshone River; *YK-SE* Yukon, southeast.)

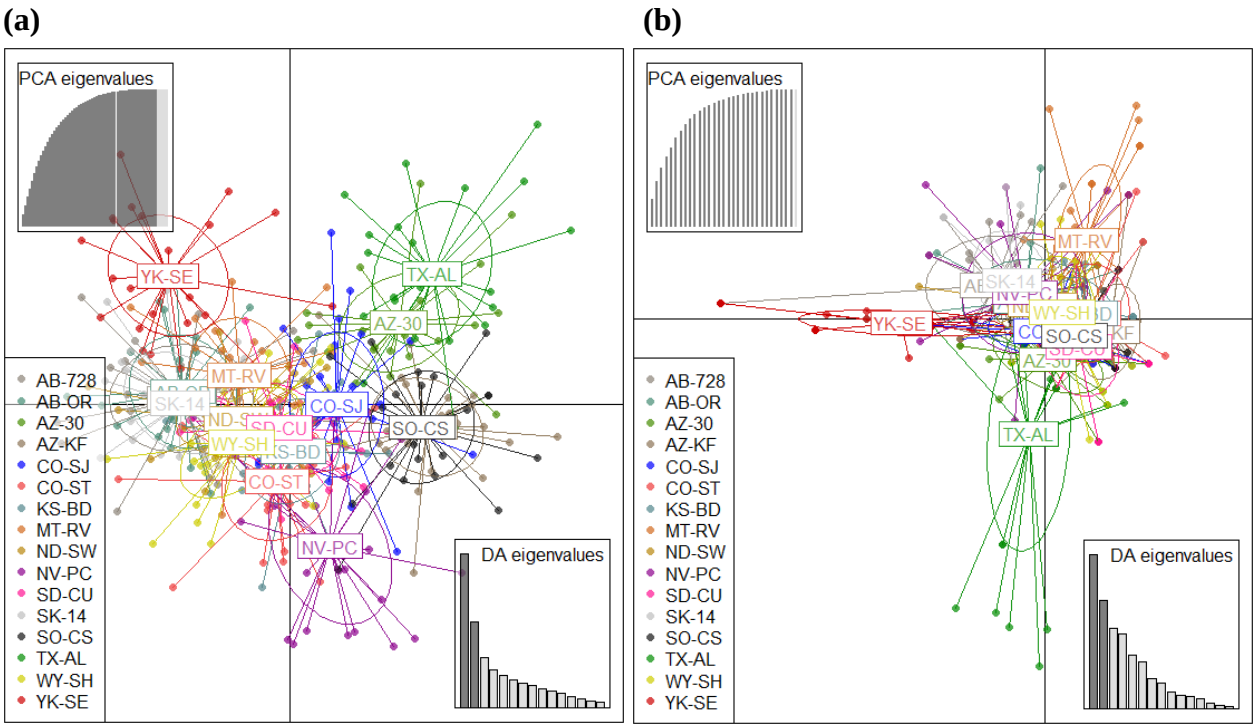


**Figure 2. Cluster analysis using Discriminant Analysis of Principal Components (DAPC).** Scatterplots show the DAPC of the 16 mule deer populations at the (a) microsatellite loci and (b) the MHC locus. Each individual is represented as a point, colored based on its population of origin, and populations are surrounded by 95% inertia ellipses.

