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MAJOR-HISTOCOMPATIBILITY-COMPLEX-ASSOCIATED VARIATION IN SECONDARY SEXUAL TRAITS OF WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*): EVIDENCE FOR GOOD-GENES ADVERTISEMENT

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Abstract.—Good-genes hypotheses predict that development of secondary sexual characters can be an honest advertisement of heritable male quality. We explored this hypothesis using a cervid model (adult, male white-tailed deer, *Odocoileus virginianus*) to determine whether antler development could provide an honest signal of a male's genetic quality and condition to adversaries. We compared antler, morphometric, hormonal, and parasitic data collected from hunter-harvested deer to characteristics of the *Mhc-DRB (Odvi),* the most widely studied gene of the major histocompatibility complex (MHC) in Artiodactyla. We detected associations between genetic characteristics at *Odvi-DRB* and antler development and body mass, suggesting that antler development and body mass may be associated with pathogen resistance in deer and thus may be an honest signal of genetic quality. We also detected associations between *Odvi-DRB* characteristics and serum testosterone during the breeding season, suggesting that certain MHC characteristics may help deer cope with stresses related to breeding activity. In addition, we observed a negative relationship between degree of antler development and overall abundance of abomasal helminths. Our observations provide support for the hypothesis that antler development in white-tailed deer is an honest signal of quality.

Key words.—Abomasal parasites, antler development, genetics, honest advertisement, major histocompatibility complex, *Odocoileus virginianus,* testosterone, white-tailed deer.

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Zahavi (1975) proposed a mechanism to explain how females can accurately assess quality of an advertiser during intersexual communication. The basis of the theory was that the best way to guard against cheating (e.g., dishonest signaling) during intersexual communication, or advertisement, is for communication to be costly such that the signaler incurs a penalty that is proportional to the intensity of the advertisement. Only the most fit males can afford to incur a heavy penalty, thus, only the most fit males can afford extravagant advertisement. Since the hypothesis (Zahavi 1975) was proposed, a plethora of studies have been published to test its various components (see Andersson 1994), and today its principles are widely accepted (Maynard Smith 1991; Collins 1993; Lotem 1993). Theory predicts that females that choose mates with extravagant ornamentation will have greater fitness, and several studies have demonstrated that offspring survival is greater when females mate with males that possess elaborate secondary characters (Norris 1993; Petrie 1994; von Schantz et al. 1994).

Zahavi (1977) and Zahavi and Zahavi (1997) expanded the original theory by proposing a handicap principle to explain honesty in all forms of advertisement. To date, most studies designed to examine the handicap principle have followed Hamilton and Zuk (1982), whose immunocompetence-handicap hypothesis proposed a mechanism whereby female mate choice is based on male sexual ornamentation and exhibited resistance to parasites. This model has been shown to explain mate choice in polygynous species where males advertise their ''good genes'' to females through sexual ornamentation and display (e.g., extravagant feathers, bright colors; Kennedy et al. 1987; Hillgarth 1990; Zuk et al. 1990b). However, almost no work has been conducted to examine the handicap principle among species where secondary sexual characters serve as weapons (e.g., antlers, horns, spurs) and are used to gain access to mates. In polygynous species where males have evolved sexual ornamentation that serves as a weapon, the handicap principle should apply to intrasexual advertisement among males (Zahavi and Zahavi 1997) to ensure that signals of quality are honest.

We examined the handicap principle in white-tailed deer (*Odocoileus virginianus*), a cervid species in which antlers serve as weapons to help gain access to mates. Courtship in white-tailed deer has been described as a tending bond (Geist 1981), whereby a dominant male remains with an estrous female and defends her until she is bred. Antler development is important for establishing dominance among males and gaining access to females and often is associated positively with breeding success (Clutton-Brock et al. 1982). Theory suggests that antlers originally evolved as weapons (Goss 1983), and studies of antler structure in species where antlers have been hypothesized to be primarily for display (e.g., Irish elk, *Megaloceros giganteus*; Gould 1974) have suggested that antlers have structural properties required for intrasexual combat (Kitchener 1987). However, the literature is replete with information describing how dominant males gain access to mates with little or no fighting, probably because it would be deleterious for immature or smaller males to challenge

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dominant individuals. This behavior suggests that antlers also serve to display strength and quality.

