How does the major histocompatibility complex influence reproductive success in white-tailed deer (*Odocoileus virginianus*)?

by

Natascha M.D. Ivy-Israel

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Approved by

Dr. Stephen Ditchkoff, Chair,
William R & Fay Ireland Distinguished Professor of Wildlife Sciences
Dr. Tonia Schwartz, Assistant Professor of Biological Sciences
Dr. Todd Steury, Associate Professor of Wildlife Science
Dr. Sarah Zohdy, Assistant Professor of Wildlife Science

Abstract

Major histocompatibility complex (MHC) gene products can influence sexual selection through their impact on the vertebrate immune system. Individuals with greater MHC diversity are generally believed to have more effective immune systems, thereby allowing these individuals to allocate more resources towards growth and reproduction. However, maximum MHC diversity may be too costly for the individual, suggesting that maximum diversity is not always optimal. This research examined how MHC diversity, measured as pairwise allelic distances between two unlinked MHC type II loci (exon 2 for the classical antigen-binding protein *MHC-DRB*, exon 2 for the accessory protein *MHC-DOB*) influenced morphology (Chapter 2), annual reproductive success (Chapter 3), and pre- and post-copulatory selection (Chapter 4) in an enclosed white-tailed deer (*Odocoileus virginianus*) population in Alabama. To generate these allelic distances, we first sequenced the second exons of *MHC-DRB* and *MHC-DOB* on the MiSeq platform (Chapter 1). Since studies conducted with domestic ruminants found a unique MHC II gene structure in which *MHC-DRB* and *MHC-DOB* were separated by a recombination hotspot due to an ancestral chromosomal inversion, we also assessed the degree of linkage between these loci in white-tailed deer.

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List of Abbreviations

ACF	Auburn Captive Facility
Alal	*Alces alces* (moose)
APC	Antigen presenting cell
Bola	*Bos taurus* (cow)
Caca	*Capreolus capreolus* (roe deer)
Ceel	*Cervus elaphus* (red deer)
CI	Confidence interval
cM	Centimorgans
DOB_aa	*MHC-DOB* allelic distance at the amino acid level
DOB_nuc	*MHC-DOB* allelic distance at the nucleotide level
DRB_aa	*MHC-DRB* allelic distance at the amino acid level
DRB_nuc	*MHC-DRB* allelic distance at the nucleotide level
GBCS	Gross Boone and Crockett antler score
Hd	Haplotype diversity
HWE	Hardy Weinberg Equilibrium
LD	Linkage disequilibrium
M	Average annual male age in non-linear model's term for growth rate
MHC	Major histocompatibility complex
MN	Average annual male age in non-linear model's terms for growth rate and asymptote

N	Average annual male age in non-linear model's term for the asymptote
N_e	Effective population size
N_f	Number of breeding females in the population
N_m	Number of breeding males in the population
Odvi	*Odocoileus virginianus* (white-tailed deer)
Ovar	*Ovis aries* (sheep)
PBR	Peptide binding region
PCR	Polymerase chain reaction
π	Nucleotide diversity
Rata	*Rangifer tarandus* (reindeer)
RS	Reproductive success
SE	Standard error
Treg	Regulatory T-cell
VBGM	Von Bertalanffy growth model
VIF	Variance inflation factor

Chapter 1: Characterization of two MHC II genes (*MHC-DOB*, *MHC-DRB*) in white-tailed deer (*Odocoileus virginianus*)

ABSTRACT

The major histocompatibility complex (MHC) is responsible for detecting and addressing foreign pathogens inside the body. While the general structure of MHC genes is relatively well conserved among mammalian species, it is notably different among ruminants due to a chromosomal inversion that splits MHC type II genes into two subregions (IIa, IIb). Recombination rates are reportedly high between these subregions, and a lack of linkage has been documented in domestic ruminants. However, no study has yet examined the degree of linkage between these subregions in a wild ruminant. The white-tailed deer (*Odocoileus virginianus*), a popular ruminant of the Cervidae family, is habitually plagued by pathogens in its natural environment (e.g. *Haemonchus contortus*, *Elaeophora*). Due to the association between MHC haplotypes and disease susceptibility, a deeper understanding of MHC polymorphism and linkage between MHC genes can further aid in this species' successful management. We sequenced *MHC-DRB* exon 2 (IIa) and *MHC-DOB* exon 2 (IIb) on the MiSeq platform from an enclosed white-tailed deer population located in Alabama. We identified 12 new *MHC-DRB* alleles, thereby generating a total of 30 *MHC-DRB* exon 2 alleles documented for white-tailed deer. Significantly less polymorphism was found for *MHC-DOB* (11 alleles), as was expected of a non-classical MHC gene. While *MHC-DRB* was under positive, diversifying selection, *MHC-DOB* was under purifying selection for white-tailed deer. *MHC-DRB* and *MHC-DOB* loci were found to be unlinked, which suggests that white-tailed deer may have the same chromosomal inversion within their MHC type II gene region as found in domestic ruminants.

KEYWORDS

Major histocompatibility complex, linkage disequilibrium, *Odocoileus virginianus*, ruminant, chromosomal inversion, MiSeq

INTRODUCTION

The major histocompatibility complex (MHC) is a well-studied group of genes whose protein products are responsible for recognizing and addressing foreign pathogens present in the body (Hedrick 1994; Schook and Lamont 1996; Kamiya et al. 2014). While type I MHC genes are present on all nucleated cells, type II MHC genes occur only on immune cells, such as dendritic cells and macrophages (Janeway et al. 2001). These class II MHC molecules consist of two membrane-spanning chains (α and β), both of which are produced by MHC genes (Wieczorek et al. 2017). Immune cells, also known as antigen presenting cells (APC), engulf exogenous particles. Once broken down, the peptide fragments are bound to MHC gene products (*MHC-DR* and *MHC-DQ*), which present them at the surface of the APC. To achieve this, non-classical MHC genes, such as *MHC-DM* and *MHC-DO*, produce accessory proteins used to effectively load antigens onto classical, peptide-binding MHC gene products, which then travel to the APC's surface to present the antigen to the immune system (Janeway et al. 2001; Poluektov et al. 2013; Mellins and Stern 2014). If an antigen is recognized as foreign, helper T-cells will bind to the presented antigen and release lymphokines, which attract other cells to the area. These helper T-cells will also bind to B-cell lymphocytes, which will ultimately stimulate the production of antibodies for this particular antigen (Janeway et al. 2001). The MHC is therefore a crucial component of a vertebrate's immune system.

The peptide binding region (PBR) determines which of the exogenous peptide fragments the MHC proteins can bind. These regions are often found to be under positive diversifying selection (Seddon and Ellegren 2002; Bernatchez and Landry 2003), increasing the probability the gene products from different alleles of an MHC gene can bind different antigens. Through this differential antigen presentation, some allele combinations (haplotypes) can confer greater susceptibility or resistance to certain pathogens. While this is strongly documented among humans (Sasazuki et al. 1983; Cucca et al. 1993; Allen et al. 1996; Casp et al. 2003) and domesticated animals (Park et al. 2004; Larruskain et al. 2010; Singh et al. 2012), it is an emerging field among wildlife species.

While the synteny of MHC genes is relatively well conserved among mammalian species (Wan et al. 2009), it is notably different among ruminants (*Bos taurus*, Andersson et al. 1988; *Ovis aries*, Gao et al. 2010). More specifically, while the MHC II genes of most placental mammals are organized as centromere – (~20 Mb of non-MHC DNA) – *MHC-DM*/*DO* – *MHC-*

DQ/*DR* – MHC III/I genes (Wan et al. 2009; Genome Reference Consortium 2019), the ruminant MHC II genes are organized as centromere – *MHC-DM*/*DO* – (~20 Mb of non-MHC DNA) – *MHC-DQ*/*DR* – MHC III/I genes (Childers et al. 2005; Rozen et al. 2017). The unique organization of MHC II genes found among ruminants is thought to be due to a chromosomal inversion in an ancestral mammal (Band et al. 1998) that has split MHC II genes into two subregions: IIa (*MHC-DR*/*DQ*) and IIb (*MHC-DM*/*DO*/*DY*, *TAP*, among others). A similar MHC II gene configuration was found in the finless Yangtze porpoise (*Neophocaena asiaeorientalis asiaeorientalis*; Ruan et al. 2016) but not in swine (*Sus scrofa*; Renard et al. 2006), which suggests the inversion occurred after the phylogenetic split between ruminants and *Suidae* but before ruminants split from *Cetacea*. The ruminant MHC II subregions are separated by at least 15 cM (centimorgans; Andersson et al. 1988; Van Eijk et al. 1995), which is markedly greater than what is found in humans (~3 cM; Termijtelen et al. 1983) and mice (~1.5 cM; *Mus musculus*; Steinmetz et al. 1986). Significant recombination rates have been observed in the interval between *MHC-DR* and *MHC-DY* genes in *Bos taurus* (Park et al. 1995, 1999), further suggesting a recombination hotspot between the two subregions in ruminants.

Studies on deer species have found the *MHC-DR* and *MHC-DQ* genotypes to be important for disease resistance. Li et al. (2014) found that one *MHC-DR*/*DQ* haplotype was associated with resistance to purulent disease, a multifactorial disease (Liu 2004), among forest musk deer (*Moschus berezovskii*), whereas two other haplotypes increased susceptibility to the disease. Different *MHC-DR* haplotypes in Iberian red deer (*Cervus elaphus hispanicus*) influenced susceptibility to a variety of different pathogens (Fernandez-de-Mera et al. 2009). For example, while one haplotype was associated with a decreased occurrence of tuberculosis but increased *Elaphostrongylus cervi* scores, deer with a different haplotype experienced the opposite trend. Similarly, when using phylogenetic groupings of *MHC-DRB* exon 2 alleles as haplotypes, Ditchkoff et al. (2005) found that white-tailed deer (*Odocoileus virginianus*) with different haplotypes experienced different levels of abomasal nematodes (including *Haemonchus contortus*) and ectoparasitism by ticks.

White-tailed deer are a well-studied wild ruminant due to their popularity as a game species (Hewitt 2011). While proper management has significantly improved their numbers, they are continually threatened by pathogens such as *Haemonchus contortus* (Prestwood and Kellogg 1971; Prestwood et al. 1973), *Elaeophora* (Couvillion et al. 1986), and the epizootic

hemorrhagic disease virus and bluetongue virus (Fletch and Karstad 1971). These diseases can have significant impacts on deer populations and a deeper understanding of factors that influence susceptibility to these diseases is crucial. While the DRB region has been previously characterized in white-tailed deer (Van Den Bussche et al. 1999, 2002), other MHC genes and the possibility of the inverted MHC genetic configuration found among other ruminants has not. To date there are 18 documented *MHC-DRB* exon 2 alleles in white-tailed deer (Van Den Bussche et al. 1999, 2002). Newer sequencing technologies (Next Generation Sequencing), however, may reveal greater polymorphism due to their greatly increased mutation detection rate (sensitivity; Jordanova et al. 1997; Chiang et al. 2015; Chennagiri et al. 2016).

To fully capture the association between MHC haplotypes and disease susceptibility, we must first have a better understanding of the polymorphism that exists at these genes. We therefore aim to further quantify *MHC-DRB* exon 2 polymorphism in white-tailed deer and characterize an additional MHC gene (*MHC-DOB* exon 2). Since these genes are predicted to lie on different MHC II subregions separated by the inversion seen in other ruminants, we also examined the degree of linkage between *MHC-DRB* and *MHC-DOB* in white-tailed deer. Since the immunological functions of *MHC-DRB* and *MHC-DOB* are vastly different, with *MHC-DRB* being the classical antigen-presenting protein and *MHC-DOB* being an accessory loading protein, selection may have influenced these genes differently. We therefore also assessed how these two genes have evolved to better understand selection pressures on MHC polymorphism of white-tailed deer.

METHODS

Study Area

This study took place at the Auburn Captive Facility (ACF) located north of Camp Hill, Alabama. The facility was part of the Piedmont Agricultural Experiment Station, which is owned by Auburn University. Deer sampled during the study were enclosed in a 174-hectare facility surrounded by a 2.6-meter fence, which was constructed in October 2007. The deer present within the ACF during the study included the original deer that inhabited this area during the fence formation in 2007 and their subsequent offspring. The population size of the adult, founding population was 71, while the effective population size was 64.8, calculated as $N_e = 4N_mN_f / (N_m+N_f)$, where N_m is the number of breeding males and N_f is the number of breeding

females in the population (Wright 1938). Subsequent to fencing the area, deer were neither introduced nor hunted within the ACF. Population size, which varied annually between 100-120 deer, was mainly regulated by natural and capture-related mortalities (Newbolt et al. 2017). Deer had access to supplemental feed in the form of food plots, corn feeders, and *ad libitum* protein feeders. A creek and its tributaries were present on the property, which provided a reliable water source year-round.

Animal handling

White-tailed deer aged 6 months and older were captured over 10 trapping seasons (October – July each year) from 2007-2017 via chemical immobilization. We administered a tranquilizer mixture into the deer's hindquarter muscle with the use of cartridge fired dart guns (Pneu-Dart model 193) and 0.22 caliber blanks. After adding 4 cc of xylazine (100 mg/ml; Lloyd Laboratories, Shenandoah, IA) to a 5 mL vial of Telazol® (100 mg/ml; Fort Dodge Animal Health, Fort Dodge, IA; Miller et al. 2003), we loaded 2 cc of this tranquilizer mixture into a telemetry dart (2.0 cc, type C, Pneu-Dart Inc., Williamsport, PA), which contained a radio transmitter (Advanced Telemetry Systems, Inc., Isanti, MN) that allowed us to locate the sedated deer via radio telemetry (Kilpatrick et al. 1996). Tolazine (100 mg/ml; Lloyd Laboratories) was injected in the shoulder and hindquarter muscle to reverse the sedation once data collection was complete (Miller et al. 2004). These methods were approved by the Auburn University Institutional Animal Care and Use Committee (2008-1417, 2008-1421, 2010-1785, 2011-1971, 2013-2372, 2014-2521, 2016-2964, and 2016-2985) and in compliance with the American Society of Mammalogists' guidelines (Sikes and Gannon 2011).

Deer received a unique 3-digit identification number at initial capture, which was displayed on ear tags and also freeze branded on the front shoulder and hind quarter of some individuals. Sex and age (tooth wear and replacement aging technique; Severinghaus 1949) were recorded and a 1-cm^2 notch of tissue was removed from their ear for genetic analysis. This tissue sample was then stored in a -80ºC freezer until DNA analysis could be performed in the laboratory.

Sequencing

A modified version of the "DNA extraction 2XCTAB Protocol for Initial DNA Isolation of *Symbiodinium*" (Santos Lab 2016) was used to extract DNA from tissue samples collected 2007-2013. DNeasy blood and tissue kits (Qiagen, Inc.) were used to extract DNA from tissue samples collected 2014-2017 and from previous samples with low DNA yield. DNA for 381 individuals were shipped to RTL Genomics (Research and Testing Laboratory, Lubbock TX, United States) for amplicon sequencing of 307 bp of the second exon of *MHC-DRB* (primers LA31 and LA32; Sigurdardottir et al. 1991; Mikko and Andersson, 1995) that targeted the peptide binding domain, and 398 bp of the second exon of *MHC-DOB* (Forward: 5' - AAAGCCCTCCTCTCCAATCC - 3'; Reverse: 5' - CCACCAAGGAGACCCCAAC - 3').

At RTL Genomics, samples were amplified via a two-step process. First, the forward primer was constructed by combining the Illumina i5 sequencing primer (5' - TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG - 3') with the specific forward primer for each *MHC-DRB* and *MHC-DOB*, and the reverse primer was created using the Illumina i7 sequencing primer (5' - GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG - 3') and the specific reverse primer for each *MHC-DRB* and *MHC-DOB*. Amplifications were performed in 25 μL reactions using Qiagen HotStar Taq master mix (Qiagen Inc, Valencia, CA), 1 μL of each 5 μM primer, and 1 μL of template on ABI Veriti thermocyclers (Applied Biosystems, Carlsbad, CA). Thermal profiles used for *MHC-DRB* and *MHC-DOB* were identical: 1) 95°C for 5 minutes; 2) 33 cycles of 95°C for 1 minute; 3) 50°C for 30 seconds; 4) 72°C for 1 minute; 5) final extension of 72°C for 10 minutes; 6) 4°C hold. Products from this first state of amplification were then added to a second PCR based on qualitatively determined concentrations. A second PCR was performed to index the sequence by individual. Primers for the second PCR used primers that were based on the Illumina Nextera PCR primers (Forward: 5' - AATGATACGGCGACCACCGAGATCTACAC[i5index]TCGTCGGCAGCGTC - 3'; Reverse: 5' - CAAGCAGAAGACGGCATACGAGAT[i7index]GTCTCGTGGGCTCGG - 3') using the following thermal profile: 1) 95°C for 5 minutes; 2) 10 cycles of 94°C for 30 seconds; 3) 54°C for 40 seconds; 4) 72°C for 1 minute; 5) final extension of 72°C for 10 minutes; 6) 4°C hold. All amplification products were visualized with eGels (Life Technologies, Grand Island, NY). The DNA extraction from one individual failed to amplify and was therefore removed from the dataset prior to sequencing. PCR products indexed by individual were pooled in equimolar. Each pool was size-selected in two rounds using SPRIselect Reagent (BeckmanCoulter,

Indianapolis, IN) in a 0.75 ratio for both rounds. These size-selected pools were then quantified using the Qubit 4 Fluorometer (Life Technologies). Successfully amplified size-selected pools were loaded on an Illumina MiSeq (Illumina, Inc., San Diego, CA) 2x300 flow cell at 10 pM. The amplicons were then sequenced for 300 bp length paired-end reads on the Illumina MiSeq platform for a targeted minimum of 10k reads per individual. Reads were demultiplexed based on the index and sorted to individual files.

To ensure the quality of the sequencing data, raw reads were trimmed using Trimmomatic (v0.35; LEADING 20, TRAILING 20, SLIDINGWINDOW: 6:20, MINLEN: 20; Bolger et al. 2014). The quality of these trimmed reads was verified using FastQC (v0.11.8). Trimmed, paired-end reads were then merged using PEAR (Zhang et al. 2014). These merged reads were further filtered by removing reads with corrupt primers, merged reads shorter than 290 base pairs, and reads whose sequence only occurred once within an individual. Once primers were removed from these filtered merged reads, the amplicon size for *MHC-DRB* and *MHC-DOB* were 250 bp and 360 bp, respectively. While the *MHC-DRB* amplicon only captured exon 2, the *MHC-DOB* amplicon captured exon 2 (270 bp) plus noncoding regions around it. Given this extra data for *MHC-DOB*, we analyzed both the full *MHC-DOB* sequences and *MHC-DOB* exon 2 for further analyses.

Defining new alleles

Alleles were defined based on nucleotide sequence variation and amino acid sequence variation. All new *MHC-DRB* and *MHC-DOB* alleles followed the nomenclature proposed by Klein et al. (1990) and Van Den Bussche et al. (1999, 2002). To define alleles and genotypes within an individual, the merged paired-end sequences were organized from the most frequent to least frequent sequence. Individuals were characterized as homozygotes when the frequency of the second most common sequence was less than 10% relative to the first sequence (90:10). Individuals were classified as heterozygotes if the ratio of the first to second most frequent sequence was 50:50 to 65:35. For *MHC-DRB*, 60 individuals out of the 380 individuals sampled (15.8%) did not classify as a homozygote or heterozygote using these guidelines, thereby we used pedigree and Sanger sequencing data to confirm their genotypes. We removed any individuals who did not have this data for confirmation (n = 5), which reduced the sample size

for *MHC-DRB* to 375 individuals. For *MHC-DOB*, all 380 individuals sampled were clear homozygotes or heterozygotes (full sequence and exon 2).

New *MHC-DRB* alleles whose frequencies were less than 0.67% [5/ (2*375)] in the population were not considered true alleles (NRC 1996) if we could not further validate them using pedigree and Sanger analysis. While 5 *MHC-DRB* alleles were at less than 0.67% in our population, we were able to validate 3 of these 5 alleles (DRB*28, DRB*29, DRB*30). The other two alleles only occurred once in the population, and these individuals were removed from our DRB dataset for further analysis (Table 1.S1), reducing our sample size further to 373 individuals for *MHC-DRB*. The minimum allele frequency threshold for *MHC-DOB* was 0.66% [5/ (2*380)]. While all *MHC-DOB* exon 2 allele frequencies were greater than this minimum, one allele from our full *MHC-DOB* sequence data was not. However, we were able to validate this allele using Sanger and pedigree data so it was not removed from the dataset.

The pedigree for our population was created by Newbolt et al. (2017). Sanger sequencing (Sanger and Coulson, 1975) was performed for *MHC-DRB* exon 2 via high throughput sequencing (htSEQ) for individuals born prior to 2008 to augment our MiSeq sequencing data for *MHC-DRB*.

Test for linkage between MHC-DRB and MHC-DOB

As *MHC-DRB* and *MHC-DOB* lie on different MHC II subregions separated by the inversion seen in other ruminants, we examined the degree of linkage between *MHC-DRB* and *MHC-DOB* among unrelated individuals in our white-tailed deer population using GenePop (Option 2, sub-option 1). We first assessed linkage disequilibrium (LD) using individuals that are least likely related to one another (individuals born before the fence was constructed in 2007 and were not offspring from this early group of deer according to pedigree data; n = 69). We then included individuals without assigned parents in our pedigree data (these individuals could be related to others in the population, but we are not 95% confident that they are; n = 122) to further assess the possibility of LD between *MHC-DRB* exon 2 and *MHC-DOB* exon 2. This method in GenePop tests the null hypothesis that the loci are independent of one another, or in other words, not linked (Weir 1996). We used the default settings where dememorization = 1000, batches = 100, and iterations per batch = 1000.

Genetic relationships among alleles

We estimated phylogenetic distances as the number of nucleotide/amino acid differences among alleles and created gene trees for each gene to understand the relationships between *MHC-DRB* and *MHC-DOB*. Nucleotide and protein alignments of *MHC-DRB* and *MHC-DOB* were created via Geneious (v11.1.5). These alignments were then used to construct phylogenetic trees for the nucleotide and amino acid sequences of both *MHC-DRB* and *MHC-DOB* using IQ-TREE (v1.6.9; Nguyen et al. 2015). IQ-TREE employed maximum likelihood (ML) for tree inference and automatically determined the best-fit model (-m TEST) via ModelFinder (Kalyaanamoorthy et al. 2017) using a standard bootstrap of 1000 replicates (-b 1000). ModelFinder identifies the best-fitting model of sequence evolution that ultimately produced our data. Phylogenetic support for tree splits was determined via bootstrap values, where a split with ≥95% support is considered statistically significant (Felsenstein 1985). Outgroups included in the *MHC-DRB* trees were moose (*Alces alces*; Mikko and Andersson, 1995), roe deer (*Capreolus capreolus*; Mikko et al. 1997a; Quemere et al. 2015), and reindeer (*Rangifer tarandus*; Mikko et al. 1999) as these are the closest related species to white-tailed deer with *MHC-DRB* data (Pitra et al. 2004; Gilbert et al. 2006). More distantly related species could result in a long branch, which may dominate the likelihood and therefore interfere with the tree inference. However, due to a lack of *MHC-DOB* data among artiodactyls, outgroups for the *MHC-DOB* trees were more distantly related species including cow (*Bos taurus*; Zimin et al. 2009), sheep (*Ovis aries*; Wright et al. 1996), and red deer (*Cervus elaphus*; Bana et al. 2016). The nucleotide tree for *MHC-DOB* used the full nucleotide sequences (360 bp), while the amino acid tree used translated *MHC-DOB* exon 2 sequences. The final ML trees were rooted using FigTree (v1.4.4). The *MHC-DRB* trees were rooted with the moose outgroup while the *MHC-DOB* trees were rooted with the cow outgroup. A heatmap was added to the trees via FigTree annotation that corresponded to the Odvi-DRB and Odvi-DOB allele frequencies present in our population.

Population Genetic Measures and Test for Selection

Allele and genotype frequencies were generated for each gene via GenePop (v4.2; Option 5, sub-option1; Raymond and Rousset, 1995; Rousset 2008) to examine the distribution of *MHC-DRB* and *MHC-DOB* alleles in our white-tailed deer population. To assess if these allele frequencies were changing over time, Hardy Weinberg Exact Tests were performed on

nucleotide data from the adult founding [2003-2007; n = 69 (*MHC-DRB*), 71 (*MHC-DOB* full sequence), 71 (*MHC-DOB* exon 2)] and adult 2016 datasets [n = 118 (*MHC-DRB*), 119 (*MHC-DOB* full sequence), 119 (*MHC-DOB* exon 2)] with GenePop (Option 1) using the probability-test (sub-option 3; Haldane 1954; Guo and Thompson, 1992). We also checked for deviations from Hardy Weinberg Equilibrium using score tests (Rousset and Raymond, 1995) to evaluate the presence of heterozygote excess (sub-option 2) or deficiency (sub-option 1) in our founding and 2016 populations. Score tests are more powerful tests than the probability test (Rousset and Raymond 1995), which will further aid in the detection of deviations from HWE. All tests employed default settings (dememorization number = 1000, number of batches = 100, number of iterations per batch = 1000).

Nucleotide (π) and haplotype (Hd) diversity (Nei 1987) were calculated for both *MHC-DRB* exon 2 and *MHC-DOB* (full sequence and exon 2) using DnaSP v5.10.00 (Rozas et al. 2003) to assess the genetic diversity present within our population. We also performed several neutrality tests (Tajima's D, Fu's Fs, Fu and Li's F*, Fu and Li's D*) in DnaSP to examine possible evidence of selection or drift at these loci in our white-tailed deer population. These tests were done using the founding, unrelated population [2003-2007; n = 69 (*MHC-DRB*), 71 (*MHC-DOB* full sequence), 71 (*MHC-DOB* exon 2)] and the adults present in the population in 2016 [n = 118 (*MHC-DRB*), 119 (*MHC-DOB* full sequence), 119 (*MHC-DOB* exon 2)]. Pairwise sequence divergence for nucleotide and amino acid sequences were calculated using MEGA X (v10.0.5; Kumar et al. 2018), using maximum composite likelihood for nucleotide distances and a Poisson correction for amino acid distances.

Lastly, to test for evidence of positive selection, SNAP (Synonymous Non-synonymous Analysis Program; v2.1.1; Korber 2000) was used to calculate synonymous and non-synonymous substitution rates and the ratio dN/dS for *MHC-DRB* exon 2 and *MHC-DOB* exon 2.

RESULTS

Sequencing

Before filtering, the average number of reads for individuals was 30,549 for *MHC-DRB* (ranged from 5,318 to 84,226 reads with a median of 24,309) and 22,341 for *MHC-DOB* (ranged from 7,029 to 49,892 reads with a median of 21,913. The number of reads recovered per

individual after filtering for quality ranged from 4,162 to 66,582 reads for *MHC-DRB* (average = 24,795.5; median = 19,214) and from 2,779 to 36,407 reads for *MHC-DOB* (average = 15,815; median = 15,522). While unfiltered read lengths ranged from 289 to 488 bp for *MHC-DRB* (average = 306, median = 307) and from 50 to 535 bp for *MHC-DOB* (average = 397, median = 398), filtered read lengths were 296 to 365 bp long for *MHC-DRB* (average = 306, median = 307) and 329 to 406 bp long for *MHC-DOB* (average = 397, median = 398). All raw sequence data is available on the NCBI Sequence Read Archive (SRA accession # PRJNA533917).

No Linkage Disequilibrium between MHC-DRB and MHC-DOB

We found no significant linkage disequilibrium between *MHC-DRB* exon 2 and *MHC-DOB* exon 2 when using only the individuals in the starting population (n = 69; p = 0.95) and when including individuals without pedigree data to this initial population (n = 122; p = 0.37). Since these loci are not linked, we present the results for each gene separately.

MHC-DRB

Defined 19 new alleles

A total of 19 *MHC-DRB* exon 2 alleles were found in our white-tailed deer population (n = 373), 12 of which were new alleles (Odvi-DRB*19 – Odvi-DRB*30; Table 1.S2). The previously identified alleles found in the population were Odvi-DRB*01, Odvi-DRB*05, Odvi-DRB*06, Odvi-DRB*10, Odvi-DRB*12, Odvi-DRB*14, and Odvi-DRB*16 (Van Den Bussche et al. 1999, 2002). The 19 *MHC-DRB* exon 2 alleles found in our population translated to 18 unique amino acid alleles (82-83 codons), as Odvi-DRB*06 and Odvi-DRB*19 differed by one synonymous substitution (Table 1.S3). Together with the other 12 previously identified *MHC-DRB* exon 2 alleles (Van Den Bussche et al. 1999, 2002) not recovered in our population, a total of 30 *MHC-DRB* exon 2 alleles have been characterized for white-tailed deer. The new *MHC-DRB* exon 2 alleles have been deposited in Genbank under accession numbers MK952679-MK952690.

Genetic relationship among alleles

The length of *MHC-DRB* exon 2 alleles was 250 bp for all alleles except for Odvi-DRB*27, which had a 3-bp frameshift deletion. Pairwise nucleotide distances among alleles

ranged from 0.40% to 21.20% (mean = 10.99%), and pairwise amino acid distances ranged from 0% to 39.35% (mean = 23.00%). The average number of nucleotide differences (k) was 24.97, and there were 85 polymorphic sites in the *MHC-DRB* exon 2 sequences.

In assessing the phylogenetic relationships using ModelFinder, the best-fit model according to BIC for the nucleotide *MHC-DRB* tree was F81+F+I+G4 (Figure 1.1). There were six near-zero internal branches (<0.0040): two in the moose outgroup, two in the roe deer outgroup, one in the reindeer outgroup, and one in the internal branch that separates the reindeer clade from the white-tailed deer sequences. The white-tailed deer/roe deer/reindeer MHC-DRB exon 2 nucleotide sequences separated fairly strongly from the moose outgroup (81% bootstrap support) but not from each other, suggesting the presence of a polytomy. While the roe deer and reindeer sequenced clustered into clear groups, the white-tailed deer sequences did not. However, there were some well-supported clades among these white-tailed deer sequences (up to 100% bootstrap support). When using translated *MHC-DRB* exon 2 sequences, the best-fit model according to BIC values was PMB+I+G4 (Figure 1.2). This tree had seven near-zero internal branches (<0.012) in terms of bootstrap support: four in the moose outgroup and three in the roe deer outgroup. As with the nucleotide tree, the white-tailed deer/roe deer/reindeer sequences separated strongly from the moose outgroup (85% bootstrap support). The roe deer sequences were further separated with 72% bootstrap support, while the white-tailed deer and reindeer sequences remained together, though there were some well-supported clades within these remaining sequences (up to 100% bootstrap support).

Population genetic measures and test for selection

When assessing the allele frequencies of the complete dataset (2003-2017), the most common *MHC-DRB* exon 2 allele was Odvi-DRB*10 (22.4%), followed by Odvi-DRB*20 (14.6%) and Odvi-DRB*14 (12.5%; Figure 1.1; Table 1.S2). The most common genotypes were Odvi-DRB*20/Odvi-DRB*10 (7.0%), Odvi-DRB*10/Odvi-DRB*10 (4.8%), and Odvi-DRB*14/Odvi-DRB*10 (4.6%; Table 1.S4). Allele frequencies changed slightly from the founding population (2003-2007) to the more recent population (2016). Frequencies for Odvi-DRB*01 (10.9-6.8%) and Odvi-DRB*14 (16.7-10.2%) decreased over time, whereas Odvi-DRB*10 (18.1-22.5%) and Odvi-DRB*19 (5.8-11.9%) became more frequent in the population. Odvi-DRB*05, a rare allele in our population, was lost from the population over time. While

MHC-DRB exon 2 was under Hardy Weinberg equilibrium using the probability-test for both the founding and 2016 populations (probability test; $p = 0.88$ and $p = 0.91$, respectively). When we specifically tested for heterozygosity excess or deficiency, the 2016 population had a heterozygote excess (score test; $p = 0.05$; Table 1.1).

Both nucleotide (π) and haplotype diversity generally stayed the same over time (0.09 to 0.10 and 0.90 to 0.88, respectively; Table 1.1). All neutrality test values were positive, and there was an increasing trend from the founding population to the more recent 2016 population (Table 1.1), suggesting that selection on *MHC-DRB* is becoming less neutral in our population. Both Fu and Li's D* and F* test statistics were significant ($p < 0.02$) for the founding (2.39 and 2.09, respectively) and 2016 (2.56 and 2.45, respectively) populations. These positive results suggest *MHC-DRB* had an excess of intermediate frequency alleles in our population, which is an indication of balancing selection and/or reduced population size. *MHC-DRB* showed evidence of positive selection based on the dN/dS ratio of 2.06 (Figure 1.3).

MHC-DOB

Defined 11 alleles

Eleven unique alleles were identified for the full *MHC-DOB* nucleotide sequences (Odvi-DOB*01 – Odvi-DOB*11) and 7 alleles for MHC-DOB exon 2 in our white-tailed deer population (Table 1.S5). Odvi-DOB*01, Odvi-DOB*02, and Odvi-DOB*11 contained the same exon 2 sequence (Odvi-DOB*010211_exon2). Odvi-DOB*03 and Odvi-DOB*10 also contained the same exon 2 (Odvi-DOB*0310_exon2), as well as Odvi-DOB*06 and Odvi-DOB*07 (Odvi-DOB*0607_exon2). Odvi-DOB*04, Odvi-DOB*05, Odvi-DOB*08, and Odvi-DOB*09 had unique exon 2 sequences (Odvi-DOB*04_exon2, Odvi-DOB*05_exon2, Odvi-DOB*08_exon2, Odvi-DOB*09_exon2, respectively). The 7 *MHC-DOB* exon 2 alleles translated into 3 unique amino acid alleles (89 codons). The *MHC-DOB* alleles have been deposited in Genbank for the full DOB sequences (accession numbers MK952691- MK952701).

Genetic relationships among alleles

In assessing the phylogenetic relationships using ModelFinder, the best-fit model according to BIC for the nucleotide *MHC-DOB* tree was K2P+I (Figure 1.4). There were seven near-zero internal branches (<0.0028%) in terms of bootstrap support, all of which occurred in

the white-tailed deer clades. The red deer and white-tailed deer sequences separated strongly from the cow and sheep sequences (97% bootstrap support). Odvi-DOB*08/Odvi-DOB*09 and Odvi-DOB*06/Odvi-DOB*07 separated from the other white-tailed deer sequences with 67% and 62% bootstrap support, respectively. The translated *MHC-DOB* tree had Blosum62 as the best-fit model (Figure 1.5). There were five near-zero internal branches (<0.0112%), which all occurred in the white-tailed deer clades. Apart from the fairly strong separation between cow/sheep sequences and the white-tailed deer/red deer sequences (66% bootstrap support), the amino acid sequences for *MHC-DOB* exon 2 did not retain the same organization as in the *MHC-DOB* nucleotide tree. The tree clearly demonstrates the three unique *MHC-DOB* exon 2 translations.