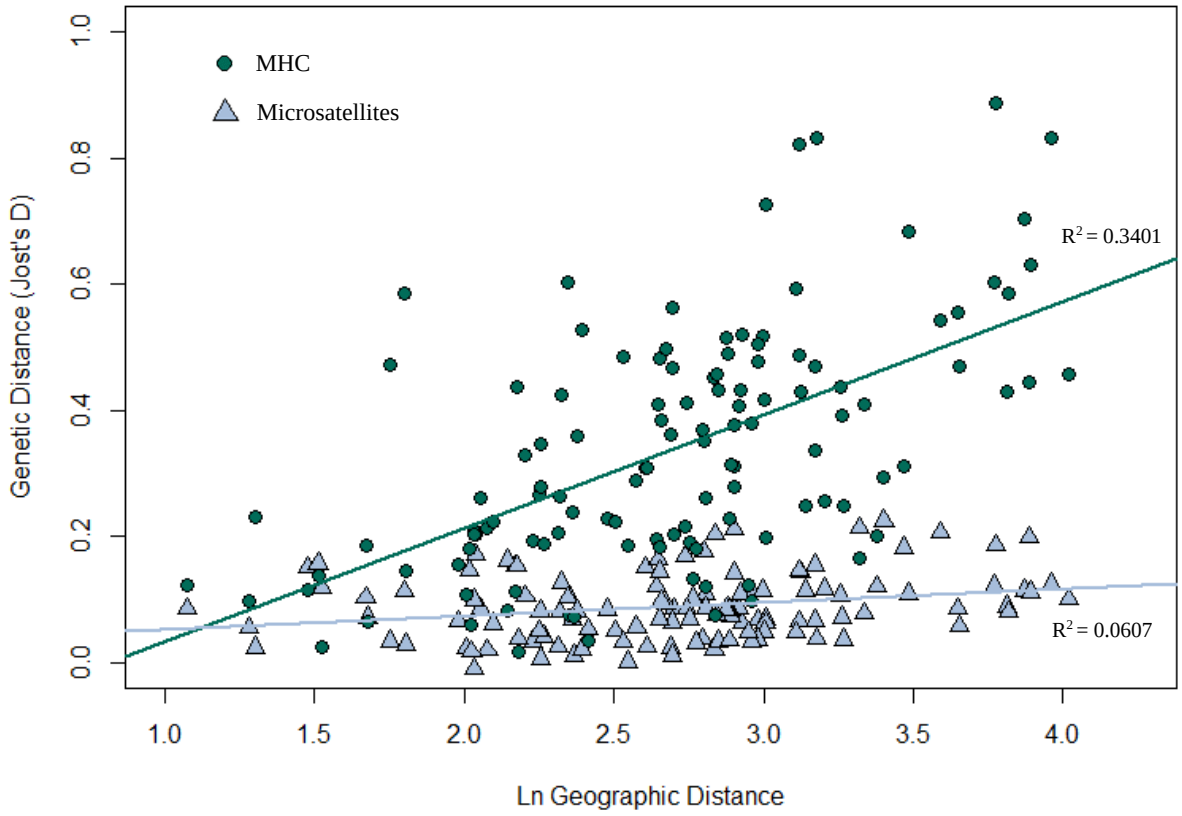


**Figure 3. Isolation by distance.** The genetic distance (Jost's *D*) between each pair of populations was calculated for microsatellites (blue triangles) and MHC (green circles) and compared to geographic distance.

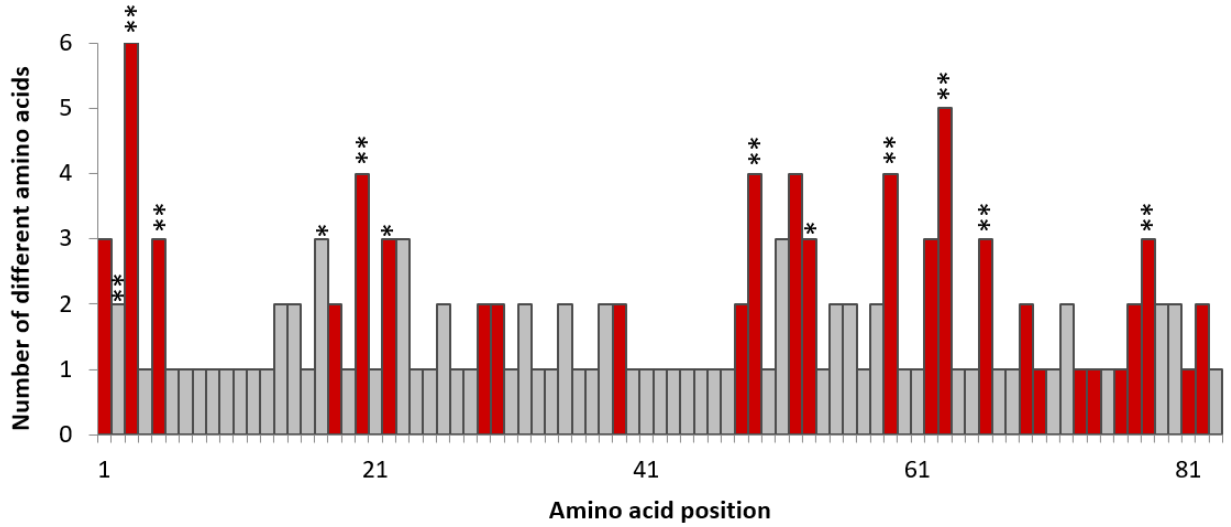


**Figure 4. Variation in amino acids for MHC class II DRB2.** Amino acid sites corresponding to human HLA antigen binding sites are shown in dark red. Positively selected sites using PAML model M8 are denoted by asterisks (* = $p < 0.5$ and ** = $p < 0.01$).