But how is antler development a handicap? Antler tissue has been described as the fastest growing tissue in the animal kingdom (Goss 1983). Considering their deciduous nature, annual growth and replacement of these organs places incredible nutritional demands on male deer (French et al. 1956; Ullrey 1983; Asleson et al. 1997). Mineral requirements are so great that male deer annually shunt calcium and phosphorus from their skeletons to meet growth requirements of antlers (Meister 1956). As a result, these ornate weapons should serve as signals of food gathering capability or metabolic efficiency.

Immunosuppression may be an additional handicap associated with development of antlers. It has been proposed that immunocompetence could be reduced indirectly through increased nutritional costs (Lochmiller 1996) associated with development of secondary sexual traits. In addition, androgenic hormones (testosterone, etc.) required to initiate antler growth and antler hardening prior to the breeding season (Morris and Bubenik 1982) have immunosuppresive effects (Grossman 1984; Alexander and Stimson 1988; Folstad et al. 1989). Therefore, antler size may serve to advertise the extent to which male deer can withstand nutritional and androgenic suppression of the immune system and may demonstrate genetic quality by signaling good physical condition throughout a period of high nutritional demand, reduced immunocompetence, and elevated susceptibility to parasitism and disease (Maynard Smith 1985; Folstad and Karter 1992).

The major histocompatibility complex (MHC) likely plays a role in the good-genes hypothesis (Howard 1991) because of its importance in antigen recognition and immune response. The MHC is a gene complex common to all vertebrates that serves to distinguish self from nonself. Class I and class II molecules of the MHC bind to antigens and aid in identifying foreign peptides (Klein 1986), thereby serving as an essential line of defense in immune response to foreign pathogens (e.g., parasites and disease). Although a plethora of studies have reported associations between MHC loci and specific pathogens, the extent of these associations are poorly understood. In addition, we have only begun to understand the influence that the MHC has on physiological processes such as growth, development, and reproduction and its role in population ecology (Lochmiller 1996). Development of secondary sexual characters, like other physiological processes, should benefit from genes that confer an immunologic advantage (Howard 1991). If individuals heterozygous for MHC loci have an immunologic advantage because of resistance to a greater variety of pathogens (Doherty and Zinkernagel 1975; Hughes and Nei 1988), MHC quality should be apparent in expression of condition-dependent traits. Taken together, it seems likely that a relationships should exist between genotypes of MHC loci and development of secondary sexual ornamentation. For example, von Schantz et al. (1996, 1997) found that spur length in male pheasants (*Phasianus colchicus*), a trait selected for by mating females (von Schantz et al. 1989) and important during intrasexual combat (Davidson 1985; Møller 1992), was associated with MHC genotype. However, no other studies have documented similar relationships.

We chose a wild population of white-tailed deer as a model to test predictions of the handicap principle (Zahavi 1975; Zahavi and Zahavi 1997) and to determine whether antler development is a good signal of genetic quality. Specifically, our objectives were to assess whether: (1) MHC characteristics were associated with antler development and body size; (2) MHC characteristics were associated with circulating levels of testosterone; and (3) developmental traits of antlers were associated with parasite burdens in adult, male deer. Previous work (Ditchkoff 2000) has reported an association between exon 2 of the *Mhc-DRB* and parasite burdens in white-tailed deer hosts and suggested that particular allelic lineages may be important for providing resistance to potential pathogens. In this study, we again examined exon 2 of *Mhc-DRB* because genotypes for this locus were available for the deer population under study, it is highly variable, and it has been the most widely studied MHC locus in white-tailed deer and Artiodactyla to date (Mikko and Anderson 1995; Swarbick et al. 1995; Mikko et al. 1997; Van Den Bussche et al. 1999).