All full *MHC-DOB* sequence alleles were 360 bp in length except for Odvi-DOB*01 (359 bp) and Odvi-DOB*02 (361 bp) due to an indel in the intronic region. The pairwise nucleotide distances ranged from 0% to 1.41% (mean = 0.58%). Odvi-DOB*01, Odvi-DOB*02, and Odvi-DOB*11 differed by one indel located in a highly repetitive region of the intron. The greatest amount of dissimilarity was found between Odvi-DOB*07 and Odvi-DOB*09 (4 synonymous substitutions, one nonsynonymous substitution; Table 1.S6). The average number of nucleotide differences (k) was 2.07, and the full *MHC-DOB* sequences had seven polymorphic sites. The full nucleotide *MHC-DOB* sequences were in Hardy Weinberg Equilibrium for both the founding and 2016 populations (probability test; $p = 0.27$ and $p = 0.55$, respectively), and there was no indication of a heterozygote deficit or excess in either populations (Table 1.2). However, there was a heterozygote deficit in the 2016 population (score test; 0.03)

All alleles for the *MHC-DOB* exon 2 region contained within our full *MHC-DOB* sequences had a length of 270 bp. The mean pairwise nucleotide distance for *MHC-DOB* exon 2 was 0.78% (0.37-1.50%), while the mean pairwise amino acid distance was 0.86% (0-2.27%). As was seen in the full *MHC-DOB* sequences, the greatest amount of dissimilarity was found between Odvi-DOB*0607_exon2 and Odvi-DOB*09_exon2 (3 synonymous substitutions, one nonsynonymous substitution; Table 1.S7). Odvi-DOB*04_exon2 and Odvi-DOB*09_exon2 also differed more than other *MHC-DOB* exon 2 alleles (2 synonymous substitutions, 2 nonsynonymous substitutions). The *MHC-DOB* exon2 sequences contained five polymorphic sites and 2.10 nucleotide differences on average. As with the full *MHC-DOB* sequences, the *MHC-DOB* exon 2 alleles were also in Hardy Weinberg Equilibrium for the founding and 2016

populations (probability test; p = 0.57 and p = 0.33, respectively), though there was statistical significance for a heterozygote deficiency in the 2016 population (score test; p = 0.01; Table 1.2).

Population genetic measures and test for selection

Odvi-DOB*08 and Odvi-DOB*08_exon2 were the most common alleles for the full *MHC-DOB* sequences (28.1%) and *MHC-DOB* exon 2 (27.8%; Figure 4; Table 1.S5). The most common genotypes all contained Odvi-DOB*08 and Odvi-DOB*08_exon2 (Table 1.S8, 1.S9). Allele frequencies increased over time for Odvi-DOB*06 (3.5 to 7.1%), Odvi-DOB*09_exon2 (4.2 to 9.7%), and Odvi-DOB*11/Odvi-DOB*010211_exon2 (9.2 to 12.6% and 11.3 to 14.3%, respectively), whereas Odvi-DOB*03 (12.7 to 10.5%), Odvi-DOB*05_exon2 (10.6 to 7.1%), and Odvi-DOB*08/Odvi-DOB*08_exon2 (both from 33.1 to 25.2%) became less frequent in the population.

While nucleotide diversity did not change over time, haplotype diversity increased slightly (Table 1.2). Neutrality test results indicated an increasing trend from the founding population to the more recent population for both full *MHC-DOB* sequences and *MHC-DOB* exon 2 (Table 1.2), with Tajima's D and Fu and Li's F* approaching statistical significance ($0.10 > p > 0.05$) for the 2016 population. Tajima's D increased from 0.87 to 1.31 for the full *MHC-DOB* sequences and from 1.38 to 1.77 for *MHC-DOB* exon 2. Fu's Fs for *MHC-DOB* was notably smaller compared to *MHC-DRB*. The dN/dS ratio for *MHC-DOB* exon 2 was 0.22, indicating no evidence of positive selection (Figure 6).

DISCUSSION

In this study we aimed to characterize *MHC-DRB* exon 2 and *MHC-DOB* exon 2 in our white-tailed deer population and to examine the extent of linkage between these two loci. No significant linkage was found between the second exons of *MHC-DRB* and *MHC-DOB* in our white-tailed deer population. While further examination is needed, this finding suggests that white-tailed deer may have the same chromosomal inversion and recombination hotspot within their MHC type II gene region as found in other ruminants (Park et al. 1995, 1999; Andersson et al. 1988; Amills et al. 1998; Childers et al. 2005; Gao et al. 2010). This MHC II inversion has been documented among cetaceans (Ruan et al. 2016; Sa et al. 2019; Zhang et al. 2019) but not

in swine (Renard et al. 2006), suggesting that this breakpoint may have occurred after the divergence of Cetartiodactyla and Suidae but before the divergence between Cetacea and ruminants (~58 million years ago; Zhou et al. 2011). A lack of linkage disequilibrium between these two loci allows them to evolve independently from one another. Because they are unlinked, we can contrast their patterns as independent loci.

Our white-tailed deer population had 19 *MHC-DRB* exon 2 alleles, which is comparable to what Van Den Bussche et al. (1999) found in a free-ranging white-tailed deer population in Oklahoma (15 *MHC-DRB* exon 2 alleles, n = 150). Even though white-tailed deer experienced severe, historical population bottlenecks (Ellsworth et al. 1994; Leberg et al. 1994), they have managed to retain more *MHC-DRB* polymorphism than other species that survived similar population reductions, such as moose (Mikko et al. 1995), bison (*Bison bison*; Mikko et al. 1997b), fallow deer (*Dama dama*) and roe deer (Mikko et al. 1999). The polymorphism found among our founding adult population was less (17 *MHC-DRB* exon 2 alleles; n = 69) than what was reported in unrelated individuals of red deer (34 *MHC-DRB* exon 2 alleles, n = 50; Swarbrick et al. 1995), Kankrej cattle (*Bos indicus*; 24 *MHC-DRB* exon 2 alleles, n = 50; Behl et al. 2007), and the Indian water buffalo (*Bubalus bubalis*; 22 *MHC-DRB* exon 2 alleles, n = 25; De et al. 2002). However, white-tailed deer have greater polymorphism than both Kankrej cattle and Indian water buffalo if all 30 *MHC-DRB* exon 2 alleles found across studies in this species are used for comparison. Red deer also have two *MHC-DRB* loci whereas white-tailed deer are believed to have only one *MHC-DRB* locus (Van Den Bussche et al. 1999).

While white-tailed deer already were known to have a reasonably high *MHC-DRB* polymorphism (18 alleles; Van Den Bussche et al. 1999, 2002), we identified 12 new *MHC-DRB* exon 2 alleles in our Alabama population. The original 18 *MHC-DRB* exon 2 alleles were identified using 7 populations from Oklahoma, Iowa, Tennessee, and New York. Alabama deer have an interesting ancestry due to restocking efforts between the 1940's and 1960's. Six white-tailed deer subspecies were used for reintroduction (Allen 1965; McDonald and Miller 1993), thereby creating an admixed white-tailed deer population in Alabama. Our study population was located in Tallapoosa county, which received deer from Georgia, Arkansas, and other counties in Alabama (Clarke, Marengo, Sumter; McDonald and Miller 2004). Additionally, reintroductions using Clarke county deer occurred after Michigan deer were introduced to Clarke county. Admixture may therefore be contributing to the large number of new *MHC-DRB* exon 2 alleles

identified in this study. Another potential explanation for identifying additional alleles may be our use of newer sequencing technology since the white-tailed deer *MHC-DRB* was last characterized via SSCP by Van Den Bussche et al. (1999, 2002). MiSeq has greater sensitivity relative to SSCP (Jordanova et al. 1997; Chiang et al. 2015; Chennagiri et al. 2016), which may have enabled us to detect more nucleotide polymorphisms in the *MHC-DRB* exon 2 sequences.

While the outgroup sequences in our *MHC-DRB* trees clustered together as monophyletic groupings, as is expected for species with reduced MHC allelic diversity due to severe population bottlenecks (Mikko et al. 1995, 1999), white-tailed deer *MHC-DRB* alleles represented paraphyletic groups. This suggests that *MHC-DRB* polymorphism is most likely greater in white-tailed deer than moose, roe deer and reindeer. Additionally, the lack of separation into geographically structured phylogenetic clades suggests that there is no, or very little, relationship between genetic and geographic distance for white-tailed deer, similar to findings reported by Van Den Bussche et al. (2002) and Leberg et al. (1994). Reintroductions may have weakened these geographic associations between *MHC-DRB* exon 2 alleles. When comparing our *MHC-DRB* nucleotide tree to the neighbor-joining tree constructed by Van Den Bussche et al. (1999, 2002), we find little similarity. This is most likely due to differences in tree construction, outgroups, and increased number of Odvi-DRB alleles included in the analyses. However, no quantification of support was provided for their trees, making a direct comparison more challenging as the level of confidence in their tree splits is unknown.

The *MHC-DOB* tree used sequences from less closely related species (cow, sheep, red deer) due to a current lack of *MHC-DOB* sequence data for Cervidae, which may have influenced tree inference. The extremely low *MHC-DOB* polymorphism found within our white-tailed deer population relative to *MHC-DRB* polymorphism may contribute to the lack of separation seen between the white-tailed deer *MHC-DOB* exon 2 alleles. Lastly, Ceel-DOB did not strongly separate from Odvi-DOB, which is similar to what Van Den Bussche et al. (1999, 2002) found for *MHC-DRB*.

MHC genes are known to evolve rapidly over time (Flajnik and Kasahara 2001; Shiina et al. 2006; Eizaguirre et al. 2012). When classifying individuals as homozygotes or heterozygotes for *MHC-DRB*, we found that the frequency of the second most common sequence was less than 35% but greater than 10% relative to the first allele (63:35 – 90:10) for 13.95% of our population. A large proportion of these individuals had either Odvi-DRB*14 or Odvi-DRB*20 as

their second allele (25.0% and 38.3%, respectively), which was further validated using pedigree and Sanger data. This could be due to allelic dropout because of a mutation in the priming region of these alleles, thereby reducing their amplification efficiency relative to the first allele. Allelic dropout is fairly common in studies that use PCR and has previously been documented in MHC genes (Sommer et al. 2013). Positive diversifying selection could also be contributing to *MHC-DRB* alleles having substitutions in the primer regions.

Varying levels of *MHC-DRB* diversity have been found among ungulates. For example, the genetic distance between *MHC-DRB* alleles can range from large (cattle, bison, red deer) to negligible (moose; Mikko et al. 1999). Within our white-tailed deer dataset, haplotype diversity (Hd) was similar between the second exons of *MHC-DRB* and *MHC-DOB*, while nucleotide diversity (π) was greater for *MHC-DRB* exon 2 than for *MHC-DOB* exon 2. Nucleotide diversity equal to 0.1 or greater is considered high (Rozas 2009), suggesting that the white-tailed deer MHC-*DRB* exon 2 alleles differed considerably from one another.

MHC-DRB exon 2, which encodes the antigen-binding site of DR molecules (Gaur and Nepom, 1996), shows strong evidence of positive, diversifying selection (e.g. dN/dS ratio) in white tailed deer. Similar results were reported by De et al. (2011) for white-tailed deer. Diversifying selection occurs when the amino acid diversity in a gene increases within a species over time (Yang et al. 2000; Cicconardi et al. 2017). As *MHC-DRB* produces peptide-binding proteins, increasing this gene's diversity may enable it to present a greater array of pathogenic antigens to the immune system (Borghans and De Boer 2000; Janeway et al. 2001; Sommer 2005; Acevedo-Whitehouse et al. 2018). Additionally, the neutrality tests indicate that there is an excess of intermediate frequency *MHC-DRB* exon 2 alleles in our population, suggesting that the *MHC-DRB* alleles are being maintained in the population via balancing selection. This balancing selection may be becoming more pronounced in our population over time as there was an increasing trend in all neutrality test values from the 2007 starting population until the 2016 adult population as well as an excess of heterozygotes in the 2016 population. This retention of *MHC-DRB* alleles may be driven by a heterozygote advantage that exists in our population, especially since MHC genes are codominant. Another explanation for the increasing Tajima's D and Fu and Li's F* and D* values found for *MHC-DRB* may be that while balancing selection acted on the population initially (before population enclosure), a reduced population size is now adding to the generation of intermediate frequency *MHC-DRB* exon 2 alleles in our population. While

demographic processes such as population size decline affect the whole genome, selection typically only affects the target loci and closely linked regions. Therefore, to fully assess the underlying reasons for the excess intermediate frequency *MHC-DRB* exon 2 alleles, future work would have to take a broader genome approach for our population.

In contrast, *MHC-DOB* exon 2 appears to have been under purifying selection (0>dN/dS>1; Biswas and Akey 2006) thereby eliminating harmful nonsynonymous substitutions from *MHC-DOB* in white-tailed deer. While haplotype diversity at the nucleotide level was high, most amino acid sequences for *MHC-DOB* exon 2 had little to no differences among them (mean pairwise difference was < 1%), and we only found 3 unique amino acid sequences. The intensity of purifying selection depends on how tolerant a genomic region is towards mutations, or how functionally constrained it is. DNA regions in which a mutation is likely to affect their gene product's function tend to be more functionally constrained and have lower substitution rates. *MHC-DOB* exon 2, which encodes the extracellular domain of the *MHC-DOB* protein (Andersson et al. 1991; NCBI 2019), may therefore be highly constrained. Neutrality tests for *MHC-DOB* were not significant, suggesting that *MHC-DOB* seems to be evolving neutrally within our white-tailed deer population. There was, however, an increasing trend in neutrality test values over time, with values for Tajima's D and Fu and Li's F* approaching statistical significance ($0.10 > p > 0.05$). This may indicate that *MHC-DOB* exon 2 is moving towards balancing selection in our population. The significant heterozygote deficiency seen in the 2016 population suggests the possibility of either selection away from heterozygote individuals or the potential effect of inbreeding due to small population size at that locus.

Overall this study identified 12 new *MHC-DRB* exon 2 alleles and characterized a new, non-classical, MHC II gene (*MHC-DOB*) for white-tailed deer. We also found a lack of significant linkage between these two loci which suggests there may be a chromosomal inversion in the MHC II region of white-tailed deer. However, more research is required to confirm this. If a chromosomal inversion is indeed found, recombination rates between the two MHC II subregions should be examined, as rates have been found to differ between individuals (Park et al. 1995). Lastly, more MHC polymorphism may be found when using next generation sequencing for white-tailed deer populations from other parts of the Americas. Improving our understanding of MHC II gene structure and polymorphism in white-tailed deer will enable to us

to further examine how these unique, highly polymorphic genes influence morphology, reproductive success, and overall population dynamics in white-tailed deer.

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TABLES

Table 1.1. Genetic diversity within our white-tailed deer population (nucleotide diversity and haplotype diversity) and neutrality tests for *MHC-DRB* exon 2 for the adult founding population [2003-2007; n = 69 (*MHC-DRB*), 71 (*MHC-DOB* full sequence), 71 (*MHC-DOB* exon 2)] and the final adult population [2016; n = 118 (*MHC-DRB*), 119 (*MHC-DOB* full sequence), 119 (*MHC-DOB* exon 2)]. P-values for the Hardy Weinberg Equilibrium (HWE) tests are also included, which shows a statistical significance for heterozygote excess in *MHC-DRB* exon 2 in the 2016 population. * $p < 0.05$.

		MHC-DRB founding population	*MHC-DRB* 2016 population
Diversity	Nucleotide diversity (π)	0.09	0.10
	Haplotype diversity	0.90	0.88
Neutrality	Tajima's D	0.92	1.47
	Fu and Li's F*	2.09*	2.45*
	Fu and Li's D*	2.39*	2.56*
	Fu's Fs	21.98	35.02
HWE	Probability test	0.88	0.91
	Heterozygote excess	0.70	0.05*
	Heterozygote deficit	0.30	0.95

Table 1.2. Genetic diversity within our white-tailed deer population (nucleotide diversity and haplotype diversity) and neutrality tests for *MHC-DOB* for the adult founding population [2003-2007; n = 71 (*MHC-DOB* full sequence, 360 bp), 71 (*MHC-DOB* exon 2, 270 bp)] and the final adult population [2016; n = 119 (*MHC-DOB* full sequence, 360 bp), 119 (*MHC-DOB* exon 2, 270 bp)]. P-values for the Hardy Weinberg Equilibrium (HWE) tests are also included, which shows a statistical significance for heterozygote deficit in *MHC-DOB* (full sequence and exon 2) in the 2016 population. * $p < 0.05$; ^ $0.10 > p > 0.05$.

		Full *MHC-DOB* Sequence		***MHC-DOB* exon 2**	
		Founding population	2016 population	Founding population	2016 population
Diversity	Nucleotide diversity (π)	0.005	0.005	0.006	0.006
	Haplotype diversity	0.83	0.86	0.80	0.83
Neutrality	Tajima's D	0.87	1.31	1.38	1.77^
	Fu and Li's F*	1.26	1.42	1.34	1.48^
	Fu and Li's D*	1.17	1.13	1.01	0.97
	Fu's Fs	-0.22	0.64	0.60	1.33
HWE	Probability Test	0.27	0.55	0.57	0.33
	Heterozygote Excess	0.77	0.97	0.44	0.99
	Heterozygote Deficit	0.24	0.03*	0.57	0.01*

Table 1.S1. *MHC-DRB* exon 2 alleles whose frequencies did not meet the minimum allele frequency (0.67%). No pedigree or Sanger data was available to validate these sequences, and they only occurred once in our white-tailed deer population

>Odvi-DRB*31 [organism=*Odocoileus virginianus*]
GGAGTATCATAAGGCCGAGTGTCATTTCTCCAACGGGACGCAGCGGGTGCGGTTCCTGGACA
GATACATCTATAACCAGGAAGAGTACGTGCGCTTCGACAGCGACGTGGGCGAGTACCGGGC
GGTGACAGAGCTGGGGCGGCCGGACGCCGAGGACTGGAACAGCCGGAAGGAGCTCCTGGA
GCAGAGGCGGGCCGAGGTGGACACGTACTGCAGACACAACTACGGGGTTATTGAGAGTTTC
ACTGTG
>Odvi-DRB*32 [organism=*Odocoileus virginianus*]
GGAGCATCATAAGGCCGAGTGTCATTTCTCCAACGGGACGCAGGGGGTGCAGTTCCTGCAG
AGATACGTCTATAACCAGGAAGAGTACGTGCGCTTCGACAGCAACGTGGGCGAGTACCGAG
CGGTGACCGAGCTGGGGCGGACGGACGCCAAGTACTATAACAGCCAGAAGGAGTTACTGGA
GCAGAAGCGGGCCTCGGTGGACACGTACTGCAGACACAACTACGGGGTCGGTGAGAGTTTC
ACTGTG

Table 1.S2. *MHC-DRB* exon 2 alleles found in our white-tailed deer population and their frequencies. (^ indicates that these alleles translated into the same amino acid sequence)

	Frequency (%)	
Allele	Nucleotide	Amino Acid
Odvi-DRB*01	7.37	7.37
Odvi-DRB*05	0.80	0.80
Odvi-DRB*06	0.94	11.66^
Odvi-DRB*10	22.39	22.39
Odvi-DRB*12	2.95	2.95
Odvi-DRB*14	12.47	12.47
Odvi-DRB*16	7.64	7.64
Odvi-DRB*19	10.72	11.66^
Odvi-DRB*20	14.61	14.61
Odvi-DRB*21	2.55	2.55
Odvi-DRB*22	2.55	2.55
Odvi-DRB*23	3.35	3.35
Odvi-DRB*24	2.28	2.28
Odvi-DRB*25	6.03	6.03
Odvi-DRB*26	1.07	1.07
Odvi-DRB*27	0.80	0.80
Odvi-DRB*28	0.40	0.40
Odvi-DRB*29	0.54	0.54
Odvi-DRB*30	0.54	0.54

Table 1.S3. Number of nucleotide (below diagonal) and amino acid (above diagonal) differences between *MHC-DRB* exon 2 alleles.

	DRB*01	DRB*02	DRB*03	DRB*04	DRB*05	DRB*06	DRB*07	DRB*08	DRB*09	DRB*10	DRB*11	DRB*12	DRB*13	DRB*14	DRB*15	DRB*16	DRB*17	DRB*18	DRB*19	DRB*20	DRB*21	DRB*22	DRB*23	DRB*24	DRB*25	DRB*26	DRB*27	DRB*28	DRB*29	DRB*30
DRB*01	-	20	12	8	11	21	21	9	3	11	20	12	12	13	15	16	9	12	21	12	14	15	10	11	13	8	23	9	8	17
DRB*02	31	-	24	21	18	23	5	18	17	18	21	21	25	26	23	18	20	23	23	25	23	20	19	23	24	21	7	21	22	25
DRB*03	19	33	-	15	18	25	24	17	10	16	22	22	7	13	11	25	10	21	25	14	14	22	12	17	15	12	26	14	13	8
DRB*04	16	35	23	-	18	23	19	13	10	14	18	16	13	13	10	20	10	15	23	12	17	15	6	8	14	8	21	7	8	17
DRB*05	17	26	23	28	-	20	19	10	8	4	18	12	20	24	22	21	17	13	20	23	15	11	16	21	16	18	21	19	19	22
DRB*06	33	39	42	40	30	-	24	20	20	20	26	22	25	27	26	27	22	21	0	27	23	21	23	25	21	22	25	23	23	27
DRB*07	32	8	34	30	26	39	-	18	18	16	19	22	24	25	22	19	19	24	24	24	23	20	17	21	24	20	2	19	20	24
DRB*08	14	25	22	22	11	30	26	-	9	10	17	10	16	20	19	17	13	14	20	19	15	12	13	17	14	15	20	15	13	20
DRB*09	4	27	15	20	13	34	28	12	-	8	22	14	12	16	14	17	9	15	20	15	17	15	8	13	16	10	20	11	11	15
DRB*10	21	31	22	22	8	34	24	15	17	-	17	14	17	21	19	23	14	15	20	20	13	13	12	17	15	15	18	15	15	19
DRB*11	28	33	31	29	24	38	31	25	31	27	-	19	20	20	17	23	23	21	26	19	15	17	20	22	15	22	21	21	21	22
DRB*12	18	31	31	25	15	32	33	15	22	21	28	-	18	17	22	17	14	11	22	16	11	11	19	14	14	12	24	12	15	22
DRB*13	18	35	9	24	24	42	35	21	16	25	30	26	-	8	10	22	7	17	25	9	11	20	12	12	14	9	26	9	13	5
DRB*14	17	37	19	22	31	45	38	28	21	32	28	24	14	-	12	24	11	15	27	1	11	20	15	5	12	10	27	9	11	12
DRB*15	21	33	14	15	27	45	34	24	19	26	26	29	15	18	-	23	15	21	26	11	16	21	10	16	15	16	24	15	17	12
DRB*16	25	25	36	32	31	41	28	26	26	37	33	29	33	35	34	-	19	21	27	23	24	20	21	21	24	19	21	19	19	26
DRB*17	17	30	13	18	23	40	27	20	15	19	34	23	10	18	21	32	-	12	22	12	12	16	9	7	16	2	21	4	8	12
DRB*18	18	34	29	24	16	33	36	19	22	22	29	13	24	21	27	33	21	-	21	15	10	12	18	13	8	11	26	11	14	21
DRB*19	34	40	43	41	31	1	40	31	35	35	39	33	43	46	46	42	41	34	-	27	23	21	23	25	21	22	25	23	23	27
DRB*20	15	35	21	20	29	43	36	26	19	30	26	22	16	2	16	33	20	21	44	-	10	19	14	6	11	11	26	10	12	13
DRB*21	19	32	20	26	17	34	33	20	23	18	21	14	17	17	22	36	19	13	35	15	-	16	19	16	6	11	25	13	16	16
DRB*22	20	28	28	24	13	31	28	15	20	19	25	13	27	28	28	29	24	16	32	26	19	-	17	16	14	16	22	15	14	24
DRB*23	21	31	21	15	25	44	27	22	17	21	32	30	21	23	17	36	15	29	45	21	30	28	-	11	18	11	19	10	11	14
DRB*24	17	37	25	15	31	44	33	27	21	27	32	22	20	7	24	34	12	21	45	9	24	26	17	-	17	5	23	4	8	16
DRB*25	18	33	23	21	18	31	33	17	22	21	21	17	22	19	21	33	26	10	32	17	9	16	29	26	-	15	26	15	14	18
DRB*26	14	33	17	14	26	38	30	24	18	22	32	19	14	15	23	32	4	18	39	17	16	24	19	8	23	-	22	2	7	14
DRB*27	36	12	38	34	30	41	4	30	32	28	35	37	39	42	38	32	21	40	42	40	37	32	31	37	37	34	-	21	22	26
DRB*28	14	34	19	14	26	38	29	24	18	22	32	19	14	15	23	32	6	18	39	17	18	24	18	8	23	2	33	-	6	12
DRB*29	14	34	20	14	27	39	29	22	18	23	33	24	21	17	26	27	13	22	40	19	24	22	21	12	21	10	33	10	-	15
DRB*30	22	34	9	28	25	43	34	25	18	26	32	30	6	18	17	37	14	28	44	20	21	31	22	24	26	18	38	16	23	-

Table 1.S4. Genotype frequencies (%) for *MHC-DRB* exon 2 alleles in our white-tailed deer population.

	DRB*01	DRB*05	DRB*06	DRB*10	DRB*12	DRB*14	DRB*16	DRB*19	DRB*20	DRB*21	DRB*22	DRB*23	DRB*24	DRB*25	DRB*26	DRB*27	DRB*28	DRB*29	DRB*30
DRB*01	0.80																		
DRB*05	0	0																	
DRB*06	0.27	0	0																
DRB*10	2.95	0.80	0.54	4.83															
DRB*12	0.54	0	0	1.88	0														
DRB*14	2.15	0	0	4.56	0.27	2.41													
DRB*16	1.07	0	0	3.75	0	1.07	0.27												
DRB*19	1.34	0	0	3.49	0.80	3.49	2.41	0.80											
DRB*20	1.07	0.54	0.54	6.97	1.61	3.49	2.68	2.41	2.15										
DRB*21	0.54	0	0.27	1.07	0	0.80	0.80	1.07	0.54	0									
DRB*22	0.27	0	0	1.07	0.27	0	0.80	0.80	1.07	0.54	0								
DRB*23	0.80	0.27	0	1.61	0	1.07	0.27	0.54	1.07	0	0.54	0							
DRB*24	0.80	0	0	1.07	0	1.07	0	0.80	0.27	0	0	0.27	0						
DRB*25	1.07	0	0.27	3.21	0.54	0.80	0.80	1.88	2.15	0	0.54	0.27	0.27	0					
DRB*26	0	0	0	1.07	0	0.27	0.27	0.54	0	0	0	0	0	0	0				
DRB*27	0	0	0	0	0	0	0.27	0.27	0.80	0	0	0	0	0.27	0	0			
DRB*28	0	0	0	0.54	0	0.27	0	0	0	0	0	0	0	0	0	0	0		
DRB*29	0	0	0	0.54	0	0.27	0	0.27	0	0	0	0	0	0	0	0	0	0	
DRB*30	0.27	0	0	0	0	0	0.27	0.27	0.27	0	0	0	0	0	0	0	0	0	0

Table 1.S5. *MHC-DOB* alleles for both the full sequence (360 bp) and exon 2 (270 bp) found in our white-tailed deer population and their frequencies. ($^{\#}$ and ^ indicates that these alleles translated into the same amino acid sequence)

***MHC-DOB* full sequence**		***MHC-DOB* exon 2**		
Allele	Frequency (%)	Allele	Nucleotide Frequency (%)	Amino Acid Frequency (%)
Odvi-DOB*01	0.53	Odvi-DOB*010211_exon2	14.61	73.42$^{\#}$
Odvi-DOB*02	1.32			
Odvi-DOB*11	12.76			
Odvi-DOB*03	10.00	Odvi-DOB*0310_exon2	20.53	73.42$^{\#}$
Odvi-DOB*10	10.26			
Odvi-DOB*04	11.84	Odvi-DOB*04_exon2	11.71	20.53^
Odvi-DOB*05	8.82	Odvi-DOB*05_exon2	8.82	20.53^
Odvi-DOB*06	7.50	Odvi-DOB*0607_exon2	10.39	73.42$^{\#}$
Odvi-DOB*07	3.03			
Odvi-DOB*08	27.89	Odvi-DOB*08_exon2	27.89	73.42$^{\#}$
Odvi-DOB*09	6.05	Odvi-DOB*09_exon2	6.05	6.05

Table 1.S6. Number of nucleotide (below diagonal) and amino acid (above diagonal) differences between the full *MHC-DOB* sequence (360 bp) alleles for white-tailed deer. The amino acid differences correspond to the amino acid differences seen in *MHC-DOB* exon 2 (Table 7).

	DOB*01	DOB*02	DOB*03	DOB*04	DOB*05	DOB*06	DOB*07	DOB*08	DOB*09	DOB*10	DOB*11
DOB*01	-	0	0	1	1	0	0	0	1	0	0
DOB*02	2	-	0	1	1	0	0	0	1	0	0
DOB*03	2	2	-	1	1	0	0	0	1	0	0
DOB*04	3	3	1	-	0	1	1	1	2	1	1
DOB*05	2	2	2	1	-	1	1	1	2	0	0
DOB*06	3	3	1	2	3	-	0	0	1	0	0
DOB*07	4	4	2	3	4	1	-	0	1	0	0
DOB*08	2	2	2	3	2	3	4	-	1	0	0
DOB*09	3	3	3	4	3	4	5	1	-	1	1
DOB*10	3	3	1	2	3	2	3	3	4	-	0
DOB*11	1	1	1	2	1	2	3	1	2	2	-

Table 1.S7. Number of nucleotide (below diagonal) and amino acid (above diagonal) differences between *MHC-DOB* exon 2 (270 bp) alleles for white-tailed deer.

	DOB*010211_exon2	DOB*0310_exon2	DOB*04_exon2	DOB*05_exon2	DOB*0607_exon2	DOB*08_exon2	DOB*09_exon2
DOB*010211_exon2	-	0	1	1	0	0	1
DOB*0310_exon2	1	-	1	1	0	0	1
DOB*04_exon2	2	1	-	0	1	1	2
DOB*05_exon2	1	2	1	-	1	1	2
DOB*0607_exon2	2	1	2	3	-	0	1
DOB*08_exon2	1	2	3	2	3	-	1
DOB*09_exon2	2	3	4	3	4	1	-

Table 1.S8. Genotype frequencies (%) for the full *MHC-DOB* sequence (360 bp) alleles in our white-tailed deer population.

	DOB*01	DOB*02	DOB*03	DOB*04	DOB*05	DOB*06	DOB*07	DOB*08	DOB*09	DOB*10	DOB*11
DOB*01	0										
DOB*02	0	0									
DOB*03	0.26	0	0.79								
DOB*04	0.26	0.26	1.58	2.37							
DOB*05	0	0	2.11	1.58	0.79						
DOB*06	0	0	1.84	1.32	3.16	0.53					
DOB*07	0	0.26	0.53	0.53	0.53	0.53	0				
DOB*08	0.26	1.32	5.53	8.68	4.74	2.37	1.05	7.90			
DOB*09	0	0	0.79	0.79	0.26	0.79	1.58	3.16	1.05		
DOB*10	0	0	2.11	0.79	1.84	1.84	0.53	7.11	1.32	2.11	
DOB*11	0.26	0.79	3.68	3.16	1.84	2.11	0.53	5.79	1.32	0.79	2.63

Table 1.S9. Genotype frequencies (%) for *MHC-DOB* exon 2 (270 bp) alleles in our white-tailed deer population.

	DOB*010211_exon2	DOB*0310_exon2	DOB*04_exon2	DOB*05_exon2	DOB*0607_exon2	DOB*08_exon2	DOB*09_exon2
DOB*010211_exon2	3.68						
DOB*0310_exon2	4.74	5.0					
DOB*04_exon2	3.68	2.63	2.11				
DOB*05_exon2	1.84	3.95	1.58	0.79			
DOB*0607_exon2	2.90	5.0	1.84	3.68	0.79		
DOB*08_exon2	7.37	12.63	8.68	4.74	3.42	7.90	
DOB*09_exon2	1.32	2.11	0.79	0.26	2.37	3.16	1.05

FIGURES

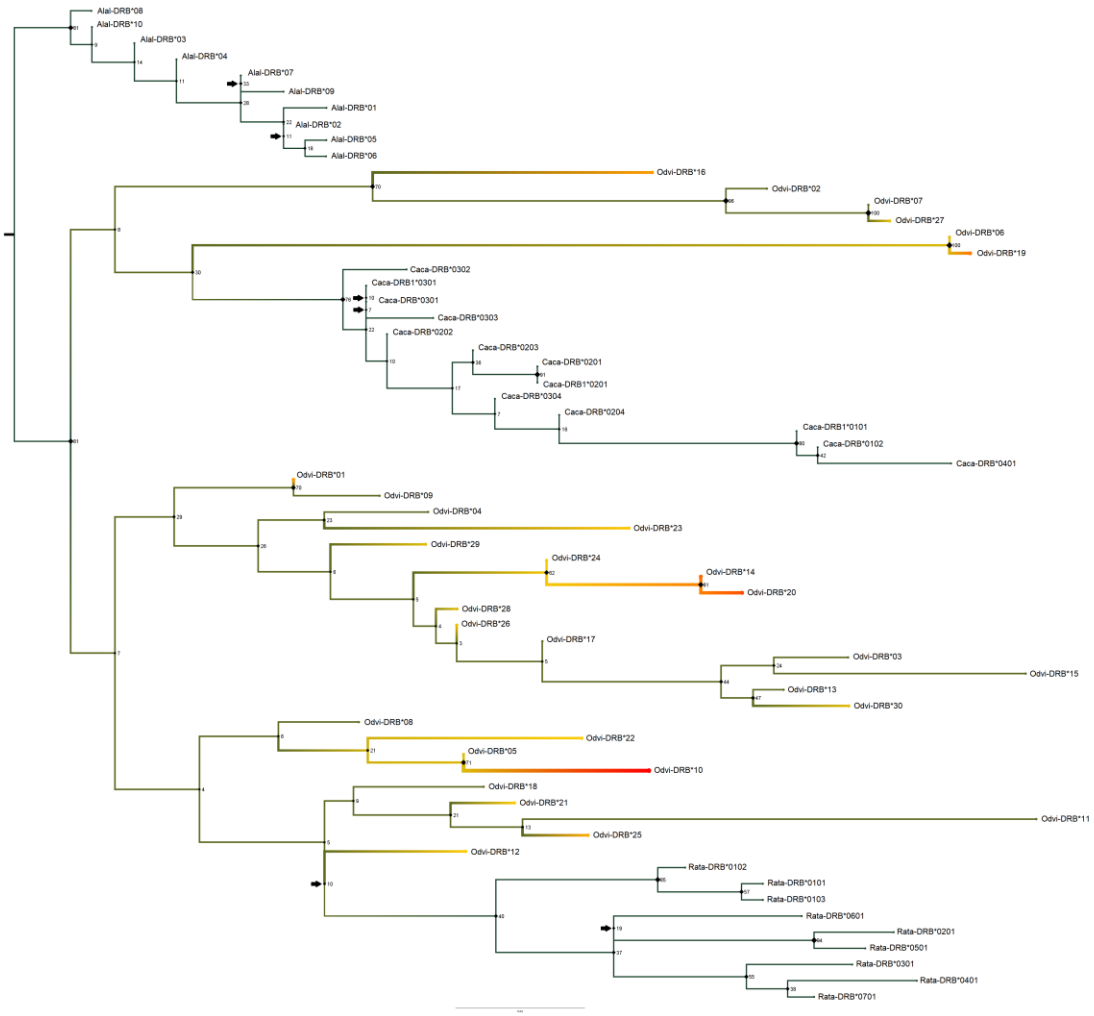

Figure 1.1. Maximum likelihood phylogenetic tree for the nucleotide sequences of *MHC-DRB* exon 2. This tree contains sequences for white-tailed deer (Odvi, *Odocoileus virginianus*; AF082161-AF082175, AF407169-AF407171; MK952679- MK952690), moose (Alal, *Alces alces*; X82398 , X83278, X83279-X83286; Mikko and Andersson, 1995), roe deer (Caca, *Capreolus capreolus*; KM488213-KM488216, KM488218, KM488220-U90925; Mikko et al. 1997a; Quemere et al. 2015), and reindeer (Rata, *Rangifer tarandus*; AF012716-AF012724; Mikko et al. 1999) as these are the closest related species to white-tailed deer with *MHC-DRB* data. It was rooted with the moose outgroup. Node labels are standard bootstrap support (%). Arrows indicate the presence of near-zero internal branch lengths (< 0.0040), which should be interpreted with caution. Heatmap colors indicate all white-tailed deer *MHC-DRB* exon 2 alleles

and further correspond to Odvi-DRB allele frequencies found in our population, where red is the most common *MHC-DRB* allele.

