Reproductive success in white-tailed deer: using structural equation modeling to understand the causal relationships of MHC, age, and morphology

by

Carolyn Elizabeth Moore

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Approved by

Stephen S. Ditchkoff, Chair, Professor of Wildlife Sciences, Auburn University
Scott R. Santos, Professor and Chair of Biological Sciences, Auburn University
Todd D. Steury, Associate Professor of Wildlife Sciences, Auburn University

Abstract

Understanding factors that influence reproductive success is of utmost importance to the study of wildlife population dynamics. There are a multitude of factors that can influence a male's ability to acquire mates, such as morphology, age, or genetic components, all of which have the potential to also influence one another. One genetic component of interest is the major histocompatibility complex (MHC). MHC genetic diversity has been linked to differences in physiology, morphology, and secondary sex characteristic production. White-tailed deer (*Odocoileus virginianus*) are an ideal model species for evaluating influences on reproductive success because they are a well-studied game species, they possess an easily measured secondary sex characteristic, and because of their high genetic diversity for MHC. Before we could begin analysis, we first characterized the *MHC-DRB* alleles present in our population. We then used structural equation modeling to examine potential causal relationships between MHC genetic diversity, morphology, age, and annual reproductive success for male white-tailed deer.

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List of Abbreviations

ACF	Auburn Captive Facility
AICc	Akaike's Information Criterion corrected for small sample size
Alal	*Alces alces,* moose
Caca	*Capreolus capreolus,* roe deer
Ceel	*Cervus elaphus,* European red deer
Ceni	*Cervus nippon,* sika deer
Dada	*Dama dama,* fallow deer
Edav	*Elaphurus davidianus,* Père David's deer
MHC	Major Histocompatibility Complex
Mucr	*Muntiacus crinifrons,* black muntjac
Mure	*Muntiacus reevesi,* Reeves's muntjac
Odvi	*Odocoileus virginianus,* white-tailed deer
Rata	*Rangifer tarandus,* reindeer
SEM	structural equation modeling

Chapter 1: Characterization of *MHC-DRB* allelic diversity in an Alabama population of white-tailed deer

INTRODUCTION

The major histocompatibility complex (MHC) remains one of the most studied regions in vertebrate genomes (Hedrick 1994, Kamiya et al. 2014). The MHC is a genetic complex composed of many genes comprised into three different classes: Class I, Class II, and Class III. Each MHC gene encodes a portion of membrane-bound molecules, which bind to and present foreign peptides to T-cells of the immune system (Schook and Lamont 1996). Specifically, molecules encoded from Class I genes are primarily involved in the presentation of intracellularly-derived peptides to cytotoxic T-cells, while molecules encoded from Class II genes present processed peptides derived from extracellular antigens to helper T-cells (Amills et al. 1998). This process allows MHC molecules to play an important role in both intracellular and extracellular pathogen recognition and subsequent immune response.

The MHC is found in all vertebrate species with consistent patterns withstanding evolutionary time, geographic space, and species (Srivastava et al. 1991, Urban and Chicz 1996, Apanius et al. 1997, Yeager and Hughes 1999, Danchin et al. 2004). The pattern with arguably the greatest importance and interest is the consistent degree of genetic diversity. Generally, MHC genes possess high levels of polymorphism (Doherty and Zinkernagel 1975, Amills et al. 1998), indicating there are multiple physical forms of a gene (alleles) available within a species. This is of most interest when the allelic polymorphism results in differences in nucleotides in regions that encode for peptide binding regions on the MHC molecule (Yeager and Hughes 1999), potentially altering the ability of a molecule in its role in mounting an immune response. Finally, this high polymorphism is often distributed at relatively equivalent frequencies across

populations within a species (Klein et al. 1990, Van Den Bussche et al. 2002). Therefore, one would expect to find high polymorphism when characterizing unstudied populations for any vertebrate species.

Alleles within MHC genes are codominantly expressed (Srivastava et al. 1991). High polymorphism within a gene translates to an array of allele combinations that a single individual could possess. Therefore, populations generally display high levels of individual heterozygosity for an MHC gene (Mitton et al. 1993). An individual that is heterozygous for an MHC gene has the potential for a genetic advantage over a homozygote within the same population (Brown 1998, Worley et al. 2010, Osborne et al. 2015). The heterozygote advantage hypothesis describes how individuals that are heterozygotes for codominant genes, like the MHC, have the potential to ward off a greater array of pathogens, thus providing a fitness advantage (Brown 1998). The heterozygote advantage hypothesis states that a heterozygous individual is able to respond to two suites of pathogens, one for each allele, while a homozygous individual would only be able to respond to one suit of pathogens (Schwensow et al. 2007). Research conducted by Doherty and Zinkernagel (1975) showed that molecules encoded from MHC alleles did differ in their ability to bind and present specific foreign peptides to the immune system, thereby altering the response to each foreign peptide. Therefore, a heterozygous individual could respond to more forms of foreign peptides and be resistant to a greater range of pathogens than a homozygous individual (Hughes et al. 1994).

To fully understand the role of the MHC at the individual level, we need to fully understand the genetic variation and characteristics present within a population. MHC associations have been studied extensively in humans (Klein et al. 1990), mice (Doherty and Zinkernagel 1975, Potts and Wakeland 1993, Potts et al. 1994), and livestock (Groenen et al.

1989; 1990, Van der Poel et al. 1990, Marello et al. 1995, Ballingall et al. 2010), because of the direct and indirect influence these studies have on human health and food. However, in order to fully understand the role of the MHC, there is a need to expand our understanding of the MHC to non-model species where less is known about the MHC structure and its ecological associations (Edwards et al. 1995, Piertney and Oliver 2006). MHC genes within the free-ranging species of the families Cervidae and Bovidae began to be sequenced within the last 20 years, particularly Class II MHC genes. The most widely studied gene for these families is the gene DRB, a Class II gene, which encodes for the beta-chain of the DR molecule (Amills et al. 1998, Yeager and Hughes 1999). Polymorphism within the DRB gene is particularly concentrated to the second exon, which is where the variable peptide binding sites are encoded (Amills et al. 1998).

Previous studies of the DRB gene in the family Cervidae showed high polymorphism in most of the studied species (Swarbrick et al. 1995, Van Den Bussche et al. 2002, Fernandez-de-Mera et al. 2009, DeYoung and Miller 2011, Wan et al. 2011, Li et al. 2013). The MHC Class II DRB gene is highly polymorphic in white-tailed deer (*Odocoileus virginianus*), the most widespread Cervidae species in North America. Eighteen alleles have been identified in white-tailed deer to date and are divided into two lineages (Van Den Bussche et al. 2002), which share sequence similarity with DRB alleles for red deer (*Cervus elaphus*) (Van Den Bussche et al. 1999, Van Den Bussche et al. 2002). However, previous studies were limited in sampling locations (Oklahoma, Iowa, Tennessee, and New York) and do not represent the whole geographic range of the species (Van Den Bussche et al. 1999, Ditchkoff et al. 2001a, Van Den Bussche et al. 2002, Ditchkoff et al. 2005). Therefore, it is suspected that the currently characterized alleles only represent a subset of all possible alleles for the species across their geographic range (Van Den Bussche et al. 1999, Van Den Bussche et al. 2002).

This research aims to build on earlier research conducted on Cervidae species, by expanding our understanding of the MHC in white-tailed deer. Specifically, the objective of this research was to examine the *MHC-DRB* allelic structure in a natural population of white-tailed deer in central Alabama, and if discovered, document uncharacterized alleles for white-tailed deer. All prior studies of the MHC in white-tailed deer used samples collected from harvested deer in only a subset of their full geographic range. This research will utilize a closed population of deer in a natural setting in central Alabama. This region of the geographic range of white-tailed deer has yet to be sampled for relationships in the MHC. Additionally, natural study populations such as this are uncommon and valuable because they provide the full spectrum of ecological processes and subsequent relationships (Edwards et al. 1995, Piertney and Oliver 2006).

METHODS

Study Area

The white-tailed deer analyzed in this study were residents of the Auburn Captive Facility (ACF), which was a 174-hectare plot of land enclosed by a 2.5-meter tall steel fence within the Piedmont Agricultural Experiment Station. The property was north of the town of Camp Hill, Alabama, and owned by Auburn University. The Auburn Captive Facility was established to study white-tailed deer and white-tailed deer management and was designed for long-term studies of mate selection and white-tailed deer genetics. The high fence was erected in October of 2007; all deer within ACF were wild deer that inhabited the land upon completion of the fence and their subsequent progeny. No outside deer were introduced to the herd. The herd was maintained at a population of 100 to 120 adult deer, and the population within ACF was not subject to hunting. The population size was regulated through natural mortality, capture related

mortalities, and selective removal of fawns by releasing the fawns outside the high fence. Outside of capture periods (mentioned later), deer were left to their natural behavior: they could roam, feed, and mate freely within the fenced area.

The habitat at ACF was approximately 40% open grass fields and 60% mixed forest, with a large creek providing a consistent water source. The mixed forest habitat was predominantly thick closed canopy with little understory growth. Approximately 70% of the mixed forest was made up of various hardwoods including oak (*Quercus* spp.), hickory (*Carya* spp.), and maple (*Acer* spp.), approximately 20% conifer primarily loblolly pine (*Pinus taeda*), and 10% regenerated thickets of *Rubus* spp., including sweetgum (*Liquidambar styraciflua*), eastern red cedar (*Juniperus virginiana*), and Chinese privet (*Ligustrun sinense*). To supplement the nutrition of the herd, two 0.4-hectare food plots were planted each spring and fall with various cool and warm season forages. Supplemental feed (18% protein pellets; "Deer Feed," SouthFresh Feeds, Demopolis, Alabama, USA) was also provided ad libitum year-round via three feeders. Finally, to attract deer for capture events, three additional feeders were deployed during the months of October through March. These feeders were on a timer to deploy approximately 2 kg of whole corn each day.

Capture Techniques

White-tailed deer were captured during the months of October through March for the years 2007 through 2013. Deer were captured by administering a sedative mixture into the muscle of the hindquarter (Miller et al. 2004). The sedative was a combination of Telazol® (Fort Dodge Animal Health, Fort Dodge, Iowa, USA) 125mg/ml given at a rate of 4.5 mg/kg and Xylazine (Lloyd Laboratories, Shenandoah, Iowa, USA) 100mg/ml given at a rate of 2.2 mg/kg (Miller et al. 2003, Miller et al. 2004). This mixture was loaded into a telemetry dart (2.0 cc, type

C, Pneu-Dart Inc., Williamsport, Pennsylvania, USA), which was shot using a Cartridge Fired Rifle (Pneu-Dart model 193) and a .22 caliber blank (Kilpatrick et al. 1996). Deer were captured in this manner at both protein and corn feeders. The dart contained a radio transmitter, which was utilized to locate the sedated deer via radio telemetry. Data collection was conducted while the deer remained in this immobilized state. While data were being collected as part of other research (Neuman et al. 2016, Newbolt et al. 2017), a tissue sample was collected. A 1-cm^2 section of tissue was removed from an ear by a notching tool and was stored in a small vile in -80° C freezer until analysis could be performed in a controlled laboratory setting. Freezing is a recommended approach to preserving and long term storage of tissue samples (Shabihkhani et al. 2014). Tissue samples were used to extract DNA for MHC genotyping. Once data collection was complete, the deer received Tolazine (1.5 mL/45.36 kg) to reverse the sedation (Miller et al. 2004). Tolazine was administered half in the muscle around the shoulder and half in the muscle around the hindquarter.

MHC Analysis

I utilized DNA sequencing to analyze the major histocompatibility complex (MHC) Class II gene DRB. DNA was extracted from the frozen tissue samples following a modification of the methods established by Coffroth et al. (1992) which utilized CTAB (hexadecyltrimethyl ammonium bromide) for isolating the DNA of Symbiodinium. See Appendix 1.1 for the full protocol. Successful DNA extraction was verified using gel electrophoresis to detect the presence or absences of a band representing a successful extraction of DNA.

After DNA was successfully extracted, samples were sent to RTL Genomics (Lubbock, Texas, USA) to perform the polymerase chain reaction (PCR) to isolate and amplify the DRB gene, in preparation for sequencing using MiSeq (Mullis et al. 1986). Primers for PCR were

designed from primers used by Sigurdardottir et al. (1991) from conserved regions of cattle MHC sequences. After PCR, RTL Genomics sequenced each sample using the Illumina MiSeq platform for a minimum of 10,000 reads per individual (Illumina, Inc., San Diego, California, USA). RTL Genomics also conducted initial bioinformatics analysis on the sequenced data to differentiate the alleles present in each individual sample. RTL Genomics completed a filtration process where the reads were first trimmed with Trimmomatic V0.36 with a Q25 cutoff. Second, the reads were merged together using PEAR V0.96, with any unassembled contigs being discarded. Next, any merged contigs less than 290 base pairs or contigs that were only sequenced once were discarded. Finally, MHC loci that were out of the reading frame were also discarded by RTL Genomics.

Before determining the alleles present for each individual, the four most prevalent sequences were analyzed in each deer to determine if the individual was a heterozygote or homozygote. If the most prevalent sequence occurred less than 50 times then the individual was cut from the analysis. A ratio was created between the two most frequent sequences in the individual. If that ratio was 99:1, then the individual was called a true homozygote, and the less common sequences found in the individual were deemed as noise from the sequencing process. If sequencing noise did not exist, a heterozygote individual's sequences would show a ratio of 50:50. To account for sequencing noise and the potential for primers to adhere to certain alleles more readily than others, we set a limit for heterozygotes to be at a ratio between 50:50 and 65:35. Individuals with ratios that did not suit the criteria for either homozygote or heterozygote were removed from the total analyzed population.

The two most prevalent sequences for each deer were compared to the known 18 alleles for white-tailed deer *MHC-DRB* gene (Van Den Bussche et al. 2002) using the program

Geneious v11.1.5 (Kearse et al. 2012) to determine the exact alleles present in the individual. If an allele did not correspond to one of the known 18 alleles, it was analyzed further and compared to other unclassified alleles to determine if the allele in question was a new allele for the white-tailed deer DRB gene. For an allele to be considered a new DRB allele it had to be observed in at least two different individuals. Allele and genotype frequencies, and expected and observed heterozygotes in the population were calculated using GenePop (web version 4.2; Option 5, sub-option 1; (Raymond and Rousset 1995, Rousset 2008)). Alleles characterized in this study that were previously undocumented for the DRB gene in white-tailed deer were published in GenBank. All alleles presented follow the nomenclature proposed by Klein et al. (1990).

To validate the accuracy of both MiSeq sequencing and my ability to correctly identify alleles, ten samples were analyzed twice and four samples were analyzed three times. After successful DNA extraction, ten samples were selected to represent a male and female individual from deer born in the years 2006 through 2013 from the population. Four of the ten selected were randomly selected to be analyzed a third time. Each duplicate/triplicate sample was analyzed by RTL Genomics and myself under a fake ID number. Only after the sample had gone through the entire process of PCR, MiSeq sequencing, initial bioinformatics, and allele identification were the duplicates/triplicates compared to their original sequences.