Materials and Methods

Study Area

We collected data from adult, male white-tailed deer (n = 128) that were harvested by hunters during October and November from 1995 to 1996 at the McAlester Army Ammunition Plant (McAAP) in southeastern Oklahoma (34°49′N, 95°55′W). The McAAP is an 18,212-ha area owned and operated by the U.S. Department of Defense and has been managed to maintain a large proportion (≥55%; Ditchkoff et al. 2000) of mature males within the population (i.e., quality deer management) since 1989 (Ditchkoff et al. 1997). Annual census indicated that the herd was below carrying capacity (density was approximately 12–13 deer/km^2) and the buck: doe ratio was 1:2.2. The McAAP was not surrounded by barriers (fence or wall) that restricted movement of deer on or off the study area, and thus our study population was contiguous with deer populations in surrounding areas. Vegetation on the area was characterized by oak uplands *(Quercus marilandica, Q. stellata)* and bottomlands *(Q. shumardii, Q. nigra)* intermixed with tallgrass prairie *(Andropogon virginicus, A. gerardii, Schizachyrium scoparium)* and brush communities *(Smilax bona-nox, Rhus copallina, Ulmus alata, Prunus angustifolia, Diospyros virginiana)*. Soils tended to be sandy, and common soil series were Chateau very fine sandy loam, Dennis-Dwight complex, Eram clay loam, and Enders-Hector complex. Mean annual rainfall was 112 cm, with a high mean temperature of 28°C during July and low mean temperature of 4.5°C between December and February.

Morphology Data

We measured chest girth immediately posterior to the front legs and body length from tip of the nose to base of the tail. Hind-foot length was measured as the distance from the tip of the hoof to the posterior end of the tuber calcis (tarsal). Skull length was measured as the tip of the nose to the base of the skull (occipital bone). Deer were weighed to the nearest 0.5 kg using a hanging scale immediately after they were

field-dressed. We removed testicles from the scrotum and weighed each to the nearest 0.1 g. Deer were aged by tooth wear and replacement (Severinghaus 1949).

Antlers were measured according to the scoring system (Boone and Crockett) described by Nesbitt and Wright (1981) to obtain an estimate of total antler development. Boone and Crockett score is a trophy scoring system where scores for each antler are summed together with the inside spread (greatest distance between the main beams) to obtain an overall estimate of antler development. This system measures length of each tine, length of the main beam, and circumferences around the main beam at the base of the antler (basal circumference) and between successive tines (not to exceed four circumferences for each antler). The Boone and Crockett scoring system also penalizes for asymmetry of antlers, but we did not include asymmetry deductions in our measures of antler score. An antler score was calculated for both the right and left antler, and a total gross score was calculated that included scores from both antlers and measurement of inside spread. We did not include scores in the analysis for animals that had broken tines or main beams. Absolute asymmetry was calculated as the difference between measurements of the right and left antlers; relative asymmetry was calculated as absolute asymmetry divided by the larger antler. Relative fluctuating asymmetry adjusts absolute asymmetry measurements for the size of the trait. Absolute and relative fluctuating asymmetry measurements were calculated for antler score, number of points, main beam length, and basal circumference.

Testosterone

We collected blood from the thoracic cavity of each deer into 10-ml evacuated serum-separating tubes (SST; Becton Dickinson Vacutainer Systems, Rutherford, NJ). Serum was separated by centrifugation within 6 h of collection and stored at −85°C. Serum testosterone levels were assayed with a direct RIA procedure (Coat-A-Count Testosterone, Diagnostics Products Corporation, Los Angeles, CA). Following the Coat-A-Count methodology, we added 50 μl of serum and 1.0 ml of ^{125}I testosterone to antibody-labeled tubes prior to incubation for 3 h at 37°C. Each Coat-A-Count tube was prelabeled with monoclonal antibodies to testosterone. Following incubation, samples were thoroughly aspirated and radioactivity of tubes was counted in a gamma counter. Each sample was assayed in duplicate and samples with error >5% were reanalyzed. This assay is sensitive to testosterone concentrations ≥4 ng/dL, and interassay percentage CV is 5–12%.