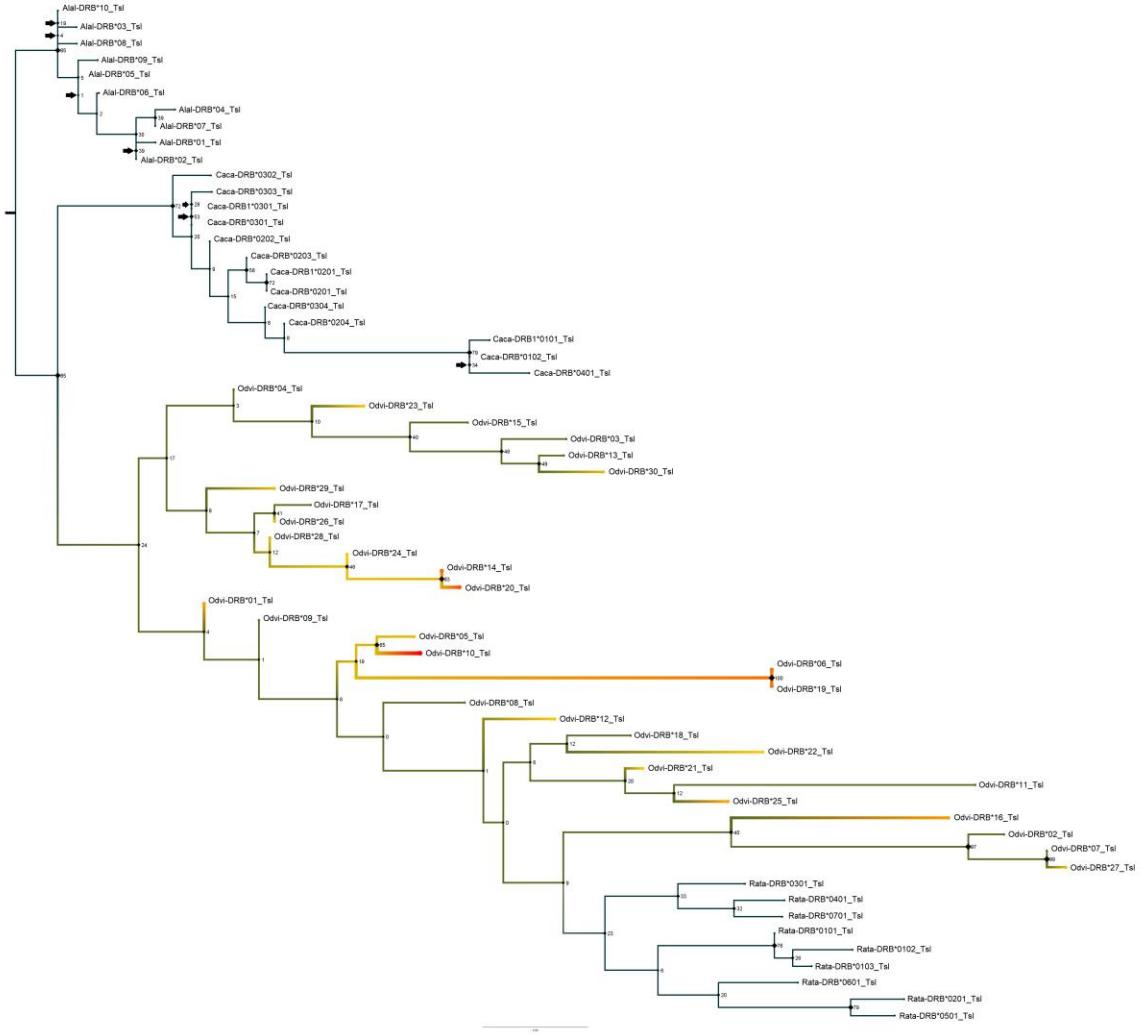

Figure 1.2. Maximum likelihood phylogenetic tree for the amino acid sequences of *MHC-DRB* exon 2. This tree contains the translated sequences for white-tailed deer (Odvi, *Odocoileus virginianus*; AF082161-AF082175, AF407169-AF407171; MK952679- MK952690), moose (Alal, *Alces alces*; X82398 , X83278, X83279-X83286; Mikko and Andersson, 1995), roe deer (Caca, *Capreolus capreolus*; KM488213-KM488216, KM488218, KM488220-U90925; Mikko et al. 1997a; Quemere et al. 2015), and reindeer (Rata, *Rangifer tarandus*; AF012716-AF012724; Mikko et al. 1999) as these are the closest related species to white-tailed deer with *MHC-DRB* data. It was rooted with the moose outgroup. Node labels are standard bootstrap support (%). Arrows indicate the presence of near-zero internal branch lengths (< 0.012), which should be interpreted with caution. Heatmap colors indicate all white-tailed deer *MHC-DRB* exon 2 alleles and further correspond to Odvi-DRB allele frequencies found in our population, where red is the most common translated *MHC-DRB* allele.

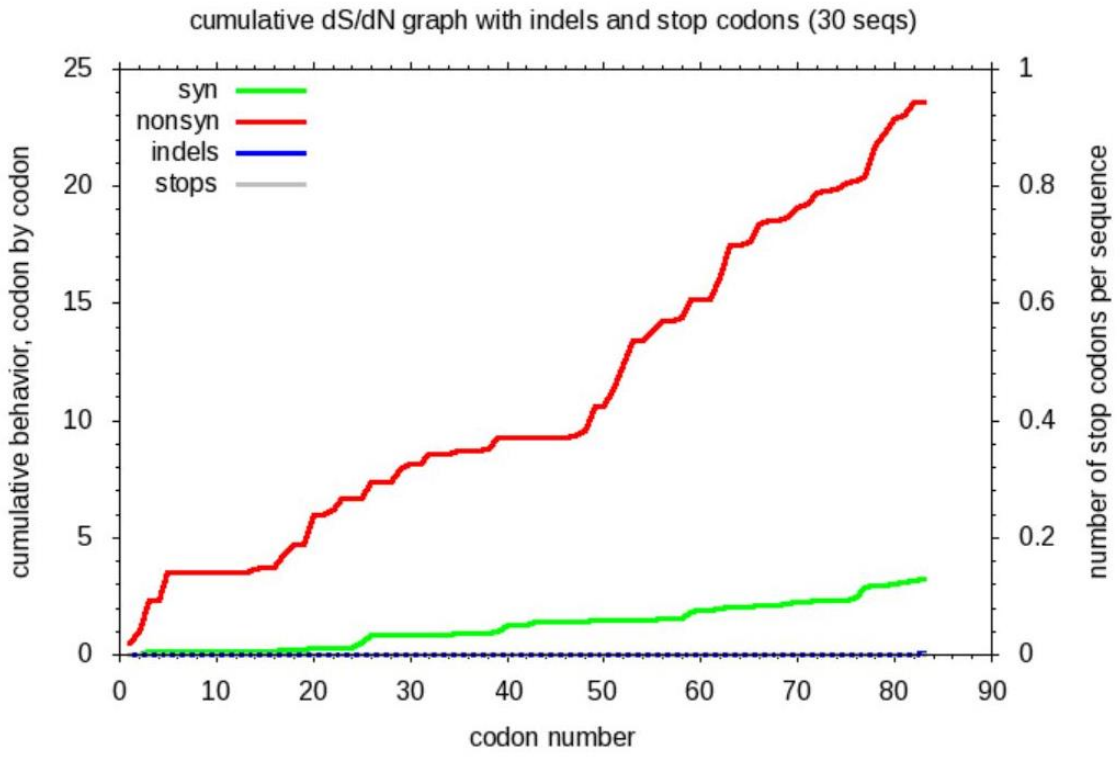

Figure 1.3. Cumulative mean codon-by-codon ratio of synonymous to nonsynonymous substitutions (dS/dN) for *MHC-DRB* exon 2. Nonsynonymous substitutions are significantly more common than synonymous substitutions for *MHC-DRB* exon 2 in white-tailed deer.

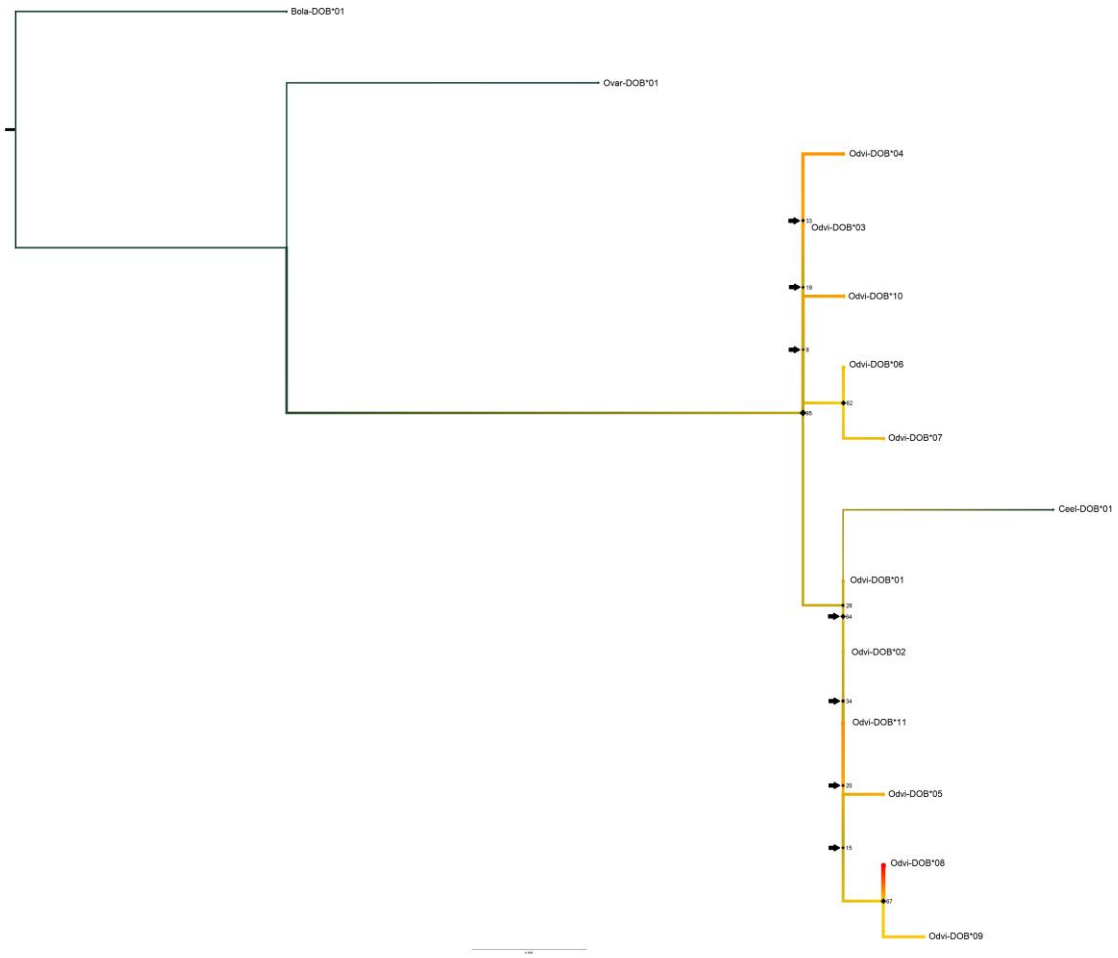

Figure 1.4. Maximum likelihood phylogenetic tree for the full nucleotide sequences of *MHC-DOB* (360 bp). This tree contains sequences for white-tailed deer (Odvi, *Odocoileus virginianus*; MK952691- MK952701), cow (Bola, *Bos taurus*; 282493; Zimin et al. 2009), sheep (Ovar, *Ovis aries*; Z49879.1; Wright et al. 1996), and red deer (Ceel, *Cervus elaphus*; CM008014.1; Bana et al. 2016). It was rooted with the cow outgroup. Node labels are standard bootstrap support (%). Arrows indicate the presence of near-zero internal branch lengths (< 0.0028), which should be interpreted with caution. Heatmap colors indicate all white-tailed deer *MHC-DOB* alleles and further correspond to Odvi-DOB allele frequencies found in our population, where red is the most common *MHC-DOB* allele.

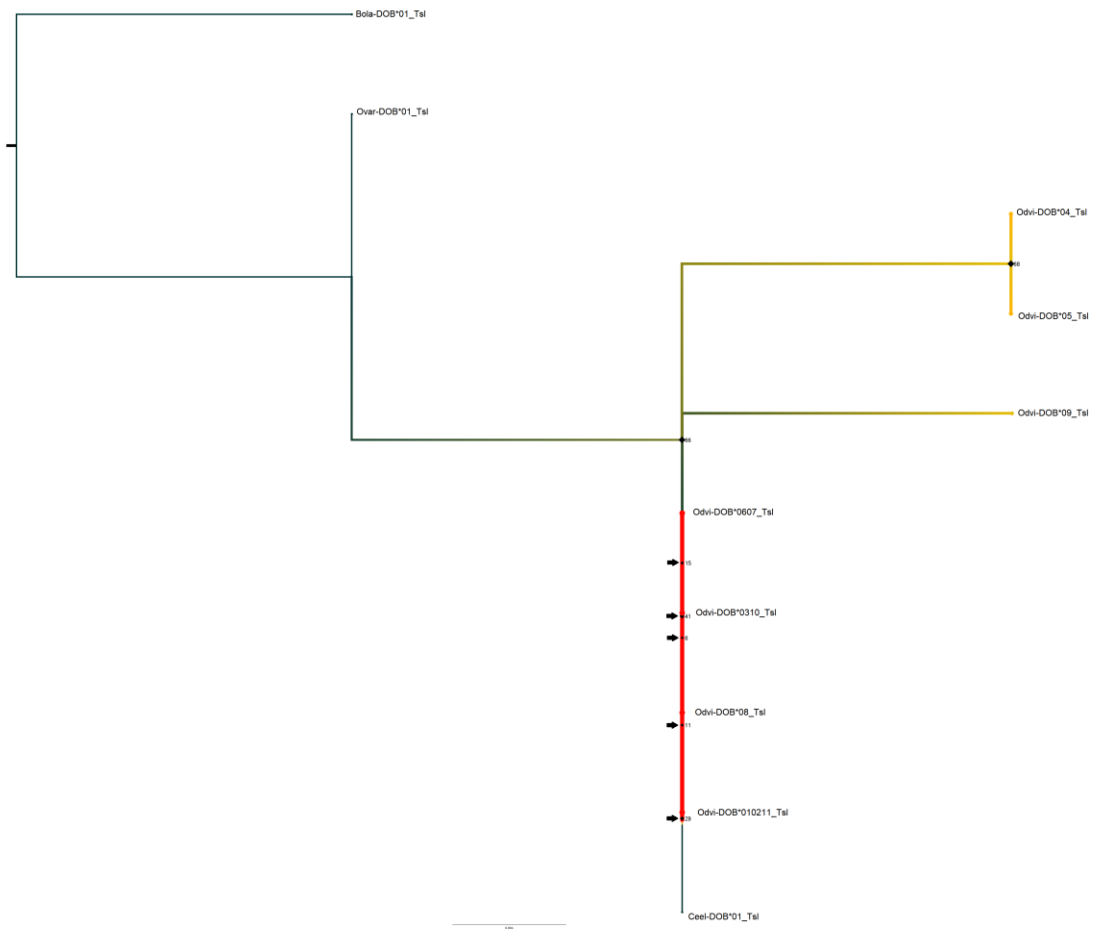

Figure 1.5. Maximum likelihood phylogenetic tree for the amino acid sequences of *MHC-DOB* exon 2. This tree contains translated sequences for white-tailed deer (Odvi, *Odocoileus virginianus*; MK952691- MK952701), cow (Bola, *Bos taurus*; 282493; Zimin et al. 2009), sheep (Ovar, *Ovis aries*; Z49879.1; Wright et al. 1996), and red deer (Ceel, *Cervus elaphus*; CM008014.1; Bana et al. 2016). It was rooted with the cow outgroup. It was rooted with the cow outgroup. Node labels are standard bootstrap support (%). Arrows indicate the presence of near-zero internal branch lengths (< 0.011), which should be interpreted with caution. Heatmap colors indicate all white-tailed deer *MHC-DOB* alleles and further correspond to Odvi-DOB allele frequencies found in our population, where red is the most common translated *MHC-DOB* allele.

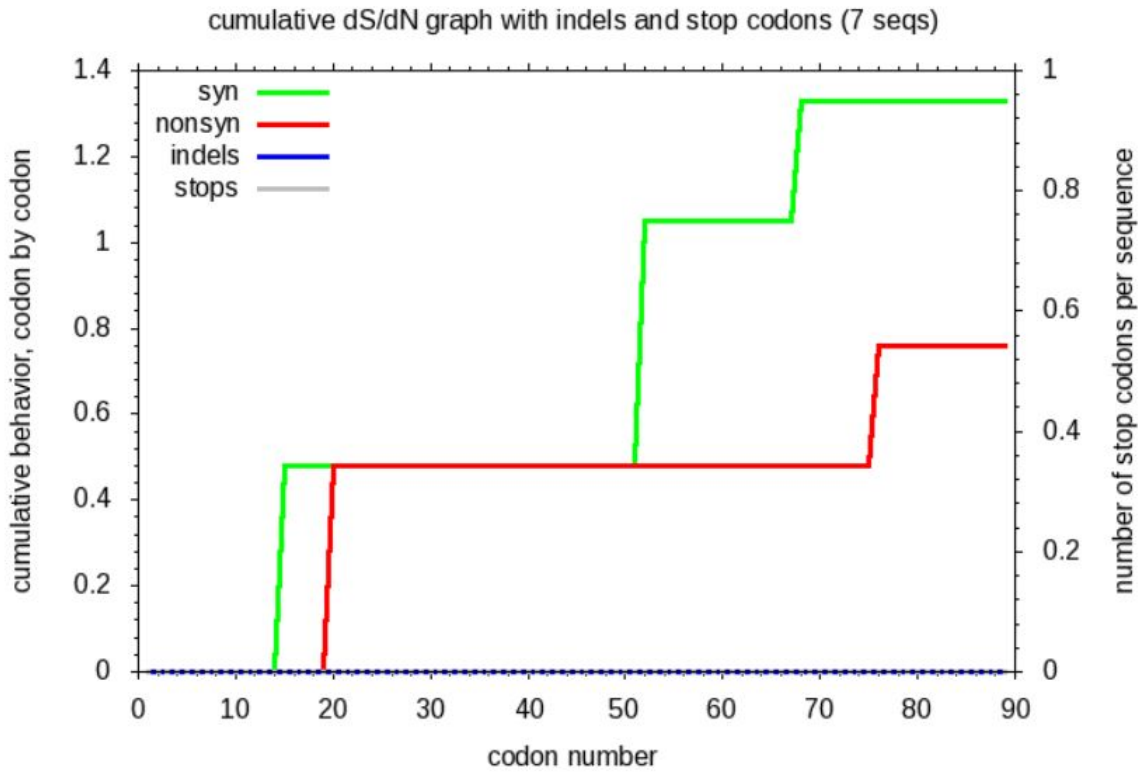

Fig 1.6. Cumulative mean codon-by-codon ratio of synonymous to non-synonymous substitutions (dS/dN) for *MHC-DOB* exon 2. Synonymous substitutions are overall more common than nonsynonymous substitutions for *MHC-DOB* exon 2 in white-tailed deer, though both are quite rare.

Chapter 2: Association between sexually selected traits and allelic distance in two unlinked MHC II loci in white-tailed deer

ABSTRACT

Body size and secondary sexual characteristics are main drivers of male reproductive success among polygynous species. A gene complex found to influence such morphology in several species is the major histocompatibility complex (MHC). However, while several studies found that greater MHC diversity enhanced male morphology, increased MHC diversity has trade-offs. Thus, maximal MHC diversity is not always optimal for the individual. This study examined if MHC diversity, measured as pairwise allelic distances at two unlinked MHC II loci (exon 2 for the classical antigen-binding protein *MHC-DRB*, exon 2 for the accessory protein *MHC-DOB*), was associated with body size or antler size in an enclosed population of white-tailed deer (*Odocoileus virginianus*). After accounting for the effect of age on morphology, we used residual analysis to assess whether MHC allelic distances explained any of the remaining variation in body and antler size. While we found no associations for MHC-DRB, we found that both male body and antler size were strongly associated with *MHC-DOB* nucleotide allelic distances. Specifically, we found a quadratic relationship between *MHC-DOB* and male body size, where body size peaked at moderate *MHC-DOB* allelic distance. However, we found a positive linear association between *MHC-DOB* nucleotide allelic distances and antler scores. Neither *MHC-DRB* nor *MHC-DOB* influenced female body size, even though the average allelic distances of males and females were not significantly different from each other. The existence of a heterozygote deficiency for *MHC-DOB* in our population might suggest that body size is more important for male reproductive success than antler size.

KEYWORDS

Major histocompatibility complex, white-tailed deer, sexual selection, morphology, secondary sexual characteristics

INTRODUCTION

Polygyny is a common breeding strategy, especially among terrestrial Artiodacyla (Geist 1974; Jarman 1974; Ralls 1977; Bubenik 1985; Clutton-Brock 1989; Weckerly 1998; Loison et al. 1999). In polygynous species, male competition is high, as only a few males may monopolize breeding access and fights are common and ensure that the strongest males are able to pass on their genetics (Clutton-Brock and Huchard, 2013). Males must therefore invest energy into body size and physical traits that positively influence reproductive success, such as teeth, horns, and antlers (West-Eberhard 1979; Clutton-Brock and Huchard, 2013). Although a polygynous strategy can grant successful males increased reproductive success, such a strategy is very costly. These costs are especially apparent with seasonal breeders, where males may cease feeding altogether during the breeding season (Thompson 1973; Clutton-Brock and Huchard 2013). Male white-tailed deer (*Odocoileus virginianus*), for example, may lose up to 30% of their body mass during the breeding season (Hewitt 2011). Unlike the strong selection for large ornamentation found among males, no such selection exists for females, as costly morphological investments are unnecessary for females to attract mates (Ditchkoff 2011). Instead, female reproductive success is determined by her ability to bear and raise offspring to reproductive age (Strassmann and Gillespie 2002).

Body and ornamentation size can be influenced by an individual's genetics. A gene complex of particular interest with respect to its association with morphology is the major histocompatibility complex (MHC; Bennett 1975; Gill and Kunz, 1979; von Schantz et al. 1996; 1997; Ditchkoff et al. 2001; Fernandez-de-Mera et al. 2009; Brambilla et al. 2015, 2018). These genes do not influence morphology directly, but instead code for proteins that are essential for the immune system to distinguish self from foreign pathogens by binding to peptide fragments (i.e. antigens) and displaying these on the surfaces of cells where they are monitored by T-cell lymphocytes (Hedrick 1994; Schook and Lamont 1996; Janeway et al. 2001; Kamiya et al. 2014). If the antigen is recognized by the body as 'self,' the T-cells will not destroy the cell. If the antigen is foreign, however, the T-cells will cause cellular destruction or initiation of a systemic immune response to clear foreign particles (Janeway et al. 2001). While gene products from MHC type I genes primarily display endogenous antigens on nucleated cells, MHC type II genes produce proteins that display exogenous antigens on immune cells such as dendritic cells and macrophages (Janeway et al. 2001). There are classical (ex. DR, DQ) and non-classical (ex.

DO, DM) MHC II genes, whose proteins serve different immunological roles. Non-classical MHC genes produce accessory proteins that are used for properly loading antigens onto classical MHC gene products, which then display these antigens at the immune cell's surface (Poluektov et al. 2013; Mellins and Stern 2014). While classical MHC genes are highly polymorphic, non-classical MHC genes are more conserved (Janeway et al. 2001; Denzin 2013).

Due to their vital role in determining the immune system's effectiveness, MHC genes may influence resources available for an individual's growth, development, and reproduction. Several studies have examined the association between the MHC and morphology. For example, the spur length of male pheasants (*Phasianus colchicus*), which correlates to male viability and female mate choice, is highly influenced by male MHC genotype characteristics (von Schantz et al. 1996; 1997). This relationship between an individual's MHC genotype and morphology may be linked to the improved immune system associated with MHC heterozygosity (von Schantz et al. 1996; Sauermann et al. 2001; Olsson et al. 2005). Specifically, the heterozygote advantage hypothesis states that individuals with greater MHC heterogeneity are able to fight off a greater diversity of pathogens (Hughes and Nei, 1992; Sommer 2005). Under this hypothesis, heterozygotes should be healthier, more successful, and consequently better able to attain larger size of sexually selected traits (Hughes and Nei, 1989; Takahata and Nei, 1990).

However, costs of increased MHC diversity exist that may define optimal levels of MHC heterozygosity for an individual (Demas and Nelson, 2011). Greater individual MHC heterozygosity is associated with increased deletion of T-cell lineages, which results in reduced T-cell repertoire diversity, and therefore a less effective immune response (Vidovic and Matzinger, 1988; Vidovic 1989; Nikolich-Zugich et al. 2004). A reduced T-cell repertoire can also lead to limited regulatory T-cell (Treg) diversity. These immune cells are responsible for controlling the intensity of an immune response (Graham et al. 2005), and reduced Treg diversity can make an individual more prone to immunopathology and autoimmune disease (Milner et al. 2007; Ferreira et al. 2009). Increased MHC heterozygosity may also increase an individual's chances of having an MHC allele that predisposes the carrier to several autoimmune diseases, such as multiple sclerosis, lupus, diabetes, and Crohn's disease in humans (Fernando et al. 2008). Similarly, certain MHC alleles may predispose the carrier to contracting infectious diseases (McClelland et al. 2003). Additionally, immune responses can be very physiologically costly and divert resources away from growth (Klasing et al. 1987; Fair et al. 1999; Bonato et al. 2009) and

reproduction (Ilmonen et al. 2000; Bonneaud et al. 2003, 2004; Hanssen 2006; Ilmonen et al. 2007; Cai et al. 2009; Bascunan-Garcia et al. 2010). Thus, as MHC heterozygous individuals typically mount a stronger immunological response than homozygotes (Doherty et al. 1975), individuals with greater MHC heterozygosity may suffer greater physiological costs that could reduce their overall fitness (Ilmonen et al. 2007). Lastly, maximum MHC heterozygosity may negatively influence T-cell activation by reducing the concentration of specific peptide-MHC complexes on the cells' surfaces (van den Berg and Rand, 2003; Woelfing et al. 2009). All of these costs of increased MHC diversity may ultimately reduce resources the individuals can devote to developing and building sexually selected morphological characteristics. Given the known trade-offs associated with increased MHC diversity, maximizing MHC diversity may not be the optimal strategy.

The MHC region of the white-tailed deer, a polygynous ruminant, has been examined by several studies. Two MHC genes have been characterized in this system, the classical *MHC-DRB* and the non-classical *MHC-DOB*. Currently, 30 unique *MHC-DRB* exon 2 alleles and 11 *MHC-DOB* alleles have been documented for this species (Van Den Bussche et al. 1999, 2002; Ivy-Israel et al. 2019). Ivy-Israel et al (2019) also found that the *MHC-DRB* and *MHC-DOB* loci are not genetically linked, suggesting that these loci occupy two different MHC II subregions on the same chromosome - possibly separated by an inversion (Band et al. 1998) - that are able to evolve independently from one another. Since 2001, when Ditchkoff et al. found a positive association between *MHC-DRB* diversity and both body mass and antler size in white-tailed deer males, 15 additional *MHC-DRB* exon 2 alleles have been identified. Therefore, in this study we examined if an association exists between morphology and *MHC-DRB* allelic distance in a previously unstudied white-tailed deer population using next-generation sequencing. We also included *MHC-DOB* in our analyses as there have been no studies assessing the role of this MHC locus on vertebrate morphology. Based on these previous findings and what is known about *MHC-DRB* and *MHC-DOB* in other species, we hypothesized that *MHC-DRB* allelic distance influences male morphology but not female morphology, as females are not investing resources towards costly sexually selected traits (Ditchkoff et al. 2011). Since *MHC-DOB*, a conservative MHC II locus, is not linked to *MHC-DRB*, we further hypothesized that there is no association between *MHC-DOB* and morphology in both male and female white-tailed deer.

METHODS

Study Area

This study took place at the Auburn Captive Facility (ACF) located north of Camp Hill, Alabama. The facility was part of the Piedmont Agricultural Experiment Station, which was owned by Auburn University. Deer sampled during the study were enclosed in a 174-hectare facility surrounded by a 2.6-meter fence, which was constructed in October 2007. The deer present within the ACF during the study included the original deer that inhabited this area during the fence formation in 2007 and their subsequent offspring. The population size and effective population size of the adult, founding population were 71 and 64.8, respectively. Effective population size was calculated as $N_e = 4N_mN_f / (N_m+N_f)$, where N_m is the number of breeding males (n = 25) and N_f is the number of breeding females (n = 46) in the population (Wright 1938). Subsequent to fencing the area, deer were neither introduced nor hunted within the ACF. Instead, population size was mainly regulated via natural and capture-related mortalities (Newbolt et al. 2017). Population size varied annually between 100-120 deer. Deer had access to supplemental feed in the form of food plots, corn feeders, and *ad libitum* protein feeders. A creek and its tributaries were present on the property, which provided a reliable water source year-round.

Animal handling

Adult white-tailed deer (≥ 6 months of age) were captured over 12 trapping seasons (October – July each year) from 2007-2018 via chemical immobilization. A tranquilizer mixture was administered into the deer's hindquarter muscle with the use of cartridge fired dart guns (Pneu-Dart model 193) and 0.22 caliber blanks. The mixture was prepared by adding 4 cc of xylaxine (100 mg/ml; Lloyd Laboratories, Shenandoah, IA) to a 5 mL vial of Telazol® (100 mg/ml; Fort Dodge Animal Health, Fort Dodge, IA; Miller et al. 2003). We then added 2 cc of this tranquilizer mixture into a telemetry dart (2.0 cc, type C, Pneu-Dart Inc., Williamsport, PA) containing a radio transmitter (Advanced Telemetry Systems, Inc., Isanti, MN). The transmitter enabled us to locate the sedated deer via radio telemetry as the dart stayed attached to the deer's hindquarter after impact (Kilpatrick et al. 1996). Sedation was reversed by injecting Tolazine (100 mg/ml; Lloyd Laboratories) into the shoulder and hindquarter muscles once data collection was complete (Miller et al. 2004). These methods were approved by the Auburn University

Institutional Animal Care and Use Committee (2008-1417, 2008-1421, 2010-1785, 2011-1971, 2013-2372, 2014-2521, 2016-2964, and 2016-2985) and in compliance with the American Society of Mammalogists' guidelines (Sikes and Gannon 2011).

All deer received a unique 3-digit identification number at initial capture, which was displayed on ear tags and also freeze branded on the front shoulder and hind quarter of some individuals. For each individual, we recorded sex, age (tooth wear and replacement aging technique; Severinghaus 1949), several body measurements (total body length, hind foot length, chest circumference; Ditchkoff et al. 2001; Newbolt et al. 2017). We also took several antler measurements for males (beam and tine lengths, antler beam circumference, inside spread) to calculate their annual gross Boone and Crocket scores (Nesbitt et al. 2009; Strickland et al. 2013). Lastly, a 1-cm^2 notch of tissue was removed from their ear for genetic analysis. This tissue sample was then stored in a -80°C freezer until DNA analysis could be performed in the laboratory.

To estimate the deer abundance and age structure of our white-tailed deer population, images of marked and unmarked deer were collected using infrared-triggered cameras at both feeders and randomly selected sites baited with corn for 14 days every February. These images were then used to calculate deer abundance using mark-recapture methods (Overton 1969; Jacobson et al. 1997). We supplemented these data with field observations and capture/mortality records to determine final population demographic estimates. Marked individuals were considered dead if not observed for two years in order to prevent a potentially ever-growing population of dead but unrecovered animals.

Genetic Analysis

Ivy-Israel et al. (2019) used the ear tissue samples collected from our population to sequence the *MHC-DRB* exon 2 (n = 373) and *MHC-DOB* exon 2 (n = 380) amplicons on the Illumina MiSeq platform (Genbank accession numbers: MK952679-MK952701; Supplementary Material 1). The characterization of these alleles in the focal population is described in detail in Ivy-Israel et al. (2019), and in brief here. While Ivy-Israel et al. (2019) only targeted exon 2 for *MHC-DRB* (250 bp), the *MHC-DOB* amplicon contained exon 2 plus noncoding regions around it. We used the full *MHC-DOB* sequence (360 bp) when analyzing nucleotide sequences and *MHC-DOB* exon 2 (270 bp) for the amino acid sequences.