An additional comparison was conducted to previous Sanger sequencing data. Sanger sequencing was conducted on 100 deer in the population. Of that subset two homozygote individuals and five heterozygote individuals were compared to the alleles called by MiSeq sequencing. Because the alleles were not cloned and separated prior to Sanger sequencing, alleles were sequenced simultaneously for an individual resulting in mixed based reads in heterozygote individuals. To utilize these data, I compared the Sanger sequencing data to the

called alleles for an individual. When the Sanger sequence resulted in a mixed base, I determined if the mixed base contained the known bases found in the two known alleles (i.e. observed mixed base R in Sanger analysis compared to the two known alleles which showed an A for one allele and a G for the other allele at that base position resulting in a match).

The number of synonymous (d_S) and nonsynonymous substitutions (d_N) per site within all pairwise comparisons of alleles were estimated using MEGA Molecular Evolutionary Genetics Analysis v10.0.5 (Kumar et al. 2018). Calculations followed the Nei and Gojobori (1986) method by applying the Jukes and Cantor (1969) correction for multiple substitutions, all positions containing gaps and missing data were eliminated, and translation began on the second base pair following Van Den Bussche et al. (1999). Hardy Weinberg Exact Tests were calculated using GenePop using both the probability-test (web version 4.2; Option 1, sub-option 3; (Haldane 1954, Guo and Thompson 1992) and score test for either heterozygote excess or deficiency (web version 4.2; Option 1, sub-option 1 and 2 respectively; (Raymond and Rousset 1995). All Hardy Weinberg Exact Tests used GenePop default settings (dememorization number = 1000, number of batches = 100, number of iterations per batch = 1000). Gene diversity, nucleotide diversity (π), and their subsequent variances were calculated in DnaSP version 6.12.03 (Rozas et al. 2017), using the equations established by Nei (1987) and Nei and Miller (1990).

All alleles characterized in the study were aligned using a global alignment with free end gaps and using the default cost matrix in Geneious v11.1.5 (Kearse et al. 2012). From the alignment, I calculated a pairwise comparison matrix table to calculate the number of nucleotide differences between each allele combination. The alignment was then translated to show the amino acid sequence for each allele. Translation used the standard genetic code and began on the

second base pair (Van Den Bussche et al. 1999). A second pairwise comparison matrix table was calculated to show the number of amino acid differences between each allele combination. Both were analyzed to understand if changes in the base nucleotide structure influenced differences in the functional amino acids and the subsequent differences between alleles.

A neighbor-joining gene tree was constructed to examine relationships between new, undocumented white-tailed deer *MHC-DRB* alleles and to observe the relationship of white-tailed deer DRB within family Cervidae. The tree was constructed using all known cervid *MHC-DRB* alleles published in GenBank. Species analyzed included white-tailed deer, moose (*Alces alces*), roe deer (*Capreolus capreolus*), European red deer (*Cervus elaphus*), sika deer (*Cervus nippon*), fallow deer (*Dama dama*), Père David's deer (*Elaphurus davidianus*), black muntjac (*Muntiacus crinifrons*), Reeves's muntjac (*Muntiacus reevesi*), and reindeer (*Rangifer tarandus*). See Appendix 1.2 for corresponding GenBank accession numbers. The gene tree was constructed in Geneious v11.1.5 using the Geneious Tree Builder neighbor-joining method using the Jukes-Cantor model without an outgroup, by using a global alignment with free end gaps and the default cost matrix (Kearse et al. 2012).

RESULTS

From 2007 to 2013, 280 white-tailed deer were captured for analysis. Of the 280, 277 were used to characterize the *MHC-DRB* gene in the population. Of the three samples not characterized, we were unable to successfully extract DNA from one, and the remaining two samples each presented one unique allele that was insufficient to be described as a new allele because of its infrequency in the population. All alleles run in duplicates and triplicates matched the alleles called for the original sample. Additionally, signatures in Sanger sequencing were

consistent with the alleles called using MiSeq sequencing for both homozygous and heterozygous individuals.

I identified 29 individuals as homozygous, and 196 individuals as heterozygotes, while 52 individuals did not adhere to our ratio requirements for heterozygote or homozygote and were subsequently removed from the analysis. This resulted in 225 deer, 110 males and 115 females (Table 1.1). GenePop calculated that the expected number of heterozygotes in the population was 199.87, while the observed number of heterozygotes was 196. The expected number of homozygotes was 25.13, while the observed number was 29.

Of the 18 previously known white-tailed deer DRB alleles (*Odvi-DRB*) (Van Den Bussche et al. 2002), we found 7 in this study population (*Odvi-DRB*01, Odvi-DRB*05, Odvi-DRB*06, Odvi-DRB*10, Odvi-DRB*12, Odvi-DRB*14,* and *Odvi-DRB*16*). In addition, we characterized 11 new DRB alleles. The new 11 new *Odvi-DRB* sequences identified were deposited in GenBank under accession numbers MK952679 – MK952686, MK952688 – MK952690 as *Odvi-DRB*19 – Odvi-DRB*26, Odvi-DRB*28 – Odvi-DRB*30*. All alleles were 250 base pairs (bp) long. Allelic frequencies for the 18 alleles in the population ranged from 0.44% for *Odvi-DRB*28* to 22.22% for *Odvi-DRB*10* (Table 1.2). A total of 79 genotypes were observed in this population (Table 1.3), with the homozygote combination of allele *Odvi-DRB*10* observed the most in 13 individuals (5.8%).

Nucleotide polymorphism and their translated amino acid polymorphism was highly polymorphic with 34.0% of nucleotide and 50.6% of amino acid positions as polymorphic sites among the 29 alleles. The number of nonsynonymous substitutions per nonsynonymous site from averaging over all sequence pairs was d_N =0.1266, while the number of synonymous substitutions per synonymous site was d_S = 0.0508. Gene diversity was 0.871 and nucleotide

diversity (π) was 0.073. The population was under Hardy Weinberg equilibrium (probability test; p = 0.881 S.E. = 0.019).

The number of nucleotide differences between comparison alleles (Table 1.4) ranged from 1 bp (*Odvi-DRB*06* vs. *Odvi-DRB*22*) to 46 bp (*Odvi-DRB*14* vs. *Odvi-DRB*19*). The number of amino acid replacements between alleles ranged from 0 amino acids (O*dvi-DRB*06* vs. *Odvi-DRB*19*) to 27 out of 81 amino acids (*Odvi-DRB*06* vs. *Odvi-DRB*14*). Notably, a difference between alleles' nucleotides did not always result in a difference in amino acids between alleles.

Monophyletic relationships were found for moose (*Alal-DRB*), fallow deer (*Dada-DRB*) and reindeer (*Rata-DRB*) alleles (Figure 1.1). Twenty-six of the 29 white-tailed deer alleles formed three clades. The clade at the top of the gene tree included the same alleles from clade 1 in Van Den Bussche et al. (2002) with the addition of seven of the new alleles found in this study (*Odvi-DRB*20, Odvi-DRB *23, Odvi-DRB *24, Odvi-DRB *26, Odvi-DRB *28, Odvi-DRB *29,* and *Odvi-DRB *30*). The clade at the top-center of the tree included the same alleles from clade 2 in Van Den Bussche et al. (2002) with the exception of alleles *Odvi-DRB*02, Odvi-DRB*06, Odvi-DRB*07, Odvi-DRB*11,* and *Odvi-DRB*16,* and the addition of three of the new alleles found in this study (*Odvi-DRB*21, Odvi-DRB*22, and Odvi-DRB*25*). Alleles *Odvi-DRB*02, Odvi-DRB*07,* and *Odvi-DRB*16* previously found in clade 2 were rooted together in a different portion of the center section of the tree. Alleles *Odvi-DRB*06, Odvi-DRB*11,* and *Odvi-DRB*19* were rooted in different sections of the tree from the three clades.

DISCUSSION

When Van Den Bussche et al. (1999) first characterized the *MHC-DRB* gene in white-tailed deer they noted that the study was restricted to a single population in a species with a vast

geographic region and proposed that their findings only represented a fraction of the allelic diversity. By characterizing a new population from an uncharacterized region for the species, we were able to describe 11 new alleles for the DRB gene. The new alleles all contained the same base pair lengths to the previously described alleles. The new alleles ranged in the number of base pair differences from 1 base pair difference when comparing allele *Odvi-DRB*06* and *Odvi-DRB*19*, to 46 base pair differences when comparing allele *Odvi-DRB*14* to allele *Odvi-DRB*19*. When comparing all 29 alleles, both new and previously described alleles show a similar distribution of base pair substitutions. The DRB gene in white-tailed deer shows consistent regions of differentiation between alleles. Van Den Bussche et al. (1999) observed that these substitutions often result in amino acid substitutions in the protein binding regions, which has the potential to influence protein shape and subsequent function in an immune response.

We had anticipated finding new alleles in an uncharacterized population from a different geographic region, however, finding 11 new alleles was more than expected. This may be an artifact of utilizing more advanced sequencing techniques. Prior to this research, all MHC research on white-tailed deer used the technique single-strand confirmation polymorphism analysis (SSCP) on all samples, cloning unique conformations, and sequencing only the unique confirmations (Van Den Bussche et al. 1999, Ditchkoff et al. 2001a, Van Den Bussche et al. 2002, Ditchkoff et al. 2005). At the time of the first white-tailed deer MHC studies, sequencing was still a costly endeavor. Due to advances in technology, we were able to sequence each individual for comparison, potentially increasing our ability to detect new alleles. Another possible reason for the high number of new alleles detected in this population is due to the difference in pathogen pressure between this region and the previously studied regions.

Numerous studies document a trend of greater parasite richness and prevalence closer to and in the tropics, suggesting that diseases and parasites are more prevalent at lower latitudes (Schemske et al. 2009). Specifically with the MHC, Dionne et al. (2009) identified an increase in MHC diversity in Atlantic salmon (*Salmo salar*) as latitude decreased. Pathogen presence and intensity may vary across the geographic range for white-tailed deer, and selection for certain immune responses has the potential to be greater in different habitats.

By introducing new white-tailed deer DRB alleles and comparing more Cervidae species, we observed changes to the gene tree. The previous trees by Van Den Bussche et al. (1999) and (2002), indicate two clear clades for white-tailed deer *MHC-DRB* alleles, whereas our tree includes three clades with three remaining alleles (Figure 1.1). The clade at the top of the tree contained the same previously characterized white-tailed deer alleles (with the addition of seven new alleles) and identified a close relation to the same red deer alleles as found in clade 1 of Van Den Bussche et al. (2002). Interestingly, what was identified as clade 2 in Van Den Bussche et al. (1999) and (2002), was split into two separate clades in our analysis. Red deer alleles *Ceel-DRB*31* and *Ceel-DRB*46* were closely linked to alleles *Odvi-DRB*02, Odvi-DRB*07*, and *Odvi-DRB*16* and remained closely linked in our gene tree. These links between alleles from different species support the conclusion of Van Den Bussche et al. (1999) that many of the allelic lineages of the *MHC-DRB* gene predate the species, supporting trans-species persistence (Klein et al. 1990).

With the addition of our central Alabama population, the total number of known alleles for the *MHC-DRB* gene in white-tailed deer increased to 29. Our population exhibits 18 of the 29 now known alleles, showing a high level of allelic diversity consistent with previous studies of the *MHC-DRB* gene in white-tailed deer (Van Den Bussche et al. 1999, Ditchkoff et al. 2001a,

Van Den Bussche et al. 2002, Ditchkoff et al. 2005) and with other Cervidae species, red deer (Swarbrick et al. 1995, Fernandez-de-Mera et al. 2009), sika deer (Li et al. 2013), and Père David's deer (Wan et al. 2011). Previous studies of the DRB gene in Cervidae showed high polymorphism in most of the studied species, with the exception being low levels in moose. Low polymorphism in moose could be attributed to a historical bottleneck (Mikko and Andersson 1995) or due to their reduced exposure to parasites from their solitary existence at higher latitudes (Mainguy et al. 2007). Eleven alleles were not characterized in this population of white-tailed deer; this is consistent with Van Den Bussche et al. (2002), in which seven study populations contained 8 to 15 of the then 18 known alleles.

Variation in specific allele frequencies between populations is likely a product of the variation in pathogen stressors among environments and regions. White-tailed deer inhabit a large geographic range and as a result a wide variety of habitat types. Subsequently, the pathogens and various strains of pathogens that are important to any population are a function of habitat and region. Across the MHC literature, we find examples of specific MHC gene alleles correlated to heightened responses to specific pathogens that may provide a potential survival advantage to individuals with the allele. For example, Ditchkoff et al. (2005) reported that individuals with the homozygotic allele combination for clade 1 had a significantly lower abundance of the nematode *Haemonchus contortus,* and in Schwensow et al. (2007) the rare MHC supertype 7 was linked to an advantage against gastrointestinal parasite burden in fat-tailed dwarf lemurs (*Cheirogaleus medius*). Ditchkoff et al. (2005) also observed a potential tradeoff, when an individual contains a genotype specific to one pathogen defense they may be more vulnerable to another pathogen. However, if that pathogen is nonexistent or provides little

immune threat in one geographic region, the genotype and alleles associated with that defense will have little selective pressure to remain in the population.

Both gene diversity (0.871) and nucleotide diversity ($\pi = 0.073$) were high for our population of white-tailed deer. This indicated that the population consisted of a high number of alleles that are considerably divergent from one another (Van Den Bussche et al. 2002). This is consistent with the relatively high gene and nucleotide diversity in the seven study populations of white-tailed deer observed by Van Den Bussche et al. (2002) and some other Cervidae species such as red deer (Swarbrick et al. 1995) and sika deer (Li et al. 2013). However, this is not the case for moose nor reindeer. In our gene tree (Figure 1.1), reindeer exhibit a monophyletic relationship, and in our tree as well as Van Den Bussche et al. (1999) and (2002), moose also exhibit a monophyletic relationship. Both moose and reindeer live specifically at high latitudes in cold climates, while other Cervidae species like white-tailed deer are broadly distributed, thus increasing the species exposure to a greater variety of pathogens (Van Den Bussche et al. 2002, Li et al. 2013). Additionally, moose exhibit a solitary lifestyle, thus reducing the interaction and spread of pathogens within the population.

This population of white-tailed deer had very little differentiation between observed and expected heterozygosity and was found to be in Hardy-Weinberg Equilibrium. This indicates that the DRB gene is not evolving in this population and that the frequencies of alleles and genotypes will remain the same over generations, given that environmental influences also remain constant. The five assumptions of the Hardy-Weinberg Equilibrium are that the population is at an infinite size, no migration occurs between neighboring populations, no mutation of alleles, random mating among members, and that alleles provide an equal fitness advantage resulting in no natural selection (Mayo 2008). While it is impossible to support an infinite population and

control the mutation and subsequent introduction of new alleles, this population is isolated by nature through the high fence's containment and does provide a situation without immigration/emigration opportunities. The roles of mate selection and natural selection of specific DRB alleles have yet to be determined and future analysis of this population comparing the generations could prove useful in understanding if the gene has evolved within the population.

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Table 1.1. The number of white-tailed deer (*Odocoileus virginianus*) analyzed from the Auburn Captive Facility, Camp Hill, Alabama study population by sex and year of birth.