Parasites

When field-dressing deer, we removed the abomasum and tied both ends with string to avoid loss of contents. Abomasa were subsequently frozen until later analysis. In the laboratory, abomasal contents were removed, diluted with water to 1000 ml, and a 50-ml aliquot examined under magnification (Prestwood et al. 1973). Total nematodes were calculated by multiplying the number of nematodes per aliquot by 20. Nematodes were identified according to Kubat et al. (1980). Ectoparasitic ticks (primarily *Amblyomma americanum* and *Ixodes scapularis*) were counted on the sternum of each deer prior to field-dressing using a template with a rectangular hole 2.5 × 12.5 cm similar to the technique described by Sams et al. (1996) for use on deer fawns. All ticks within the template were counted and the count was used as an index of the degree of infestation with ectoparasites.

We calculated abundance, intensity, and prevalence of each species of abomasal nematode according to Margolis et al. (1982). Abundance was defined as the mean number of abomasal nematodes per deer. We estimated intensity of infection by calculating mean number of nematodes per deer with at least one nematode, whereas prevalence was the percentage of deer with at least one nematode. For ticks, we calculated abundance only (not intensity or prevalence) because prevalence approached 100%.

Genetics

Genomic DNA was isolated from approximately 1 g of liver tissue following the protocol of Longmire et al. (1997). Amplification of the second exon of *Mhc-DRB* was accomplished via polymerase chain reaction (PCR) using primers LA31 an LA32 (Sigurdardottir et al. 1991; Mikko and Andersson 1995). These primers flank the functionally critical antigen-binding site and produce a product of 390 base pairs that encompasses 83 amino acid residues. PCR was accomplished by using approximately 400 ng of DNA in a final reaction volume of 50 μl consisting of 1 unit of *Taq* DNA polymerase (Promega, Madison, WI), 0.5 μM of each primer, 0.07 mM deoxynucleotides, 1.0 μCi α^{32}P-dCTP, and 2.0 mM $MgCl_2$. The thermal profile consisted of 95°C for 60 sec, 50°C for 30 sec, and 72°C for 30 min and was conducted using a GeneAmp PCR System 9600 (Perkin Elmer, Norwalk, CT).

Following amplification, all individuals were genotyped via single-stranded conformation polymorphism (SSCP) analysis (Orita et al. 1989). PCR amplicons were denatured by heating and immediately placed into ice water, loaded onto 5% nondenaturing acrylamide gels (acrylamide:bisacrylamide = 49:1) containing 10% glycerol, and subjected to electrophoresis at 300 V for 24 h with a fan blowing on the gel. Following electrophoresis, acrylamide gels were transferred to Whatman paper, dried, and exposed to autoradiographic film.

All unique conformations were cloned using the pGEM-T cloning system (Promega) for subsequent analyses. For each cloned allele, SSCP analysis was performed on PCR products of several recombinant clones using reaction conditions and thermal profiles described above. PCR amplicons from cloned inserts were run alongside amplified products from the individual. This approach enabled verification of which recombinant clones contained the correct allele with no PCR-induced error prior to DNA sequencing. After verification, PCR amplicons of each unique allele were cleaned using the Wizard PCR Prep DNA Purification System (Promega) and sequenced in both directions on a Perkin Elmer Applied Biosystems 373 automated DNA sequencer. To aid genotyping individuals across gels, the 15 alleles (*Odvi-DRB**01–15) for this population were run on every gel.