For each individual, the pairwise genetic distance between the alleles at a locus (*MHC-DRB* and *MHC-DOB*) was calculated as the number of differences at either the nucleotide level (DRB_nuc and DOB_nuc, respectively) or the amino acid level (DRB_aa and DOB_aa, respectively). Distance matrices were generated via Geneious (v11.1.5).

Statistical Analysis

Ruminants that form tending bonds, such as white-tailed deer, display great sexual-size dimorphism (Weckerly 1998). We therefore analyzed female and male body size separately. As male fawns did not have antlers, we excluded this age group from our antler size analyses. We therefore analyzed three separate datasets: male body size (n = 366), male antler size (n = 313), and female body size (n = 183). Allelic distances for *MHC-DRB* and *MHC-DOB* were compared between unique males (n = 156) and females (n = 134) using unpaired t-tests in Program R (v3.6.0; lm function) to explore differences between the sexes.

The average age of adult males (≥ 6 months) present in the population was calculated for each sampling season to account for observed changes in population demographics (Newbolt et al. 2017). Average annual male age, individual age, body size, antler size, *MHC-DRB* nucleotide distance, *MHC-DRB* amino acid distance, *MHC-DOB* nucleotide distance, and *MHC-DOB* amino acid distance were standardized (i.e. subtracted mean and divided by standard deviation) prior to analysis. We used Program R to perform a principal component analysis (PCA) of the 3 standardized body measurements to generate a single term (first principal component) for annual body size. Gross Boone and Crocket antler scores were used to represent an individual's annual antler size. Collinearity was assessed for all variables by calculating variance inflation factors (VIFs) and pairwise correlation coefficients. We only found collinearity between the nucleotide and amino acid sequences for *MHC-DRB* exon 2 [VIF scores for DRB_nuc and DRB_aa: 37.96 and 37.84 (male body size dataset); 38.98 and 38.74 (antler size dataset); 32.81 and 32.86 (female body size dataset)].

We first accounted for the strong effect of age on body and antler size using non-linear models (nls function in Program R; R Core Team, 2019). Mammalian growth is typically asymptotic, thereby making sigmoid growth functions more realistic (Leberg et al. 1989). Popular growth models include the Von Bertalanffy asymptotic growth, Logistic, and Gompertz models (Zullinger et al. 1984; Lesage et al. 2001; Canaza-Cayo et al. 2015; Thalmann et al.

2015; Table 2.1), where y_{ij} is the observed principal component score for body size of individual i (i=1,…,n) at measurement time j (j=1,…,n_i) for animal i, t_{ij} is the age of animal i (years) at time j, and ε_{ij} is the random residual term. There are three growth curve parameters for these curves: A, the asymptotic mature body size; B, the proportion of asymptotic mature weight obtained after birth; and k, the maturation rate or how fast individuals approach adult weight. The Von Bertalanffy growth model (VBGM) for body length data is often cubed for weight data. As we were using principal component scores for body size, we considered both the regular VBGM and the cubed VBGM in our set of *a priori* models. We also considered variations of these models by incorporating average annual buck age (α) into the non-linear model's term for growth rate (M), the asymptote (N), or both (MN).

The Akaike's Information Criterion adjusted for sample size (AIC_c) in Program R (package bbmle; Bolker 2017) was used to select our most competitive models. We closely examined all models that were within 2 AICc units of the top-supported model to assess the presence of uninformative parameters (Arnold 2010). If uninformative variables were identified in our growth curve models, we re-examined model weights for models without these uninformative variables. We examined the need to include a term for autocorrelation (moving average) in our top models via extra-sum-of-squares *F* (hereafter 'drop tests'; Murtaugh 2008) as some individuals were captured multiple times in their lifetime.

Residuals from our top models were used in analyses to examine if MHC variables could explain the remaining variation in body and antler size variables after accounting for age. Analyses were performed using mixed-effects models with package nlme in Program R (lme function; Pinheiro et al. 2018). The support for including non-linear (quadratic) effects for our predictors in our models was evaluated via drop tests (Murtaugh 2008). All models included a random term for individual as some individuals were captured more than once in their lifetime. To aid in model interpretations, we back transformed the data to assess how average body/antler size residuals differ at varying levels of MHC diversity.

RESULTS

Allelic Distance Comparison for Males and Females

There was no difference ($p > 0.097$) in MHC allelic distances between males and females for all MHC variables examined in this study (Table 2.2). Females had a greater number of

unique *MHC-DRB* alleles (19 alleles) compared to males (17 alleles), though the two sexes shared the same most common alleles (DRB*10, DRB*14, DRB*20). While females had a greater range of possible allelic distance values for DOB_nuc, males had more unique full *MHC-DOB* sequence alleles (11 alleles) than females (10 alleles). DOB*08 was the most common full MHC-DOB allele for both sexes, though the order of remaining allele frequencies differed slightly.

Male Body Size

Our male body size dataset contained 156 unique males for a total of 366 entries (Table 2.3). Ages ranged from 0 to 12.5 years with an average of 3.5 years. The most frequently captured male was measured nine times.

Among the unique males, 21 were homozygous for *MHC-DRB* and 135 were heterozygous for *MHC-DRB* at both the nucleotide and amino acid level. *MHC-DRB* allelic distances ranged from 0 to 46 at the nucleotide level (mean ± SE = 24.6 ± 0.7), and from 0 to 27 for translated *MHC-DRB* amino acid sequences (mean ± SE = 15.7 ± 0.5). While allelic distances were fairly large for the full *MHC-DOB* nucleotide sequences (33 homozygotes, 123 heterozygotes), the majority of males were homozygous for translated MHC-DOB exon 2 sequences (98 homozygotes, 58 heterozygotes). *MHC-DOB* allelic distances ranged from 0 to 4 at the nucleotide level (mean ± SE = 1.62 ± 0.06), and from 0 to 2 at the amino acid level (mean ± SE = 0.37 ± 0.03).

Principal component analysis

The first principal component explained 87.90% of the variation in our three body measurements. Each measurement contributed equally to the first principal component: 0.58 (chest), 0.59 (body length), 0.56 (hind foot). Scores ranged from -7.66 (newborn fawn) to 2.18 (6.5-year-old male).

Nonlinear age model selection

The top model was the regular VBGM with male mean age in the asymptote term (Table 2.4; Figure 2.1). While the second-best model did have $\Delta AICc \leq 2$, the additional parameter (mean age in growth rate term) did not provide a net reduction in AICc and was therefore

uninformative (Arnold 2010). When we re-examined model weights for models without uninformative parameters, we found that the new weight for the top model was 0.78. Drop tests between the top model and the same model with a term for autocorrelation indicated accounting for autocorrelation in our model significantly improved the fit to the data ($p = 0.001$).

Residual analysis with MHC variables

The average male body size residuals doubled from 0.04 (± 0.83; 95% CI) at a small *MHC-DRB* allelic distance (DRB_aa = 1) to 0.08 (± 0.63; 95% CI) at a large *MHC-DRB* allelic distance (DRB_aa = 27) for translated MHC-DRB sequences, though the relationship was not statistically significant ($p = 0.99$; Table 2.5). Quadratic effects were required for DOB_nuc according to drop test results ($p = 0.04$). When including all MHC variables and the quadratic effect for DOB_nuc in the model, we found that the residuals for male body size were greater for individuals with a moderate *MHC-DOB* allelic distance (DOB_nuc = 2) compared to homozygotes and individuals with greater DOB_nuc allelic distances ($p = 0.04$). Body size residuals peaked at 0.09 (± 0.12; 95% CI) when DOB_nuc = 2 and then continually decreased to -0.13 (± 0.33; 95% CI) at DOB_nuc = 4 (Figure 2.2). However, body size residuals were smallest for homozygous males at -0.25 (± 0.18; 95% CI).

Antler Size

Our antler size dataset contained 124 unique white-tailed deer males for a total of 313 entries (Table 2.3). Ages ranged from 1.5 to 12.5 years with an average of 4 years, while gross antler scores ranged from 0 (1.5-year-old male) to 168.4 (5.5-year-old male). As with our male body size dataset, the most frequently captured male was measured nine times.

Seventeen of the unique males were homozygous for *MHC-DRB* and 107 were *MHC-DRB* heterozygotes at both the nucleotide and amino acid level. *MHC-DRB* allelic distances ranged from 0 to 46 at the nucleotide level (mean ± SE = 24.8 ± 1.3) and from 0 to 27 for translated *MHC-DRB* sequences (mean ± SE = 15.8 ± 0.8). As with our male body size dataset, the majority of individuals were homozygous for translated *MHC-DOB* exon 2 (78 homozygotes, 46 heterozygotes) while allelic distances were larger at the full *MHC-DOB* nucleotide sequences (24 homozygotes, 100 heterozygotes). *MHC-DOB* allelic distances ranged from 0 to 4 at the

nucleotide level (mean ± SE = 1.7 ± 0.1) and from 0 to 2 (mean ± SE = 0.4 ± 0.05) at the amino acid level.

Nonlinear age model selection

The top model (ΔAICc = 0) was the regular (i.e. not cubed) VBGM without mean age (Table 2.6; Figure 2.3). While the VBGM models with parameters for mean age had ΔAICc ≤ 2, additional parameters did not provide a net reduction in AICc and were therefore considered uninformative (Arnold 2010). The weight of the top model increased to 1.0 when excluding models with mean age parameters. Drop tests between the top model and the same model with a term for autocorrelation indicated that accounting for autocorrelation in our model significantly improves the fit to the data ($p < 0.001$).

Residual analysis with MHC variables

No quadratic effects were needed for MHC variables when assessing the antler size residuals. As with male body size, no significant associations were found between antler size residuals and *MHC-DRB* allelic distance. However, there was a considerable effect size for DRB_nuc, where antler size residuals increased from -0.28 (± 0.60; 95% CI) at a small *MHC-DRB* allelic distance (DRB_nuc = 2) to 0.25 (± 0.56; 95% CI) at a large *MHC-DRB* allelic distance (DRB_nuc = 46; $p = 0.37$; Table 2.7). Standard errors were elevated for *MHC-DRB* variables (SE = 0.189 for DRB_nuc), which may be attributed to the variance inflation found for the *MHC-DRB* variables (38.98 and 38.74 for DRB_nuc and DRB_aa, respectively). There was a positive association between DOB_nuc and the antler size residuals ($p = 0.02$), where antler size residuals increased from -0.12 (± 0.11; 95% CI) for homozygous males (DOB_nuc = 0) to -0.05 (± 0.07; 95% CI) at a small *MHC-DOB* allelic distance (DOB_nuc = 1) to 0.17 (± 0.15; 95% CI) at a large *MHC-DOB* allelic distance (DOB_nuc = 4; Figure 2.4).

Female Body Size

Our female body size dataset contained 134 unique white-tailed deer females for a total of 183 entries (Table 2.8). Ages ranged from 0 to 10.5 years with an average of 2.5 years. The most frequently captured female was measured four times.

Thirteen unique individuals were homozygous for *MHC-DRB* and 121 individuals were heterozygous for *MHC-DRB* at both the nucleotide and amino acid level. *MHC-DRB* allelic distances ranged from 0 to 44 at the nucleotide level (mean ± SE = 22.6 ± 3.0) and from 0 to 27 for translated *MHC-DRB* sequences (mean ± SE = 14.9 ± 2.0). Less than a quarter of individuals were homozygous for the full *MHC-DOB* nucleotide sequences (22 homozygotes, 112 heterozygotes), while the majority of individuals were homozygous for the translated *MHC-DOB* exon 2 sequences (78 homozygotes, 56 heterozygotes). *MHC-DOB* allelic distances ranged from 0 to 5 at the nucleotide level (mean ± SE = 2.1 ± 0.2) and from 0 to 2 at the amino acid level (mean ± SE = 0.4 ± 0.1).

Principal component analysis

The first principal component explained 93.31% of the variation in our three body measurements. Each measurement contributed equally to the first principal component: 0.58 (chest), 0.58 (body length), 0.57 (hind foot). Scores ranged from -8.22 (newborn fawn) to 2.17 (9.5-year-old female).

Nonlinear age model selection

The top model (ΔAICc = 0) was the regular VBGM without mean age (Table 2.9; Figure 2.5). While the VBGM models with parameters for mean age had ΔAICc $\leq$ 2, the additional parameters did not provide a net reduction in AICc and were therefore uninformative (Arnold 2010). The weight of the top VBG model increased to 1.0 when excluding models with uninformative parameters. Drop tests between the top model and the same model with a term for autocorrelation indicated that accounting for autocorrelation in our model did not significantly improve the fit to the data ($p = 0.14$). However, most females were only captured once in their lifetime.

Residual analysis with MHC variables

No quadratic effects were needed for the MHC variables when assessing the residuals for our female body size dataset. Unlike male body size, we found no significant association between female body size residuals and the MHC variables (Table 2.10). Female body size residuals only increased from -0.06 (± 0.98; 95% CI) at a small *MHC-DRB* allelic distance

(DRB_nuc = 2) to 0.04 (± 0.97; 95% CI) at a large *MHC-DRB* allelic distance (DRB_nuc = 44; p = 0.92), while *MHC-DOB* increased from -0.01 (± 0.11; 95% CI) at a small *MHC-DOB* allelic distance (DOB_nuc = 1) to 0.004 (± 0.28; 95% CI) at a large *MHC-DOB* allelic distance (DOB_nuc = 5; p = 0.93). Standard errors were fairly similar for *MHC-DRB* and *MHC-DOB* variables.

DISCUSSION

While previous studies reported an association between *MHC-DRB* diversity and morphology, we did not find similar results. Ditchkoff et al. (2001) found a positive association between *MHC-DRB* diversity and both body mass and antler size in male white-tailed deer. When examining body size, they only found significance for field-dressed body weights and skull length. They did not find statistical significance, however, for the body measurements that we used for generating our principal component scores (i.e. body length, chest girth, hind foot length). Our results for body size and *MHC-DRB* allelic distances are therefore consistent, although we do not have the field-dressed body weights and skull lengths to test if we would also find the reported association with these measures. Antler size, on the other hand, was measured similarly (gross Boone and Crockett scores). Ditchkoff et al. (2001) reported that gross antler scores for heterozygotes were greater than homozygote deer. We found no association between *MHC-DRB* allelic distances (nucleotide and amino acid level) and antler size. This difference in findings may be attributed to our methodological differences. For example, while Ditchkoff et al. (2001) classified individuals as either heterozygotes or homozygotes using the phylogenetic clades determined by Van Den Bussche et al. (1999), we calculated pairwise allelic distances between an individual's *MHC-DRB* alleles based on next generation sequencing (Ivy-Israel et al. 2019). We also included several newly identified *MHC-DRB* alleles in our analyses that were not available in 2001. Standard errors for our *MHC-DRB* variables were quite large, however, which may have also attributed to our lack of significance. Ivy-Israel et al (2019) reported that the white-tailed deer population sampled in this study was experiencing a heterozygote excess for *MHC-DRB*, and that *MHC-DRB* may be under balancing selection in our population. If true, the results would indicate that *MHC-DRB* heterozygosity is selected for in our population, even though we did not see a significant association between *MHC-DRB* and morphology.

We found a strong association between *MHC-DOB* nucleotide sequences and male morphology. Male body size was greatest when the pairwise nucleotide distance between an individual's *MHC-DOB* alleles was equal to 2 (from range of 0 - 4); whereas smaller body sizes were associated with homozygosity and increased pairwise distance of *MHC-DOB* alleles. Greater *MHC-DOB* allelic distance may be too costly to combine with maintaining a larger body size, as costly immune responses associated with greater MHC diversity can divert resources away from growth and survival (Klasing et al. 1987; Møller and Saino, 1994; Fair et al. 1999; Norris and Evans, 2000; Hanssen et al. 2004; Barribeau et al. 2008; Bonato et al. 2009). Antler size, on the other hand, had a positive linear association with *MHC-DOB* nucleotide allelic distances, where greater *MHC-DOB* allelic distances were associated with greater antler scores. Both body and antler size are strong determining factors for a male's reproductive success, especially among polygynous species (Geist 1966; Clutton-Brock et al. 1988; Rose 1995; Pelabon et al. 1999; McElligott et al. 2001; Mysterud et al. 2004; Johnson et al. 2007). Indeed, Newbolt et al. (2017) found that the annual reproductive success of males in our white-tailed deer population was positively associated with both male body size and antler size. However, antler size mainly influenced a male's reproductive success when the population had an older male age structure. Male body size positively influences reproductive success in several ways. First, males with larger body sizes are capable of storing more nutrients and energy reserves than smaller males (Lindstedt and Boyce, 1985), which enables larger males to maintain good body condition while tending and chasing receptive females. Second, body size may also influence post-copulatory selection as larger males have greater sperm volume per ejaculate than smaller males (Møller 1991). Lastly, females tend to prefer to mate with larger males (Clutton-Brock et al. 1989; Byers et al. 1994). Antlers, on the other hand, are mainly used for intraspecific competition for breeding access and for providing visual cues for a male's overall strength (Clutton-Brock 1979; Andersson 1994; McElligott et al. 1998). Antler size primarily determines a male's reproductive success when there is an abundance of older males, which is when males engage in intense intrasexual competition for breeding opportunities (Newbolt et al. 2017), while body size is consistently important regardless of the population's demographic processes. Ivy-Israel et al. (2019) found evidence of heterozygote deficiency for *MHC-DOB* in this population and that *MHC-DOB* is under purifying selection. This result is consistent with our findings that larger males, who have moderate *MHC-DOB* allelic distance, produce the majority of offspring

in our population. Body size may therefore be more important to a male's reproductive success than antler size, as selecting larger individuals with intermediate *MHC-DOB* allelic distances favors males with moderate antler scores. Another explanation is that the immunological cost of having greater *MHC-DOB* allelic distance, and larger antlers, is too costly for our male white-tailed deer.

While *MHC-DOB* is not linked to *MHC-DRB* in white-tailed deer (Ivy-Israel et al. 2019), it is still in linkage disequilibrium with other MHC II genes in that chromosomal subregion that may be driving this association with the nucleotide sequence diversity at *MHC-DOB*. The TAP1 and TAP2 genes, for example, are part of the MHC II subregion with *MHC-DOB* in cattle (Childers et al. 2005). Numerous studies have identified the role of these genes in diseases such as Dengue fever (Soundravally and Hoti, 2007), sarcoidosis (Foley et al. 1999), multiple sclerosis (Moins-Teisserenc et al. 1995), and celiac disease (Djilali-Saiah et al. 1994) in humans. Therefore, while our results suggest that *MHC-DOB* may be influencing white-tailed deer morphology, it may be serving as a marker for another locus that is genetically linked to *MHC-DOB*. The lack of significance for the translated *MHC-DOB* exon 2 sequences, which represents the functional extracellular domain of the *MHC-DOB* protein (Andersson et al. 1991; NCBI 2009), further suggests that another part of the *MHC-DOB* gene or another locus linked to *MHC-DOB* is driving our results. Future work should therefore take a functional genomic approach in this population to clarify which MHC II locus is truly influencing male morphology.

We did not find a significant association between either *MHC-DRB* or *MHC-DOB* and female body size. Among white-tailed deer, adult females differ little physically. Body size typically plateaus when females reach an age of two to three years (Ditchkoff 2011). Therefore, we observed very little variation for body size in our female dataset, which may contribute to our lack of significant findings. The majority of studies that searched for associations between MHC II and morphology focused on males. In fact, the evolution of female morphology and ornaments as a whole are poorly understood, especially for species in which intrasexual competition is most intense among males and where females are responsible for all parental care (i.e. polygynous species; Kokko et al. 2006; Clutton-Brock 2007, 2009). Huchard et al. (2010) did find that certain MHC supertypes are associated with poor body condition and less developed sexual signals (size and shape of sexual swellings) in female baboons (*Papio ursinus*), though they did not find an effect of MHC diversity on the females' sexual swellings. A female's MHC diversity

can influence her fecundity (Smith et al. 2010; Grogan 2014). For example, Smith et al. (2010) found that heterozygous European brown hares (*Lepus europaeus*) had greater fecundity compared to homozygotes. Females with larger litters have to invest more nutrients towards lactation (Kounig et al. 1988), which can significantly reduce the female's body mass and condition (Parker et al. 1990; Cook et al. 2004) and subsequent fitness and offspring survival (Allaye Chan 1991; Russell et al. 1998; Thaker and Bilkei, 2005). Lactating females also divert more of their resources away from parasite defense (Festa-Bianchet 1989), thereby making them more susceptible to pathogens. Given this, future research on female body size should also include lactation and/or litter size data to better account for variation in female body size, especially since MHC II diversity may particularly influence female size of lactating females by governing their immune responses.

An association between MHC II genes and morphology has been reported for several non-ruminant species, such as pheasants (*Phasianus colchicus*; von Schantz et al. 1996, 1997), common yellowthroats (*Geothlypis trichas*; Dunn et al. 2012), baboons (Huchard et al. 2010), neotropical lesser bulldog bats (*Noctilio albiventris*; Schad et al. 2012), and montane water voles (*Arvicola scherman*; Charbonnel et al. 2010). Ruminants, such as the white-tailed deer, have a unique MHC II organization due to a proposed chromosomal inversion that has split this typically linked region into two subregions (*Bos taurus*, Andersson et al. 1988; *Ovis aries*, Gao et al. 2010; white-tailed deer, Ivy-Israel et al. 2019). As *MHC-DRB* and *MHC-DOB* occupy different MHC II subregions, these loci are evolving independently in ruminants. The synteny of other mammalian species, however, is relatively well-conserved (Wan et al. 2009). The lack of significant linkage disequilibrium seen in the MHC II region of ruminants can enable us to study these two subregions separately, which may highlight where, and why, these studies found a significant association between MHC II and morphology in these non-ruminant species. The majority of studies examining this association in ruminants was primarily focused on the *MHC-DRB* region, as this locus is known for its extreme variability and polymorphism (Mikko and Anderson, 1995; Swarbrick et al. 1995; Mikko et al. 1997; Van Den Bussche 1999, 2002; Ditchkoff et al. 2001, 2005; Fernandez-de-Mera et al. 2009; Brambilla et al. 2015, 2018). More research is therefore needed on the other MHC II subregion (i.e. the one containing *MHC-DOB*) to fully capture which MHC II locus, or perhaps loci, is influencing vertebrate morphology.

In this study we examined the potential association between *MHC-DRB* and *MHC-DOB* heterozygosity and morphology in white-tailed deer. While we found no associations for *MHC-DRB*, we found a strong positive association between *MHC-DOB* nucleotide sequences and antler size and a quadratic relationship between *MHC-DOB* nucleotide sequences and male body size. Our results suggest that *MHC-DOB*, or a gene genetically linked to this locus, may influence male morphological characteristics in white-tailed deer. A broader genome approach is needed to reveal which MHC II locus is actually responsible for this association. Future research should examine whether *MHC-DOB* also influences male white-tailed deer reproductive success as both body and antler size have been found to determine a male's annual reproductive success in our population (Newbolt et al. 2017). Neither *MHC-DRB* nor *MHC-DOB* influenced female body size, even though the average allelic distances of males and females were not significantly different from each other. Female white-tailed deer morphology may therefore not be dependent on their MHC diversity, though future studies should include female-specific variables that may better explain the slight variations seen in female body size.

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TABLES

Table 2.1. *a priori* growth models used to account for the effect of age on body and antler size.

Model	Formula	Reference
Von Bertalanffy (length) - with average annual buck age in growth rate - with average annual buck age in asymptote - with average annual buck age in growth rate and asymptote	$y_{ij} = A_i\left(1 - B_i e^{-k_i t_{ij}}\right) + \varepsilon_{ij}$ $y_{ij} = A_i\left[1 - \left(B_i + M_i\alpha_j\right) e^{-k_i t_{ij}}\right] + \varepsilon_{ij}$ $y_{ij} = \left(A_i + N_i\alpha_j\right)\left(1 - B_i e^{-k_i t_{ij}}\right) + \varepsilon_{ij}$ $y_{ij} = \left(A_i + N_i\alpha_j\right)\left[1 - \left(B_i + M_i\alpha_j\right) e^{-k_i t_{ij}}\right] + \varepsilon_{ij}$	Von Bertalanffy 1938
Von Bertalanffy (weight) - with average annual buck age in growth rate - with average annual buck age in asymptote - with average annual buck age in growth rate and asymptote	$y_{ij} = A_i\left(1 - B_i e^{-k_i t_{ij}}\right)^3 + \varepsilon_{ij}$ $y_{ij} = A_i\left[1 - B_i\left(B_i + M_i\alpha_j\right) e^{-k_i t_{ij}}\right]^3 + \varepsilon_{ij}$ $y_{ij} = \left(A_i + N_i\alpha_j\right)\left(1 - B_i e^{-k_i t_{ij}}\right)^3 + \varepsilon_{ij}$ $y_{ij} = \left(A_i + N_i\alpha_j\right)\left[1 - \left(B_i + M_i\alpha_j\right) e^{-k_i t_{ij}}\right]^3 + \varepsilon_{ij}$	Von Bertalanffy 1957
Gompertz - with average annual buck age in growth rate - with average annual buck age in asymptote - with average annual buck age in growth rate and asymptote	$y_{ij} = A_i e^{-B_i e^{-k_i t_{ij}}} + \varepsilon_{ij}$ $y_{ij} = A_i e^{-\left(B_i + M_i\alpha_j\right) e^{-k_i t_{ij}}} + \varepsilon_{ij}$ $y_{ij} = \left(A_i + N_i\alpha_j\right) e^{-B_i e^{-k_i t_{ij}}} + \varepsilon_{ij}$ $y_{ij} = \left(A_i + N_i\alpha_j\right) e^{-\left(B_i + M_i\alpha_j\right) e^{-k_i t_{ij}}} + \varepsilon_{ij}$	Laird 1965
Logistic - with average annual buck age in growth rate - with average annual buck age in asymptote - with average annual buck age in growth rate and asymptote	$y_{ij} = A_i \,/\, \left(1 + B_i e^{-k_i t_{ij}}\right) + \varepsilon_{ij}$ $y_{ij} = A_i \,/\, \left[1 + \left(B_i + M_i\alpha_j\right) e^{-k_i t_{ij}}\right] + \varepsilon_{ij}$ $y_{ij} = \left(A_i + N_i\alpha_j\right) / \left(1 + B_i e^{-k_i t_{ij}}\right) + \varepsilon_{ij}$ $y_{ij} = \left(A_i + N_i\alpha_j\right) / \left[1 + \left(B_i + M_i\alpha_j\right) e^{-k_i t_{ij}}\right] + \varepsilon_{ij}$	Nelder 1961

Table 2.2. *MHC-DRB* and *MHC-DOB* allelic distance differences between standardized MHC variables for unique males and females using male body size dataset and female body size dataset.

DRB_nuc			**DRB_aa**		
difference	SE	p	difference	SE	p
0.150	0.118	0.204	0.095	0.118	0.421
DOB_nuc			**DOB_aa**		
difference	SE	p	difference	SE	p
-0.196	0.118	0.097	-0.107	0.118	0.366

Table 2.3. Mean (±SE) gross Boone and Crockett antler scores (GBCS) and body measurements separated by age for male white-tailed deer.

	GBCS (cm)			**Body length (cm)**			**Hind Foot length (cm)**			**Chest girth (cm)**		
Age (yrs)	n	x	SE	n	x	SE	n	x	SE	n	x	SE
0^	NA	NA	NA	8	50	1.3	8	22	0.4	8	30	0.5
0.5	NA	NA	NA	48	108	1.0	48	37	0.4	48	67	0.7
1.5	73	74	3.7	75	128	0.8	75	42	0.2	75	79	0.5
2.5	53	193	5.2	55	140	0.9	55	43	0.2	55	87	0.8
3.5	51	252	6.2	48	147	1.0	48	44	0.4	48	92	0.8
4.5	42	291	6.4	40	149	1.6	40	43	0.7	40	96	0.7
5.5	28	319	8.2	28	153	1.3	28	44	0.2	28	99	0.9
6.5 +	66	310	4.8	64	151	1.0	64	44	0.2	64	96	1.1

^ We collected several newborn fawns (n = 8) using Vaginal Implant Transmitters in the summer of 2010 (Neuman et al. 2016)

Table 2.4. AICc scores and weights for the a priori growth curve models used to describe the effect of age on body size for male white-tailed deer using the male body size dataset.

Model	AICc	ΔAICc	df	Weight
Von Bertalanffy (length) with MeanAge in asymptote (N)	688.4	0.0	5	0.439
Von Bertalanffy (length) with MeanAge in asymptote and growth rate (MN)	688.7	0.4	6	0.367
Von Bertalanffy (length)	690.9	2.5	4	0.124
Von Bertalanffy (length) with MeanAge in growth rate (M)	692.1	3.7	5	0.069
Von Bertalanffy (weight) with MeanAge in asymptote and growth rate (MN)	702.2	13.9	6	< 0.001
Von Bertalanffy (weight) with MeanAge in growth rate (M)	708.6	20.2	5	< 0.001
Von Bertalanffy (weight)	719.3	30.9	4	< 0.001
Von Bertalanffy (weight) with MeanAge in asymptote (N)	720.1	31.8	5	< 0.001
Gompertz model	1319.2	630.8	4	< 0.001
Logistic model	1320.4	632.0	4	< 0.001
Gompertz model with MeanAge in asymptote (N)	1320.7	632.4	5	< 0.001
Gompertz model with MeanAge in growth rate (M)	1321.3	632.9	5	< 0.001
Logistic model with MeanAge in asymptote (N)	1321.9	633.6	5	< 0.001
Logistic model with MeanAge in growth rate (M)	1322.4	634.0	5	< 0.001
Gompertz model with MeanAge in asymptote and growth rate (MN)	1322.8	634.4	6	< 0.001
Logistic model with MeanAge in asymptote and growth rate (MN)	1324.0	635.6	6	< 0.001

Table 2.5. Residual analysis, using residuals from top growth curve model (Von Bertalanffy with male mean age in asymptote, Table 2.1, 2.4), for examining possible associations between MHC variables and male white-tailed deer body size.

Parameter	Value (β)	SE	df	t-value	p-value
DRB_nuc	0.002	0.249	206	0.009	0.993
DRB_aa	0.014	0.248	206	0.055	0.956
DOB_nuc	0.095	0.046	206	2.077	0.039
(DOB_nuc)^2	-0.091	0.045	206	-2.016	0.045
DOB_aa	-0.013	0.046	154	-0.286	0.775

Table 2.6. AICc scores and weights for the a priori growth curve models used to describe the effect of age on antler size of male white-tailed deer using the male antler size dataset.

Model	**AICc**	**ΔAICc**	**df**	**Weight**
Von Bertalanffy (length)	310.9	0.0	4	0.35
Von Bertalanffy (length) with MeanAge in asymptote (N)	311.2	0.3	5	0.31
Von Bertalanffy (length) with MeanAge in asymptote and growth rate (MN)	312.0	1.1	6	0.20
Von Bertalanffy (length) with MeanAge in growth rate (M)	312.9	2.0	5	0.13
Von Bertalanffy (weight)	340.4	29.5	4	<0.001
Von Bertalanffy (weight) with MeanAge in asymptote (N)	340.8	29.9	5	<0.001
Von Bertalanffy (weight) with MeanAge in growth rate (M)	341.7	30.8	5	<0.001
Von Bertalanffy (weight) with MeanAge in asymptote and growth rate (MN)	342.7	31.8	6	<0.001
Gompertz model	781.6	470.7	4	<0.001
Gompertz model with MeanAge in growth rate (M)	782.5	471.6	5	<0.001
Logistic model	782.9	472.0	4	<0.001
Gompertz model with MeanAge in asymptote (N)	783.3	472.4	5	<0.001
Logistic model with MeanAge in growth rate (M)	783.9	473.0	5	<0.001
Gompertz model with MeanAge in asymptote and growth rate (MN)	784.5	473.6	6	<0.001
Logistic model with MeanAge in asymptote (N)	784.7	473.8	5	<0.001
Logistic model with MeanAge in asymptote and growth rate (MN)	785.1	474.2	6	<0.001

Table 2.7. Residual analysis, using residuals from top growth curve model (Von Bertalanffy, Table 2.1, 2.6), for examining possible associations between MHC variables and male white-tailed deer antler size.

Parameter	**Value (β)**	**SE**	**df**	**t-value**	**p-value**
DRB_nuc	0.171	0.189	120	0.906	0.367
DRB_aa	-0.134	0.187	120	-0.715	0.476
DOB_nuc	0.083	0.034	120	2.442	0.016
DOB_aa	-0.035	0.033	188	-1.059	0.291

Table 2.8. Mean (±SE) body measurements separated by age for female white-tailed deer.

	Body length (cm)			**Hind Foot length (cm)**			**Chest girth (cm)**		
Age (yrs)	n	x	SE	n	x	SE	n	x	SE
0^	6	46	1.8	6	20	1.2	6	26	1.7
0.5	47	105	0.8	47	35	0.2	47	64	0.5
1.5	44	119	1.2	44	40	0.2	44	76	0.6
2.5	33	126	1.1	33	40	0.3	33	78	0.7
3.5	16	129	1.2	16	41	0.2	16	81	0.8
4.5	16	129	1.6	16	40	0.7	16	82	0.7
5.5	8	132	3.2	8	40	0.5	8	83	1.1
6.5 +	13	131	2.3	13	41	0.3	13	82	0.9

^ We collected several newborn fawns (n = 6) using Vaginal Implant Transmitters in the summer of 2010 (Neuman et al. 2016)

Table 2.9. AICc scores and weights for the a priori growth curve models used to describe the effect of age on body size of female white-tailed deer using the female body size dataset

Model	**AICc**	**ΔAICc**	**df**	**Weight**
Von Bertalanffy (length)	312.2	0.0	4	0.38
Von Bertalanffy (length) with MeanAge in asymptote (N)	312.6	0.3	5	0.33
Von Bertalanffy (length) with MeanAge in growth rate (M)	314.2	1.9	5	0.15
Von Bertalanffy (length) with MeanAge in asymptote and growth rate (MN)	314.2	2.0	6	0.14
Von Bertalanffy (weight) with MeanAge in growth rate (M)	326.9	14.7	5	<0.001
Von Bertalanffy (weight) with MeanAge in asymptote and growth rate (MN)	328.8	16.6	6	<0.001
Von Bertalanffy (weight) with MeanAge in asymptote (N)	330.0	17.8	5	<0.001
Von Bertalanffy (weight)	330.2	17.9	4	<0.001
Gompertz model	680.3	368.0	4	<0.001
Logistic model	681.3	369.1	4	<0.001
Gompertz model with MeanAge in growth rate (M)	682.0	369.8	5	<0.001
Gompertz model with MeanAge in asymptote (N)	682.4	370.1	5	<0.001
Logistic model with MeanAge in growth rate (M)	683.0	370.8	5	<0.001
Logistic model with MeanAge in asymptote (N)	683.4	371.2	5	<0.001
Gompertz model with MeanAge in asymptote and growth rate (MN)	684.1	371.9	6	<0.001
Logistic model with MeanAge in asymptote and growth rate (MN)	687.2	375.0	6	<0.001

Table 2.10. Residual analysis, using residuals from top growth curve model (Von Bertalanffy, Table 2.1, 2.9), for examining possible associations between MHC variables and female white-tailed deer body size.