Year	Males	Females
2003	0	4
2004	2	4
2005	0	4
2006	7	4
2007	6	12
2008	13	7
2009	13	16
2010	17	13
2011	16	17
2012	15	15
2013	21	19
Total	110	115

Table 1.2. Frequency of *MHC-DRB* alleles found in white-tailed deer (*Odocoileus virginianus*) at the Auburn Captive Facility, Camp Hill, Alabama study population (*Odvi-DRB*).

Previously Characterized Alleles	Frequency (n)	New Alleles	Frequency (n)
*Odvi-DRB*01*	0.0822 (37)	*Odvi-DRB*19*	0.0844 (38)
*Odvi-DRB*05*	0.0111 (5)	*Odvi-DRB*20*	0.1356 (61)
*Odvi-DRB*06*	0.0111 (5)	*Odvi-DRB*21*	0.0244 (11)
*Odvi-DRB*10*	0.2222 (100)	*Odvi-DRB*22*	0.0289 (13)
*Odvi-DRB*12*	0.0333 (15)	*Odvi-DRB*23*	0.0333 (15)
*Odvi-DRB*14*	0.1267 (57)	*Odvi-DRB*24*	0.0267 (12)
*Odvi-DRB*16*	0.0756 (34)	*Odvi-DRB*25*	0.0733 (33)
		*Odvi-DRB*26*	0.0089 (4)
		*Odvi-DRB*28*	0.0044 (2)
		*Odvi-DRB*29*	0.0089 (4)
		*Odvi-DRB*30*	0.0089 (4)

[a] Previously characterized alleles were published by Van Den Bussche et al. (2002).
[b] New alleles are alleles that were first found in this Auburn Captive Facility, Camp Hill, Alabama population.

Table 1.3. Frequency of genotypes observed in white-tailed deer (*Odocoileus virginianus*) at the Auburn Captive Facility, Camp Hill, Alabama study population (*Odvi-DRB*).

	Odvi DRB 01*	*Odvi DRB* 05*	*Odvi DRB* 06*	*Odvi DRB* 10*	*Odvi DRB* 12*	*Odvi DRB* 14*	*Odvi DRB* 16*	*Odvi DRB* 19*	*Odvi DRB* 20*	*Odvi DRB* 21*	*Odvi DRB* 22*	*Odvi DRB* 23*	*Odvi DRB* 24*	*Odvi DRB* 25*	*Odvi DRB* 26*	*Odvi DRB* 28*	*Odvi DRB* 29*	*Odvi DRB* 30*
*OdviDRB*01*	3																	
*OdviDRB*05*	0	0																
*OdviDRB*06*	0	0	0															
*OdviDRB*10*	8	3	1	13														
*OdviDRB*12*	1	0	0	4	0													
*OdviDRB*14*	3	0	0	9	1	6												
*OdviDRB*16*	4	0	0	8	0	1	1											
*OdviDRB*19*	3	0	0	3	3	7	5	1										
*OdviDRB*20*	1	1	2	12	3	11	6	4	5									
*OdviDRB*21*	2	0	1	1	0	2	1	2	2	0								
*OdviDRB*22*	1	0	0	3	1	1	2	1	1	0	0							
*OdviDRB*23*	3	1	0	3	0	3	1	1	0	0	1	0						
*OdviDRB*24*	2	0	0	4	0	3	0	0	1	0	0	1	0					
*OdviDRB*25*	2	0	1	10	2	2	2	4	6	0	2	1	1	0				
*OdviDRB*26*	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0			
*OdviDRB*28*	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0		
*OdviDRB*29*	0	0	0	2	0	1	0	1	0	0	0	0	0	0	0	0	0	
*OdviDRB*30*	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0

Table 1.4. Pairwise comparison matrix among 29 MHC *Odvi-DRB* allele sequences from white-tailed deer (*Odocoileus virginianus)*; values above the diagonal are the number of nucleotide differences between alleles' sequences, while values below the diagonal are the number of amino acid differences between each comparison.

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

[a] Matrix table was calculated in program Geneious v11.1.5 (Kearse et al. 2012)

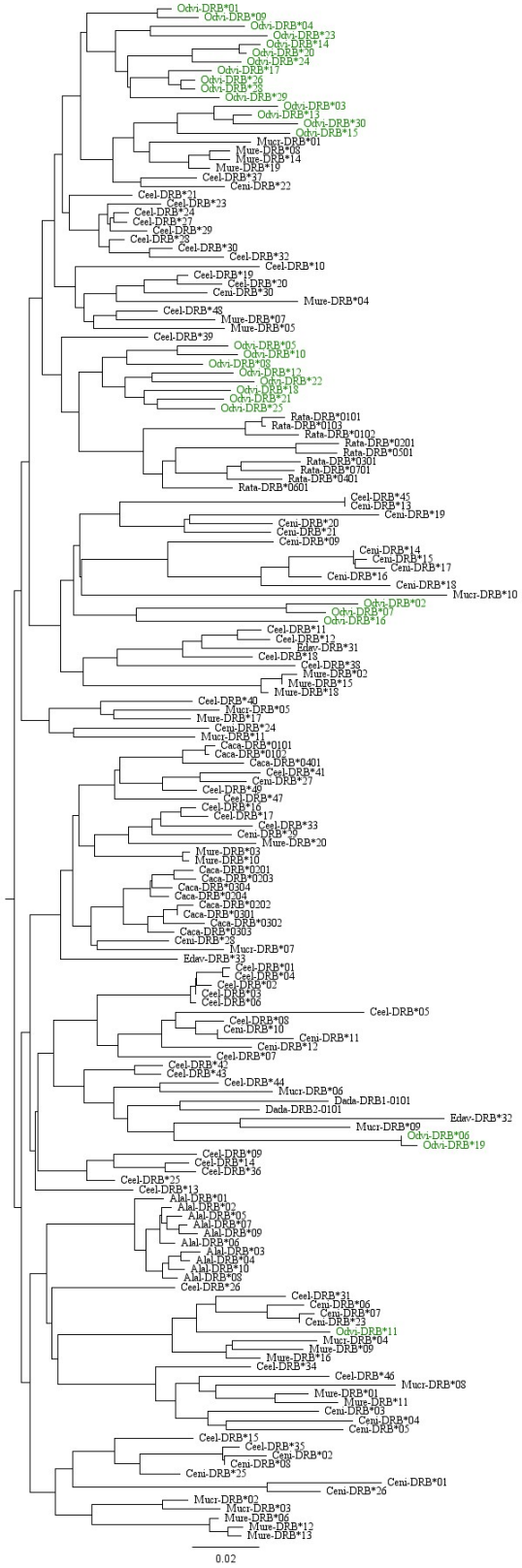

Figure 1.1. Neighbor-joining gene tree depicting relationships of new *Odvi-DRB* alleles detected in this study to all known cervid *MHC-DRB* alleles found in GenBank. See Appendix 1.2 for a list of alleles and their corresponding GenBank Accession Numbers. Abbreviations: *Alal*, moose (*Alces alces*); *Caca*, roe deer (*Capreolus capreolus*); *Ceel*, European red deer (*Cervus elaphus*); *Ceni*, sika deer (*Cervus nippon*); *Dada*, fallow deer (*Dama dama*); *Edav*, Père David's deer (*Elaphurus davidianus*); *Mucr*, black muntjac (*Muntiacus crinifrons*); *Mure*, Reeves's muntjac (*Muntiacus reevesi*); *Odvi*, white-tailed deer (*Odocoileus virginianus*); and *Rata*, reindeer (*Rangifer tarandus*). *Odvi-DRB* alleles highlighted in green.

Chapter 2: Utilizing structural equation modeling to understand the causal relationships of MHC, age, morphology, and reproductive success

INTRODUCTION

Understanding the factors that can influence reproductive success is of utmost importance to the study of population dynamics, selection, and adaptation (Brown 1988, Clutton-Brock 1988). Reproductive success of an individual within a population is best defined as the successful production (or recruitment) of viable offspring, measured by breeding season or across an individual's lifetime (Bekoff and Goodall 2004). Reproductive success varies greatly between the sexes and by the reproductive strategy of the species, i.e. polygynous versus polyandrous (Clutton-Brock 1988). In most mating systems, reproductive success is highly variable in males, and less so in females (Bekoff and Goodall 2004, DeYoung et al. 2009). In polygynous species, males provide little to no parental care, and as a result, their reproductive success is believed to be based solely on their ability to acquire mates during the breeding season (Trivers and Campbell 1972, Sorin 2004), while females are restricted to their body's physical limitations in litter/clutch size. In these circumstances, an individual male has the potential to produce considerably more offspring than a female.

There are a multitude of factors that can influence a male's ability to acquire mates, such as body size, an individual's age, or social dominance in the population. For example, in bullfrogs (*Rana catesbeiana*), larger males are observed to achieve more mating opportunities and their offspring have increased hatching success (Howard 1988). Similarly, Pyle et al. (2001) found a positive relationship between age and reproductive success in male Cassin's auklets (*Ptychoramphus aleuticus*). In northern elephant seals (*Mirounga angustirostris*) social rank describes the most variance between individual breeding males; Le Boeuf and Reiter (1988)

estimated that four dominant males inseminated 75% of the females mated in the population. Additionally, factors that influence reproductive success also have the potential to influence one another (Bekoff and Goodall 2004). For example, male red deer reproductive success depends on social dominance, which is influenced by increased body size (Clutton-Brock et al. 1988).

White-tailed deer (*Odocoileus virginianus*) are an ideal model species for evaluating the relative important of factors that influence reproductive success. The species is a polygynous species, where males attempt to mate with multiple females over the course of the breeding season (DeYoung and Miller 2011). White-tailed deer have an easily observed and measured secondary sex characteristic: antlers. Additionally, basic aspects of reproduction in white-tailed deer are well-documented (Hewitt 2011). Typically, white-tailed deer breed once a year, with the breeding season spanning from fall to early winter depending on the region. Males exhibit a tending-bond mating strategy, where males search out and guard or tend an individual female in estrus until copulation or until he is displaced by a rival male (Hirth 1977, Newbolt et al. 2017). While females typically produce twins, it is unclear how many offspring a single male can sire in one breeding season. Finally, white-tailed deer are of great importance as a game species, and as such are well-studied, particularly in regard to management.

Prior to genetic studies, researchers believed that breeding opportunities were dictated by a dominance hierarchy, where a few dominant males sired the majority of fawns in a population (DeYoung and Miller 2011). Indeed, previous studies demonstrated that dominance in white-tailed deer is generally determined by age and body size, where mature, large-bodied males are more dominant (DeYoung and Miller 2011). However, more recent studies indicate that trends in reproductive success are more complex. Specifically, body size and antler size appear to have a positive influence on reproduction (Newbolt et al. 2017). However, both of those factors are also

influenced by an individual's age. In controlled populations representing a range of sex ratios and age structures, DeYoung et al. (2009) observed that male reproductive success was distributed among a large number of males with no single male monopolizing breeding. Additionally, Newbolt et al. (2017) observed that young males reproduced more frequently when the male age structure of the population was younger.

Additionally, an individual's genetics may influence reproductive success directly or indirectly by influencing factors such as body size and/or antler size (Ditchkoff et al. 2001a). A genetic component more recently studied in white-tailed deer is the major histocompatibility complex (MHC). MHC genes encode a portion of membrane-bound molecules that bind to and present foreign peptides to T-cells of the immune system (Schook and Lamont 1996). In several species, correlations have been documented between heterozygosity in MHC genes, and greater pathogen resistance, an increase in growth rate, increased viability, and differences in physiology, morphology and secondary sex characteristic production (Zouros and Foltz 1987, Brown 1998). In white-tailed deer, Ditchkoff et al. (2001a) observed positive associations between *MHC-DRB* gene characteristics with antler development and body mass in a population of white-tailed deer in Oklahoma. From the observations of these individual studies on white-tailed deer, we notice that a number of factors were observed to influence reproductive success, but when you analyze the factors concurrently which factor or factors provide the greatest influence on reproductive success? Additionally, can genetic components, such as the MHC, play a direct or indirect role in an individual's reproductive success?

One method to analyze these interrelated causal relationships is through structural equation modeling (SEM). Over the last 20 years, ecologists have begun to use SEM as a method to address studies with multiple confounding factors, like those that influence reproductive

success (Fan et al. 2016). SEM is a technique used to evaluate multivariate causal relationships by testing both direct and indirect effects of relationships simultaneously (Grace 2006, Fan et al. 2016). SEMs can be better suited than traditional univariate statistical models, which focus on a single process or response because SEM can identify multiple processes that control the behavior of a system. With SEM, models are designed from existing knowledge of the system to represent competing hypotheses about the causal relationships in the system (Grace 2008). In contrast, conventional multivariate procedures, though useful in examining complex data, are largely exploratory by nature and are not suitable for evaluating hypotheses of the entire system (McCune et al. 2002, Grace 2008). Finally, structural equation models are a better fit for calculating variance, because they allow for common variance found with each individual variable, as well as variance due to other unaccounted factors such as errors in measurement (McCoach et al. 2007).

This research aims to build on earlier research by Newbolt et al. (2017) on the reproductive success of white-tailed deer in central Alabama. The objective of this project was to utilize structural equation modeling (SEM) to examine the potential causal relationships and the direct and indirect effects of genetic diversity of the *MHC-DRB* gene, individual sire age, herd age, body size, and antler size on the annual reproductive success of male white-tailed deer.

METHODS

Study Area

The white-tailed deer analyzed in this study were residents of the Auburn Captive Facility (ACF) within the Piedmont Agricultural Experiment Station owned by Auburn University. The Piedmont Station was north of the town of Camp Hill, Alabama, USA. ACF is a 174-hectare plot of land enclosed by a 2.5-meter tall steel fence, which was erected in October of

2007. Consequently, all deer within ACF were wild deer that inhabited the land upon completion of the facility and their subsequent offspring. No outside deer were introduced to the herd. The herd was maintained at a population of 100 to 120 adult deer. Deer within ACF were not subject to hunting, therefore, the population was regulated through natural mortality, capture related mortalities, and selective removal of fawns. Outside of capture periods, deer were left to their natural behavior and could roam, feed, and mate freely within the fenced area.

The habitat at ACF was approximately 40% open grass fields and 60% mixed forest, with a large creek providing a consistent water source. The mixed forest habitat was predominantly thick closed canopy with little understory growth. Approximately 70% of the mixed forest was made up of various hardwoods including oak (*Quercus* spp.), hickory (*Carya* spp.), and maple (*Acer* spp.), approximately 20% was made up of conifer species primarily loblolly pine (*Pinus taeda*), and 10% consisted of regenerated thickets of *Rubus* spp., including sweetgum (*Liquidambar styraciflua*), eastern red cedar (*Juniperus virginiana*), and Chinese privet (*Ligustrun sinense*). To supplement the nutrition of the herd, two 0.4-hectare food plots were planted each spring and fall with various cool and warm season forages. Supplemental feed (18% protein pellets; "Deer Feed," SouthFresh Feeds, Demopolis, Alabama, USA) was also provided ad libitum year-round via three feeders. Finally, to attract deer for capture events, three additional feeders were deployed during the months of October through March. These feeders were on a timer to deploy approximately 2 kg of whole corn each day.