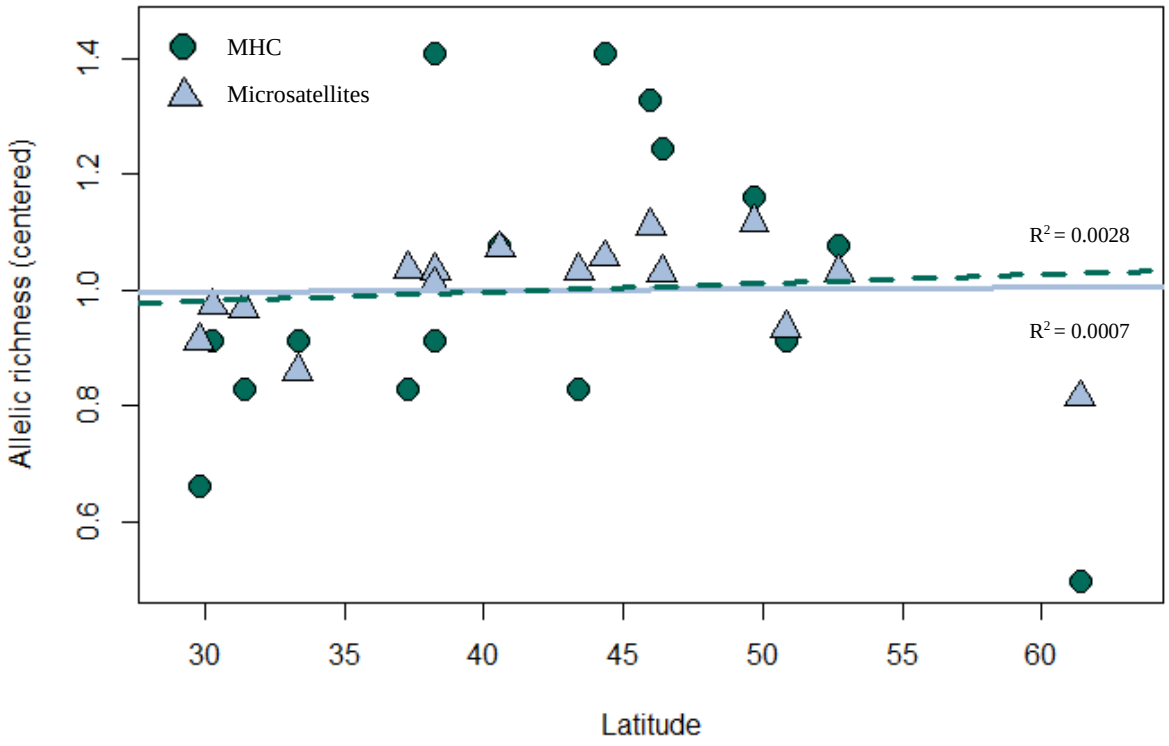


**Figure 5. Mean-centered allelic richness and latitude.** Mean-centered allelic richness is similar across latitudes for both MHC (green circles, dashed line) and for microsatellites (averaged across loci; blue triangles, solid line).