Parameter	Value (β)	SE	df	t-value	p-value
DRB_nuc	0.024	0.254	129	0.096	0.923
DRB_aa	-0.032	0.254	129	-0.126	0.900
DOB_nuc	0.005	0.053	129	0.084	0.933
DOB_aa	0.004	0.053	129	0.083	0.934

FIGURES

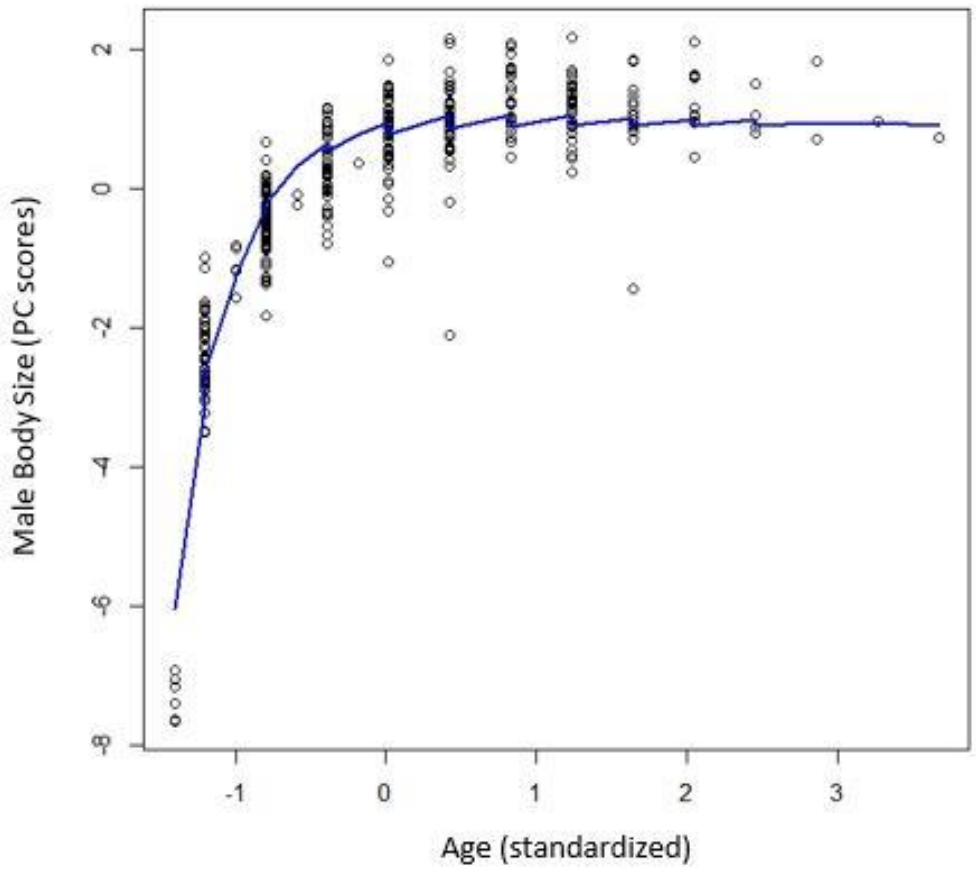

Figure 2.1. The Von Bertalanffy (length) model with male mean age in the asymptote term was the top model for capturing the relationship between age and body size of male white-tailed deer (Table 2.4).

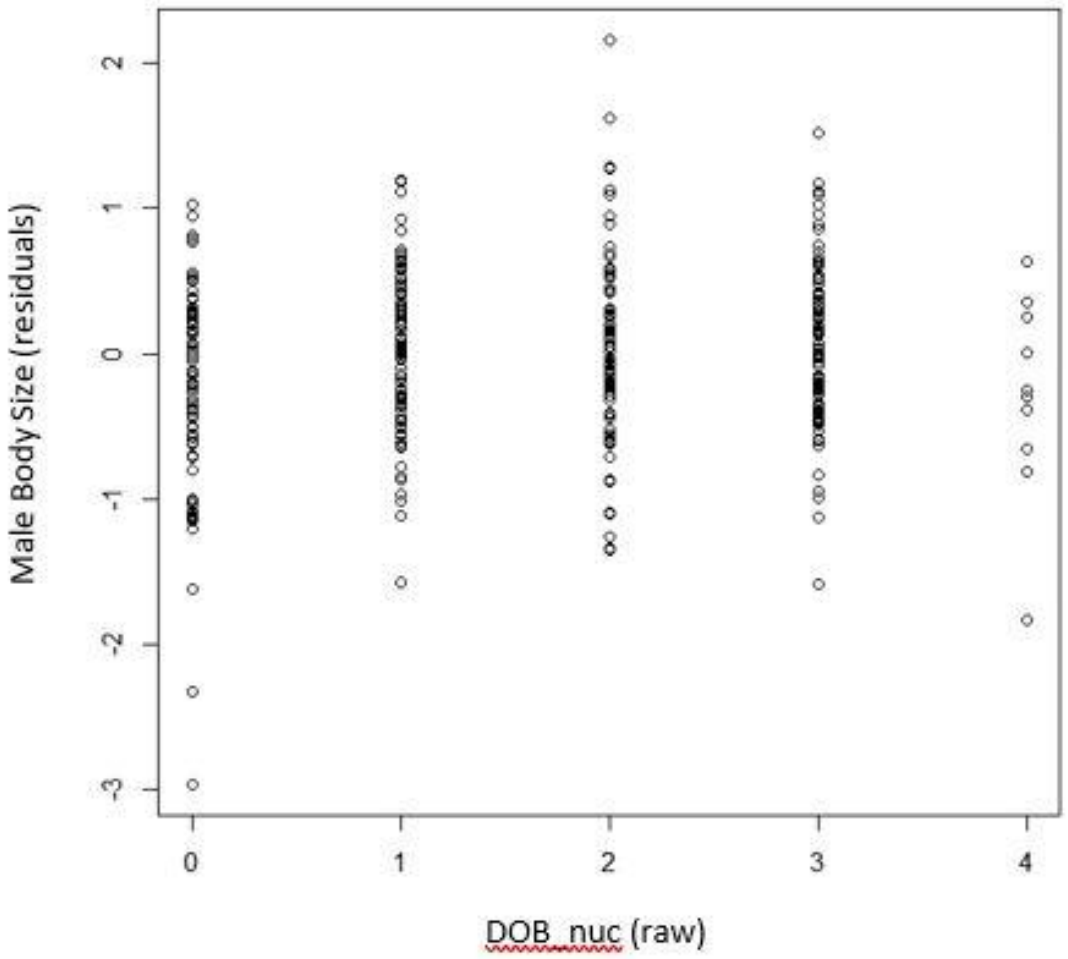

Figure 2.2. Relationship between DOB_nuc and the residuals for male body size from the model that best explained the effect of age on body size for white-tailed deer (Von Bertalanffy model, Table 2.4). Male body size peaks when DOB_nuc is equal to 2 and then gradually decreases with further increases in DOB_nuc heterozygosity.

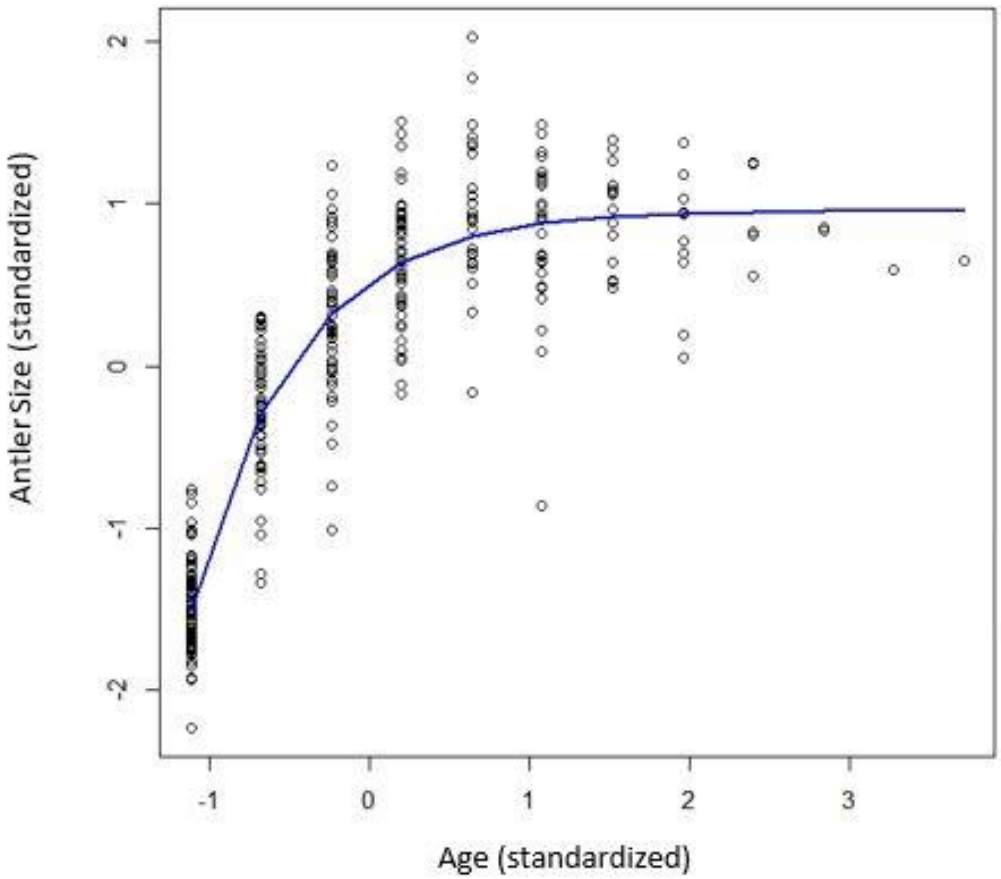

Figure 2.3. The Von Bertalanffy (length) model was the top model for capturing the relationship between age and antler size of male white-tailed deer (Table 2.6).

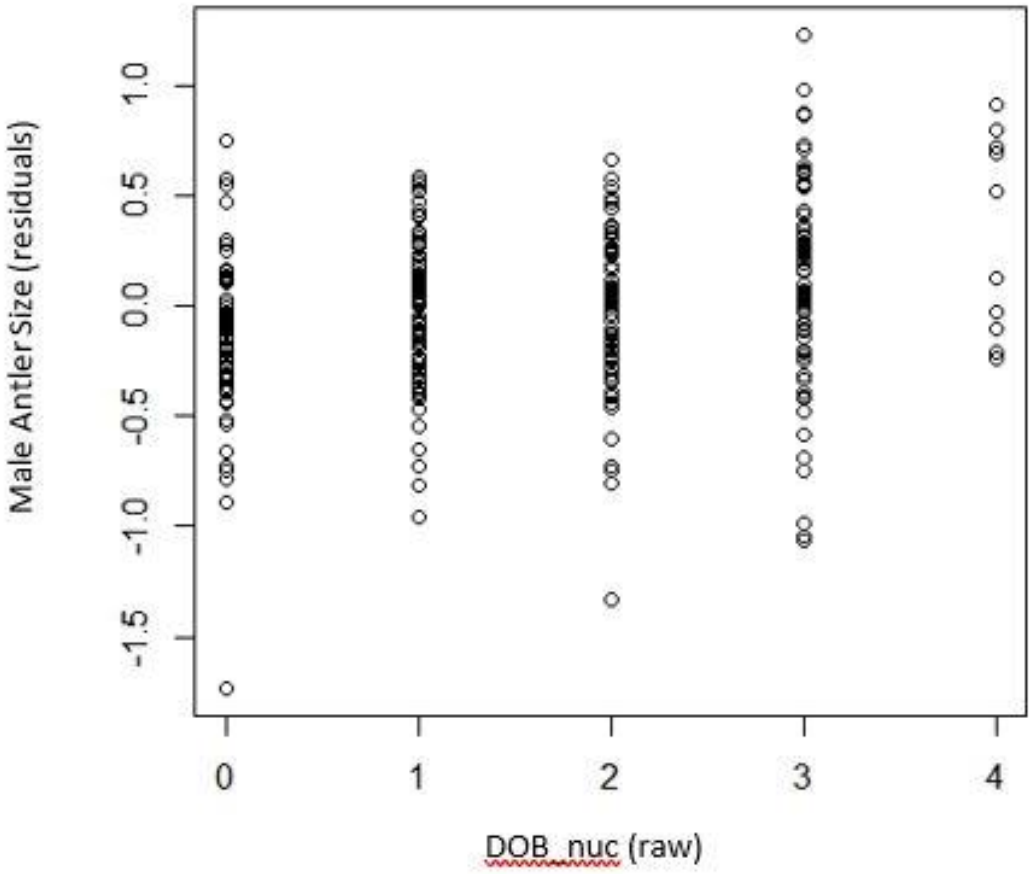

Figure 2.4. Relationship between DOB_nuc and the residuals for male antler size from the model that best explained the effect of age on body size for white-tailed deer (Von Bertalanffy model, Table 2.6). There is a positive association between antler size and DOB_nuc allelic distance.

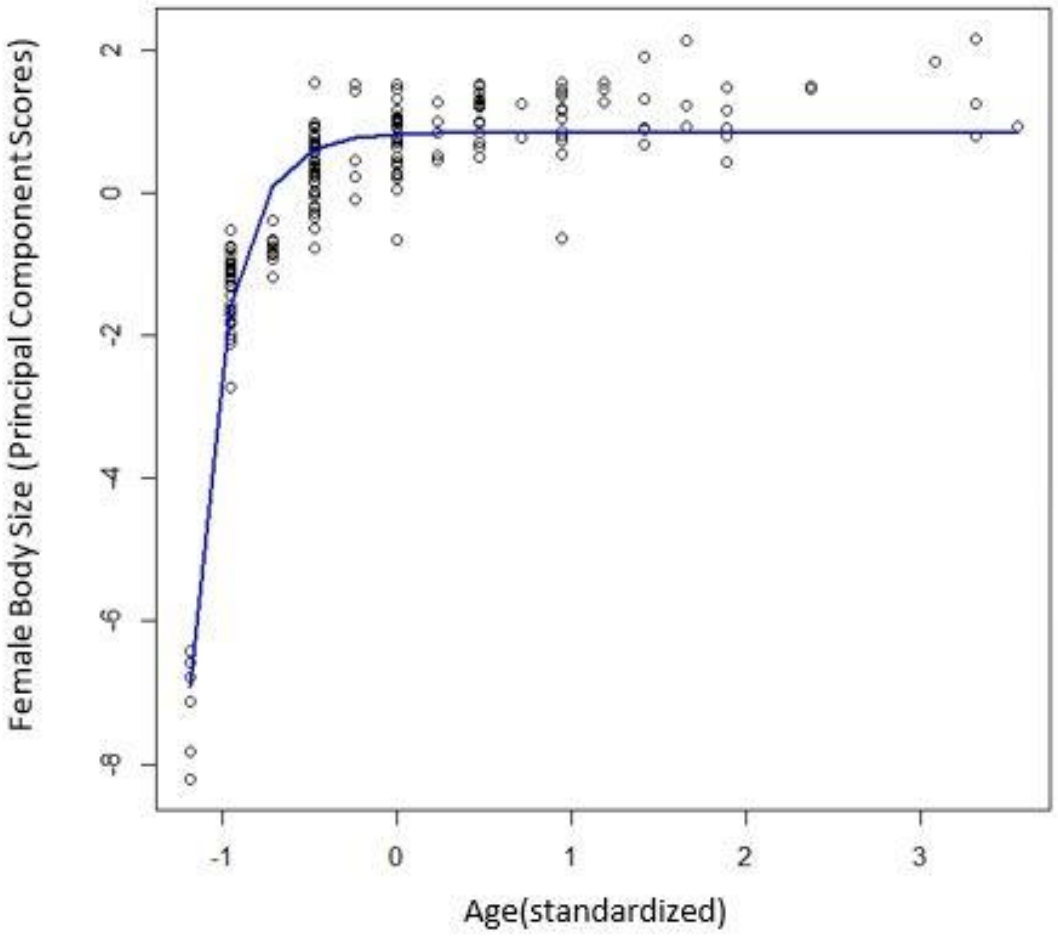

Figure 2.5. The Von Bertalanffy (length) model was the top model for capturing the relationship between age and body size of female white-tailed deer (Table 2.9)

Chapter 3: Allelic diversity in two unlinked MHC II loci influences reproductive success of white-tailed deer

ABSTRACT

Reproductive success (RS) is strongly influenced by morphology. However, recent studies suggest that gene products of the major histocompatibility complex (MHC) can also influence an individual's RS through its impact on the immune system, morphology, behavior, and mate selection. Greater MHC diversity and specific MHC alleles have been found to improve immune function, thereby allowing greater allocation of resources towards reproduction. This study examined if there is an association between annual RS and 1) pairwise allelic distance at two unlinked MHC II loci (exon 2 for the classical antigen-binding protein *MHC-DRB*, exon 2 for the accessory protein *MHC-DOB*), and 2) specific *MHC-DRB* and *MHC-DOB* alleles that are becoming more or less frequent in an enclosed population of white-tailed deer (*Odocoileus virginianus*). We found that male RS is positively associated with nucleotide *MHC-DRB* allelic distance but negatively associated with amino acid MHC-DRB allelic distance. Nucleotide *MHC-DRB* allelic distance may be acting like a marker that reflects the positive influence of the genomic region linked to *MHC-DRB* exon 2 on male RS. Greater amino acid allelic distance at *MHC-DRB* exon 2 specifically, however, was detrimental to annual RS. Furthermore, male RS had a weak positive association with amino acid *MHC-DOB* allelic distance, while female RS had a weak negative association with nucleotide *MHC-DOB* allelic distance. Lastly, males heterozygous with allele DRB*10 had greater RS than males without DRB*10, whereas females heterozygous for allele DRB*01 had lower RS than females without DRB*01. Both MHC allelic distance and specific alleles therefore influence RS in white-tailed deer.

KEYWORDS

Reproductive success, sexual selection, major histocompatibility complex, white-tailed deer, heterozygote advantage, immunological cost

INTRODUCTION

Reproductive success (RS), defined as the number of successful offspring produced by an individual during their lifetime, has a considerable impact on population dynamics by influencing population size and structure and determining how these variables change over time and under varying conditions (Brys et al. 2003; Seyboth et al. 2016). The individual variance that exists in RS within a population drives which phenotypes are passed to the next generation and in what abundance (Bouteiller and Perrin, 2000; Rosenbaum et al. 2002). Genotypes associated with the most successful phenotypes are therefore imparted most frequently to offspring in the population. An individual's RS is linked to a wide variety of physical, physiological, and immunological characteristics. Physical traits, such as body size and secondary sexual characteristics, play an important role in determining RS (Berglund and Rosenqvist, 2001; Preston et al. 2003). For example, increased body size and horn length allow male Soay sheep (*Ovis aries*) to monopolize access to receptive females (Preston et al. 2003). As reproduction is costly, immunological effectiveness is also important in deciding how much energy and resources an individual can invest towards reproductive activities (Apanius et al. 1994). Several studies have therefore examined the role of the major histocompatibility complex (MHC), a group of genes whose products are vital to having an effective immune system, on RS (Sauermann et al. 2001; Setchell et al. 2010; Smith et al. 2010; Thoß et al. 2011; Grogan 2014), especially MHC type II. MHC II proteins, which are expressed on all antigen-presenting cells (ex. dendritic cells, macrophages), bind to antigens and are displayed on the surfaces of cells where T-cell lymphocytes recognize the proteins as 'self' or 'non-self' (Janeway et al. 2001). If the antigen is foreign, T-cells will cause cellular destruction or initiation of a systemic immune response. MHC II gene products therefore help protect the body from foreign invaders such as bacteria, viruses, and parasites.

Several MHC II loci have been found to be associated with an individual's RS through their impact on the immune system, morphology, behavior, and mate selection. Individuals who are heterozygous at their MHC II genes are generally believed to have an immunological advantage as their cells can recognize a greater array of pathogens to the body's immune cells (i.e. heterozygote advantage hypothesis; Hughes and Nei, 1989; Takahata and Nei, 1990; Hughes and Nei, 1992; Sommer 2005; Lenz 2011). For instance, heterozygous white-tailed deer (*Odocoileus virginianus*) had moderate levels of parasitism by both ectoparasitic ticks and

abomasal nematodes whereas homozygous deer had very low levels of parasitism by one of these pathogens (Ditchkoff et al. 2005). Similarly, heterozygous water voles (*Arvicola terrestris*) experienced less parasitism than homozygous individuals (Oliver et al. 2009). MHC II heterogeneity has also been found to be associated with larger body sizes and more elaborate secondary sexual characteristics for several vertebrate species (von Schantz et al. 1996, 1997; Sauermann et al. 2001; Olsson et al. 2005). For example, male white-tailed deer who had greater MHC diversity generally had greater overall body size and antler size compared to homozygous males (Ditchkoff et al. 2001). Elaborate physical characteristics, while important for attracting mates, are costly and may be a handicap to the individual displaying them. A more effective immune system due to greater MHC heterozygosity may allow for greater investments into costly morphological traits. Males with more elaborate physical characteristics generally have the highest quality genotypes and are typically more preferred by females (Zahavi 1975). Hence, MHC II genes play a key role in determining RS by influencing the outcomes of sexual selection.

Previous research found that the MHC can influence mate selection due to the link between an individual's immune and olfactory systems. By modifying body odor, MHC II proteins can act as olfactory cues that enable individuals to select for mates who will complement their own genetic material (Leinders-Zufall et al. 2004; Santos et al. 2005; Boehm and Zufall, 2006). For example, female sticklebacks (*Gasterosteus aculeatus*) selected mates with a complementary set of MHC alleles and produced offspring with optimal MHC II diversity (Aeschlimann et al. 2003). These MHC olfactory cues can help decrease the occurrence of inbreeding and increase the population's overall genetic diversity by maintaining a species' MHC polymorphism (Potts et al. 1991). Offspring with greater MHC II heterozygosity will be more successful due to improved immunocompetence, which may translate to greater RS for both parents (Bernatchez and Landry 2003; Ziegler et al. 2005).

Factors influencing RS of white-tailed deer have recently been examined by Newbolt et al. (2017), who found that male annual RS was associated with both body and antler size. While the association between morphology and MHC diversity in white-tailed deer has been examined (Ditchkoff et al. 2001; Ivy-Israel et al. 2019b), no study has yet examined the role of MHC diversity on white-tailed deer RS. Additionally, while several studies have found that certain MHC alleles and/or supertypes significantly influence an individual's RS (grey mouse lemur,

Microcebus murinus, Schwensow et al. 2007a; neotropical lesser bulldog bat, *Noctilio albiventris*, Schad et al. 2012; great tits, *Parus major*,Sepil et al. 2013), no such study has been conducted using white-tailed deer. White-tailed deer have two characterized MHC II loci (*MHC-DRB* exon 2 and *MHC-DOB* exon 2; Van Den Bussche et al. 1999, 2002; Ivy-Israel et al. 2019a), which are genetically unlinked (Ivy-Israel et al. 2019a). We therefore examined if there was an association between annual RS and pairwise allelic distances for *MHC-DRB* and for *MHC-DOB* as well as test for associations with specific *MHC-DRB* and *MHC-DOB* alleles in our white-tailed deer population. Given the positive influence of *MHC-DOB* allelic distances on male morphology (Ivy-Israel et al. 2019b), we hypothesized that *MHC-DOB* allelic distances would be positively associated with male RS. We hypothesized that there would be a positive association between *MHC-DRB* and male RS since other studies have reported this association in other species (Sauermann et al. 2001; Lenz et al. 2013). There is less literature on the influence of MHC diversity on female RS, and studies that did examine this relationship did not find a significant association (Sauermann et al. 2001; Huchard et al. 2010). We therefore hypothesized that MHC diversity would not influence female RS. Lastly, Ivy-Israel et al. (2019a) found that several *MHC-DRB* and *MHC-DOB* allele frequencies changed over time, which could be driven by differential RS among individuals with these fluctuating alleles. Given this, we further hypothesized that alleles with decreasing frequencies would be associated with lower RS whereas alleles with increasing frequencies would be associated with greater RS.

METHODS

Study Area

Our study was conducted at the Auburn Captive Facility (ACF), which is part of the Piedmont Agricultural Experiment Station owned by Auburn University, located north of Camp Hill, Alabama. The facility had 100-120 white-tailed deer in a 174-hectare area enclosed by a 2.6-meter fence, which was constructed in October 2007. The deer present within the ACF included the original deer that inhabited this area during the fence formation in 2007 and their subsequent offspring. The founding population consisted of 71 individuals, 46 of which were female. This slightly uneven sex ratio decreased the effective population size to 64.8, which was calculated as $N_e = 4N_mN_f / (N_m+N_f)$, where N_m is the number of breeding males (n = 25) and N_f

is the number of breeding females (n = 46) in the population (Wright 1938). Subsequent to fencing the area, no deer were either introduced or hunted within the facility. Population size was therefore mainly regulated via natural and capture-related mortalities (Newbolt et al. 2017). Supplemental feed was available in the form of food plots, corn feeders, and *ad libitum* protein feeders. The facility also contained a creek and its tributaries were present on the property, which provided a reliable water source year-round.

Abundance and age structure of our white-tailed deer population were measured using images of marked and unmarked deer collected by infrared-triggered cameras at both feeders and randomly selected sites baited with corn for 14 days each February via mark-recapture methods (Overton 1969; Jacobson et al. 1997). We supplemented this data with field observations and capture/mortality records to determine final population demographic estimates. We considered marked individuals deceased if not observed for two years to prevent a potentially ever-growing population of dead but unrecovered animals.

Animal handling

From 2007 to 2015 we captured adult white-tailed deer (≥ 6 months of age) over 8 trapping seasons (October through July each year). We chemically immobilized deer by administering a tranquilizer mixture into the deer's hindquarter using cartridge fired dart guns (Pneu-Dart model 193) and 0.22 caliber blanks. We loaded 2 cc of the tranquilizer mixture, which was prepared by adding 4 cc of xylaxine (100 mg/ml; Lloyd Laboratories, Shenandoah, IA) to a 5 mL vial of Telazol® (100 mg/ml; Fort Dodge Animal Health, Fort Dodge, IA; Miller et al. 2003), into a telemetry dart (2.0 cc, type C, Pneu-Dart Inc., Williamsport, PA). We also inserted a radio transmitter (Advanced Telemetry Systems, Inc., Isanti, MN) in the dart, which enabled us to locate the sedated deer after tranquilizer administration (Kilpatrick et al. 1996). After data collection was complete, we reversed the sedation by injecting the shoulder and hindquarter muscles of the deer with Tolazine (100 mg/ml; Lloyd Laboratories; Miller et al. 2004). All methods employed during our study were approved by the Auburn University Institutional Animal Care and Use Committee (2008-1417, 2008-1421, 2010-1785, 2011-1971, 2013-2372, 2014-2521, 2016-2964, and 2016-2985) and followed the American Society of Mammalogists' guidelines (Sikes and Gannon 2011).

We gave all deer a unique 3-digit identification number at initial capture in the form of ear tags. Deer were aged using the tooth wear and replacement aging technique by Severinghaus (1949). A 1-cm^2 notch of tissue was removed from their ears for genetic analysis, which was stored in a -80°C freezer prior to DNA analysis.

Genetic Analysis

MHC-DRB exon 2 (n = 373) and *MHC-DOB* exon 2 (n = 380) amplicons were sequenced on the Illumina MiSeq platform using ear tissue samples collected from our population as described in Ivy-Israel et al. (2019a; Genbank accession numbers: MK952679-MK952701). While only exon 2 was captured for *MHC-DRB* (250 bp), the *MHC-DOB* amplicon contained exon 2 plus noncoding regions around it. We used the full *MHC-DOB* sequence (360 bp) when analyzing nucleotide sequences and *MHC-DOB* exon 2 (270 bp) for the amino acid sequences.

Pairwise genetic distances between the alleles at each locus (*MHC-DRB* and *MHC-DOB*) were calculated as the number of differences between the sequences at both the nucleotide level (DRB_nuc and DOB_nuc, respectively) and the amino acid level (DRB_aa and DOB_aa, respectively). Distance matrices used to assess the number of differences were generated via Geneious (v11.1.5).

A pedigree was created for our population by Newbolt et al. (2017), which assigned parentage (dam and/or sire) with a minimum 95% reliability threshold. Using this pedigree, we were able to calculate the annual RS (using offspring that survived to ≥ 6 months of age) of adult deer that were used as candidate parents when assigning parentage to offspring born between 2008 and 2015.

Statistical Analysis

While male morphology is known to influence male RS in our population (Newbolt et al. 2017), little is known about female RS. Ivy-Israel et al. (2019b) found that the association between morphology and MHC allelic distances differs between the two sexes. We therefore analyzed annual RS of males and females separately. We standardized *MHC-DRB* allelic distances and *MHC-DOB* allelic distances prior to analysis (i.e. subtracted mean and divided by standard deviation). Allelic distances for *MHC-DRB* and *MHC-DOB* were compared between

unique males (n = 93) and females (n = 105) using unpaired t-tests in Program R (v3.6.0; lm function; R Core Team 2019) to explore differences between the sexes.

Ivy-Israel et al. (2019a) reported that the frequencies of several *MHC-DRB* and *MHC-DOB* alleles were changing in the population over time. Specifically, while alleles DRB*10, DRB*19, DOB*09, and DOB*11 increased over time, DRB*01, DRB*14, DOB*03, and DOB*08 decreased over time in our population. To examine if these allelic frequency trends were driven by differential RS among individuals with these alleles, we included a variable for each allele that indicated the frequency of that specific allele in the individual (i.e. 0, 1, or 2). Collinearity was assessed for all variables by calculating variance inflation factors (VIFs) and pairwise correlation coefficients.

Generalized linear mixed effects models were run in Program R (package glmmTMB; Brooks et al. 2017), with annual RS as our dependent variable. Our independent variables were either the four MHC allelic distance variables (DRB_nuc, DRB_aa, DOB_nuc, DOB_aa) or the specific allele variables. All models included a random term for individual as some individuals were captured more than once in their lifetime. Prior to running the models, we assessed the need to include non-linear (quadratic) effects for the predictors in our models via extra-sum-of-squares *F* (hereafter 'drop tests'; Murtaugh 2008). We also tested for overdispersion in our two datasets to aid in the selection of the appropriate family of error distribution (i.e. Poisson, negative binomial), as well as the presence of zero-inflation in our data using Akaike's Information Criterion adjusted for sample size (AIC_c; package bbmle; Bolker 2017) and drop tests (Murtaugh 2008).

RESULTS

A total of 19 *MHC-DRB* exon 2 alleles were found in our white-tailed deer population. The most common MHC-DRB alleles were DRB*10 (22.17%), DRB*14 (14.11%), and DRB*20 (14.11%), while the least frequent MHC-DRB alleles were DRB*28 (0.25%), DRB*26 (0.50%), DRB*29 (0.50%), and DRB*30 (0.50%). DOB*08 was the most common MHC-DOB allele (29.80%), while alleles DOB*01 (0.76%) and DOB*02 (1.77%) were the least frequent MHC-DOB alleles.

Allelic Distance Comparison for Males and Females

There was no difference ($p > 0.30$) in MHC allelic distances between males and females for all MHC variables examined in this study (Table 3.1). Males and females had the same number of unique *MHC-DOB* alleles. However, there was one *MHC-DRB* allele (DRB*29) found in males but not females and two *MHC-DRB* alleles (DRB*28, DRB*30) found in females but not males.

Male Annual Reproductive Success

We had annual RS data for 93 unique males aged 1.5 to 10.5 (average ± SE = 3.51 ± 0.11) years. As several males were reproductively active in multiple years, we had a total of 316 entries. Annual RS ranged from 0 to 10, though 69% of entries had zero annual RS (average RS ± SE = 0.64 ± 0.07). Of the 93 unique individuals, 10 were homozygous at the *MHC-DRB* locus, 21 were homozygous for the *MHC-DOB* nucleotide sequences, and 60 were homozygous for the *MHC-DOB* amino acid sequences. Only two individuals were homozygous at all MHC loci. Within males, the *MHC-DRB* allelic distances ranged from 0 to 46 (average ± SE = 23.71 ± 1.42) for the nucleotide sequences and 0 to 27 (average ± SE = 15.28 ± 0.89) for the translated *MHC-DRB* sequences. Within males, the allelic distance for the *MHC-DOB* nucleotide sequences ranged from 0 to 4 (average ± SE = 1.62 ± 0.12), while distances for amino acid sequences of *MHC-DOB* only ranged from 0 to 1 (average ± SE = 0.35 ± 0.05). We only found collinearity between the nucleotide and amino acid sequences for *MHC-DRB* exon 2 (VIF scores for DRB_nuc and DRB_aa were 33.24 and 33.63, respectively).

While there was no need to account for quadratic effects or overdispersion ($p > 0.05$), we did need to include a term for zero inflation in our male RS model (AICc weight for model with zero-inflation term = 1.0; drop test p-value = 1.24E-08). We found a strong positive association between DRB_nuc and annual RS in males ($p = 0.01$), but a similarly strong negative association between DRB_aa and annual RS ($p = 0.02$; Table 3.2). Specifically, for each standard deviation increase in DRB_nuc, males produced 6.69 as many offspring while each standard deviation increase in DRB_aa resulted in males producing 0.16 as many offspring (Figure 3.1). A male's annual RS was therefore greatest when *MHC-DRB* nucleotide allelic distances were large but *MHC-DRB* amino acid distances were small. There was also a trend towards significance for the amino acid sequences of *MHC-DOB* ($p = 0.08$), where each standard deviation increase in

DOB_aa resulted in males producing 1.28 as many offspring (Figure 3.2). Heterozygous males (DOB_aa = 1) therefore had greater annual RS than homozygous males (DOB_aa = 0).

Males who were heterozygous with DRB*10 (n = 30) had greater RS than males without DRB*10 (n = 59; p = 0.014; Table 3.3; Figure 3.3). Annual RS of males homozygous for DRB*10 (n = 4) did not differ significantly from either DRB*10 heterozygous males (p = 0.614) or males without DRB*10 (p = 0.673).