Capture Techniques

White-tailed deer were captured by administering a sedative mixture via a telemetry dart (2.0 cc, type C, Pneu-Dart Inc., Williamsport, Pennsylvania, USA), which was shot using a Cartridge Fired Rifle (Pneu-Dart model 193) and a .22 caliber blank (Kilpatrick et al. 1996). The

dart was fired into the muscle of the hindquarter (Miller et al. 2004). The sedative was a combination of Telazol® (Fort Dodge Animal Health, Fort Dodge, Iowa, USA) 125mg/ml given at a rate of 4.5 mg/kg and Xylazine (Lloyd Laboratories, Shenandoah, Iowa, USA) 100mg/ml given at a rate of 2.2 mg/kg (Miller et al. 2003, Miller et al. 2004). The dart also contained a radio transmitter, which was utilized to locate the sedated deer via radio telemetry. Data collection was conducted while the deer remained in this immobilized state. Once data collection was complete, the deer received Tolazine (1.5 mL/45.36 kg) to reverse the sedation (Miller et al. 2004). Tolazine was administered half in the muscle around the shoulder and half in the muscle around the hindquarter.

Data Collection

Capture events took place during the months of October through March starting in the year 2007. During a deer's first capture event, the deer received ear tags and freeze brands with a unique identification number. Each deer received three ear tags, two displaying the unique identification number, and the third tag was a unique electronic ID tag (EID). Ear tags and freeze brands aided camera surveys to monitor the population's size and sex ratio (Newbolt et al. 2017), as well as allowing for multiyear analysis of each individual without the need for genetic samples to be collected each capture. During their first capture, the deer was aged by tooth replacement and wear following Severinghaus (1949). An exhaustive effort was made to capture deer before the age of 2.5 years old when aging can be easily determined by the shape and number of molars present.

During a deer's first capture, a 1-cm^2 section of tissue was removed from one ear by a notching tool. Tissue samples were used for DNA analysis for paternity and maternity

assignment, as well as MHC genotyping. Tissue samples were stored in a small vile in -80° C freezer until analysis could be performed in a controlled laboratory setting.

An attempt was made to capture each male deer every year to observe the changes in body and antler structure as deer age. Body measurements, antler measurements, and additional samples for other studies were recorded at every capture (Neuman et al. 2016, Newbolt et al. 2017). Skeletal body measurements were taken in centimeters using a flexible measuring tape. Measurements included: body length measured from the tip of the snout following the backbone to the proximal end of the tail bone; hind foot length measured from the tip of the hoof to the posterior end of the tuber calcis; and chest girth measured by the circumference of the chest immediately posterior to the front legs (Ditchkoff et al. 2001a). To generate a single term to represent an individual's body size, we conducted a principal component analysis (PCA) of the three body measurement variables using Program R (RStudioTeam 2016)(Package stats; R core development team, R Studio, version 1.1.456 accessed 30 June 2019). Because these variables were highly correlated, component 1 from the PCA was used to represent annual body size (Neuman et al. 2016, Newbolt et al. 2017).

Antlers were measured according to the Boone & Crockett scoring system (Wright et al. 1997). However, official Boone & Crockett scoring penalizes asymmetrical antlers; instead of applying these deductions, we calculated a gross antler score using both antler scores and the measurement of inside spread (Ditchkoff et al. 2001a).

Parentage Assignment and Reproductive Success

Genetic analysis to determine parentage was conducted by DNA Solutions, Inc. (Oklahoma City, Oklahoma, USA) using an analysis of microsatellite markers. A small (5 mm) section of tissue was taken from the original sample and sent to DNA Solutions. DNA Solutions

determined the allele composition for eighteen separate microsatellite loci for each deer sampled. Microsatellites were tested using FSTAT to determine their capability at discerning individual identities (Pearson and Lipman 1988). The microsatellite markers for each deer were entered into Cervus 3.0 (Field Genetics, London, UK) to assign parentage. Cervus 3.0 was also used to estimate the level of confidence of each parentage assignments (Neuman et al. 2016, Newbolt et al. 2017).

We defined annual reproductive success as the number of offspring sired by a male that survived to at least 6 months of age. Male and female white-tailed deer can sire or conceive offspring as young as 6 months (DeYoung 2011, Neuman 2014). We only included offspring that lived to this age, given that at that age they were also able to reproduce and contribute to the population. Reproductive data was observed for fawns born in 2008 thru 2014. For each year a collection of all potential candidate fathers and mothers were compiled. Parentage assignments were made using the likelihood-ratio method in Cervus 3.0 to assign parentage using a parent pair analysis (Neuman et al. 2016, Newbolt et al. 2017). We assigned parentage based on the proportions and thresholds established in Neuman et al. (2016) and Newbolt et al. (2017). Parentage was assigned to both candidate parents of the most likely parent-offspring trios with ≤4 mismatching loci when the delta statistic reached a minimum 95% reliability threshold. If the trio failed to reach the 95% level because of mismatches between the dam and offspring, we assigned candidate paternity only in instances where the individual delta statistic reached the 95% level and the pair contained ≤2 mismatching loci. From the parentage analysis, a total annual offspring value was calculated for each male in the population each year.

MHC Analysis

DNA sequencing was utilized to analyze the major histocompatibility complex (MHC) Class II gene DRB. DNA was extracted from the frozen tissue samples following a modification of the methods established by Coffroth et al. (1992) which utilized CTAB (hexadecyltrimethyl ammonium bromide) for isolating the DNA of Symbiodinium. The full protocol is found in Appendix A. After completing the DNA extraction, samples were tested using gel electrophoresis to identify the presence of DNA. Final DNA extraction materials were stored at -80° C.

After DNA was successfully extracted from the frozen tissue, samples were sent to RTL Genomics (Lubbock, Texas, USA) to perform the polymerase chain reaction (PCR) in preparation for sequencing using MiSeq (Mullis et al. 1986). PCR primers were designed from primers used by Sigurdardottir et al. (1991) from conserved regions of cattle MHC sequences to isolate and amplify the DRB gene. RTL Genomics sequenced each sample using the Illumina MiSeq platform for a minimum of 10,000 reads per individual (Illumina, Inc., San Diego, California, USA).

Additionally, RTL Genomics conducted bioinformatics analysis on the sequenced data to differentiate the alleles present in each individual sample. To do so, RTL Genomics first completed a filtration process where the reads were trimmed with Trimmomatic V0.36 with a Q25 cutoff. Second, the reads were merged together using PEAR V0.96. Any unassembled contigs, any merged contigs less than 290 base pairs, or contigs that were only sequenced once were discarded. Finally, MHC loci that were out of the reading frame were discarded by RTL Genomics.

From the bioinformatics analysis provided by RTL Genomics, the two most prevalent sequences were isolated for each deer. Both alleles were compared to the known 18 alleles for white-tailed deer *MHC-DRB* gene from Van Den Bussche et al. (2002) and the 11 new alleles characterized in chapter 1, using the program Geneious v11.1.5 (Kearse et al. 2012) to determine the exact allele(s) present in each individual sire. If one or more alleles did not match the known 29 alleles than the deer was not included in further analysis.

In this study, we used *MHC-DRB* sequence data to calculate genetic distance between the alleles present in the individual as a way to quantify MHC characteristics in an individual. First, all known *MHC-DRB* alleles for white-tailed deer were aligned using a global alignment with free end gaps and using the default cost matrix in Geneious v11.1.5 (Kearse et al. 2012). The alignment was then translated to show the amino acid sequence for each allele. The translation used the standard genetic code and began on the second base pair (Van Den Bussche et al. 1999). A pairwise comparison matrix table was calculated to show the number of amino acid differences between each allele combination and provide a metric of genetic distance. We decided to use this metric instead of a comparison between the numbers of nucleotide differences between alleles because calculating changes between nucleotides would include both synonymous and nonsynonymous substitutions. We were interested in calculating differences between alleles that could change the amino acid structure and thus potentially change the function of the protein. We used Program R (RStudioTeam 2016) (Packages tidyverse & dplyr; R core development team, R Studio, version 1.1.456 accessed 30 June 2019) to load the pairwise comparison matrix, select the corresponding pairwise comparison value from the matrix based on the two alleles for each individual, and import the value of genetic distance for an individual into the overall data table for each individual sire (Table 2.1).

SEM Design

We designed eight hypotheses to examine the proposed causal relationships and correlation assumptions of six variables: body size, antler size, MHC genetic distance, sire age, herd age, and annual reproductive success. As described earlier, body size was determined from the PCA component 1 output, antler size was calculated following gross Boone & Crockett scoring system (Wright et al. 1997), and MHC genetic distance was described as the number of amino acid differences between the two alleles in an individual. Sire age was determined as the age of the male during the breeding season corresponding to that year's offspring production. Herd age was the average age of all males present in the population during the corresponding breeding season. Finally, annual reproductive success was the number of offspring sired by a male that survived to at least 6 months of age.

The hypotheses were designed from variable associations previously published for white-tailed deer by Ditchkoff et al. (2001a) and Newbolt et al. (2017), and from basic life history characteristics for white-tailed deer. See Appendix 2.1 for a graphical representation of each model. Model 8 or the Global Model included all six variables and proposed relationships. Models 1 thru 7 were designed as more parsimonious models, with model 7 as the second most complex and models 1 and 2 as the simplest models. The variables antler size and body size were proposed to have a correlated relationship, as were the variables sire age and herd age.

All parameters in the models were estimated using maximum likelihood. One of the assumptions of maximum likelihood states that the data set must be complete without missing variables or data points (Grace 2006, Fan et al. 2016, Shipley 2016). Unfortunately, despite our best efforts, every potential sire was not captured each year and subsequently missing data points existed in the body size and antler size measurements. Additionally, a few captures occurred

after the male had dropped his antlers and an antler size was unable to be calculated. To address this assumption, we omitted any observation that included a missing variable, for this data an observation is defined as an individual male during a single breeding year.

Another assumption of maximum likelihood states that all variables are normally distributed. To address this, we applied natural log transformations to improve normality. We then compared the distribution of log-transformed variables to their raw data to determine if transformation improved the variable and was warranted. Ultimately we fit models with and without transformations of various variables to assess if transformation of the variable influenced the outcome of the model. From these results, we determined that log transformation of the variables sire age, herd age, MHC genetic distance, body size, and antler size did not influence the results or improve variable normality and thus these variables were left in their original state. Only the variable annual reproductive success was log transformed to ensure normality. Other estimation methods, like the generalized least squares method, exist that do not require variables to follow the assumption of normality as strictly. However, maximum likelihood methods are best suited for model comparison methods such as AICc (Akaike Information Criterion corrected for small sample size). We compared the results of the global model estimated using maximum likelihood estimation and the generalized least squares estimation and found no qualitative or quantitative difference between the two estimators.

We used Program R (RStudioTeam 2016)(Package sem; R core development team, R Studio, version 1.1.456 accessed 30 June 2019) to run each model and compare model fit. We determined model fit by using the indices χ^2 and AICc (Akaike information criterion corrected for small sample size). We used χ^2 goodness of fit tests as a robust measure of model fitness (Fan et al. 2016), and we used AICc and model weight (ω) for model comparison. We calculated

standardized path coefficients and their significance at a level of $p < 0.05$ level for each hypothesized causal influence. Finally, we calculated 95% bias-correlated confidence limits (C.L.) for the standardized path coefficients of each causal relationship by running a bootstrap with 1000 replications.

RESULTS

Our original data set consisted of 377 observations (defined earlier as a single sire during a single breeding year), across 7 breeding seasons. (Figure 2.1). After filtering for missing data, 145 observations remained for analysis. Thirty-one deer were available as potential sires once in the extent of the study, while the remaining 114 were available during two or more breeding seasons (Figure 2.2). The age structure of the herd changed as the study progressed, with the average age of the herd increasing from 1.4 years in 2008 to 3.2 years in 2014 (Figure 2.3). Annual reproductive success among individuals varied from 0 to 9 offspring. With the exception of 2008, there was a consistent trend across the years where over 50% of potential sires did not produce any offspring (Figure 2.4). Individual MHC genetic distance ranged from 0 amino acid differences between alleles in an individual to 27 differences.

Goodness-of-fit indices, specifically AICc, and model weight (ω), suggested that our simple models (Models 1-4) best fit the data (Table 2.2). Models 1-4 and model 7 all were saturated and contained paths between all pairs of variables, resulting in no degrees of freedom for testing the overall model fit (Grace 2006). Consequently, the χ^2 values were all 0 and no model p-value could be calculated. Models 5 ($p = 0.003$) and 6 ($p = 0.008$) both were significant and thus were deemed to have an inadequate fit (Grace 2006, Fan et al. 2016). When comparing the results of each model, consistent trends of significance and size of standardized path coefficients existed. For example, in all models where sire age was included as a variable, there

was a significant ($p < 0.05$) and strong effect on antler size. Because of these trends, we analyzed the global model to interpret all potential relationships.

In the global model, the proportion of variance not explained by the model for each of the endogenous variables were 0.99 (95% C.L. = 0.95, 1.00) for sire age, 0.31 (95% C.L. = 0.22, 0.39) for antler size, and 0.43 (95% C.L. = 0.34, 0.52) for body size. Importantly, the model only explained 26% (95% C.L. = 0.19, 0.44) of the variation in annual reproductive success. The direct effect of antler size had the greatest path coefficient and strongest influence on annual reproductive success (rho or $\rho = 0.42$; 95% C.L. = 0.00, 0.71; $p = 0.01$; Figure 2.5). The indirect effect of sire age through antler size had a greater influence ($\rho = 0.83 \times 0.42 = 0.35$) than the direct effect of sire age on reproductive success ($\rho = -0.07$; 95% C.L. = -0.31, 0.26; $p = 0.62$; Figure 2.5). The indirect effect of sire age through body size also had a greater influence ($\rho = 0.75 \times 0.17 = 0.13$) than the direct effect of sire age on reproductive success ($\rho = -0.07$; 95% C.L. = -0.31, 0.26; $p = 0.62$; Figure 2.5). Herd age had a significant direct influence on annual reproductive success ($\rho = -0.20$; 95% C.L. = -0.35, -0.04; $p = 0.01$; Figure 2.5). Finally, MHC genetic diversity had the weakest direct influence on annual reproductive success ($\rho = 0.05$; 95% C.L. = -0.09, 0.18; $p = 0.52$; Figure 2.5), and was weaker than sire age on its influencing of antler size and body size ($\rho = -0.02$; 95% C.L. = -0.11, 0.06; $p = 0.62$; and $\rho = 0.002$; 95% C.L. = -0.11, 0.12; $p = 0.97$ respectively). Parameter estimates and their corresponding standard error and p values can be found in Table 2.3.