TABLE 1. Categorization of alleles of the *Mhc-DRB (Odvi)* exon 2 found in white-tailed deer from southeastern Oklahoma into evolutionary lineages based on phylogenetic analysis.[1]

Allelic lineage 1	Frequency (%)	Allelic lineage 2	Frequency (%)
*Odvi-DRB*01*	1.9	*Odvi-DRB*02*	0.4
*Odvi-DRB*03*	1.9	*Odvi-DRB*05*	32.8
*Odvi-DRB*04*	26.9	*Odvi-DRB*06*	0.8
*Odvi-DRB*09*	0.8	*Odvi-DRB*07*	13.8
*Odvi-DRB*13*	0.8	*Odvi-DRB*08*	1.1
*Odvi-DRB*14*	6.9	*Odvi-DRB*10*	1.9
*Odvi-DRB*15*	0.4	*Odvi-DRB*11*	5.4
		*Odvi-DRB*12*	4.2

[1] Evolutionary groupings were based upon phylogenetic analysis of Van Den Bussche et al. (1999).

Statistics

The detection of 15 alleles among 128 individuals sampled from this population indicated 120 possible *Odvi-DRB* genotypes, thereby limiting the statistical power available for statistical analyses. To increase statistical power to test for associations between *Mhc-DRB* exon 2 genotype and morphologic and physiologic variables, we classified alleles into one of two allelic lineages (Table 1) based on evolutionary lineages reported by Van Den Bussche et al. (1999). Using this phylogenetic classification, we were able to categorize the *Odvi-DRB* profiles of our deer into three categories. Deer with both *Odvi-DRB* alleles from lineage 1 were categorized as type 11, and deer with both alleles from lineage 2 were categorized as type 22. Deer with alleles from both lineages were categorized as type 12. Because Van Den Bussche et al. (1999) reported that evolution of *Odvi-DRB* alleles typically occurs by point mutations rather than recombination, we felt that alleles from the same lineage were likely to be similar from a functional standpoint. Although this classification of alleles falsely inflates the number of homozygotes, we felt that this would be a conservative approach to test for statistically significant trends between *Odvi-DRB* exon 2 and morphologic or physiologic variables.

We compared total Boone and Crockett score, antler score, number of points, beam length, and basal circumference of deer with different genetic types using analysis of covariance (ANCOVA), with age as a covariate to control for age-related variation in antler size. We also used ANCOVA with age as a covariate to test for differences in body size between deer with different genetic types. We used analysis of variance (ANOVA) to test for differences in relative and absolute asymmetry of antlers between deer with different genetic types. We did not use ANCOVA to control for age in this analysis because relative asymmetry accounts for age-related differences in antler size. We examined genetic influences on serum testosterone using ANCOVA with age and date of harvest as covariates. We tested for differences in least squared means to determine differences between genetic types if genetic effects were statistically significant ($P < 0.05$) in ANCOVAs. We used multiple regression to assess relationships between antler size and testosterone. Actual measures of antler size were the dependent variable and age was included with serum testosterone as an independent variable to control for age-related changes in antler size and testosterone. We used simple linear regression analyses to assess relationships between serum testosterone and parasite measures (e.g., abundance, intensity, and prevalence), and multiple regression to evaluate relationships between antler development and parasite burdens.

TABLE 2. Number (%) of adult, male white-tailed deer with particular genetic characteristics of the *Mhc-DRB.*

Age	*Odvi-DRB* type 11	*Odvi-DRB* type 12	*Odvi-DRB* type 22
1.5	9 (19%)	12 (25%)	27 (56%)
2.5	7 (17%)	19 (45%)	16 (38%)
3.5	4 (20%)	9 (45%)	7 (35%)
4.5	1 (11%)	6 (67%)	2 (22%)
≥5.5	6 (67%)	3 (33%)	0 (0%)
Total	27 (21%)	49 (38%)	52 (41%)

RESULTS

Allelic frequencies for the 15 unique alleles (*Odvi-DRB**01–15; GenBank accession numbers AF082161–AF082175) detected for this population ($n = 128$) ranged from 0.8% to 57.0% (Table 1). Sequences were confirmed either by sequencing the same allele for more than one individual or by checking SSCP banding patterns between cloned PCR products and PCR products of the individual from which the allele was isolated. As discussed by Van Den Bussche et al. (1999), nucleotide and amino acid polymorphism at this locus was characteristic of highly polymorphic MHC loci. Thirty percent of nucleotide and 47% of amino acid positions were polymorphic among these 15 alleles. Nucleotide sequence variation among all pairwise comparisons of *Odvi-DRB* alleles, corrected for multiple substitutions, ranged from 3.27% to 20.60%, whereas amino acid replacements among all pairwise comparisons ranged from four to 27 (Van Den Bussche et al. 1999).