Female Annual Reproductive Success

We had annual RS data for 105 unique females aged 1.5 to 11.5 (average ± SE = 4.00 ± 0.12). As with males, several females were reproductively active in multiple years thereby increasing our total number of data entries to 349. Annual RS for females ranged from 0 to 2, though only 12% of entries were of females producing twins (average RS ± SE = 0.54 ± 0.04). Thirteen of the 105 unique females were homozygous for *MHC-DRB*, 21 were homozygous for the nucleotide *MHC-DOB* sequences, and 65 were homozygous for the amino acid *MHC-DOB* sequences. Five individuals were homozygous at all MHC loci. *MHC-DRB* allelic distances ranged from 0 to 44 (average ± SE = 21.84 ± 1.15) at the nucleotide level and from 0 to 27 (average ± SE = 14.46 ± 0.75) for the amino acid sequences. Females had a greater range of possible allelic distance values for *MHC-DOB* at both the nucleotide level (0 to 5; average ± SE = 1.81 ± 0.13) and the amino acid level (0 to 2; average ± SE = 0.40 ± 0.05). We found collinearity between the nucleotide and amino acid sequences for *MHC-DRB* exon 2 (VIF scores for DRB_nuc and DRB_aa were 33.13 and 33.15, respectively).

No MHC variables needed quadratic effects (p > 0.05). As there was no overdispersion, we used the poisson family in our models. Unlike the male model, there was no zero-inflation for our female annual RS dataset (p > 0.05). While there were no strong associations between *MHC-DRB* and female annual RS (DRB_nuc, p = 0.815; DRB_aa, p = 0.637), there was a trend towards significance for DOB_nuc (p = 0.056; Table 3.4; Figure 3.4). Specifically, greater allelic distances for *MHC-DOB* nucleotide sequences were associated with lower female annual RS, where each standard deviation increase in DOB_nuc resulted in females producing 0.83 as many offspring.

Females who were heterozygous for DRB*01 (n = 15) had lower annual RS compared to females without DRB*01 (n = 87; p = 0.011; Table 3.5; Figure 3.5). Annual RS of females

homozygous for DRB*01 (n = 3) did not differ significantly from DRB*01 heterozygous females (p = 0.701) or females without DRB*01 (p = 0.389).

DISCUSSION

Annual RS of male white-tailed deer was positively associated with *MHC-DRB* allelic distance at the nucleotide level but negatively associated with *MHC-DRB* allelic distance at the amino acid level. While *MHC-DRB* is not in linkage disequilibrium with *MHC-DOB* (Ivy-Israel et al. 2019a), it is still genetically linked to other MHC II loci. Our nucleotide *MHC-DRB* variable may therefore be acting like a marker that captured the positive influence of a different locus (or loci) linked to *MHC-DRB*, or a different part of the *MHC-DRB* gene itself, on male RS. For example, heterozygosity at *MHC-DQB* has been found to significantly increase annual RS in free-ranging rhesus macaques (*Macaca mulatta*; Sauermann et al. 2001). The negative association between the amino acid *MHC-DRB* distances and male RS suggests that greater allelic distance at *MHC-DRB* exon 2 specifically may negatively influence annual RS in male white-tailed deer, since the amino acid *MHC-DRB* variable captures the functionality of *MHC-DRB* exon 2 (i.e. antigen-binding site of DR molecules; Gaur and Nepom, 1996). Although this is not what we predicted based on previous findings in white-tailed deer and other species, this finding is consistent with some other findings reported for Galapagos sea lions (*Zalophus vollebaeki*) in a study that also used pairwise allelic distances between translated *MHC-DRB* exon 2 sequences (Lenz et al. 2013). Lenz et al. (2013) found that intermediate *MHC-DRB* distances were associated with the greatest levels of RS in both male and female sea lions, which suggests that maximum *MHC-DRB* diversity is not optimal for annual RS. Specifically, they predicted that peak male RS was attained when *MHC-DRB* distance was 4. While the maximum allelic distance for translated *MHC-DRB* sequences was 10 for sea lions, the maximum distance found in our white-tailed deer population was 27. Although MHC diversity can be beneficial, it can also be costly to the individual. Greater individual MHC heterozygosity is associated with reduced T-cell repertoire and regulatory T-cell diversity (Vidovic and Matzinger, 1988; Vidovic 1989; Nikolich-Zugich et al. 2004), which enhances the risk of autoimmune disease as these individuals have a stronger immune response to all antigens, including self-antigens (Milner et al. 2007; Ferreira et al. 2009). Stronger immunological responses of heterozygotes (Doherty et al. 1975) can also be very costly physiologically and divert resources away from growth (Klasing

et al. 1987; Fair et al. 1999; Bonato et al. 2009) and reproduction (Ilmonen et al. 2000, 2007; Bonneaud et al. 2003, 2004; Hanssen 2006; Cai et al. 2009; Bascunan-Garcia et al. 2010), thereby negatively impacting the individual's RS. Given the extremely high values of *MHC-DRB* allelic distance in our population, maximum *MHC-DRB* allelic distance may be detrimental due to costs associated with increased MHC diversity.

Since there was no association between *MHC-DRB* allelic distance and male morphology in our population (Ivy-Israel et al. 2019b), the associations found between *MHC-DRB* allelic distance and male RS may be attributed to a different biological process responsible for determining a male's RS. Mate selection can determine an individual's RS by influencing which individuals breed. While male morphology strongly affects the outcome of mate selection (West-Eberhard 1979; Clutton-Brock et al. 1980; Clutton-Brock and Huchard, 2013), a potential mate's olfactory cues can also serve as an important signal of genetic quality. Peptide ligands of MHC molecules can act as olfactory cues by affecting body odor (Leinders-Zufall et al. 2004; Santos et al. 2005; Boehm and Zufall, 2006). Vomeronasal sensory neurons are stimulated by these MHC ligands, which leads to a behavioral response such as mate selection. Yamazaki et al (1976) noticed that male mice (*Mus musculus*) preferred females with a different H-2 type. They concluded that there are two linked genes in the mouse's H-2 region that govern olfactory mating preference. Female sticklebacks (*Gasterosteus aculeatus*) selected mates who complemented their own set of MHC alleles to produce offspring with optimum MHC diversity (Aeschlimann et al. 2003). Similar findings were found in studies with humans (Wedekind et al. 1995; Jacob et al. 2002; Santos et al. 2005).

MHC-DRB allelic distance may also influence post-copulatory selection, which ultimately determines which sperm cell successfully fertilizes the female's available oocyte (Eberhard 1996; Schwensow et al. 2007b; Firman and Simmons, 2008; Løvlie et al. 2013). Female white-tailed deer can breed with multiple males during the breeding season, which often results in twins having different fathers (DeYoung et al. 2002, 2009; Sorin 2004; Neuman et al. 2016; Newbolt et al. 2017). Post-copulatory selection therefore seems possible in white-tailed deer. Mature spermatozoa express MHC II gene products in humans (Fellous and Dausset, 1970; Bishara et al. 1987; Barbieri et al. 1990; Obashi et al. 1990; Scofield et al. 1992; Martin-Villa et al. 1996, 1999; Paradisi et al. 2000), though this topic was quite controversial (Rodriguez-Cordoba et al. 1990; Schaller et al. 1993). Given this, females may select the sperm that best

complements her available oocyte's MHC haplotype via cryptic female choice. A male's *MHC-DRB* allelic distance may also influence his RS by controlling other variables important for sperm competition, such as volume of sperm in ejaculate, sperm motility, and copulation duration (Reinhold et al. 2002; Olsson et al. 2004; Burger et al. 2015a, 2015b). For example, Fitzpatrick and Evans (2009) found that reduced MHC heterozygosity impairs sperm quality, thereby putting these males at a disadvantage. Supplying greater quantities of competitive sperm to a female ensures that more of the male's sperm reaches the oocyte, thereby increasing his chance of successful fertilization (Parker 1982; Parker et al. 1996, 1997; Snook 2005).

There was a weak positive association between male RS and pairwise allelic distances for translated *MHC-DOB* sequences. Since there were only two possible values for *MHC-DOB* amino acid sequences (0 or 1), this suggests that heterozygous males had slightly greater annual RS than homozygous males. Ivy-Israel et al. (2019b) found an association between morphology of male white-tailed deer and pairwise allelic distances for *MHC-DOB* at the nucleotide level, where both body and antler size were significantly smaller for homozygous males compared to heterozygous males. Additionally, Newbolt et al. (2017) reported a positive association between RS of male white-tailed deer and both body and antler size in our population, though antler size only influenced RS when the population was under an older male age structure. Given that *MHC-DOB* heterozygous males are larger and that larger males have greater annual RS, it follows that *MHC-DOB* heterozygous males would have greater RS. Possible values for *MHC-DOB* allelic distance are considerably less than *MHC-DRB* allelic distance in our white-tailed deer population. Costs associated with maximum *MHC-DOB* allelic distance may therefore not have detrimental effects for the individual.

While male RS was positively associated with *MHC-DOB* allelic distance at the amino acid level, female RS was negatively associated with *MHC-DOB* allelic distance at the nucleotide level. Females in polygynous species, such as white-tailed deer, do not invest in costly secondary sexual traits as these features are generally unnecessary for attracting mates (Ditchkoff 2011). However, females face stringent nutrient requirements during gestation and especially during lactation (Allaye Chan-McLeod et al. 1994; Pekins et al. 1998; Barboza and Parker, 2008). Energy and protein requirements are substantially greater when a female is lactating: female white-tailed deer have been reported to have metabolic rates during lactation that are almost 2.5 times the basal metabolic rate (Moen 1973). This metabolic rate is greater

than during activity, growth, or gestation. Given this, maximum nucleotide allelic distance at *MHC-DOB* may cause the costs associated with maximum *MHC-DOB* allelic distance to be detrimental to successful gestation and/or lactation. Since female RS was influenced by *MHC-DOB* at the nucleotide level but not at the amino acid level, it may be that a different MHC II locus linked to *MHC-DOB* is driving this association. Takakuwa et al (1999) found that *MHC-DP* is associated with recurrent abortion in women. MHC-DP is an MHC II gene that is part of the same MHC II subregion as *MHC-DOB* in cattle (Andersson and Rask, 1988; Childers et al. 2005). The negative association between female RS and nucleotide MHC-DOB allelic distance may therefore be driven by MHC-DP. Inability to carry a fetus to term would significantly reduce a female's RS, though more research is required to further examine the effect of *MHC-DP* on fetal loss in white-tailed deer. Therefore, while our results suggest that *MHC-DOB* may be influencing female RS in white-tailed deer, it may be serving as a marker for another locus that is genetically linked to *MHC-DOB*. Future work should therefore take a functional genomic approach in this population to clarify which MHC II locus is truly influencing female annual RS.

Two *MHC-DRB* alleles were found to influence annual RS in our study population. Ivy-Israel et al. (2019a) found that frequencies for DRB*01 were decreasing in our population whereas DRB*10 was becoming more abundant over time. These trends may be explained by our finding that females with DRB*01 were less likely to recruit offspring while males with DRB*10 were more likely to successfully produce offspring. More research is needed to identify how these alleles are specifically influencing annual RS, as it could occur due to differential individual survival, mate selection, post-copulatory selection, and/or fawn survival (*in utero* or post-partum). Fluctuating MHC allelic frequencies may also be explained by the frequency-dependent hypothesis, which states that individuals with rare MHC alleles are more successful at fighting off pathogens due to host-parasite dynamics (Sommer 2005). Future studies on this population should examine how pathogen exposures are changing over time and how different *MHC-DRB* alleles perform under varying exposure conditions as pathogen-driven selection is known to vary spatiotemporally (Nevo and Beiles, 1992; Hedrick 2002; Hedrick 2004).

We found that while *MHC-DRB* allelic distance at the nucleotide level positively influenced male RS, *MHC-DRB* allelic distance at the amino acid level negatively influenced it. The positive association at the nucleotide level is likely attributed to another MHC II locus linked to *MHC-DRB* exon 2 having a positive influence on male RS. However, further research

is required to confirm this. As expected, *MHC-DOB* allelic diversity was positively associated with male RS, most likely through its effect on male morphology (Ivy-Israel et al. 2019a). As morphology is just one factor that determines RS, future studies should further examine how *MHC-DRB* and *MHC-DOB* allelic distances influence the other factors of RS, such as mate selection and post-copulatory selection. Lastly, while there was no association between *MHC-DRB* allelic distance and female RS, there was a mild negative association between *MHC-DOB* allelic distance and female RS. This should be further examined, especially since this could suggest that greater values of allelic distance at *MHC-DOB*, or an MHC II locus linked to *MHC-DOB*, may be too costly to combine with gestation and/or lactation.

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TABLES

Table 3.1. Differences in MHC allelic distance between standardized MHC variables for unique males and females. Positive values for difference indicate that average allelic distance for that MHC variable were greater in males than females, though none of these differences were statistically significant.

DRB_nuc			**DRB_aa**		
difference	SE	p	difference	SE	p
0.147	0.143	0.304	0.101	0.143	0.479
DOB_nuc			**DOB_aa**		
difference	SE	p	difference	SE	p
-0.149	0.143	0.299	-0.089	0.143	0.533

Table 3.2. Summary of fixed MHC effects on male white-tailed deer annual reproductive success.

Parameter	**Value (β)**	**SE**	**z-value**	**p-value**
DRB_nuc	1.8986	0.7516	2.526	0.0115
DRB_aa	-1.8285	0.7612	-2.402	0.0163
DOB_nuc	0.1142	0.1506	0.759	0.4480
DOB_aa	0.2488	0.1442	1.726	0.0844

Table 3.3. Summary of DRB*10's effect on male white-tailed deer annual reproductive success, where the difference indicates how the RS of individuals with DRB*10 differs from individuals without DRB*10. Positive values for difference indicate that RS for the second comparison entry was greater than the first.

Comparison		**Difference**	**SE**	**p-value**
No DRB*10	DRB*10 heterozygote	0.7620	0.3093	0.01375
No DRB*10	DRB*10 homozygote	0.3445	0.8160	0.673
DRB*10 heterozygote	DRB*10 homozygote	-0.4175	0.8268	0.614

Table 3.4. Summary of fixed MHC effects on female white-tailed deer annual reproductive success.

Parameter	Value (β)	SE	z-value	p-value
DRB_nuc	-0.1057	0.4506	-0.235	0.815
DRB_aa	0.2128	0.4505	0.472	0.637
DOB_nuc	-0.1849	0.0968	-1.911	0.056
DOB_aa	0.0080	0.0973	0.082	0.934

Table 3.5. Summary of DRB*01's effect on female white-tailed deer annual reproductive success, where the difference indicates how the RS of individuals with DRB*01 differs from individuals without DRB*01. Positive values for difference indicate that RS for the second comparison entry was greater than the first.

Comparison		Difference	SE	p-value
No DRB*01	DRB*01 heterozygote	-0.79402	0.31241	0.011
No DRB*01	DRB*01 homozygote	-0.53206	0.61757	0.389
DRB*01 heterozygote	DRB*01 homozygote	0.2620	0.6831	0.701

FIGURES

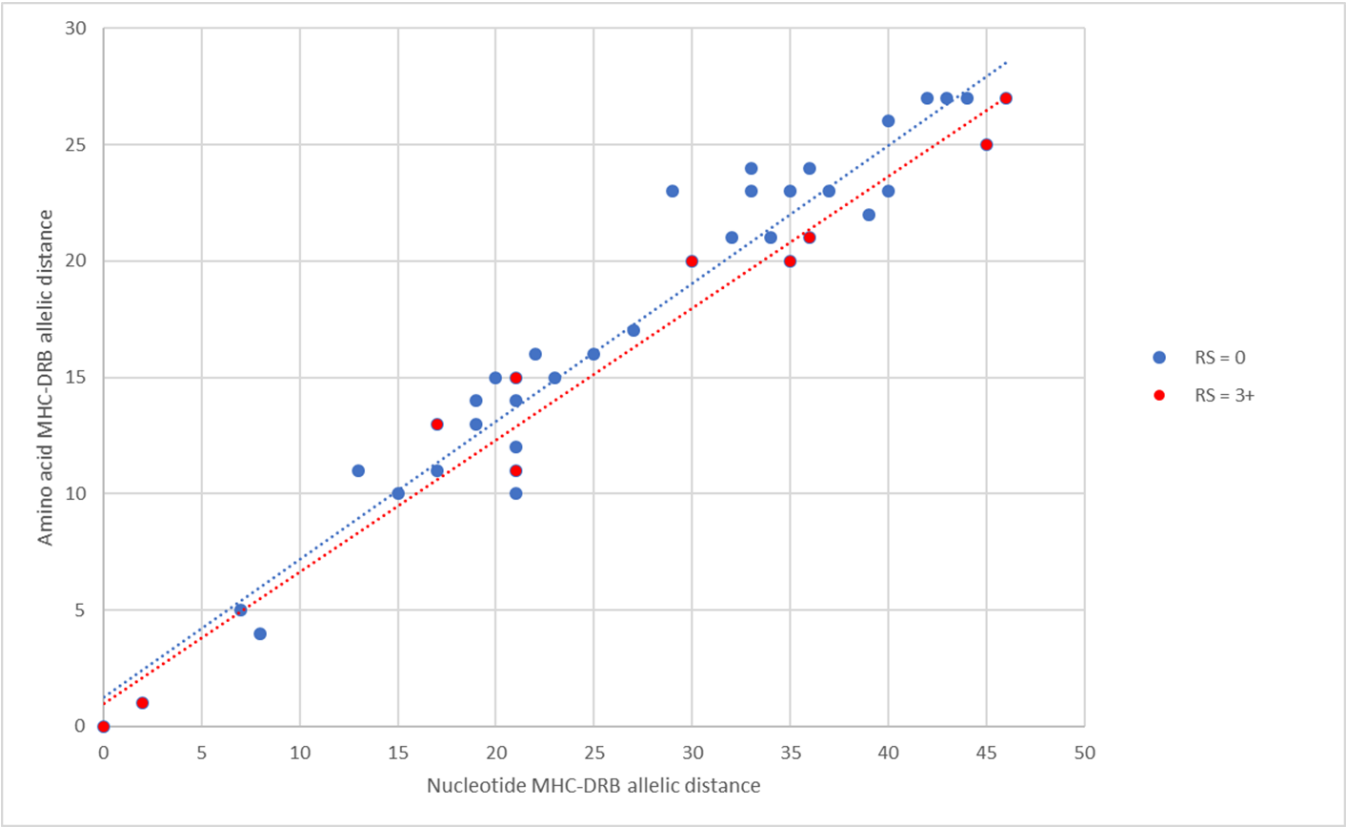

Figure 3.1. Annual reproductive success (RS) of male white-tailed deer was positively associated with nucleotide *MHC-DRB* allelic distance but negatively associated with amino acid *MHC-DRB* allelic distance. Though values for nucleotide and amino acid allelic distance were highly collinear, males with high RS (produced 3 or more offspring) had smaller values of amino acid *MHC-DRB* allelic distance associated with the corresponding nucleotide *MHC-DRB* allelic distance than males that did not produce any offspring (RS = 0).

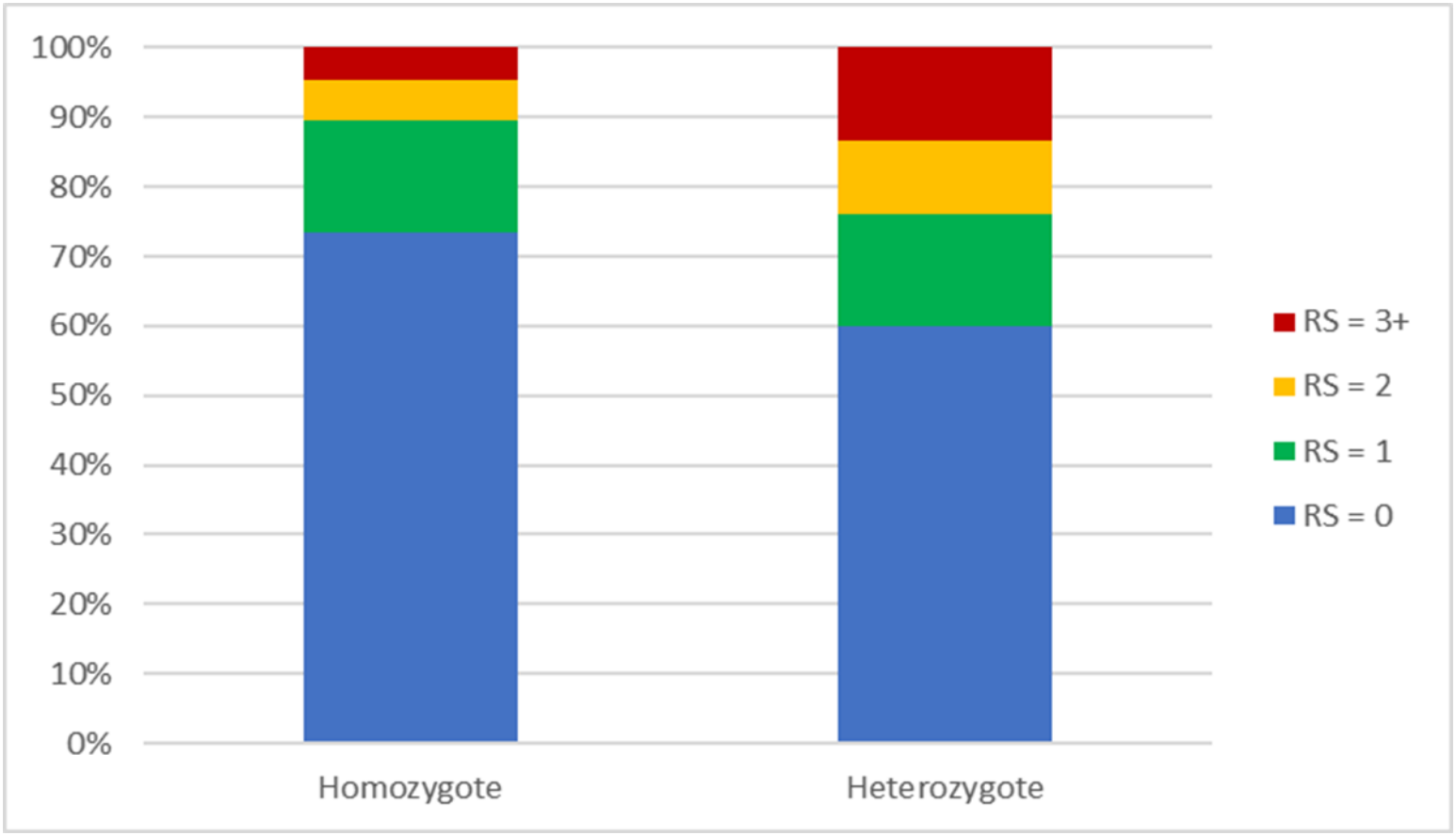

Figure 3.2. Male annual reproductive success (RS) was positively associated with amino acid *MHC-DOB* allelic distance. A greater percentage of homozygous males (*MHC-DOB* allelic distance = 0) produced no offspring (RS = 0; blue) compared to heterozygous males (*MHC-DOB* allelic distance = 1). High RS (produced 3 or more offspring; red) was more common in heterozygous males than homozygous males.

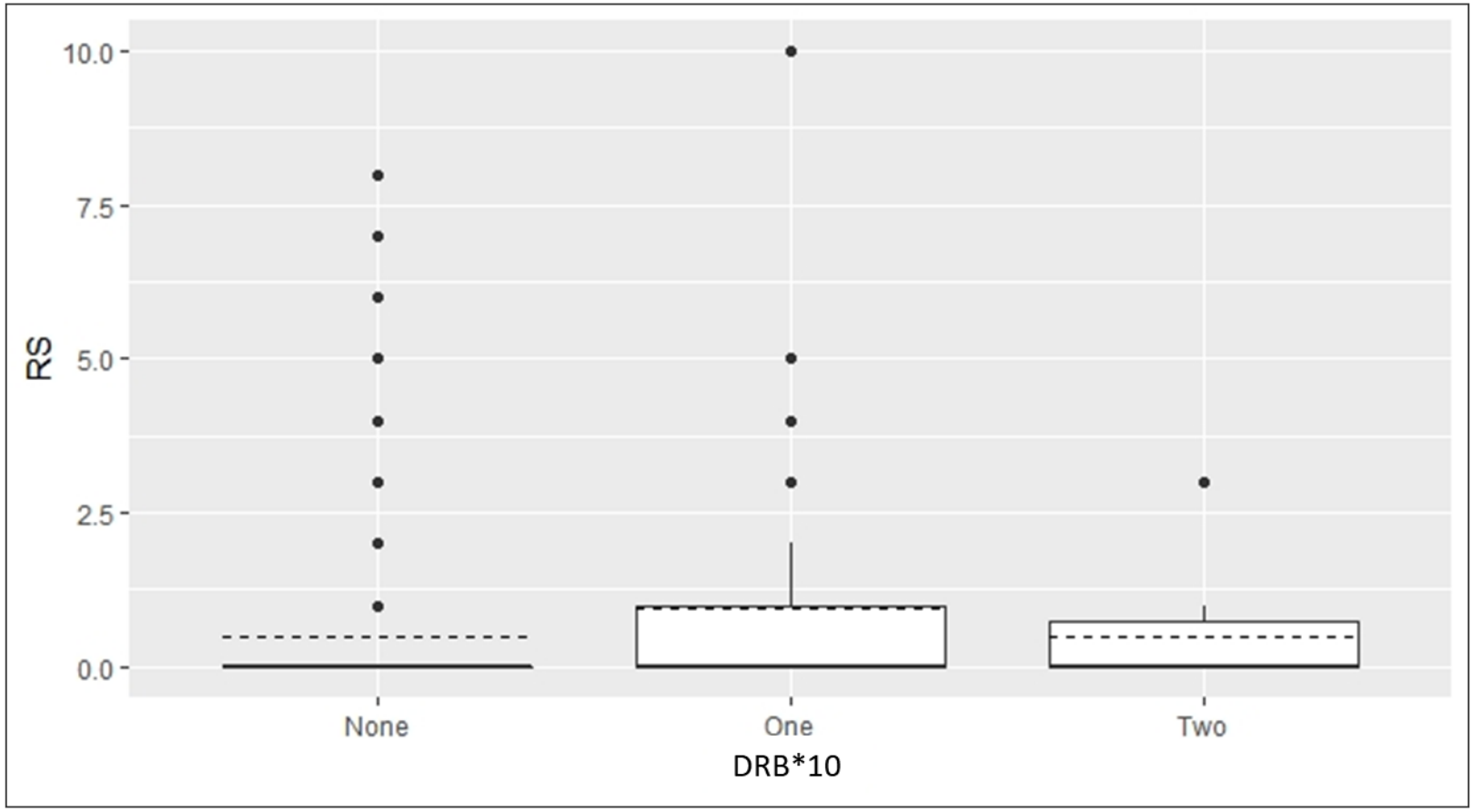

Figure 3.3. Males who were heterozygous with DRB*10 (n = 30) had greater reproductive success (RS) than males without DRB*10 (n = 59). The dashed horizontal lines indicate the mean RS values.

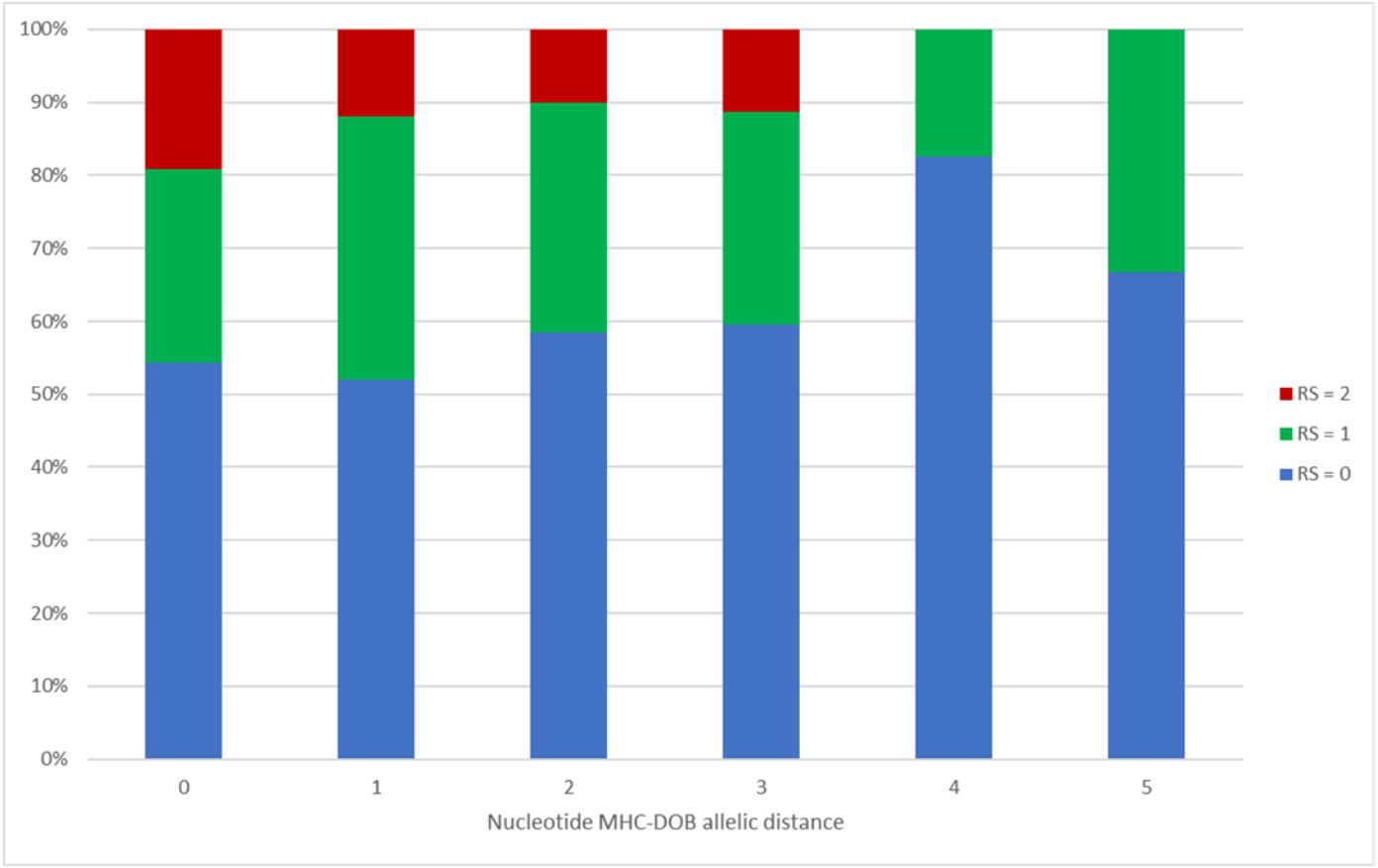

Figure 3.4. Female annual reproductive success was negatively associated with nucleotide *MHC-DOB* allelic distance. A greater percentage of females with no or low *MHC-DOB* allelic distance produced twins (RS = 2; red) compared to females with high values for *MHC-DOB* allelic distance.

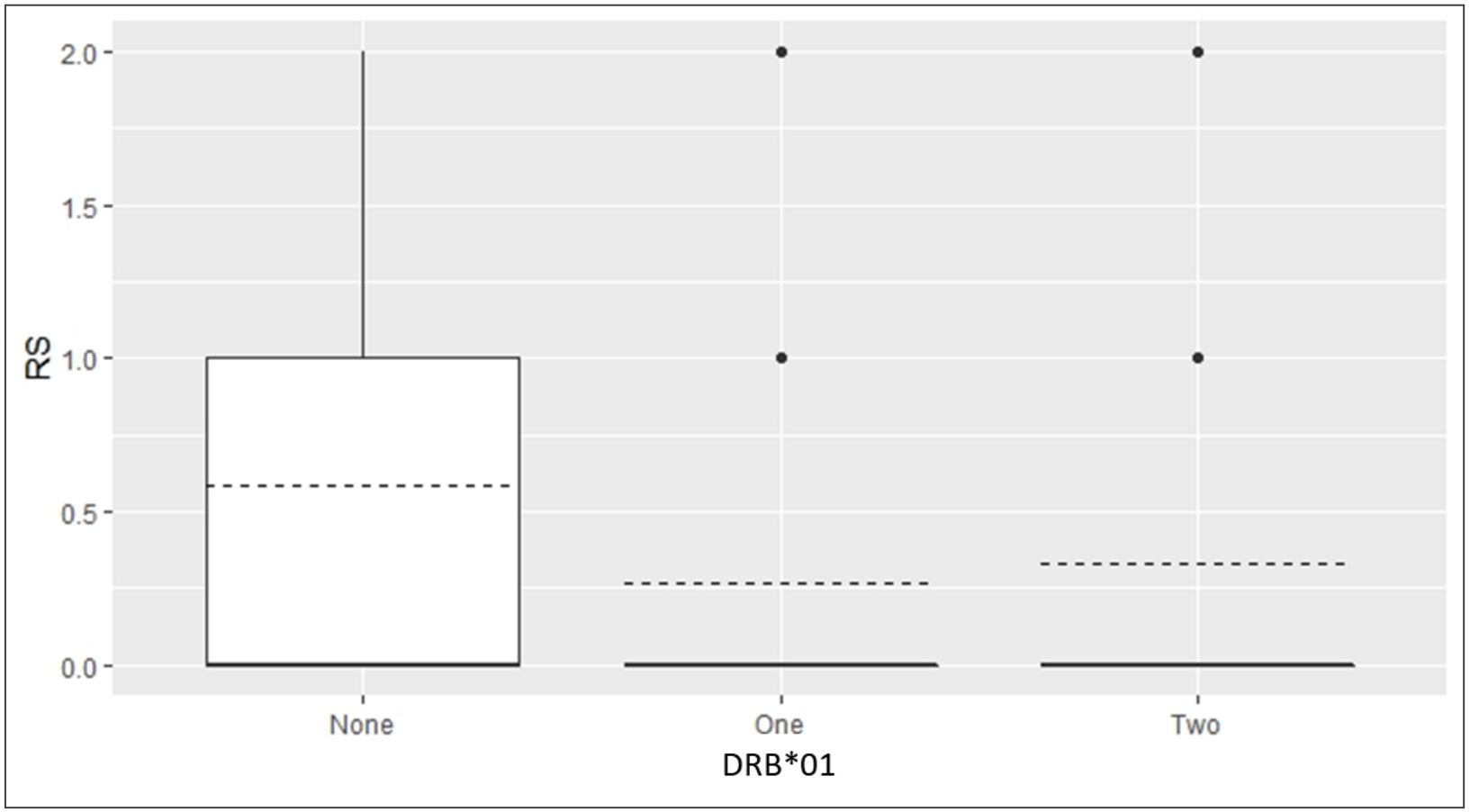

Figure 3.5. Females who were heterozygous with DRB*01 (n = 15) had greater annual reproductive success (RS) than females without DRB*01 (n = 87). The dashed horizontal lines indicate the mean RS values.