DISCUSSION

Antler size appeared to have the greatest influence on annual reproductive success. This is consistent with findings from Newbolt et al. (2017) who reported that antler and body size were positively associated with annual reproductive success. However, our findings illustrated

that the influence of antler size was almost 2.5 times greater than body size ($\rho = 0.42$; $p = 0.01$ and $\rho = 0.17$; $p = 0.21$ respectively). Antler size could play a role in annual reproductive success through its role as weaponry and/or as a signal of phenotypic quality. Specifically, antlers can be used as a weapon to combat other males for access to females (Andersson 1994, Demarais and Strickland 2011), and also as a visual cue to other males of their dominance, minimizing the need for costly fights (Demarais and Strickland 2011). In red deer (*Cervus elaphus),* antler size influences a male's probability of becoming a harem holder and dominating access to breeding females (Bartoš and Bahbouh 2006), thus increasing their potential reproductive success. Antlers may also serve as a signal to breeding females. Antlers are a condition-dependent secondary sex characteristic and as such are influenced by the age, health, and nutritional status of the individual (Kruuk et al. 2002, Demarais and Strickland 2011). Zahavi (1975) postulated that because secondary sex characteristics, like antlers, are costly to produce, only individuals of superior health and genetics can afford to risk their production. Therefore, antlers become an honest signal of an individual's quality (Ditchkoff et al. 2001b). Vanpe et al. (2007) identified this in roe deer (*Capreolus capreolus*) by observing that antlers were an honest signal of individual's quality as a sire.

Sire age was more strongly associated with antler size and body size than it was with annual reproductive success. Sire age had its greatest influence on reproductive success as an indirect relationship through antler size. Newbolt et al. (2017) also did not find individual sire age to be a factor influencing reproductive success. Rather, age was highly correlated with factors that were directly associated with reproductive success, such as body and antler size. However, DeYoung et al. (2009) observed that 70% of offspring were sired by males 3.5 years and older and that older males were more successful breeders than younger males. But, their

study focused only on age structures and sex ratios and did not include factors such as body size or antlers size. Our findings suggest that a male's age influences antler production, and thus indirectly influences reproductive success in this manner.

The next greatest influence on annual reproductive success was the direct impact of herd age. The relationship was negative, which suggests that as the average age of the herd increased, the amount of offspring an individual male sired decreased. In populations with a diverse age structure, older physically mature bucks are more likely to be successful sires and produce a greater portion of the fawns born in a breeding season (DeYoung et al. 2009, DeYoung and Miller 2011). DeYoung et al. (2009) observed that physically mature males (3.5 years and older) sired 70% of fawns in populations with a natural variation of age structure. However, in situations where populations lack mature males, breeding tends to be more evenly distributed across all available males, increasing the reproductive success for the majority of males (DeYoung et al. 2009, DeYoung and Miller 2011). Newbolt et al. (2017) observed an influence of the average age of the herd on reproductive success. They found an interaction between antler size and herd age, indicating that the influence of antler size on reproductive success was greater in scenarios with older male age structures. Our findings suggest that the age structure of males in a herd influences the proportion of males who successfully sire offspring and the amount of offspring each individual sires.

The genetic distance between MHC alleles in an individual showed the least influence on annual reproductive success. Additionally, in comparison to individual sire age, MHC genetic distance showed considerably less influence than individual age on antler and body size. This conflicts with findings by Ditchkoff et al. (2001a), who found a relationship between the *MHC-DRB* gene in white-tailed deer and body and antler size. However, our study differed from

Ditchkoff et al. (2001) in the metric used to describe the characteristics of the MHC. Ditchkoff et al. (2001a) used the phylogenetic analysis by Van Den Bussche et al. (1999) of known cervid *MHC-DRB* alleles and characterized individuals based on allelic lineages found for white-tailed deer on the phylogenetic tree. They observed that individuals characterized as heterozygotes had larger body and antler sizes.

Our study characterized the MHC by analyzing the genetic distance between alleles in an individual by calculating the number of amino acid differences between the alleles. With the discovery of new alleles for the *MHC-DRB* gene in white-tailed deer (see chapter 1), the updated gene tree no longer consisted of 2 distinct lineages. We developed the comparison of amino acid differences as a characterization method to calculate dissimilarity between alleles. We decided to compare amino acids instead of nucleotide difference to focus on differences between the alleles that had the potential to change the protein formed from the amino acids, potentially changing the function of the protein. Because genes in the MHC are codominantly expressed, alleles that produce different functioning proteins are hypothesized to have an immunologic advantage because the differing proteins provide resistance to a greater variety of pathogens (Brown 1998, Penn et al. 2002, Knapp 2007, Osborne et al. 2015).

Another factor to consider with this analysis is that the MHC is a complex of many genes working together to provide an immune response to pathogens. Our analysis only compared the genetic distance found in one gene within MHC. According to this analysis, only the genetic distance between alleles in the *MHC-DRB* shows little influence on white-tailed deer annual reproductive success. Characterization of other MHC genes not analyzed in this study may still influence reproductive success. Ultimately, analyzing the combined effect of all MHC genes

would provide greater insight into the influence that genes involved in immune response have on reproductive success.

Due to the design of structural equation modeling, no missing values can occur in the data set. As a result, our sample size was reduced to 145 observations (defined earlier as a single sire during a single breeding year). Literature recommendations for adequate sample size for structural equation modeling vary from 5 to 10 to 20 observations per model parameter (Grace 2006; 2008, Fan et al. 2016). Our global model contains 18 parameters, therefore our sample size does meet the minimum recommendations. However, this sample size is limited and there was ample uncertainty in path coefficients and variance for the endogenous variables. Increasing complete observations would benefit by reducing uncertainty.

The global model and relationships examined within accounted for 26% of the variation in annual reproductive success in this population of white-tailed deer, resulting in 74% of the variation being unexplained. Other factors that could influence male reproductive success that this study could not account for include other genetic components, behavior, environmental factors, and the effects influenced by the female. Foley et al. (2018) postulated that an individual's behavior, specifically time management, may also play a role in successfully acquiring mating opportunities. Examples of environmental factors that could be analyzed in future studies include the influence that varying sex ratios may play, population size, and population distribution (DeYoung et al. 2009). All of these factors could influence the amount of time a male spends searching for receptive mates (Clutton-Brock 1988). Variation due to the influence of factors associated with the female could be vast. For example, our dataset does not take into account the potential influence of the female's health, nutrition, and underlying genetics (Clutton-Brock 1988).

Finally, this study, as with all structural equation modeling, provides inference into variables and their causal relationships: SEM does not prove causality. Our structural equation modeling revealed that antler size, the average age of the herd, and the indirect effect of individual sire age through antler size all influenced annual reproductive success in male white-tailed deer.

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Table 2.1. Pairwise comparison matrix table of the number of amino acid differences among 29 MHC *Odvi-DRB* allele sequences from white-tailed deer (*Odocoileus virginianus)*.

	*Odvi-DRB*01*	*Odvi-DRB*02*	*Odvi-DRB*03*	*Odvi-DRB*04*	*Odvi-DRB*05*	*Odvi-DRB*06*	*Odvi-DRB*07*	*Odvi-DRB*08*	*Odvi-DRB*09*	*Odvi-DRB*10*	*Odvi-DRB*11*	*Odvi-DRB*12*	*Odvi-DRB*13*	*Odvi-DRB*14*	*Odvi-DRB*15*	*Odvi-DRB*16*	*Odvi-DRB*17*	*Odvi-DRB*18*	*Odvi-DRB*19*	*Odvi-DRB*20*	*Odvi-DRB*21*	*Odvi-DRB*22*	*Odvi-DRB*23*	*Odvi-DRB*24*	*Odvi-DRB*25*	*Odvi-DRB*26*	*Odvi-DRB*28*	*Odvi-DRB*29*	*Odvi-DRB*30*
*Odvi-DRB*01*	--																												
*Odvi-DRB*02*	20	--																											
*Odvi-DRB*03*	12	24	--																										
*Odvi-DRB*04*	8	21	15	--																									
*Odvi-DRB*05*	11	18	18	18	--																								
*Odvi-DRB*06*	21	23	25	23	20	--																							
*Odvi-DRB*07*	21	5	24	19	19	24	--																						
*Odvi-DRB*08*	9	18	17	13	10	20	18	--																					
*Odvi-DRB*09*	3	17	10	10	8	20	18	9	--																				
*Odvi-DRB*10*	11	18	16	14	4	20	16	10	8	--																			
*Odvi-DRB*11*	20	21	22	18	18	26	19	17	22	17	--																		
*Odvi-DRB*12*	12	21	22	16	12	22	22	10	14	14	19	--																	
*Odvi-DRB*13*	12	25	7	13	20	25	24	16	12	17	20	18	--																
*Odvi-DRB*14*	13	26	13	13	24	27	25	20	16	21	20	17	8	--															
*Odvi-DRB*15*	15	23	11	10	22	26	22	19	14	19	17	22	10	12	--														
*Odvi-DRB*16*	16	18	25	20	21	27	19	17	17	23	23	17	22	24	23	--													
*Odvi-DRB*17*	9	20	10	10	17	22	19	13	9	14	23	14	7	11	15	19	--												
*Odvi-DRB*18*	12	23	21	15	13	21	24	14	15	15	21	11	17	15	21	21	12	--											
*Odvi-DRB*19*	21	23	25	23	20	0	24	20	20	20	26	22	25	27	26	27	22	21	--										
*Odvi-DRB*20*	12	25	14	12	23	27	24	19	15	20	19	16	9	1	11	23	12	15	27	--									
*Odvi-DRB*21*	14	23	14	17	15	23	23	15	17	13	15	11	11	11	16	24	12	10	23	10	--								
*Odvi-DRB*22*	15	20	22	15	11	21	20	12	15	13	17	11	20	20	21	20	16	12	21	19	16	--							
*Odvi-DRB*23*	10	19	12	6	16	23	17	13	8	12	20	19	12	15	10	21	9	18	23	14	19	17	--						
*Odvi-DRB*24*	11	23	17	8	21	25	21	17	13	17	22	14	12	5	16	21	7	13	25	6	16	16	11	--					
*Odvi-DRB*25*	13	24	15	14	16	21	24	14	16	15	15	14	14	12	15	24	16	8	21	11	6	14	18	17	--				
*Odvi-DRB*26*	8	21	12	8	18	22	20	15	10	15	22	12	9	10	16	19	2	11	22	11	11	16	11	5	15	--			
*Odvi-DRB*28*	9	21	14	7	19	23	19	15	11	15	21	12	9	9	15	19	4	11	23	10	13	15	10	4	15	2	--		
*Odvi-DRB*29*	8	22	13	8	19	23	20	13	11	15	21	15	13	11	17	19	8	14	23	12	16	14	11	8	14	7	6	--	
*Odvi-DRB*30*	17	25	8	17	22	27	24	20	15	19	22	22	5	12	12	26	12	21	27	13	16	24	14	16	18	14	12	15	--

[1]Matrix table was calculated in program Geneious v11.1.5 (Kearse et al. 2012)

Table 2.2. Goodness-of-fit statistics comparing 8 models demonstrating hypotheses of variables that influence male annual reproductive success of white-tailed deer (*Odocoileus virginianus*) constructed via structural equation modeling (SEM) across study years 2008 to 2014 at the Auburn Captive Facility, Camp Hill, Alabama.

Model	**AICc**	**ω**	**χ^2**	**d.f.**	**P***
Model 1: Simple model with MHC and body size	0.6087	0.2913	0.0000	0	NA
Model 2: Simple model with antler size and body size	0.6087	0.2913	0.0000	0	NA
Model 3: Simple model with sire age, antler size, and body size	1.6418	0.1738	0.0000	0	NA
Model 4: Simple model with MHC, antler size, and body size	1.6418	0.1738	0.0000	0	NA
Model 7: Global model without herd age	3.7209	0.0615	0.0000	0	NA
Model 8: Global model	7.9532	0.0074	2.5246	3	0.4709
Model 5: Global model without sire age and MHC	12.8375	0.0006	11.7787	2	0.0028
Model 6: Global model without sire age	14.2227	0.0003	11.8590	3	0.0079

[a] AICc, Akaike's Information Criterion corrected for small sample size; ω, Akaike weight; χ2, Chi-square test; d.f., degree of freedom; P, p-value

*significance < 0.05; NA represents that p-value could not be calculated due to the degrees of freedom value being 0

Table 2.3. Parameter estimates, standard error, and p values for all proposed relationships expressed by the global model for structural equation modeling (SEM) analysis of variables influencing male annual reproductive success in white-tailed deer (*Odocoileus virginianus*) from 2008 to 2014 at the Auburn Captive Facility, Camp Hill, Alabama.

Parameter	**β**	**SE**	**P***
MHC → Sire Age	0.016	0.017	0.329
MHC → Antler Size	-0.102	0.207	0.620
MHC → Body Size	0.003	0.089	0.972
MHC → Reproductive Success	0.003	0.005	0.516
Sire Age → Antler Size	17.564	0.973	< 0.001
Sire Age → Body Score	5.762	0.420	< 0.001
Sire Age → Reproductive Success	-0.020	0.040	0.618
Herd Age → Reproductive Success	-0.212	0.079	0.007
Antler Size → Reproductive Success	0.006	0.002	0.008
Body Size → Reproductive Success	0.006	0.005	0.211

* significance < 0.05

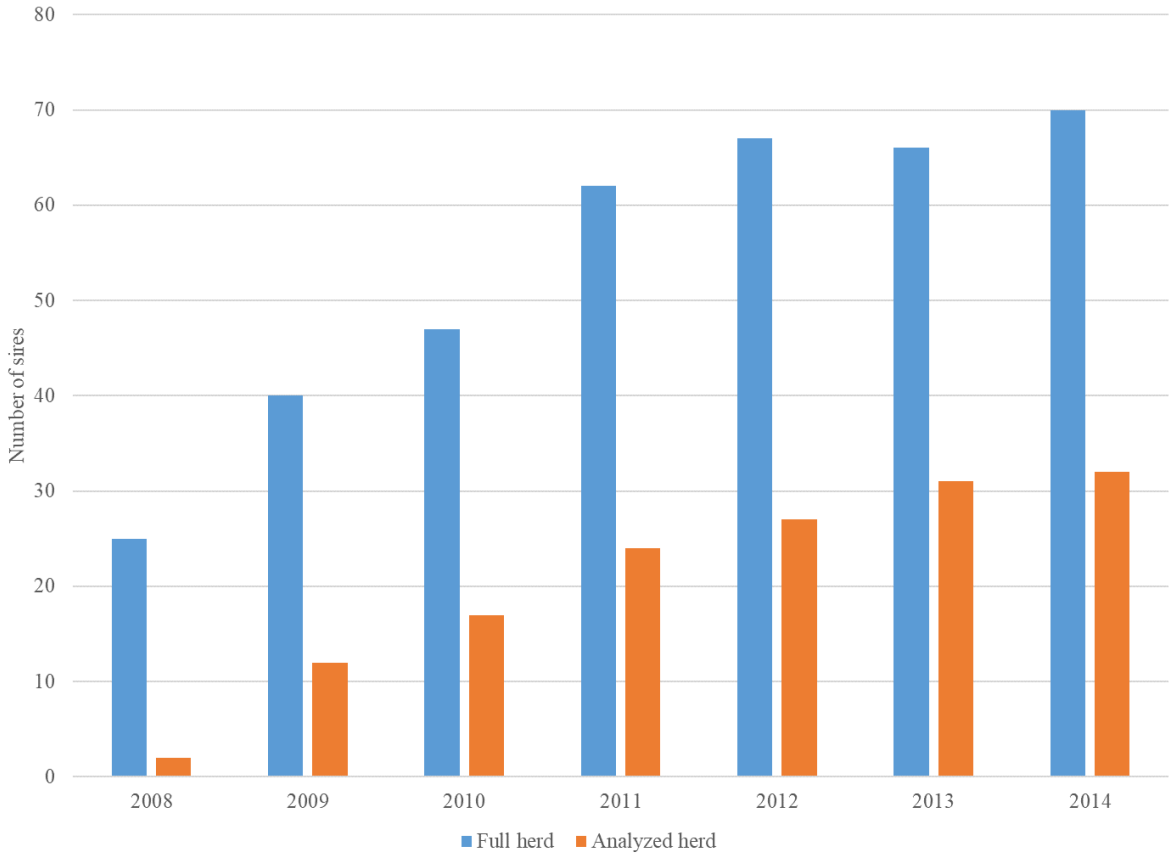


Figure 2.1. Number of potential white-tailed deer (*Odocoileus virginianus*) sires available (Full herd), compared to the number of sires with complete observations (Analyzed herd) analyzed via structural equation modeling (SEM) across study years 2008 to 2014 at the Auburn Captive Facility, Camp Hill, Alabama.