As discussed by Van Den Bussche et al. (1999), white-tailed deer appear to have only a single *DRB* gene (i.e., no pseudo-*DRB* genes). All deer were examined using SSCP analysis and at no time were more than four prominent SSCP bands detected in any individual. Subsequent cloning and SSCP analysis of PCR amplicons confirmed that less prominent bands were alternative conformations of the same allele. Although we cannot be completely certain that white-tailed deer do not contain more than a single expressed *DRB* gene, Van Den Bussche et al. (1999) suggested that if additional *DRB* loci are present in white-tailed deer, the primers used did not amplify additional loci to the extent that they were observable in SSCP or cloning and sequence analyses.

Grouping alleles into the two evolutionary lineages detected by the phylogenetic analysis of Van Den Bussche et al. (1999) resulted in mean number of amino acid differences of 11.3 within allelic lineage 1, 17.2, within allelic lineage 2, and 19.0 between allelic lineages. Type 11 deer accounted for 22% of the deer sampled, 37% of the deer were type 12, and 41% of the deer were type 22 (Table 2). Deer with *Odvi-DRB* alleles from both allelic lineages (type 12) had 20% and 13% greater ($P < 0.05$) Boone and Crockett antler scores than deer with *Odvi-DRB* types 11 and 22, respectively (Table 3). Similarly, mean number of antler points and mean basal

TABLE 3. Least-square means of antler measurements from white-tailed deer with different *Mhc-DRB (Odvi)* characteristics calculated from an analysis of covariance, with age as a covariate. Least-square means in a column with different letters are different ($P \leq 0.05$).

Odvi-DRB	Gross Boone and Crockett score	Mean antler score	Mean number of antler points	Mean main beam length (cm)	Mean basal circumference (cm)
Type 11	69.0 a	28.9 a	2.95 a	32.7 a	7.55 a
Type 12	83.0 b	34.9 b	3.46 b	36.9 b	8.25 b
Type 22	73.7 a	31.4 ab	3.03 a	34.0 ab	7.61 a

TABLE 4. Least-square means of body measurements from white-tailed deer with different *Mhc-DRB (Odvi)* characteristics calculated from an analysis of covariance, with age as a covariate. Least-square means in a column with different letters are different ($P \leq 0.05$).

Odvi-DRB	Field-dressed body mass (kg)	Body length (cm)	Skull length (mm)	Chest girth (mm)	Testicle mass (g)
Type 11	44.5 a	133.3 a	309.3 a	870.9 a	36.0 a
Type 12	47.9 b	135.8 a	324.0 b	870.9 a	36.4 a
Type 22	45.7 ab	134.5 a	317.2 ab	814.6 a	32.7 a

circumference were more than 14% and 8% greater ($P < 0.05$) for type 12 deer than types 11 or 22 deer, respectively. Type 12 deer had a 21% greater ($P < 0.05$) mean antler score and a 13% greater length of main beams than type 11 deer, whereas these antler traits did not differ from type 22 deer. Although measures of antler size tended to be greater for type 22 than type 11 deer, statistical significance was not apparent ($P > 0.05$). Relative and absolute asymmetry of antlers (score, number of points, length of main beam, and basal circumference) did not differ ($P > 0.10$) among groups of deer with different genetic characteristics. Field-dressed body mass and skull length were about 5% greater ($P < 0.05$) for type 12 than type 11 deer, but were similar ($P > 0.05$) between types 12 and 22 deer (Table 4). Body length, chest girth, and mean testicular mass did not differ ($P > 0.05$) among deer from the three MHC types.