CHAPTER 4: Post-copulatory selection in white-tailed deer favors offspring with greater *MHC-DRB* allelic distance

ABSTRACT

The major histocompatibility complex (MHC) is a highly polymorphic region in vertebrates. Biological processes that drive this increased genetic diversity include MHC-dependent mate choice, cryptic female choice, MHC-determined abortion, and differential survival among offspring. This study examined if an enclosed white-tailed deer population recruited offspring with greater allelic distance at two genetically unlinked loci (*MHC-DRB* exon 2, *MHC-DOB* exon 2). We further assessed how this potential difference was driven by pre- and/or post-copulatory processes. All potential sires and dams were used to generate a complete list of possible breeding pairs for 2008-2015. We found that successfully recruited offspring had greater *MHC-DRB* allelic distance than expected given the average allelic distances of all possible breeding pairs. While allelic distances between successful breeding pairs (those that successfully recruited offspring) and non-successful breeding pairs did not differ, suggesting a lack of pre-copulatory selection, we did find that successful breeding pairs had fewer DRB*01 alleles than non-successful breeding pairs. However, when comparing allelic distance between recruited offspring and average allelic distance of their confirmed parents to assess post-copulatory selection, we found that offspring had greater *MHC-DRB* allelic distance than expected. Furthermore, offspring from older sires had greater *MHC-DRB* allelic distance than offspring from younger sires. Our findings suggest that post-copulatory processes are selecting for offspring with greater *MHC-DRB* allelic distance in white-tailed deer.

KEYWORDS

Major histocompatibility complex, white-tailed deer, pre-copulatory selection, post-copulatory selection, cryptic female choice, divergent allele advantage

INTRODUCTION

The major histocompatibility complex (MHC) is a group of genes that plays a vital role in the vertebrate immune system by coding for proteins that help the immune system recognize and address foreign pathogens. Type I MHC genes are expressed on all nucleated cells, whereas type II MHC genes only occur on certain immune cells, such as dendritic cells and macrophages (Janeway et al. 2001). Classical MHC proteins (ex. *MHC-DR*, *MHC-DQ*) bind to peptide fragments, with assistance from non-classical accessory MHC gene products (ex. *MHC-DO*, *MHC-DM*), and display these antigens on the surfaces of cells where they are checked by T-cell lymphocytes (Janeway et al. 2001; Poluektov et al. 2013; Mellins and Stern 2014). If antigens presented by MHC proteins to the immune system are foreign, T-cells will cause cellular destruction or initiate a systemic immune response (Janeway et al. 2001).

The MHC is well-known for its incredibly high rates of polymorphism. There are several hypotheses for how the MHC retains such high levels of genetic diversity in vertebrates, one of which is the heterozygote advantage hypothesis, which states that individuals with greater MHC heterogeneity are able to fight off a greater array of pathogens (Hughes and Nei, 1992; Sommer 2005). Therefore, heterozygotes should be healthier and more successful (Hughes and Nei, 1989; Takahata and Nei, 1990). Improved immune function of MHC heterozygotes also enables greater investments into costly morphological traits. For example, male white-tailed deer (*Odocoileus virginianus*) with greater MHC diversity had greater overall body size and antler size than homozygous males (Ditchkoff et al. 2001; but see Ivy-Israel et al. 2019b). Similarly, a secondary sexually selected trait among male pheasants (*Phasianus colchicus*), spur length, is also influenced by the male's MHC genotype characteristics (von Schantz et al. 1996; 1997). Two separate models are suggested to exist within the heterozygote advantage hypothesis: symmetric balancing selection (Takahata 1990) and divergent allele advantage (Wakeland et al. 1990). The symmetric model states that all heterozygotes experience a similar selective advantage, while the divergent model suggests some heterozygotes have more divergent allelic sequences than others. Those whose alleles are more dissimilar are expected to have a greater advantage as they present a greater spectrum of antigens to their immune system. The divergent allele advantage has recently drawn more attention as several studies reported a positive association between mate choice and MHC allelic divergence (Landry et al. 2001; Forsberg et al. 2007; Schwensow et al. 2008; Juola and Dearborn, 2012; Evans et al. 2012; Lenz et al. 2013).

MHC-dependent mate choice may also maintain MHC polymorphism. Individuals are typically more attracted to potential mates with different MHC characteristics, which results in offspring with greater MHC heterogeneity (Yamazaki et al. 1976; Wedekind et al. 1995; Jacob et al. 2002; Santos et al. 2005). For example, female sticklebacks (*Gasterosteus aculeatus*) selected mates who would complement their own set of MHC alleles as heterozygous offspring generally had lower parasite burdens (Aeschlimann et al. 2003). Copulation frequency between MHC-similar mates is typically found to be lower compared to MHC-dissimilar mates (Apanius et al. 2017). Olfactory cues, generated from MHC proteins, can stimulate vomeronasal sensory neurons, which may lead to a behavioral response such as mate selection (Leinders-Zufall et al. 2004; Santos et al. 2005; Boehm and Zufall, 2006). For example, Grogan et al. (2018) found that both male and female ring-tailed lemurs (*Lemur catta*) can signal their MHC diversity and pairwise MHC similarity via genital secretions, thereby enabling MHC-associated mate selection. Different MHC alleles produce unique olfactory cues (Lenz 2011). For example, the concentrations of a subset of volatile compounds in urine were found to differ between MHC haplotypes (Willse et al. 2005; Eggert et al. 1996; Singer et al. 1997; Montag et al. 2001). Specifically, a study done on mice (*Mus musculus*) found that individuals with different MHC haplotypes produced different concentrations of 28 unique components in their urine (Willse et al. 2005). Overall, the MHC genes are believed to account for approximately 50% of individual variance in odor (Beauchamp and Yamazaki, 2001), thereby enabling individuals to select mates who best complement their own MHC haplotype.

Even if a female is bred by an MHC-similar male, selection can still occur within the female reproductive tract for sperm with optimum MHC characteristics via a type of post-copulatory selection known as cryptic female choice (Dewsbury 1988; Eberhard and Cordero, 1995). When a female is bred by multiple males, there may be bias during fertilization so that sperm from the MHC-dissimilar male successfully fertilizes her available oocyte (Trivers 1972; Thornhill 1983; Eberhard 1996; Olsson et al. 2003; Freeman-Gallant et al. 2003). Several studies in humans found that mature spermatozoa express MHC II gene products (Fellous and Dausset, 1970; Bishara et al. 1987; Barbieri et al. 1990; Obashi et al. 1990; Scofield et al. 1992; Martin-Villa et al. 1996, 1999; Paradisi et al. 2000). Expression of MHC II molecules on sperm may enable post-copulatory selection benefitting sperm with MHC-dissimilar MHC II haplotypes relative to the egg. Cryptic female choice has also been found to favor males with greater MHC

diversity (Richardson et al. 2005; Skarstein et al. 2005; Promerova et al. 2011), though this may be attributed to reduced sperm quality in males with less MHC diversity (Fitzpatrick and Evans, 2009). Male phenotypic cues may also influence cryptic female choice, as Løvlie et al. (2013) found that cryptic female choice in red junglefowl (*Gallus gallus*) occurred during natural matings but not following artificial insemination.

MHC-dependent abortion also favors increased MHC polymorphism. Since offspring with greater MHC variation generally have greater survival (Paterson et al. 1998; Brouwer et al. 2010), a female can differentially abort MHC homozygotes while keeping heterozygous fetuses to term (Palm 1969; 1970; Hamilton and Hellstrom, 1978). A study with humans found that couples that share the same HLA-DR alleles have significantly lower fertility than couples that differ at this locus (Ober 1992). Shared HLA-DR alleles were associated with a 27% incidence of recognized miscarriage, whereas this percentage was only 12% when HLA-DR alleles were different (Ober et al. 1985). Some MHC homozygosity may be lethal for the fetus (the lethal gene hypothesis; Gill 1994; Kostyu 1994). Homozygosity of recessive, lethal MHC genes can negatively affect fetal growth and differentiation in rats (*Rattus norvegicus*; Gill 1994). Lastly, odors may also block implantation via a post-copulatory mate choice adaptation known as the Bruce effect (Schwagmeyer 1979; Huck 1982). For example, female mice (*Mus musculus*) are more likely to block pregnancy if they encounter, and smell, a newly introduced male whose MHC characteristics are even more complementary to her own than the current sire (Yamazaki et al. 1983; 1986).

White-tailed deer are a popular polygynous species. While males invest greatly in costly morphological traits to attract mates, females do not (Ditchkoff et al. 2011). Female white-tailed deer are often bred by multiple males, which can result in multiple paternity for single litters (DeYoung et al. 2002). Cryptic female choice may therefore occur in white-tailed deer, though no research has yet explored this. Two genetically unlinked MHC II loci (*MHC-DRB* exon 2 and *MHC-DOB* exon 2) have been characterized in white-tailed deer (Van Den Bussche et al. 1999, 2002; Ivy-Israel et al. 2019a). Previous work on this population found that male annual reproductive success (RS) is positively associated with the genomic region linked to MHC-DRB. However, greater amino acid allelic distance at MHC-DRB exon 2 specifically was negatively associated with male RS (Ivy-Israel et al. 2019b). Furthermore, the genomic region that contains MHC-DOB was found to influence male morphology, where body size peaked at moderate

nucleotide MHC-DOB allelic distance and antler size peaked at maximum MHC-DOB allelic distance. While male RS had a weak positive association with amino acid MHC-DOB allelic distance, female RS had a weak negative association with nucleotide MHC-DOB allelic distance

This study tested if observed *MHC-DRB* and *MHC-DOB* allelic distances of offspring that survived to sexual maturity were greater than expected given the average allelic distances of all possible offspring based on the potential breeding pairs. To further understand this difference, we assessed whether selection for greater or lower MHC allelic distance occurred pre- or post-copulatory. We hypothesized that offspring captured in our enclosed white-tailed deer population would have greater MHC diversity than expected due to both pre- and post-copulatory selection. We further hypothesized that post-copulatory selection would be strongest in older females due to their previous breeding experience (Bateman et al. 2001, 2004).

METHODS

Study Area

Our study was conducted at the Auburn Captive Facility (ACF), which was part of the Piedmont Agricultural Experiment Station owned by Auburn University, located north of Camp Hill, Alabama. The facility had 100-120 white-tailed deer in a 174-hecatre area enclosed by a 2.6-meter fence, which was constructed in October 2007. The deer present within the ACF during the study included the original deer that inhabited this area during the fence formation in 2007 and their subsequent offspring. The founding population consisted of 71 individuals, 46 of which were female. This slightly uneven sex ratio decreased the effective population size to 64.8, which was calculated as $N_e = 4N_mN_f / (N_m+N_f)$, where N_m is the number of breeding males (n = 25) and N_f is the number of breeding females (n = 46) in the population (Wright 1938). Subsequent to fencing the area, no deer were either introduced or hunted within the facility, with natural and capture-related mortalities mainly regulating population size during our study (Newbolt et al. 2017). Supplemental feed was available in the form of food plots, corn feeders, and *ad libitum* protein feeders. The facility also contained a creek and its tributaries were present on the property, which provided a reliable water source year-round.

Deer abundance and age structure of our white-tailed deer population were measured using images of marked and unmarked deer collected by infrared-triggered cameras at both feeders and randomly selected sites baited with corn for 14 days each February via mark-

recapture methods (Overton 1969; Jacobson et al. 1997). We supplemented this data with field observations and capture/mortality records to determine final population demographic estimates. We considered marked individuals deceased if not observed for two years in order to prevent a potentially ever-growing population of dead but unrecovered animals.

Animal handling

From 2007 to 2015 we captured adult white-tailed deer (≥ 6 months of age) over 8 trapping seasons (October through July each year). We chemically immobilized deer by administering a tranquilizer mixture into the deer's hindquarter using cartridge fired dart guns (Pneu-Dart model 193) and 0.22 caliber blanks. We added 4 cc of xylazine (100 mg/ml; Lloyd Laboratories, Shenandoah, IA) to a 5 mL vial of Telazol® (100 mg/ml; Fort Dodge Animal Health, Fort Dodge, IA; Miller et al. 2003), and loaded 2 cc of this tranquilizer mixture a telemetry dart (2.0 cc, type C, Pneu-Dart Inc., Williamsport, PA) together with a radio transmitter (Advanced Telemetry Systems, Inc., Isanti, MN), which enabled us to locate the sedated deer after tranquilizer administration (Kilpatrick et al. 1996). After data collection was complete, we reversed the sedation by injecting the shoulder and hindquarter muscles of the deer with Tolazine (100 mg/ml; Miller et al. 2004). All methods employed during our study were approved by the Auburn University Institutional Animal Care and Use Committee (2008-1417, 2008-1421, 2010-1785, 2011-1971, 2013-2372, 2014-2521, 2016-2964, and 2016-2985) and were in compliance with the American Society of Mammalogists' guidelines (Sikes and Gannon 2011).

We gave all deer a unique 3-digit identification number at initial capture in the form of ear tags. We also freeze branded these identification numbers on the front shoulder and hind quarter of some individuals. Deer were aged using the tooth wear and replacement aging technique by Severinghaus (1949). A 1-cm^2 notch of tissue was removed from their ears for genetic analysis, which was stored in a -80°C freezer prior to DNA analysis.

Genetic Analysis

MHC-DRB exon 2 (n = 373) and *MHC-DOB* exon 2 (n = 380) amplicons were sequenced on the Illumina MiSeq platform using ear tissue samples collected from our population as described in Ivy-Israel et al. (2019a). The 19 MHC-DRB and 11 MHC-DOB alleles found in this

population are in NCBI (Genbank accession numbers: AF082161, AF082165, AF082166, AF082170, AF082172, AF082174, AF407169, MK952679-MK952701). While only exon 2 was captured for *MHC-DRB* (250 bp), the *MHC-DOB* amplicon contained exon 2 plus noncoding regions around it. We used the full *MHC-DOB* sequence (360 bp) when analyzing nucleotide sequences and *MHC-DOB* exon 2 (270 bp) for the amino acid sequences.
Pairwise genetic distances between the alleles at each locus (*MHC-DRB* and *MHC-DOB*) were calculated as the number of differences between the sequences at both the nucleotide level (DRB_nuc and DOB_nuc, respectively) and the amino acid level (DRB_aa and DOB_aa, respectively). Distance matrices used to assess the number of differences were generated via Geneious (v11.1.5).

A pedigree was created for our population by Newbolt et al. (2017), which assigned parentage (dam and/or sire) with a minimum 95% reliability threshold.

Statistical Analysis

The available dams and sires in the population that could have produced offspring born between 2008 and 2015 were used to generate a combined list of all possible breeding pairs for those years. We first assessed if MHC allelic distances of successfully recruited offspring were different from expected MHC allelic distance of all possible offspring (i.e. if all potential breeding pairs had successfully produced offspring) using Program R (v3.6.0; lm function; R Core Team 2019). Expected MHC allelic distance was estimated as the average allelic distance between the four alleles of each possible breeding pair following the Mendelian laws of inheritance (Mendel 1866) since MHC alleles are codominant (Janeway et al. 2001). We also examined if age of the dam and/or sire influenced the difference between observed and expected offspring MHC diversity (lm function in R). To further dissect why offspring may have greater or lesser MHC allelic distance than expected, we tested two mechanistic hypotheses: 1) pre-copulatory selection (i.e. mate selection) by testing if observed (successful) breeding pairs had greater average allelic distance than expected from randomized breeding pairs (non-successful breeding pairs; lm function), and 2) post-copulatory / early life selection (i.e. cryptic female choice, MHC-determined abortion, fawn survival) by testing if successfully recruited offspring (i.e. those who survived to sexual maturity at 6 months of age) had on average greater MHC allelic distance than expected by Mendelian inheritance given their parents' MHC alleles (lme

function in nlme package; Pinheiro et al. 2018). We accounted for parent/offspring pairings as a random effect when assessing post-copulatory selection. As with the initial analysis, we assessed the influence of dam/sire age on the difference between observed and expected MHC allelic distance (lme function in R)

Since DRB*01 and DRB*10 influence annual reproductive success of female and male white-tailed deer, respectively (Ivy-Israel et al. 2019b), we also examined how the frequency of these *MHC-DRB* alleles differed between successful breeding pairs and non-successful breeding pairs (pre-copulatory selection), and between the observed frequencies of these alleles in the recruited offspring and expected frequencies of these alleles in the offspring given the parents' *MHC-DRB* alleles (post-copulatory selection).

We standardized dam and sire age, expected/observed *MHC-DRB* allelic distances, and expected/observed *MHC-DOB* allelic distances (i.e. subtracted mean and divided by standard deviation) prior to analysis. Collinearity was assessed for all variables by calculating variance inflation factors (VIFs) and pairwise correlation coefficients.

RESULTS

There were 25,288 possible breeding pairs that could have produced an offspring between 2008 and 2015. Among these potential breeding pairs, 167 successfully produced an offspring that survived to sexual maturity (6 months). As several breeding pairs produced twins (n = 21), the total number of offspring produced by these successful breeding pairs was 188. *MHC-DRB* allelic distance and *MHC-DOB* allelic distance of dams and sires did not differ between those from successful and non-successful breeding pairs, except for sire DRB_nuc and DOB_aa (Table 4.1). Specifically, sires from successful breeding pairs had greater allelic distance for DRB_nuc ($p = 0.047$) and DOB_aa ($p < 0.001$). Additionally, both dams ($p = 0.001$) and sires ($p < 0.001$) from successful breeding pairs were older compared to dams and sires from non-successful breeding pairs.

Observed offspring MHC allelic distance

Observed, successfully recruited offspring had greater *MHC-DRB* allelic distance than expected offspring that could have been produced from all possible breeding pairs at both the nucleotide and amino acid levels (DRB_nuc, $p < 0.001$; DRB_aa, $p < 0.001$; Table 4.2).

Specifically, we found that the nucleotide and amino acid MHC-DRB allelic distance of observed offspring were 0.32 (± 0.14; ± 95% CI) standard deviations greater than expected. Additionally, the difference between observed and expected *MHC-DRB* allelic distance increased with increasing sire (p = 0.001) and dam age (p = 0.022; Table 4.3). In other words, for each 1-year increase in sire age, the difference between observed and expected nucleotide and amino acid MHC-DRB allelic distance increased by 0.28 (± 0.17; ± 95% CI) and 0.29 (± 0.17; ± 95% CI) standard deviations, respectively (Figure 4.1). Similarly, for each 1-year increase in dam age, the difference between observed and expected nucleotide and amino acid MHC-DRB allelic distance increased by 0.18 (± 0.16; ± 95% CI) and 0.20 (± 0.16; ± 95% CI), respectively (Figure 4.2). Therefore, successful breeding pairs produced offspring with greater *MHC-DRB* allelic distance than expected when both parents were older compared to younger successful breeding pairs. There was no difference between offspring observed and expected *MHC-DOB* allelic distance (DOB_nuc, p = 0.262; DOB_aa, p = 0.333).

Pre-copulatory selection

There was no difference in *MHC-DRB* or *MHC-DOB* allelic distance between successful and non-successful breeding pairs at either the nucleotide (p = 0.414 and p = 0.579, respectively) or amino acid level (p = 0.594 and p = 0.809, respectively; Table 4.4). There was also no significant difference in average allelic distances between successful breeding pairs and non-successful breeding pairs for *MHC-DOB*. However, successful breeding pairs had fewer DRB*01 alleles than non-successful breeding pairs (p = 0.007; Table 4.5). Specifically, successful breeding pairs had 0.13 (± 0.09; ± 95% CI) fewer DRB*01 alleles on average compared to non-successful breeding pairs. While both dams and sires of successful breeding pairs had fewer DRB*01 alleles than dams and sires from non-successful breeding pairs, this was only statistically significant for dams (dams, p = 0.016; sires, p = 0.159).

Post-copulatory selection

Successfully recruited offspring had greater *MHC-DRB* allelic distance compared to the expected, average MHC-DRB allelic distance given the *MHC-DRB* alleles of their parents (DRB_nuc, p = 0.051; DRB_aa, p = 0.034; Table 4.6). Specifically, we found that the nucleotide and amino acid MHC-DRB allelic distances were 0.14 (± 0.14; ± 95% CI) and 0.15 (± 0.14; ±

95% CI) standard deviations greater than expected, respectively. There was no significant difference in *MHC-DOB* allelic distances. Offspring born from breeding pairs with older sires had greater *MHC-DRB* allelic distance than expected compared to offspring born from breeding pairs with younger sires (DRB_nuc, p = 0.008; DRB_aa, p = 0.005; Table 4.7; Figure 4.3). In other words, for each 1-year increase in sire age, the difference between observed and expected nucleotide and amino acid MHC-DRB allelic distance increased by 0.16 (± 0.15; ± 95% CI) standard deviations. Specific *MHC-DRB* allele frequencies for DRB*01 and DRB*10 did not differ from expected allelic frequencies given their parents (p = 0.497).

DISCUSSION

We found that successfully recruited offspring in our white-tailed deer population had greater *MHC-DRB* allelic distance than expected. There is an exhaustive list of literature that has reported similar results, where greater individual MHC allelic divergence is strongly selected for (She et al. 1990; Landry et al. 2001, Richman et al. 2001; Consuegra and Garcia de Leaniz, 2008; Neff et al. 2008; Lenz et al. 2009, 2013), which further supports the heterozygote advantage hypothesis (Hughes and Nei, 1989, 1992; Takahata and Nei, 1990; Sommer 2005), specifically the divergent allele advantage (Wakeland et al. 1990). Additionally, we found that successfully recruited offspring with older parents had greater *MHC-DRB* allelic distance than expected compared to offspring with younger parents. In other words, older sires and dams produced offspring with greater allelic distance than younger sires and dams. Since this initial analysis captured the effects of several different processes (i.e. mate selection, cryptical female choice, fawn survival), it is unclear which process, or processes, were responsible for these results. We therefore ran two more specific analyses to examine how these results were influenced by pre- and post-copulatory processes.

Our pre-copulatory analyses showed no significant difference in average MHC allelic distance between breeding pairs that successfully recruited offspring and breeding pairs that did not. While MHC-assortative mating has been reported for birds (Richardson et al. 2005; Bonneaud et al. 2006), rodents (Yamazaki et al. 1976, 1988; Potts et al. 1991; Penn and Potts, 1998), reptiles (Olsson et al. 2003), fish (Olsen et al. 1998; Landry et al. 2001; Aeschlimann et al. 2003), and humans (Wedekind et al. 1995; Jacob et al. 2002; Santos et al. 2005), our findings suggest that this may not be true for white-tailed deer. Similar findings were reported for another

polygynous ruminant, the Soay sheep (*Ovis aries* L.; Paterson and Pemberton, 1997). Specifically, the breeding system of the St Kildan Soay sheep population was not influenced by MHC diversity as there was no evidence for non-random mating with respect to *MHC-DRB* markers. Outcomes of intrasexual competition are the main determining factor by which males gain breeding access to receptive females among Soay sheep (Grubb 1974). Female choice using MHC-associated body odors may therefore not exist at the pre-copulatory level among polygynous ruminants. However, while our results suggest that there was no pre-copulatory selection for MHC diversity in our population, we should point out that there are limitations to our dataset that hinder a comprehensive analysis of the effect of pre-copulatory selection on MHC diversity in our population. Mainly, we have no record of which individuals copulated since we only use confirmed parentage from successfully recruited offspring as proof of copulation. We therefore have no data on which deer mated but did not produce offspring that survived to maturity.

Results from the post-copulatory analyses showed that offspring had greater *MHC-DRB* allelic distance compared to average *MHC-DRB* allelic distance given the parent's *MHC-DRB* alleles. There are several post-copulatory processes that could drive this result, including 1) cryptic female choice, where sperm with *MHC-DRB* dissimilar haplotypes are more successful at fertilizing the oocyte; 2) MHC-dependent abortion, where females do not carry fetuses with lower *MHC-DRB* allelic distances to term; and 3) a positive association between fawn survival and *MHC-DRB* allelic distance. Cryptic female choice is a well-studied phenomenon, which enables a female's reproductive system to bias sperm used for fertilization even after copulation (Thornhill 1983; Eberhard 1996; reviewed in Firman et al. 2017). Evidence of MHC-dependent gamete fusion has been reported for mice (Rulicke et al. 1998), salmon (*Salmo salar*; Yeates et al. 2009), red jungle fowl (Løvlie et al. 2013), and guppies (*Poecilia reticulata*; Gasparini et al. 2015). Rulicke et al. (1998) found that mice infected with mouse hepatitis virus produced more MHC-heterozygous embryos than uninfected parents. Cryptic female choice may therefore depend on the male's infection status. A proposed mechanism for how cryptic female choice favors sperm with optimum MHC diversity is that they gain information about her mate's MHC and overall quality via male phenotypic cues. For example, Løvlie et al. (2013) reported that cryptic female choice only occurred in red jungle fowl following natural copulation but not after artificial insemination. Male phenotypic cues, such as MHC-associated body odors (Leinders-

Zufall et al. 2004; Santos et al. 2005; Broehm and Zufall, 2006), may therefore be necessary for a female to bias a male's sperm and engage in cryptic choice.

Once fertilization is complete, MHC-determined abortion can further influence MHC diversity of successfully recruited offspring. Couples with greater MHC similarity, especially at the *MHC-DRB* locus (Unander and Olding, 1983; Reznikoff-Etievant et al. 1984; Beer et al. 1985; McIntyre et al. 1986), are more likely to experience recurrent fetal loss than MHC-dissimilar couples (Komlos et al. 1979; Schacter et al. 1979; Gill 1983; Reznikoff-Etievant et al. 1988; Ho et al. 1990). MHC antigen sharing among couples may be positively associated with MHC-linked genetic defects, congenital abnormalities, and/or increased susceptibility to cancer (Gill 1984, 1987, 1992). Females may therefore lose offspring *in utero* if their fetus inherited suboptimal MHC diversity or lethal MHC homozygosity (Gill 1994; Kostyu 1994). MHC diversity may also influence the survival of offspring post-partum. For example, Paterson et al. (1998) found that *MHC-DRB* alleles associated with parasite resistance in lambs greatly influenced juvenile survival in Soay sheep. Similarly, Brouwer et al. (2010) found that MHC diversity was positively associated with juvenile survival in the Seychelles warbler (*Acrocephalus sechellensis*). Since we do not have fawn survival data from our population, either *in utero* or post-partum, we are not able to confirm that our post-copulatory results are due to differential fawn survival. However, selection for greater *MHC-DRB* allelic distance in early life (< 6 months of age) may be contributing to the balancing selection found for *MHC-DRB* in our population (Ivy-Israel et al. 2019).

The difference between observed and expected *MHC-DRB* allelic distance for successfully recruited offspring was positively associated with the sire's age. In other words, older males produced offspring with greater *MHC-DRB* allelic distance than expected compared to younger sires. Male senescence has been reported to decrease sperm quality (lower motility and more deleterious mutations), which ultimately reduces male reproductive success (Hansen and Price, 1995, 1999; Radwan 2003; Pizzari et al. 2008; Garcia-Palomares et al. 2009; Møller et al. 2009; Gasparini et al. 2010). The oldest male in our population was 10.5 years old. Since white-tailed deer generally do not begin to reproductively senesce until after 10 years of age (Ditchkoff et al. 2011), our males may not have reached these detrimental effects of senescence. So how do older males produce offspring with greater *MHC-DRB* allelic distance? Since male white-tailed deer typically leave the female after copulation (DeYoung and Miller, 2011), they

generally do not make any parental investments towards their offspring. The association between male age and offspring *MHC-DRB* allelic distance is therefore most likely attributed to his sperm. It is possible that older males may be better at allocating more sperm into their ejaculate when breeding with MHC-dissimilar females. For example, Reinhold et al. (2002) found that males can divert more sperm in their ejaculate when breeding high quality females. MHC gene products of MHC-dissimilar females are detected via a male's vomeronasal sensory neurons (Leinders-Zufall et al. 2004; Santos et al. 2005; Boehm and Zufall, 2006), which may then stimulate the male to invest more sperm during copulation. This MHC signal could influence copulation duration and/or frequency. Longer copulation can improve a male's chances of successful fertilization, as a positive association between copula duration and ejaculate volume has been reported for sand lizards (*Lacerta agilis*; Olsson et al. 2004), Mallee dragons (*Ctenophorus fordi*; Olsson 2001), common garter snakes (*Thamnophis sirtalis parietalis*; Shine et al. 2000) and guppies (Pilastro et al. 2007). Likewise, breeding a female multiple times supplies the female with more sperm, thereby aiding in sperm competition and fertilization (Parker 1982). Older males may also be in better condition. Male white-tailed deer can lose up to 30% of their body fat during the breeding season (Hewitt 2011). Therefore, males in better condition can better absorb the costs associated with tending does, allowing him to successfully copulate and fertilize a receptive female. Lastly, several studies have found that mature spermatozoa express MHC II genes in humans (Fellous and Dausset, 1970; Bishara et al. 1987; Barbieri et al. 1990; Obashi et al. 1990; Scofield et al. 1992; Martin-Villa et al. 1996, 1999; Paradisi et al. 2000), though this topic used to be controversial (Rodriguez-Cordoba et al. 1990; Schaller et al. 1993). These protein markers on sperm cells may change with age, where sperm from older males may enhance cryptic female choice that favors greater MHC diversity in the zygote. However, these are all theoretical explanations for our results.

MHC-DOB allelic distance did not differ between 1) recruited offspring and all potential offspring given the available dams and sires (initial analysis), 2) successful breeding pairs and non-successful breeding pairs (pre-copulatory), and 3) recruited offspring and average *MHC-DOB* allelic distance of their parents (post-copulatory). Ivy-Israel et al. (2019a) found that there was an overall trend towards a heterozygote deficit for *MHC-DOB* during our period of study. While we did find that nucleotide *MHC-DOB* allelic distances were smaller for successfully recruited offspring than expected, it was not statistically significant. Since *MHC-DOB* allelic

distance is also positively associated with both male morphology and annual reproductive success (Ivy-Israel et al. 2019b), it is possible that retaining some polymorphism at this locus is favorable.

We found that successful breeding pairs had significantly fewer DRB*01 alleles between them compared to non-successful breeding pairs. Ivy-Israel et al. (2019b) reported that annual reproductive success of female white-tailed deer was significantly less for females heterozygous for DRB*01. We also found that both dams and sires from successful breeding pairs had significantly fewer DRB*01 alleles than dams and sires from non-successful breeding pairs, though this was only statistically significant for dams. There are several possible explanations for this result. First, DRB*01 gene products may alter a female's body odor so that she becomes less attractive to searching males. Females with DRB*01 may also suffer from greater parasitism, as greater levels of parasitism were found be associated with certain MHC alleles in Soay sheep (Paterson et al. 1998). Since parasitized individuals are often selected against as potential mates (Kavaliers and Colwell, 1992, 1995a, 1995b; Willis and Poulin, 2000), it may be that these females are at a disadvantage at the pre-copulatory stage. Specific MHC supertypes have also been found to negatively influence survival (Sepil et al. 2013), thereby further reducing her potential lifetime reproductive success.

In conclusion, this study found that our enclosed white-tailed deer population is successfully recruiting offspring that have greater *MHC-DRB* allelic distance than expected. There seems to be greater evidence of post-copulatory selection, suggesting that cryptic female choice, MHC-determined abortion, and/or fawn survival are mostly responsible for this difference in *MHC-DRB* allelic distance. Future studies should include several other variables in their analyses to further examine how MHC allelic distance influences pre- and post-copulatory selection in white-tailed deer, such as copulation records, fawn survival, and parasite data.

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TABLES

Table 4.1. Non-successful breeding pairs (n = 25,121) and successful breeding pairs (n = 167) in our enclosed white-tailed deer population from 2008 to 2015. Sire age, sire nucleotide *MHC-DRB* allelic distance, sire amino acid *MHC-DOB* allelic distance, and dam age were significantly greater for successful breeding pairs. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

	Non-successful breeding pairs		Successful breeding pairs	
	Dam	Sire	Dam	Sire
Age	3.34 ± 2.51 **	2.66 ± 2.10 ***	3.97 ± 2.37 **	4.41 ± 1.88 ***
DRBnuc	22.77 ± 11.99	23.85 ± 13.95 *	23.19 ± 12.32	26.00 ± 13.02 *
DRBaa	15.03 ± 7.77	15.29 ± 8.63	15.44 ± 7.98	16.25 ± 7.80
DOBnuc	1.86 ± 1.28	1.63 ± 1.18	1.70 ± 1.20	1.70 ± 1.00
DOBaa	0.43 ± 0.53	0.33 ± 0.47 ***	0.41 ± 0.52	0.47 ± 0.50 ***

Table 4.2. Successfully recruited offspring had greater average *MHC-DRB* allelic distance than expected given all potential offspring, whereas there was no significant difference for *MHC-DOB*. Positive values for difference indicate that observed allelic distances were greater than expected allelic distances.

DRB_nuc			**DRB_aa**		
difference	SE	p	difference	SE	p
0.321	0.073	1.24E-05	0.317	0.073	1.57E-05
DOB_nuc			**DOB_aa**		
difference	SE	p	difference	SE	p
-0.082	0.073	0.262	0.071	0.073	0.333

Table 4.3. The difference between observed and expected *MHC-DRB* allelic distances increased with increasing sire and dam age at both the nucleotide and amino acid level. Positive values for difference indicate that observed allelic distances were greater than expected allelic distances.

	DRB_nuc			**DRB_aa**		
	difference	SE	p	difference	SE	p
Sire age	0.277	0.085	0.001	0.289	0.085	0.0006
Dam age	0.184	0.080	0.022	0.196	0.080	0.014

Table 4.4. There was no statistically significant difference in *MHC-DRB* or *MHC-DOB* allelic distance between successful and non-successful breeding pairs. Positive values for difference indicate that observed allelic distances were greater than expected allelic distances.

DRB_nuc			**DRB_aa**		
difference	SE	p	difference	SE	p
0.064	0.078	0.414	0.042	0.078	0.594
DOB_nuc			**DOB_aa**		
difference	SE	p	difference	SE	p
-0.043	0.078	0.579	0.019	0.078	0.809

Table 4.5. While successful breeding pairs had significantly fewer DRB*01 alleles than non-successful breeding pairs at the pre-copulatory stage, there was no difference between observed and expected DRB*01 frequency in successfully recruited offspring.

	difference	SE	p
Pre-copulatory	-0.130	0.048	0.006
Post-copulatory	-0.011	0.016	0.497

Table 4.6. Observed offspring had greater *MHC-DRB* allelic distance compared to the average MHC-DRB allelic distance given the *MHC-DRB* alleles of their parents. Positive values for difference indicate that observed allelic distances were greater than expected allelic distances.

DRB_nuc			**DRB_aa**		
difference	SE	p	difference	SE	p
0.136	0.069	0.051	0.148	0.069	0.034
DOB_nuc			**DOB_aa**		
difference	SE	p	difference	SE	p
-0.021	0.073	0.774	0.050	0.074	0.503

Table 4.7. The difference between observed and expected *MHC-DRB* allelic distances among successfully recruited offspring increased with increasing sire age at both the nucleotide and amino acid level. Positive values for difference indicate that observed allelic distances were greater than expected allelic distances.