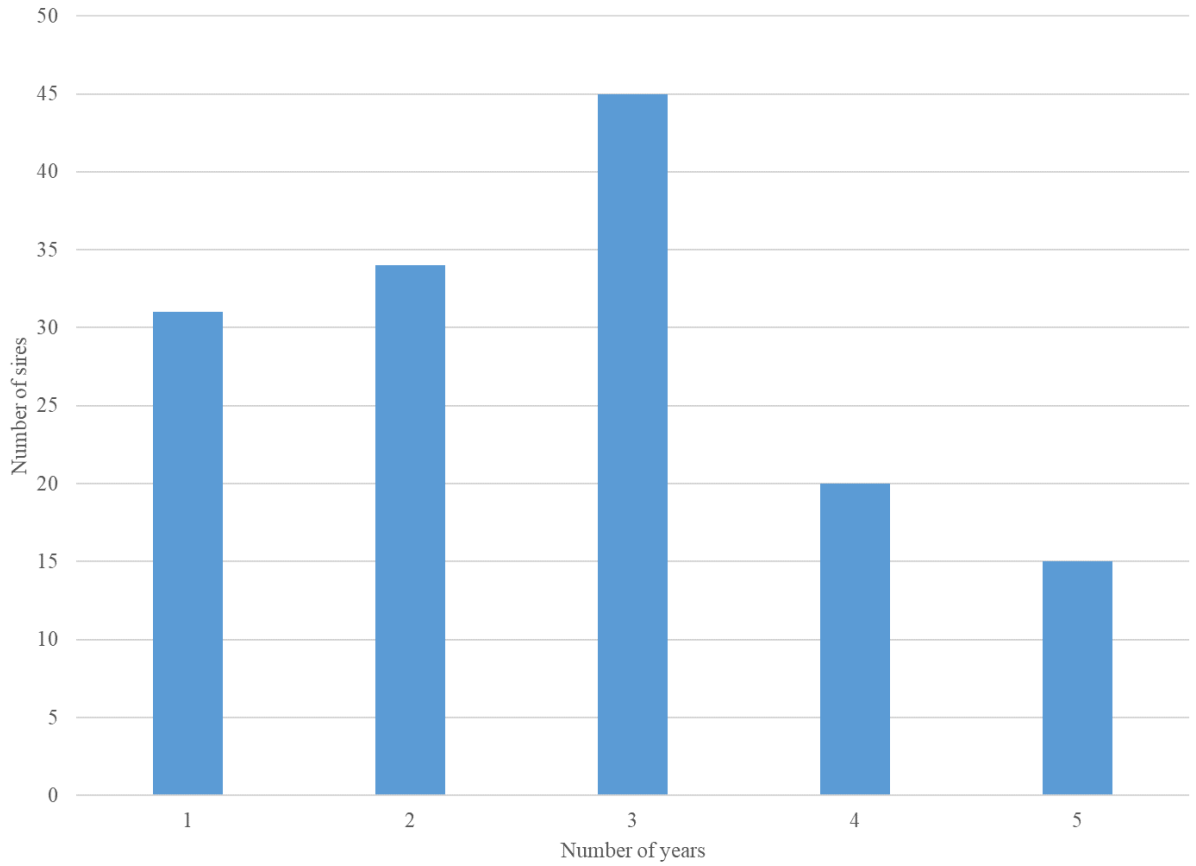


Figure 2.2. The frequency (years) that male white-tailed deer (*Odocoileus virginianus*) were used in structural equation modeling (SEM) analysis as potential sires across study years 2008 to 2014, at the Auburn Captive Facility, Camp Hill, Alabama.

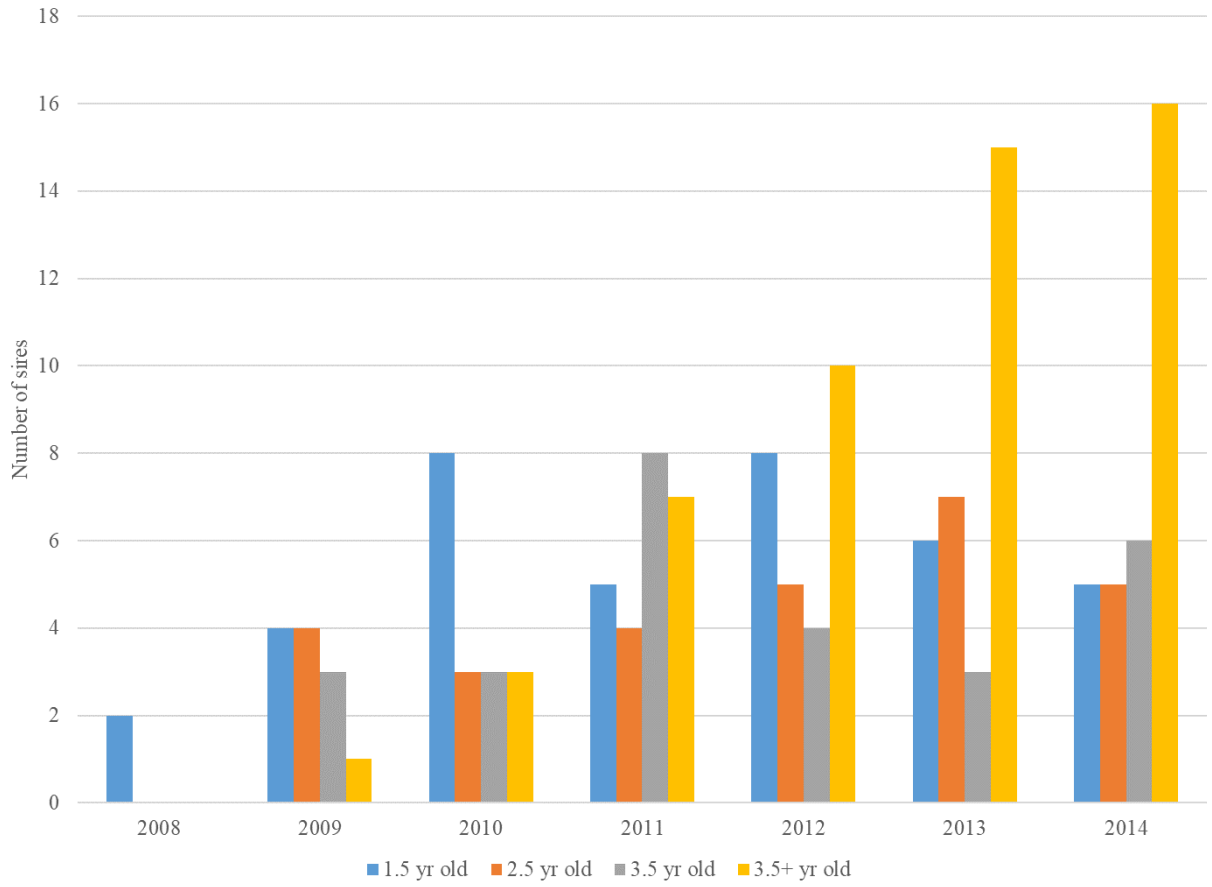


Figure 2.3. Number of potential white-tailed deer (*Odocoileus virginianus*) sires used in structural equation modeling (SEM) analysis by age class across study years 2008 to 2014, at the Auburn Captive Facility, Camp Hill, Alabama.

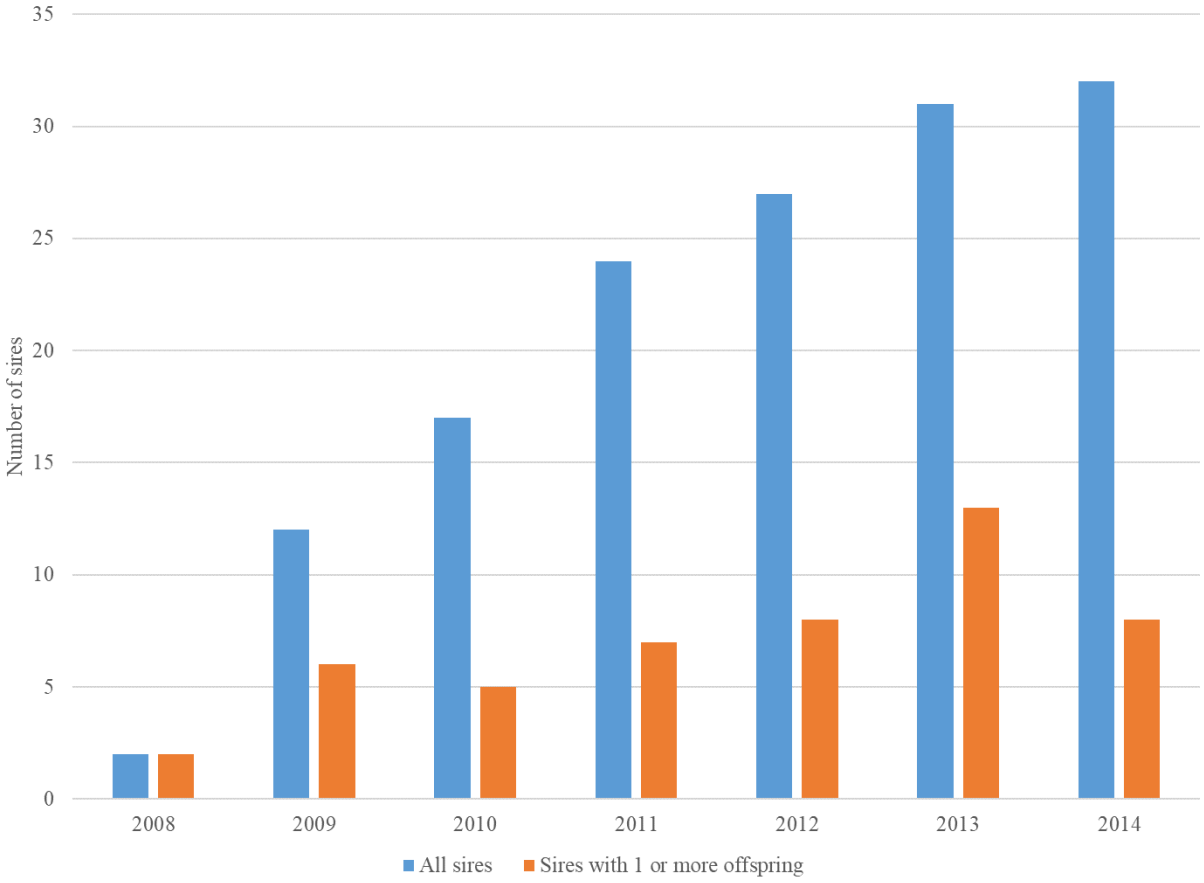


Figure 2.4. All potential white-tailed deer (*Odocoileus virginianus*) sires used in structural equation modeling (SEM) analysis compared to those that sired 1 or more offspring across study years 2008 to 2014, at the Auburn Captive Facility, Camp Hill, Alabama.

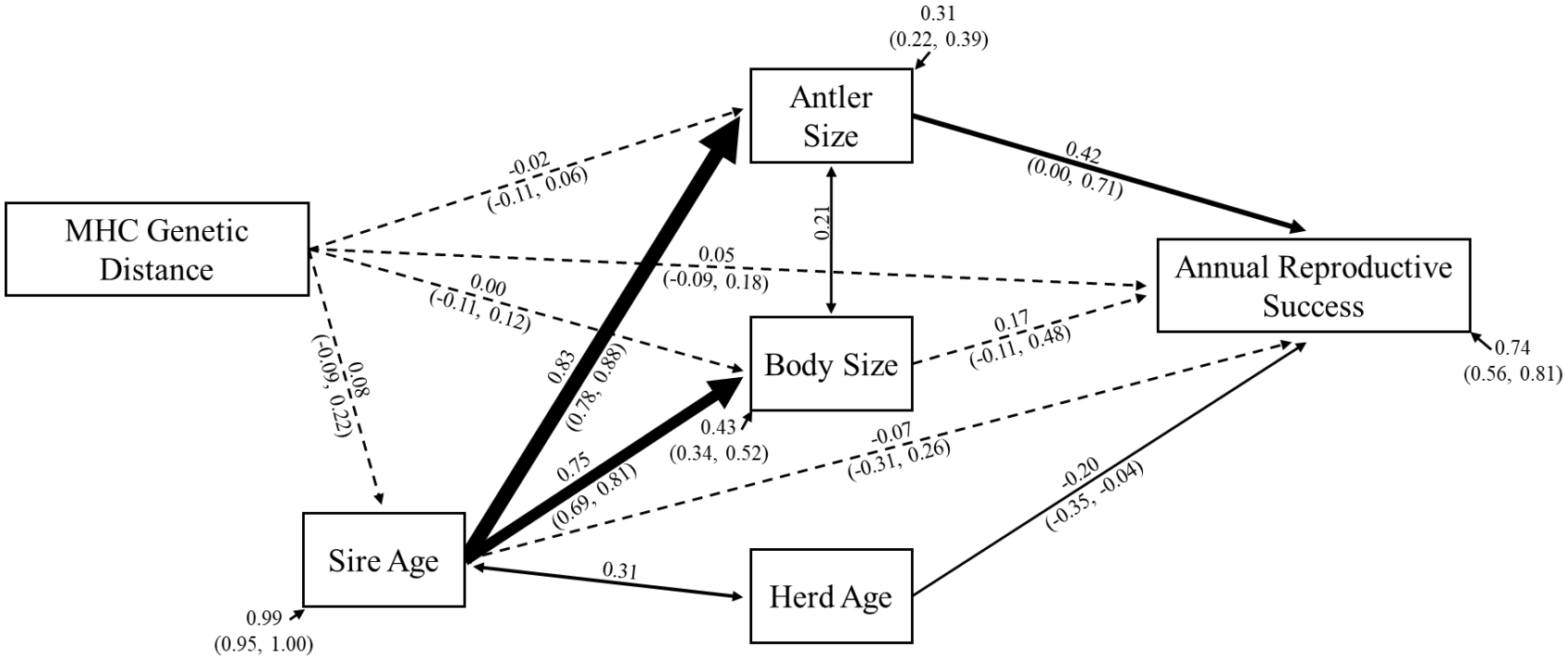


Figure 2.5. Structural equation model (SEM) for the global model on the influences of annual reproductive success in male white-tailed deer (*Odocoileus virginianus*) across study years 2008 to 2014, at the Auburn Captive Facility, Camp Hill, Alabama. Standardized path coefficients are indicated by long single-headed arrows, correlations are indicated by double-headed arrows, and short single-headed arrows pointing towards variables indicate the proportion of unexplained variance not accounted for by the effects of the independent variables. Solid arrows represent significant paths ($p < 0.05$), and effect strength is also indicated by the thickness of the arrow. 95% confidence limits of path coefficients and unexplained variance are shown in parentheses.