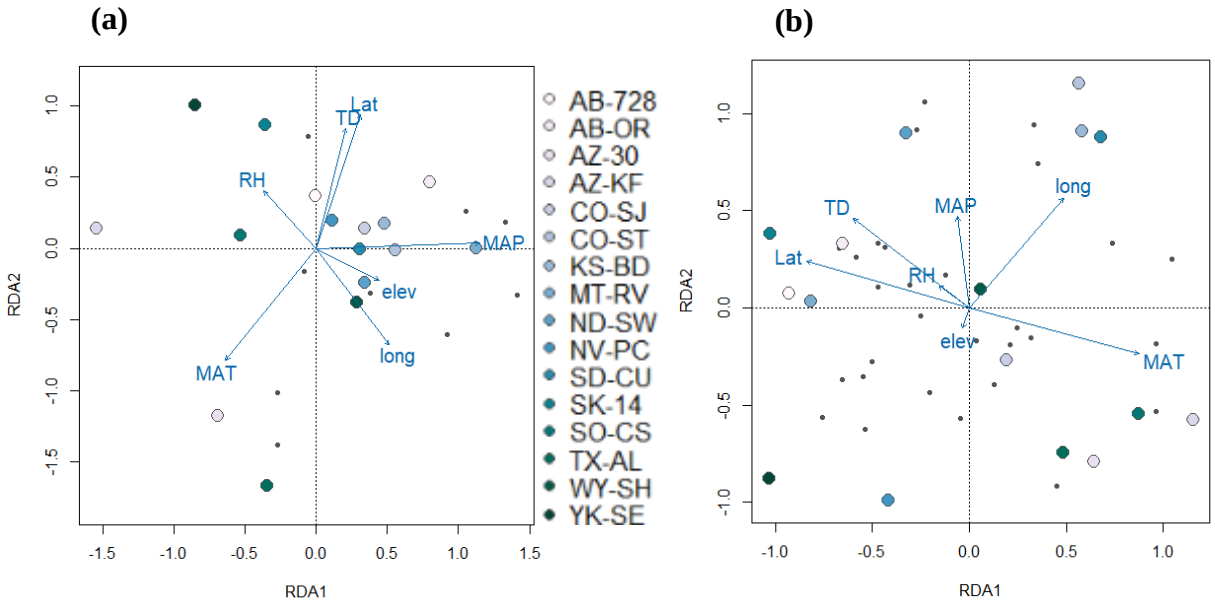


**Figure 6. Redundancy Analysis (RDA) for microsatellites and MHC.** (a) Axes 1 and 2, explaining 46% and 23% of the variance, respectively, in allelic richness for 9 microsatellite loci across 16 populations. (b) Axes 1 and 2, explaining 27% and 21% of the variance in allele frequencies for the MHC locus across 16 populations. Points for each plot are colored by population. Vectors in blue show constraining axes, or environmental predictors, pointing in the direction of positive contribution.