	DRB_nuc			**DRB_aa**		
	difference	SE	p	difference	SE	p
Sire age	0.156	0.074	0.034	0.156	0.074	0.035
Dam age	0.056	0.074	0.451	0.066	0.074	0.371

FIGURES

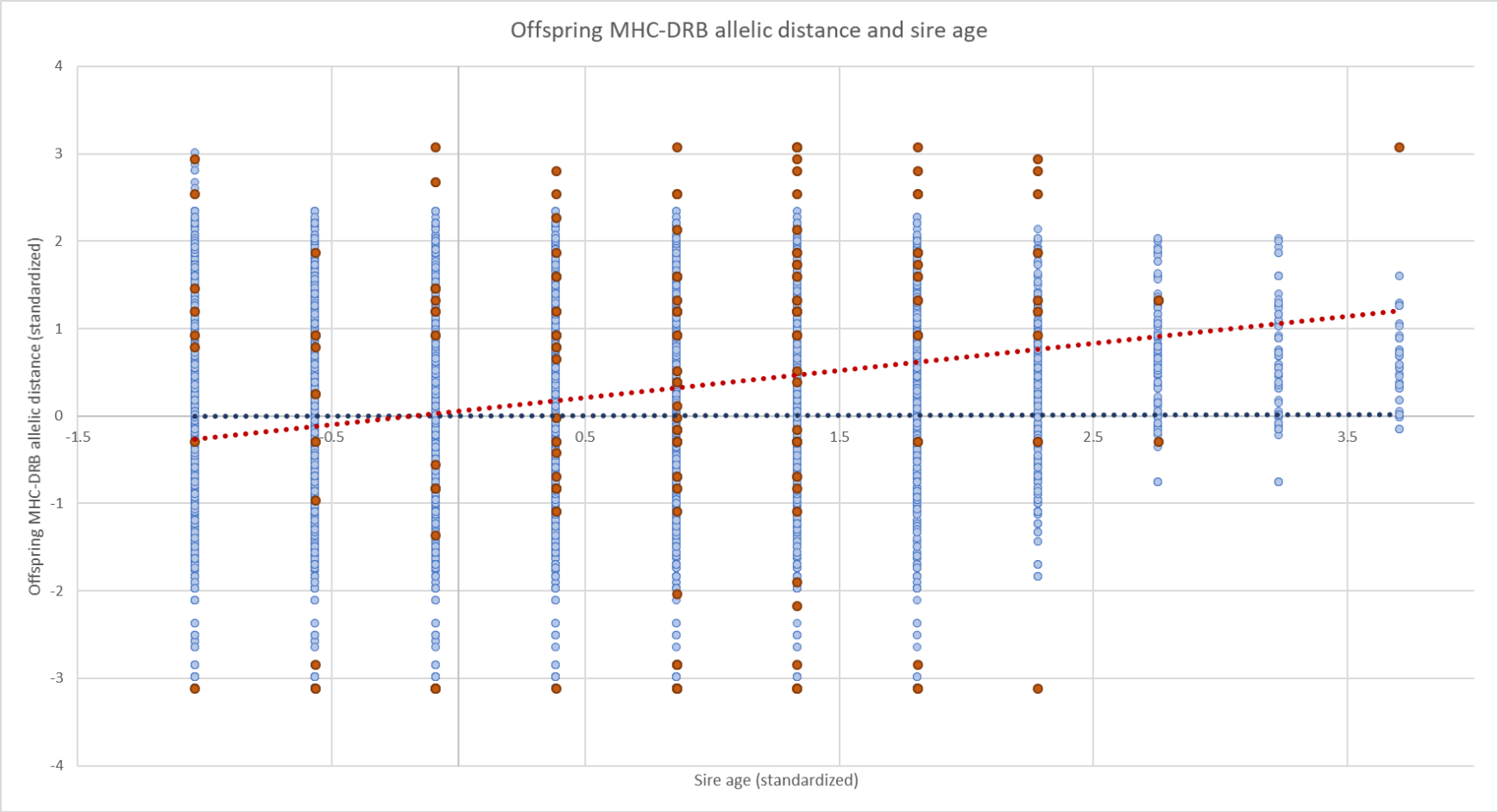

Figure 4.1. The difference between observed (red) and expected (blue) values of *MHC-DRB* allelic distance increases with increasing sire age. While expected values are not influenced by sire age (blue linear trendline), observed allelic distances become greater as sire age increases (red linear trendline). As this association is similar for nucleotide and amino acid *MHC-DRB* allelic distances, we are only showing this association for *MHC-DRB* allelic distances at the nucleotide level.

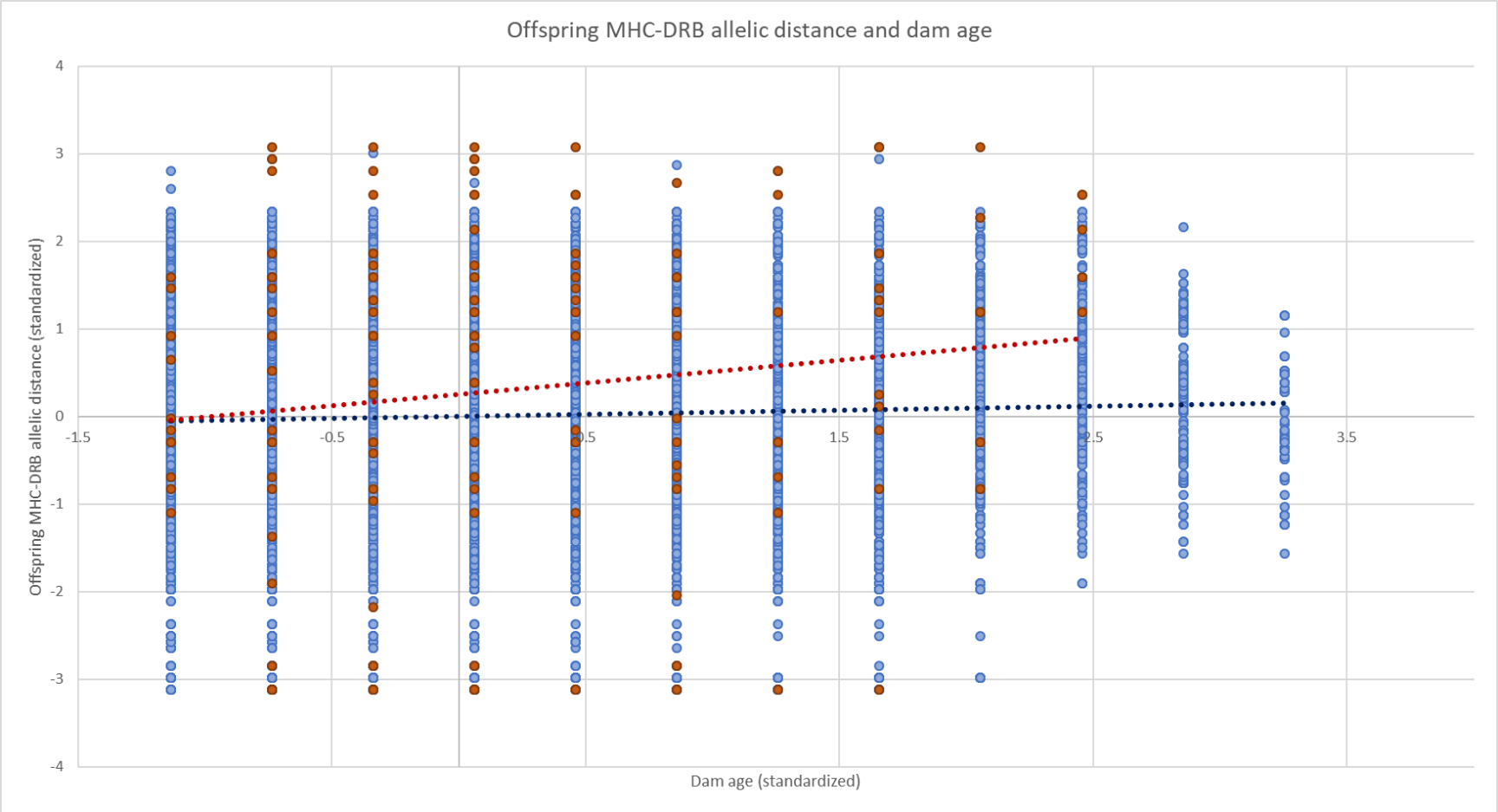

Figure 4.2. The difference between observed (red) and expected (blue) values of *MHC-DRB* allelic distance increases with increasing dam age. While expected values are only slightly influenced by dam age (blue linear trendline), observed allelic distances become greater as dam age increases (red linear trendline). As this association is similar for nucleotide and amino acid *MHC-DRB* allelic distances, we are only showing this association for *MHC-DRB* allelic distances at the nucleotide level.

Sire age influences post-copulatory selection on offspring MHC-DRB

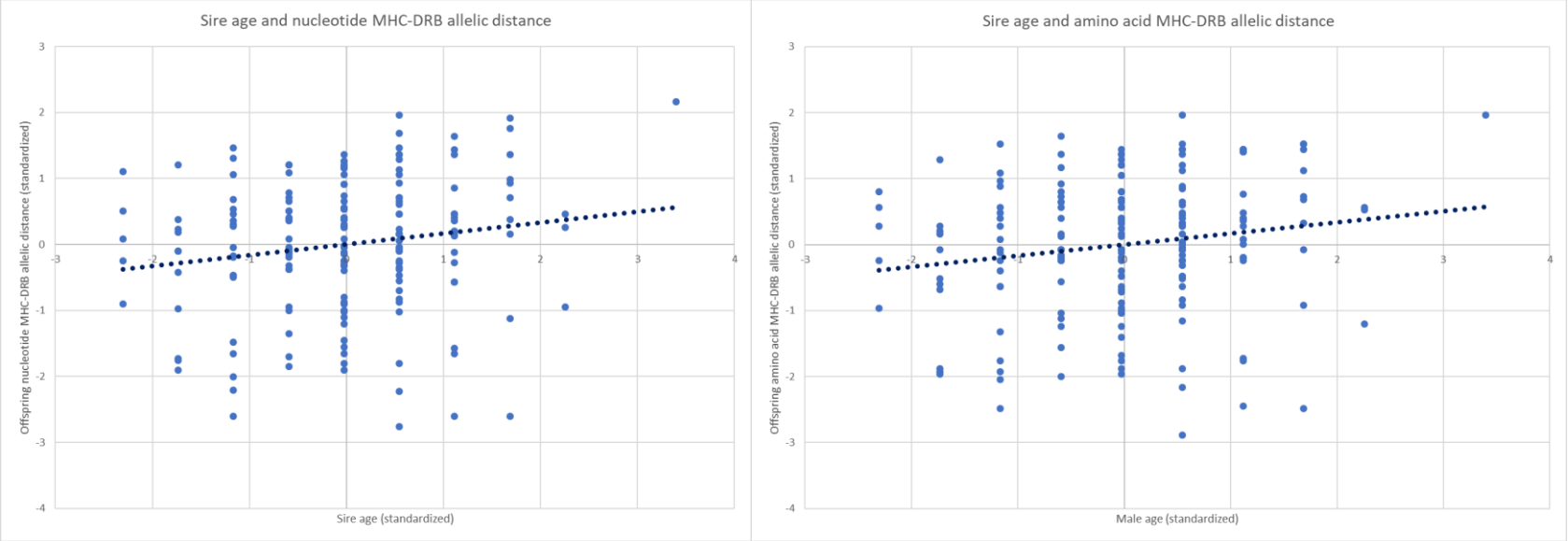

Figure 4.3. The *MHC-DRB* allelic distance of successfully recruited offspring become increasingly greater than expected average *MHC-DRB* allelic distance given their parents' *MHC-DRB* alleles with increasing sire age. As this association is similar for nucleotide and amino acid *MHC-DRB* allelic distances, we are only showing this association for *MHC-DRB* allelic distances at the nucleotide level.

Conclusion

We sequenced two genetically unlinked MHC II loci (*MHC-DRB* exon 2, antigen-binding protein; *MHC-DOB* exon 2, accessory protein) for our enclosed white-tailed deer population in Alabama. *MHC-DRB* was under positive diversifying selection while *MHC-DOB* was under purifying selection in white-tailed deer at the species level.

There was an excess of intermediate frequency *MHC-DRB* alleles in our population, which may suggest that *MHC-DRB* alleles are being maintained in the population via balancing selection. This retention of *MHC-DRB* alleles may be driven by selection for *MHC-DRB* heterozygous individuals, as we found significant heterozygote excess for *MHC-DRB* in our population. To better understand how balancing selection is being maintained in our population, we examined how *MHC-DRB* allelic distance is influencing fitness at different stages of life. We found that greater allelic distance at both the genomic region that contains *MHC-DRB* (nucleotide level) and *MHC-DRB* specifically (amino acid level) was selected for in the early stages of life (fertilization to 6 months post-partum). While *MHC-DRB* allelic distance did not influence white-tailed deer morphology, it was associated with male annual reproductive success (RS). Specifically, we found that allelic distance of the genomic region linked to *MHC-DRB* positively influenced male RS, whereas allelic distance at *MHC-DRB* specifically (at the amino acid level) negatively influenced male RS. This suggest that individuals with divergent *MHC-DRB* proteins have lower RS compared to individuals with more similar *MHC-DRB* proteins, which indicates that greater *MHC-DRB* allelic distance is no longer selected for once an individual reaches sexual maturity. Selection on *MHC-DRB* may therefore differ with age, where early life selection for greater diversity in the *MHC-DRB* protein may contribute to the *MHC-DRB* heterozygote excess present in our population. We also found that specific *MHC-DRB* alleles were associated with greater or lesser RS. Specifically, DRB*10, an *MHC-DRB* allele whose frequency is increasing over time, seems to be beneficial for male RS whereas DRB*01, an *MHC-DRB* allele whose frequency is decreasing over time, is detrimental to female RS. Moreover, we found that successful breeding pairs (i.e. breeding pairs that successfully recruited

an offspring into the population) had fewer DRB*01 alleles, which suggests that this *MHC-DRB* allele may influence RS at the pre-copulatory stage (mate selection). Sexual selection on particular alleles may therefore be driving the changing *MHC-DRB* allele frequencies seen at the population level.

MHC-DOB polymorphism was considerably lower than *MHC-DRB* polymorphism, which was expected for a conservative, non-classical MHC II gene. We found that our population became heterozygote deficient for *MHC-DOB* over time, suggesting that we either have selection away from heterozygote individuals in our population or effects of inbreeding at *MHC-DOB* due to small population size. The genomic region that contains *MHC-DOB* significantly influenced male morphology, where body size peaked at moderate nucleotide *MHC-DOB* allelic distance and antler size peaked at maximum nucleotide *MHC-DOB* allelic distance. This genomic region was also negatively associated with female RS, where females with less nucleotide *MHC-DOB* allelic distance had greater RS than females with greater nucleotide *MHC-DOB* allelic distance. While allelic distance at *MHC-DOB* exon 2 specifically (amino acid level) did not influence male morphology, it was weakly associated with male RS, where heterozygous males (amino acid *MHC-DOB* allelic distance = 1) had slightly greater RS than homozygous males (amino acid *MHC-DOB* allelic distance = 0). However, *MHC-DOB* allelic distance of successfully recruited offspring did not differ from expected *MHC-DOB*. Additionally, no specific *MHC-DOB* alleles were associated with male or female RS. Together, this may suggest that the heterozygote deficit found at the population level at the *MHC-DOB* exon 2 locus may be due to genetic drift and/or inbreeding.

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Appendix 1: Are the most common *MHC-DRB* exon 2 alleles in our population also most different compared to other *MHC-DRB* alleles found in our population?

We found that the five most common *MHC-DRB* exon 2 alleles (DRB*10, DRB*14, DRB*16, DRB*19, DRB*20) occurred on different clades in our nucleotide *MHC-DRB* phylogenetic tree (see Chapter 1). To assess whether the allelic distance between these five common *MHC-DRB* alleles was significantly different from the allelic distance between all *MHC-DRB* alleles found in our population (DRB*01, DRB*05, DRB*06, DRB*10, DRB*12, DRB*14, DRB*16, DRB*19 - DRB*30) , we performed a Wilcoxon Rank Sum test in Program R (v3.6.0; wilcox.test function; R Core Team, 2019). We found a significant difference in allelic distance between the five common *MHC-DRB* alleles and all *MHC-DRB* alleles at both the nucleotide ($W = 1272$; $p = 0.01$) and amino acid level ($W = 1175.5$; $p = 0.005$). The mean and median nucleotide allelic distance for the common *MHC-DRB* alleles were 33.6 and 35, respectively, whereas the mean and median nucleotide allelic distance for all *MHC-DRB* alleles were 25.9 and 25, respectively (Figure A1.1). Amino acid *MHC-DRB* allelic distance was also greater for the common *MHC-DRB* alleles compared to all *MHC-DRB* alleles (Figure A1.2). Mean and median amino acid allelic distance were 21.3 and 23 for the common *MHC-DRB* alleles, respectively, whereas mean and median amino acid allelic distance were 16.3 and 16 for all *MHC-DRB* alleles, respectively. This suggests that the most common *MHC-DRB* alleles in our population are also more divergent compared to the other *MHC-DRB* alleles found in our white-tailed deer population.

Given that there is also balancing selection for *MHC-DRB* in our population, we may specifically have balancing selection for the most distant *MHC-DRB* alleles rather than balancing selection for just different *MHC-DRB* alleles.

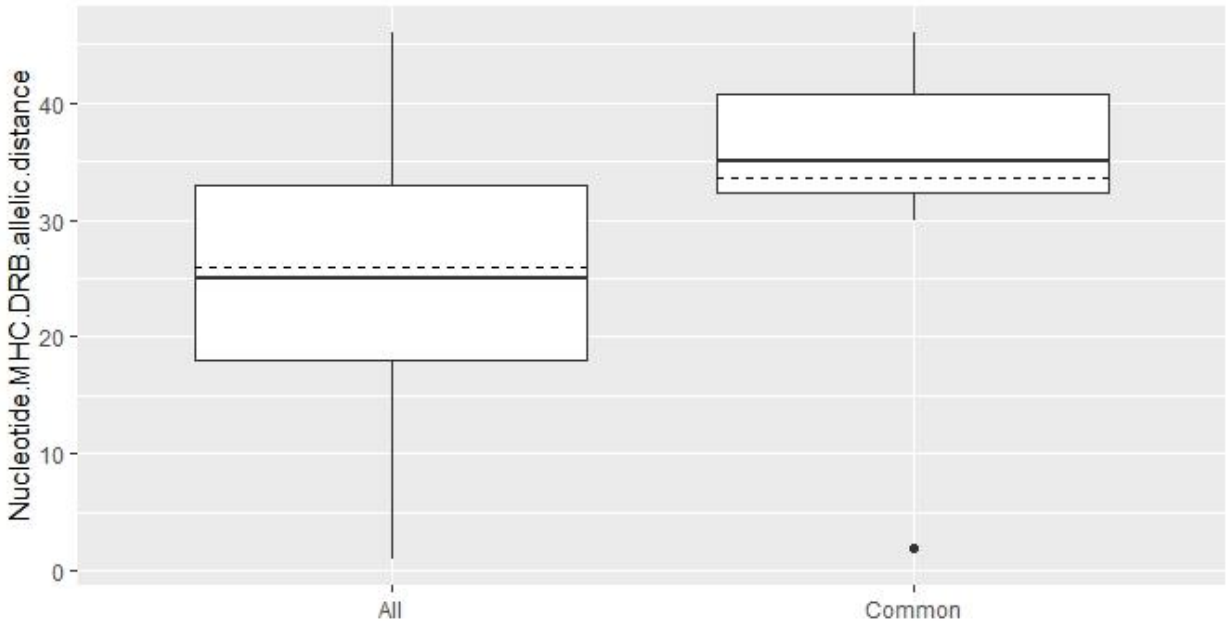

Figure A1.1. Nucleotide *MHC-DRB* allelic distance was greater among the five most common *MHC-DRB* alleles in our population compared to *MHC-DRB* allelic distance of all *MHC-DRB* alleles present in our population. The dashed horizontal lines indicate the mean allelic distance values, while the solid black horizontal lines indicate median allelic distance.

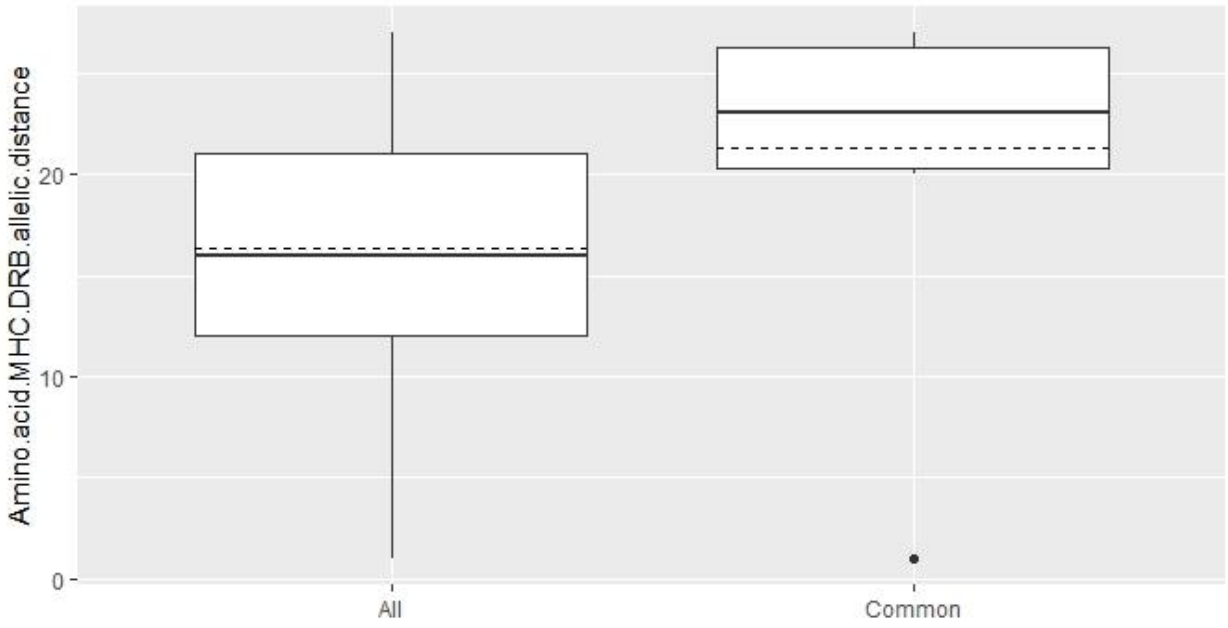

Figure A2.1. Amino acid *MHC-DRB* allelic distance was greater among the five most common *MHC-DRB* alleles in our population compared to *MHC-DRB* allelic distance of all *MHC-DRB* alleles present in our population. The dashed horizontal lines indicate the mean allelic distance values, while the solid black horizontal lines indicate median allelic distance.

Appendix 2: Do older individuals in our white-tailed deer population have more rare *MHC-DRB* exon 2 alleles than younger individuals?

The frequency-dependent hypothesis states that individuals with rare MHC alleles are better able to fight off pathogens due to host-parasite dynamics (Sommer 2005). Pathogens generally adapt to infect the most common host genotypes while leaving the more rare alleles alone (Lively and Dybdahl, 2000). When the rare alleles become more common in the host population, however, parasite antigenicity will change accordingly so that it can adapt to the new common allele of their hosts (Sommer 2005). Some studies suggest that this pathogen-driven selection should lead to varying spatiotemporal selection (Nevo and Beiles, 1992; Hedrick 2002; Hedrick 2004). For instance, while one particular host MHC allele is favored at one time or in one habitat, it may be selected against at another time or in another environment. We found that several *MHC-DRB* alleles are becoming less frequent in our population over time (chapter 1). One of these *MHC-DRB* alleles, DRB*01, was negatively associated with female annual reproductive success (RS; chapter 3) and was selected against at the pre-copulatory level (especially among the dams of the successful breeding pairs; chapter 4). It is possible that DRB*01 and other rare *MHC-DRB* alleles were favored in our founding population (2007) but are now no longer selected for due to pathogen-host coevolution. If this were true, we would find that older individuals, especially females due to our previous findings, are more likely to have *MHC-DRB* alleles that are now considered to be rare and/or decreasing in frequency. We therefore examined if there was an association between age and the frequencies of their *MHC-DRB* alleles for male and female white-tailed deer.

We used a mixed-effects model in Program R (lme function; Pinheiro et al. 2018), where our response variable was the summed frequency of an individual's two *MHC-DRB* alleles (standardized). Individuals with two common (high frequency) *MHC-DRB* alleles would therefore have a greater value for the response variable than an individual with two rare (low

frequency) *MHC-DRB* alleles. Our independent variable was the pairwise allelic distance (standardized) between *MHC-DRB* alleles present in our white-tailed deer population. Since some individuals were captured more than once in their lifetime, our model included a random term for individual. Box and whisker plots were created in Program R (ggplot2; Wickham 2016) to demonstrate potential differences in *MHC-DRB* allele frequency between young (1.5-2.5 years old), adult (3.5-4.5 years old), and old (5.5+ years) individuals.

Summed *MHC-DRB* allele frequency ranged from 5.9 to 44.78% for females, and from 5.5 to 44.78% for males. Male age was not associated with the summed *MHC-DRB* allele frequency ($p = 0.266$). However, there was a strong negative association between female age and allele frequency ($p = 0.003$). Specifically, we found that for each 1-year increase in female age, the summed allele frequency decreased by 0.20 (± 0.11; ± 95% CI) standard deviations. Older females therefore have more rare alleles than young females in our population, which may suggest that there was a recent shift in favorable *MHC-DRB* alleles due to the shifting pathogen antigenicity. Additionally, older females may be contributing to the balancing selection on *MHC-DRB* in our population by maintaining rare *MHC-DRB* alleles over time.

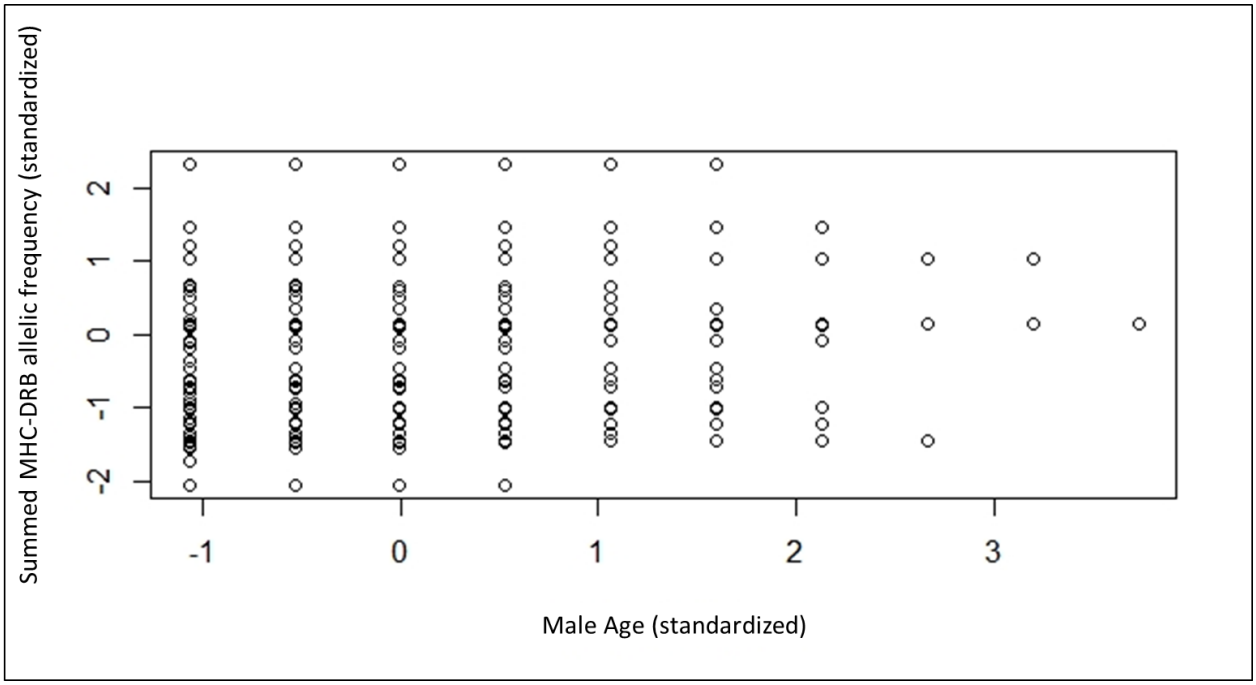

Figure A2.1. We found no association between male age and summed *MHC-DRB* allelic frequency. Older males therefore do not have more rare alleles than younger males.

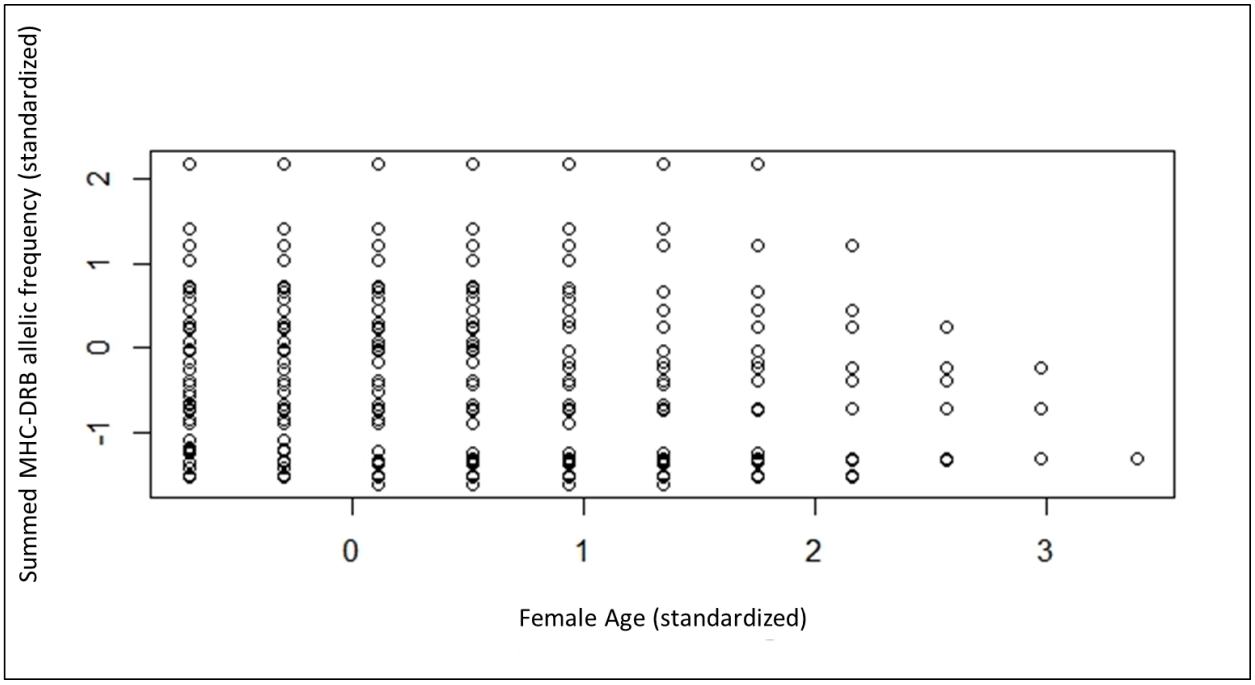

Figure A2.2. Female age was negatively associated with summed *MHC-DRB* allelic frequency. Older females therefore have more rare alleles than younger females.

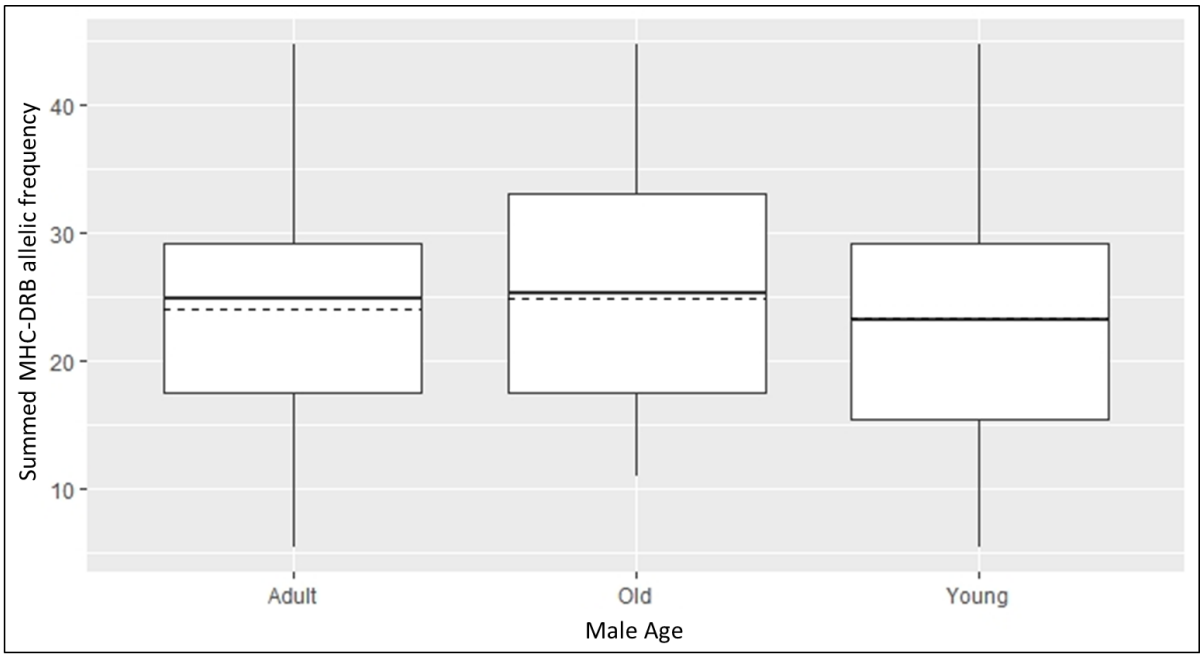

Figure A2.3. Older males (5.5+ years) do not have a lower summed *MHC-DRB* allelic frequency compared to young (1.5-2.5 years) or adult (3.5-4.5 years) male white-tailed deer in our population. The dashed horizontal lines indicate the mean allelic frequency, while the solid black horizontal lines indicate median allelic frequency.

Figure A2.4. Older females (5.5+ years) have a lower summed *MHC-DRB* allelic frequency compared to young (1.5-2.5 years) or adult (3.5-4.5 years) female white-tailed deer in our population. Older females therefore have more rare *MHC-DRB* alleles. The dashed horizontal lines indicate the mean allelic frequency, while the solid black horizontal lines indicate median allelic frequency.