APPENDIX 1.1. 2XCTAB adjusted protocol for DNA isolation of white-tailed deer (*Odocoileus virginianus*) tissue samples

Sample Preparation for Frozen Samples:

1. Freeze samples in individual tubes in a -80° freezer.
2. Label 1.5ml tubes.
3. Fill tubes with 600µl 2XCTAB. Close caps immediately to avoid losing CTAB to evaporation.
4. Cut a small (5-7mm) strip of tissue from main sample. Finely cut up tissue then place in CTAB tube. Clean equipment thoroughly with 70% EtOH for every use. Keep original samples on ice to prevent DNA degradation.
5. Grind tissue into CTAB using a dounce till solution is cloudy.

With Sample in 600µl 2xCTAB:

1. Add 3.6µl of Proteinase K (20mg/ml) directly in ground up tissue. Use a new tip for each tube.
2. Incubate in water bath at 65° for 120-180 minutes. Invert tubes every 30 minutes while incubating.
3. Add 600µl of CIA (Chloroform-isoamyl alcohol). Buzz on vortex machine till milky color.
4. Centrifuge for 15 minutes at 13K. Label second/final set of 1.5ml tubes.
5. Remove aqueous phase (top), and transfer to new labeled tube. Discard bottom organic layer in CIA waste container. Be careful not to transfer hair or CIA to new tubes. Use a new tip for each tube.
6. Add 1 ml of cold 95% EtOH and invert tubes 2-3 times.
7. Place in -20° freezer to precipitate overnight. Can stay in freezer for multiple nights.

After Overnight Precipitation:

1. Centrifuge for 30 minutes at 13K. Orient tubes so hinge is up and DNA pellet will be easy to find.
2. Discard supernatant using vacuum, being very careful not to lose the pellet (it is acceptable to leave a small amount of EtOH in this step). Add 500µl of cold 70% EtOH, invert to mix and spin at 13K for 5 minutes. (Place EtOH back in fridge between uses)
3. Repeat previous step.
4. Discard supernatant using vacuum. Spin for 30 seconds at 13K and remove the rest of the cold EtOH (leave as little EtOH as possible without losing the pellet).
5. Dry the pellet in the speed-vacuum for 15 minutes with caps open at 30°. Do no open speed-vacuum if still spinning because dry pellets will be suctioned out. Check that pellets look flakey and dry if not speed-vacuum further.
6. Re-suspend pellet in 25µl of ddH_2O. If pellet doesn't dissolve immediately, incubate in water bath at 65° for 5 minutes.
7. Sample can now be run on a gel and then stored at -20° or -80°.

Running a Gel:

1. Clean jar for TAE, rinse gel box and gel tray.
2. Set up gel tray in box with rubber sides against wall and the black adaptor in back.

3. Make 1% TAE gel: weigh 1 gram of agarose powder in jar, fill to 100 mL with TAE (use the left container), heat in microwave with lid loosely on till dissolved (take jar out and swirl periodically, do not let it boil over). Extra TAE gel can go in warming tray.
4. Add 4.5µL of Ethidium Bromide to 45mL of TAE gel (Ethidium Bromide in fridge, put back immediately).
5. Pass Ethidium Bromide and TAE back and forth between two 50mL tubes till mixed.
6. Pour mixture into gel tray to set with two 12 slot combs, insure that combs are set so to insure the biggest lane. Pop bubbles if any. Let gel solidify.
7. Once gel has solidified, pull out combs, then rotate tray so slots are in the back.
8. Fill gel box with TAE to fill line. Make sure that TAE covers the gel.
9. In PCR box covered with parafilm wax, add 7µL of 1x LB BB dye, then 7µL of ddH_2O, then 2µL of sample DNA (use new tips for DNA).
10. Use 20 µL tip to suck up and mix sample/dye mixture then load into gel. Make sure to load gels quickly and use a new tip for each sample.
11. Run gel: set time for about 20 minutes, 120 volts, and 100 amps. Take a picture of gel and save to computer file and lab journal.

APPENDIX 1.2. All Cervidae *MHC-DRB* alleles used for analysis and their subsequent GenBank information.

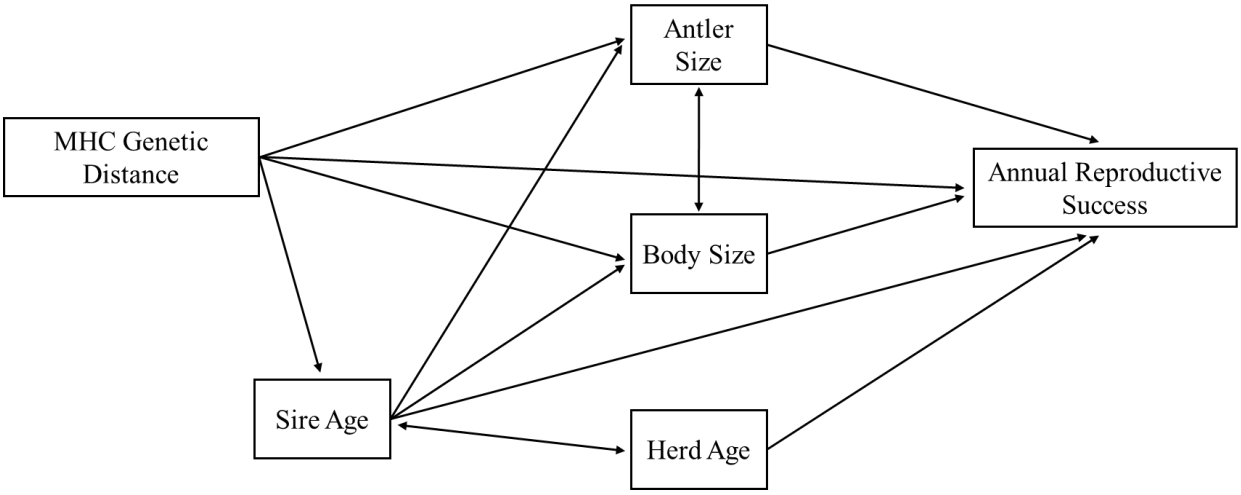
Species	*MHC-DRB* Allele Number	GenBank Accession Number	Author(s)	Year
Alces alces	1	X82398	Mikko and Andersson	1995
Alces alces	2	X83278	Mikko and Andersson	1995
Alces alces	3	X83279	Mikko and Andersson	1995
Alces alces	4	X83280	Mikko and Andersson	1995
Alces alces	5	X83281	Mikko and Andersson	1995
Alces alces	6	X83282	Mikko and Andersson	1995
Alces alces	7	X83283	Mikko and Andersson	1995
Alces alces	8	X83284	Mikko and Andersson	1995
Alces alces	9	X83285	Mikko and Andersson	1995
Alces alces	10	X83286	Mikko and Andersson	1995
Capreolus capreolus	0302	KM488213	Quemere et al.	2015
Capreolus capreolus	0102	KM488214	Quemere et al.	2015
Capreolus capreolus	0304	KM488215	Quemere et al.	2015
Capreolus capreolus	0303	KM488216	Quemere et al.	2015
Capreolus capreolus	0401	KM488218	Quemere et al.	2015
Capreolus capreolus	0203	KM488220	Quemere et al.	2015
Capreolus capreolus	0204	KM488221	Quemere et al.	2015
Capreolus capreolus	0202	KM488222	Quemere et al.	2015
Capreolus capreolus	0101	U90923	Mikko, Lewin, and Andersson	1997
Capreolus capreolus	0201	U90924	Mikko, Lewin, and Andersson	1997
Capreolus capreolus	0301	U90925	Mikko, Lewin, and Andersson	1997
Cervus elaphus	1	U11101	Swarbrick et al.	1995
Cervus elaphus	2	U11102	Swarbrick et al.	1995
Cervus elaphus	3	U11103	Swarbrick et al.	1995
Cervus elaphus	4	U11104	Swarbrick et al.	1995
Cervus elaphus	5	U11105	Swarbrick et al.	1995
Cervus elaphus	6	U11106	Swarbrick et al.	1995
Cervus elaphus	7	U11107	Swarbrick et al.	1995
Cervus elaphus	8	U11108	Swarbrick et al.	1995
Cervus elaphus	9	U11109	Swarbrick et al.	1995
Cervus elaphus	10	U11110	Swarbrick et al.	1995
Cervus elaphus	11	U11111	Swarbrick et al.	1995
Cervus elaphus	12	U11112	Swarbrick et al.	1995
Cervus elaphus	13	U11113	Swarbrick et al.	1995
Cervus elaphus	14	U11114	Swarbrick et al.	1995
Cervus elaphus	15	U11115	Swarbrick et al.	1995
Cervus elaphus	16	U11116	Swarbrick et al.	1995
Cervus elaphus	17	U11117	Swarbrick et al.	1995
Cervus elaphus	18	U11118	Swarbrick et al.	1995
Cervus elaphus	19	U11119	Swarbrick et al.	1995
Cervus elaphus	20	U11120	Swarbrick et al.	1995

Species	*MHC-DRB* Allele Number	GenBank Accession Number	Author(s)	Year
Cervus elaphus	21	U11121	Swarbrick et al.	1995
Cervus elaphus	23	U11122	Swarbrick et al.	1995
Cervus elaphus	24	U11123	Swarbrick et al.	1995
Cervus elaphus	25	U11210	Swarbrick et al.	1995
Cervus elaphus	26	U11211	Swarbrick et al.	1995
Cervus elaphus	27	U11212	Swarbrick et al.	1995
Cervus elaphus	28	U11213	Swarbrick et al.	1995
Cervus elaphus	29	U11214	Swarbrick et al.	1995
Cervus elaphus	30	U11215	Swarbrick et al.	1995
Cervus elaphus	31	U11216	Swarbrick et al.	1995
Cervus elaphus	32	U11217	Swarbrick et al.	1995
Cervus elaphus	33	U11218	Swarbrick et al.	1995
Cervus elaphus	34	U11219	Swarbrick et al.	1995
Cervus elaphus	35	U11220	Swarbrick et al.	1995
Cervus elaphus	36	U11221	Swarbrick et al.	1995
Cervus elaphus	37	U11222	Swarbrick et al.	1995
Cervus elaphus	38	U11223	Swarbrick et al.	1995
Cervus elaphus	39	U11224	Swarbrick et al.	1995
Cervus elaphus	40	U11225	Swarbrick et al.	1995
Cervus elaphus	41	U11226	Swarbrick et al.	1995
Cervus elaphus	42	U11227	Swarbrick et al.	1995
Cervus elaphus	43	U11228	Swarbrick et al.	1995
Cervus elaphus	44	U11229	Swarbrick et al.	1995
Cervus elaphus	45	U11230	Swarbrick et al.	1995
Cervus elaphus	46	U11231	Swarbrick et al.	1995
Cervus elaphus	47	U11232	Swarbrick et al.	1995
Cervus elaphus	48	U11233	Swarbrick et al.	1995
Cervus elaphus	49	U11234	Swarbrick et al.	1995
Cervus nippon	1	DQ225340	Li and Xu	2005
Cervus nippon	2	DQ225341	Li and Xu	2005
Cervus nippon	3	DQ225342	Li and Xu	2005
Cervus nippon	4	DQ225343	Li and Xu	2005
Cervus nippon	5	DQ225344	Li and Xu	2005
Cervus nippon	6	DQ225345	Li and Xu	2005
Cervus nippon	7	DQ225346	Li and Xu	2005
Cervus nippon	8	DQ225347	Li and Xu	2005
Cervus nippon	9	DQ225348	Li and Xu	2005
Cervus nippon	10	DQ225349	Li and Xu	2005
Cervus nippon	11	DQ225350	Li and Xu	2005
Cervus nippon	12	DQ225351	Li and Xu	2005
Cervus nippon	13	DQ225352	Li and Xu	2005
Cervus nippon	14	DQ225353	Li and Xu	2005
Cervus nippon	15	DQ225354	Li and Xu	2005
Cervus nippon	16	DQ225355	Li and Xu	2005

Species	*MHC-DRB* Allele Number	GenBank Accession Number	Author(s)	Year
Cervus nippon	17	DQ225356	Li and Xu	2005
Cervus nippon	18	DQ225357	Li and Xu	2005
Cervus nippon	19	DQ225358	Li and Xu	2005
Cervus nippon	20	DQ225359	Li and Xu	2005
Cervus nippon	21	DQ225360	Li and Xu	2005
Cervus nippon	22	FJ864326	Li, Xu, and Ma	2009
Cervus nippon	23	FJ864327	Li, Xu, and Ma	2009
Cervus nippon	24	FJ864328	Li, Xu, and Ma	2009
Cervus nippon	25	FJ864329	Li, Xu, and Ma	2009
Cervus nippon	26	FJ864330	Li, Xu, and Ma	2009
Cervus nippon	27	FJ864331	Li, Xu, and Ma	2009
Cervus nippon	28	FJ864332	Li, Xu, and Ma	2009
Cervus nippon	29	FJ864333	Li, Xu, and Ma	2009
Cervus nippon	30	FJ864334	Li, Xu, and Ma	2009
Dama dama	DRB1*0101	AF012725	Mikko et al.	1997
Dama dama	DRB2*0101	AF012726	Mikko et al.	1997
Elaphurus davidianus	DRB31	EF195649	Zeng and Jiang	2006
Elaphurus davidianus	DRB32	EF195650	Zeng and Jiang	2006
Elaphurus davidianus	DRB33	EF195651	Zeng and Jiang	2006
Muntiacus crinifrons	5	GQ871811	Jian	2009
Muntiacus crinifrons	11	GQ871812	Jian	2009
Muntiacus crinifrons	1	GQ871813	Jian	2009
Muntiacus crinifrons	2	GQ871814	Jian	2009
Muntiacus crinifrons	3	GQ871815	Jian	2009
Muntiacus crinifrons	4	GQ871816	Jian	2009
Muntiacus crinifrons	6	GQ871817	Jian	2009
Muntiacus crinifrons	9	GQ871818	Jian	2009
Muntiacus crinifrons	8	GQ871819	Jian	2009
Muntiacus crinifrons	7	GQ871820	Jian	2009
Muntiacus crinifrons	10	GQ871821	Jian	2009
Muntiacus reevesi	4	GQ871794	Jian	2009
Muntiacus reevesi	7	GQ871795	Jian	2009
Muntiacus reevesi	6	GQ871795	Jian	2009
Muntiacus reevesi	5	GQ871796	Jian	2009
Muntiacus reevesi	13	GQ871796	Jian	2009
Muntiacus reevesi	17	GQ871797	Jian	2009
Muntiacus reevesi	12	GQ871797	Jian	2009
Muntiacus reevesi	8	GQ871798	Jian	2009
Muntiacus reevesi	19	GQ871799	Jian	2009
Muntiacus reevesi	14	GQ871800	Jian	2009
Muntiacus reevesi	9	GQ871801	Jian	2009
Muntiacus reevesi	16	GQ871802	Jian	2009
Muntiacus reevesi	3	GQ871803	Jian	2009
Muntiacus reevesi	10	GQ871804	Jian	2009