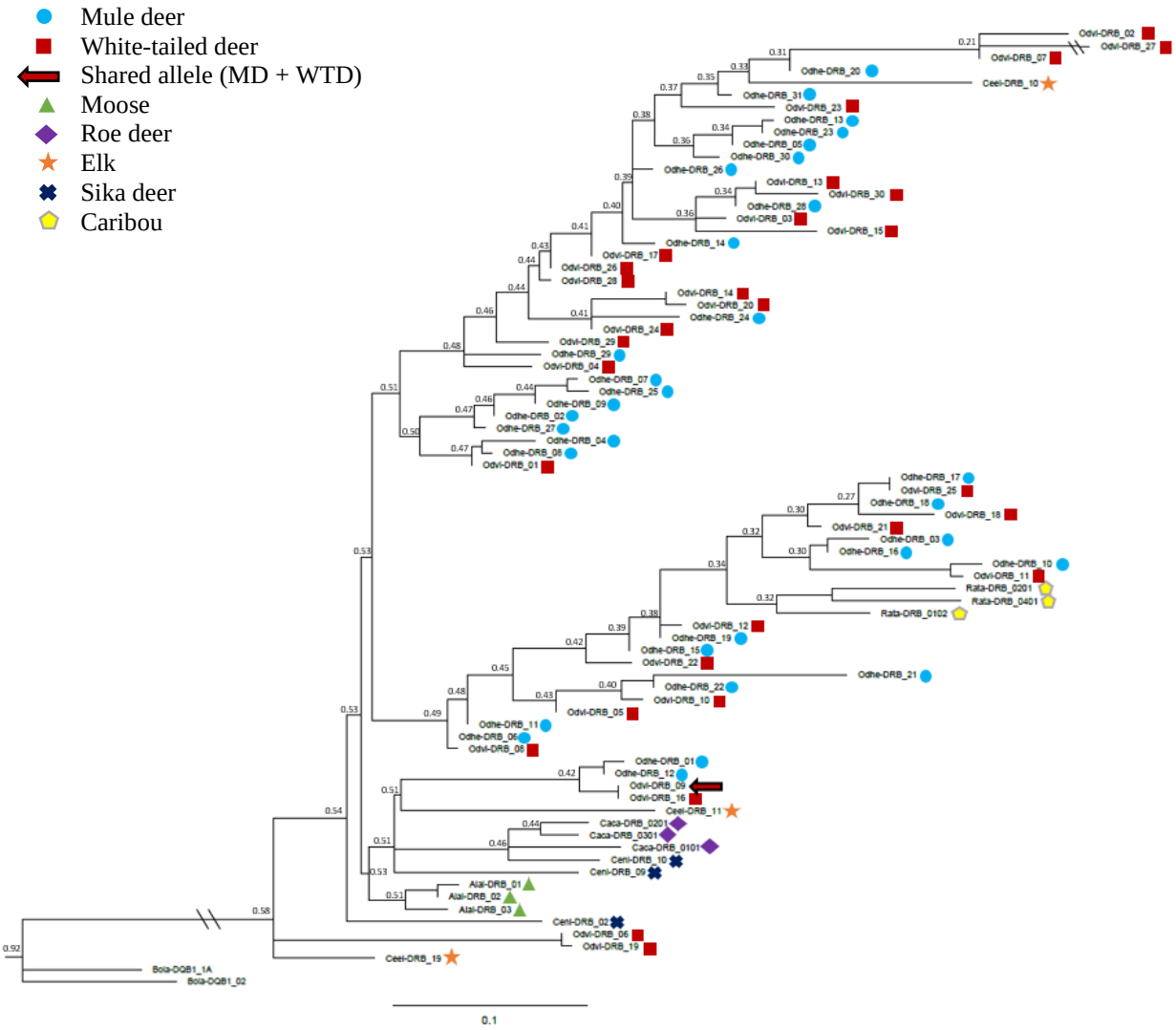


**Figure 7. Maximum likelihood phylogenetic tree of MHC DRB exon 2 allele sequences.** Sequences included here are the 31 new mule deer alleles (Odhe, *Odocoileus hemionus*) shown by blue circles, 30 white-tailed deer alleles (Odvi, *Odocoileus virginianus*) shown by red squares, and outgroups including moose (Alal, *Alces alces*; green triangles), roe deer (Caca, *Capreolus capreolus*; purple diamonds), elk (Ceel, *Cervus elaphus*; orange stars), sika deer (Ceni, *Cervus nippon*; navy crosses), and caribou (Rata, *Rangier tarandus*; yellow pentagons), rooted with cattle DQB sequences (Bola, *Bos taurus*). Nodes are labeled with bootstrap support values. The shared allele between mule deer and white-tailed deer (Odvi-DRB*09) is shown with a red arrow.

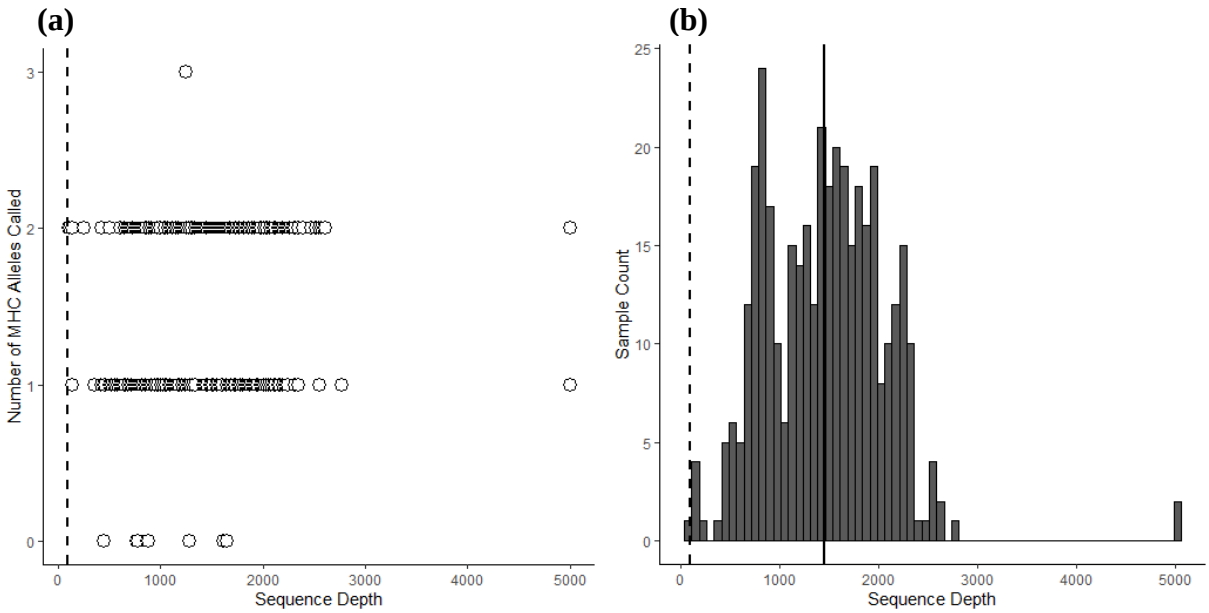


**Figure S1. Sequencing depth and allele calls.** (a) Sequencing depth compared with number of alleles called per individual sample. (b) Sequencing depth distribution; the average amplicon depth per individual after filtering was 1452, shown with the solid line. The minimum amplicon depth was set at 100, shown with the dashed line in both plots.

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