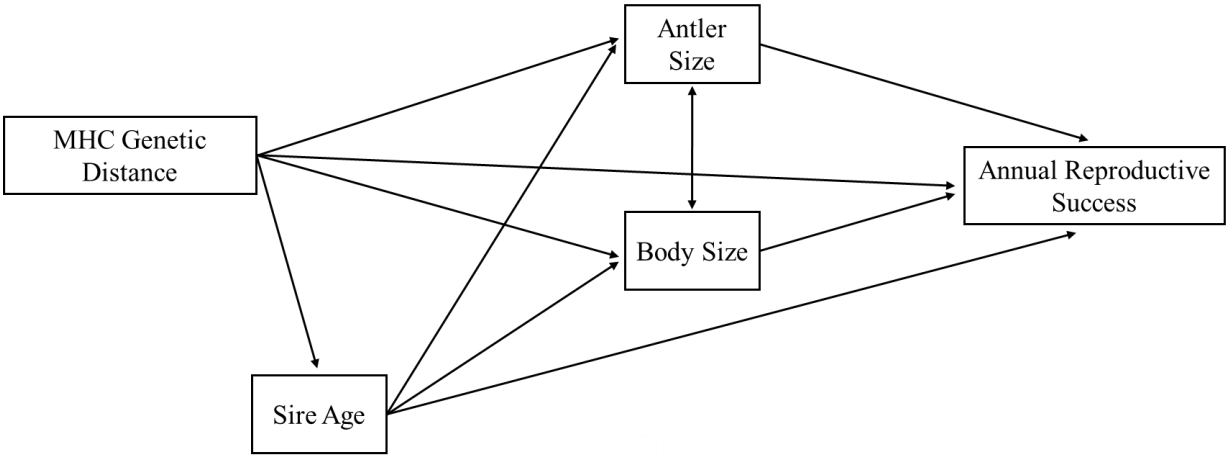
Species	*MHC-DRB* Allele Number	GenBank Accession Number	Author(s)	Year
Muntiacus reevesi	20	GQ871805	Jian	2009
Muntiacus reevesi	1	GQ871806	Jian	2009
Muntiacus reevesi	11	GQ871807	Jian	2009
Muntiacus reevesi	2	GQ871808	Jian	2009
Muntiacus reevesi	15	GQ871809	Jian	2009
Muntiacus reevesi	18	GQ871810	Jian	2009
Odocoileus virginianus	1	AF082161	Van Den Bussche, Hoofer, and Lochmiller	1999
Odocoileus virginianus	2	AF082162	Van Den Bussche, Hoofer, and Lochmiller	1999
Odocoileus virginianus	3	AF082163	Van Den Bussche, Hoofer, and Lochmiller	1999
Odocoileus virginianus	4	AF082164	Van Den Bussche, Hoofer, and Lochmiller	1999
Odocoileus virginianus	5	AF082165	Van Den Bussche, Hoofer, and Lochmiller	1999
Odocoileus virginianus	6	AF082166	Van Den Bussche, Hoofer, and Lochmiller	1999
Odocoileus virginianus	7	AF082167	Van Den Bussche, Hoofer, and Lochmiller	1999
Odocoileus virginianus	8	AF082168	Van Den Bussche, Hoofer, and Lochmiller	1999
Odocoileus virginianus	9	AF082169	Van Den Bussche, Hoofer, and Lochmiller	1999
Odocoileus virginianus	10	AF082170	Van Den Bussche, Hoofer, and Lochmiller	1999
Odocoileus virginianus	11	AF082171	Van Den Bussche, Hoofer, and Lochmiller	1999
Odocoileus virginianus	12	AF082172	Van Den Bussche, Hoofer, and Lochmiller	1999
Odocoileus virginianus	13	AF082173	Van Den Bussche, Hoofer, and Lochmiller	1999
Odocoileus virginianus	14	AF082174	Van Den Bussche, Hoofer, and Lochmiller	1999
Odocoileus virginianus	15	AF082175	Van Den Bussche, Hoofer, and Lochmiller	1999
Odocoileus virginianus	16	AF407169	Van Den Bussche, Ross, and Hoofer	2002
Odocoileus virginianus	17	AF407170	Van Den Bussche, Ross, and Hoofer	2002
Odocoileus virginianus	18	AF407171	Van Den Bussche, Ross, and Hoofer	2002
Odocoileus virginianus	**19**	**MK952679**	**Ivy-Israel, Moore, Schwartz, and Ditchkoff**	**2019**
Odocoileus virginianus	**20**	**MK952680**	**Ivy-Israel, Moore, Schwartz, and Ditchkoff**	**2019**
Odocoileus virginianus	**21**	**MK952681**	**Ivy-Israel, Moore, Schwartz, and Ditchkoff**	**2019**
Odocoileus virginianus	**22**	**MK952682**	**Ivy-Israel, Moore, Schwartz, and Ditchkoff**	**2019**
Odocoileus virginianus	**23**	**MK952683**	**Ivy-Israel, Moore, Schwartz, and Ditchkoff**	**2019**
Odocoileus virginianus	**24**	**MK952684**	**Ivy-Israel, Moore, Schwartz, and Ditchkoff**	**2019**
Odocoileus virginianus	**25**	**MK952685**	**Ivy-Israel, Moore, Schwartz, and Ditchkoff**	**2019**
Odocoileus virginianus	**26**	**MK952686**	**Ivy-Israel, Moore, Schwartz, and Ditchkoff**	**2019**
Odocoileus virginianus	**28**	**MK952688**	**Ivy-Israel, Moore, Schwartz, and Ditchkoff**	**2019**
Odocoileus virginianus	**29**	**MK952689**	**Ivy-Israel, Moore, Schwartz, and Ditchkoff**	**2019**
Odocoileus virginianus	**30**	**MK952690**	**Ivy-Israel, Moore, Schwartz, and Ditchkoff**	**2019**
Rangifer tarandus	0101	AF012716	Mikko, S.	1997
Rangifer tarandus	0102	AF012717	Mikko, S.	1997
Rangifer tarandus	0103	AF012718	Mikko, S.	1997
Rangifer tarandus	0201	AF012719	Mikko, S.	1997
Rangifer tarandus	0301	AF012720	Mikko, S.	1997
Rangifer tarandus	0401	AF012721	Mikko, S.	1997
Rangifer tarandus	0501	AF012722	Mikko, S.	1997
Rangifer tarandus	0601	AF012723	Mikko, S.	1997
Rangifer tarandus	0701	AF012724	Mikko, S.	1997

APPENDIX 2.1. A priori models developed to represent the eight hypothesized relationships among variables influencing annual reproductive success in male white-tailed deer (*Odocoileus virginianus*). Variables are defined on page 41 and include MHC genetic distance, herd age, sire age, body size, antler size, and annual reproductive success. Potentially correlated variables are indicated by two-way arrows.



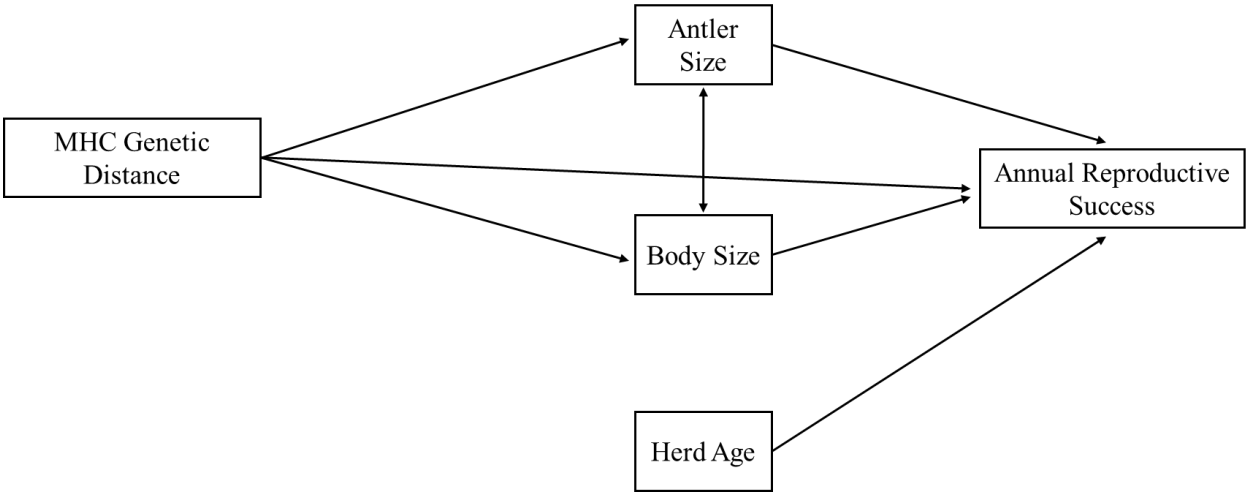
Model 8: Global model

APPENDIX 2.1 Continued



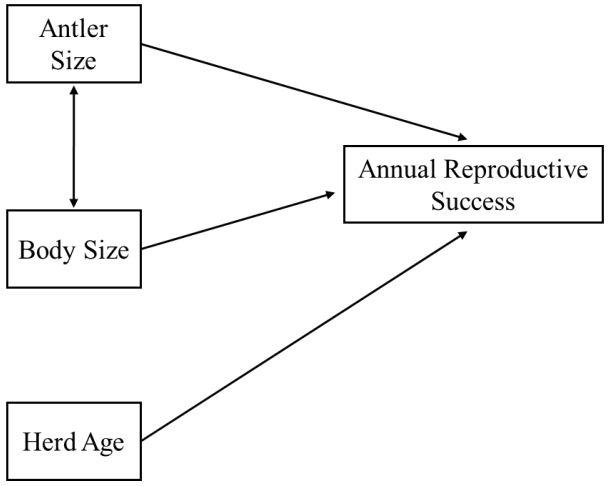
Model 7: Global model without herd age

APPENDIX 2.1 Continued



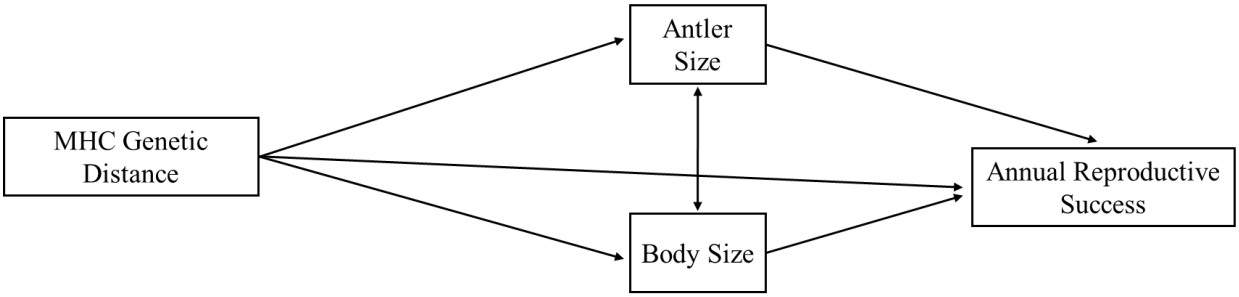
Model 6: Global model without sire age

APPENDIX 2.1 Continued



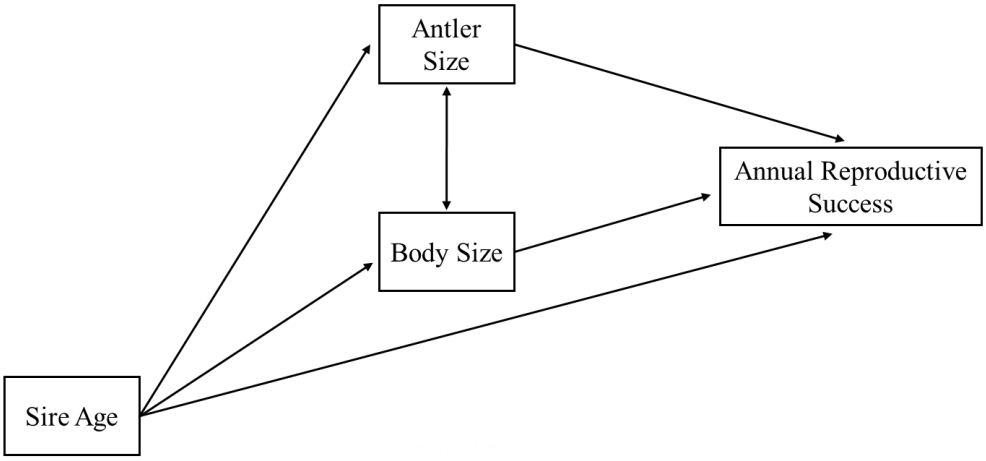
Model 5: Global model without sire age and MHC

APPENDIX 2.1 Continued



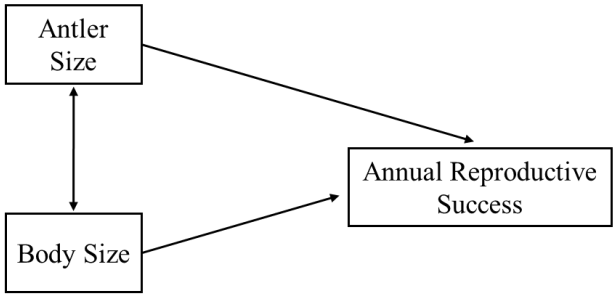
Model 4: Simple model with MHC, antler size, and body size

APPENDIX 2.1 Continued



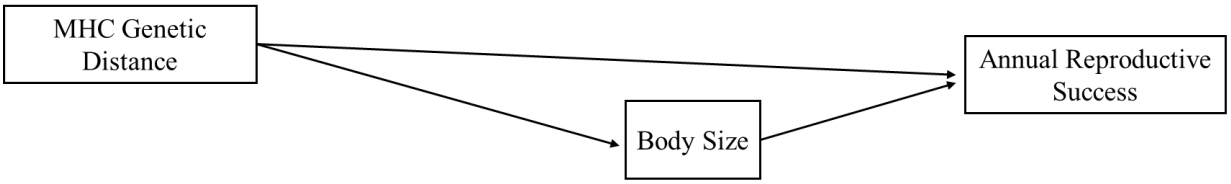
Model 3: Simple model with sire age, antler size, and body size

APPENDIX 2.1 Continued



Model 2: Simple model with antler size and body size

APPENDIX 2.1 Continued



Model 1: Simple model with MHC and body size