We found that both age and testosterone were positively associated with ($P < 0.05$) and accounted for significant variation in antler characteristics (Table 5). Mean antler score increased 0.26 ± 0.09 (mean ± SE) points, main beam length increased 0.16 ± 0.07 cm, number of antler points increased by 0.02 ± 0.01, basal circumference increased 0.04 ± 0.01 cm, and Boone and Crockett score increased 0.57 ± 0.21 points for each nanogram increase in serum testosterone. Regressions of Boone and Crockett score, mean antler score, main beam length, and basal circumference with age and testosterone produced $R^2 \geq 0.62$, whereas number of antler points had $R^2 = 0.48$ when regressed against age and testosterone. Concentrations of serum testosterone in deer with *Odvi-DRB* type 12 were 75% greater ($P = 0.031$) than type 22 deer (Fig. 1). Type 12 deer that were 2.5 years old also had greater ($P = 0.043$) concentrations of serum testosterone than 2.5-year-old deer from with type 22 genetics. This trend was not apparent in other age classes. Concentrations of serum testosterone in type 11 deer did not differ ($P > 0.05$) from those deer with type 12 or type 22 genetic characteristics within age classes or overall.

Total abundance of abomasal nematodes was 735.7 ± 52.7, with abundance estimates ranging from 20 to 3200 nematodes. We identified six species of abomasal nematodes and mean abundances ranged from 3.0 to 504.0; *Haemonchus contortus* (25.8 ± 11.0; mean ± SE), *Apteragia odocoilei* (504.0 ± 35.0), *A. pursglovei* (3.3 ± 1.5), *Ostertagia dikmansi* (148.3 ± 16.7), *O. mossi* (58.4 ± 9.8), and *O. ostertagia* (3.0 ± 1.6). Statistical analyses of *A. pursglovei* and *O. ostertagia* were not possible because each were found in fewer than five deer. Mean number of nematode species within the abomasum of each deer was 2.6.

Levels of serum testosterone were not associated with abundances of total abomasal nematodes ($P = 0.934$), *H. contortus* ($P = 0.564$), *A. odocoilei* ($P = 0.368$), *O. mossi* ($P = 0.174$), *O. dikmansi* ($P = 0.738$), or sternum ticks ($P = 0.367$). Similarly, intensity or prevalence of infection were not associated with ($P > 0.05$) levels of serum testosterone. In contrast, multiple regression analysis revealed that total abundance of abomasal nematodes was negatively associated ($P = 0.050$) with mean antler score (Table 6). However, individual species of nematodes were not associated ($P > 0.05$) with antler size. Our index of ectoparasitic ticks was positively associated with antler size ($P = 0.033$) and body mass ($P = 0.001$).

DISCUSSION

We observed significant associations between the *Mhc-DRB* and development of secondary sexual characters (e.g., antlers) in white-tailed deer. Deer that possessed *Mhc-DRB* alleles from both evolutionary clades (type 12 deer) generally had greater age-adjusted development of antlers. Previous studies using electrophoretic analysis have suggested that

TABLE 5. Parameter estimates of multiple regressions comparing age and concentration serum testosterone to measures of antler size in male white-tailed deer ($n = 128$) from a population in southeastern Oklahoma.

Dependent variable	R^2	Intercept			Age (years)			Testosterone (ng/dL)		
		Est.	SE	*P*	Est.	SE	*P*	Est.	SE	*P*
Antler score[1]	0.63	7.47	1.93	0.001	8.44	0.63	0.001	0.26	0.09	0.007
Beam length	0.62	16.02	1.49	0.001	6.51	0.48	0.001	0.16	0.07	0.028
Antler points	0.48	1.46	0.19	0.001	0.60	0.06	0.001	0.02	0.01	0.016
Basal circum.	0.66	4.34	0.26	0.001	1.21	0.08	0.001	0.04	0.01	0.001
B & C score[2]	0.63	20.08	4.26	0.001	18.87	1.39	0.001	0.57	0.21	0.008

[1] Antler score was determined as the mean of individual Boone and Crockett scores (Nesbitt and Wright 1981) for each antler.
[2] Boone and Crockett scores were measured according to Nesbitt and Wright (1981).

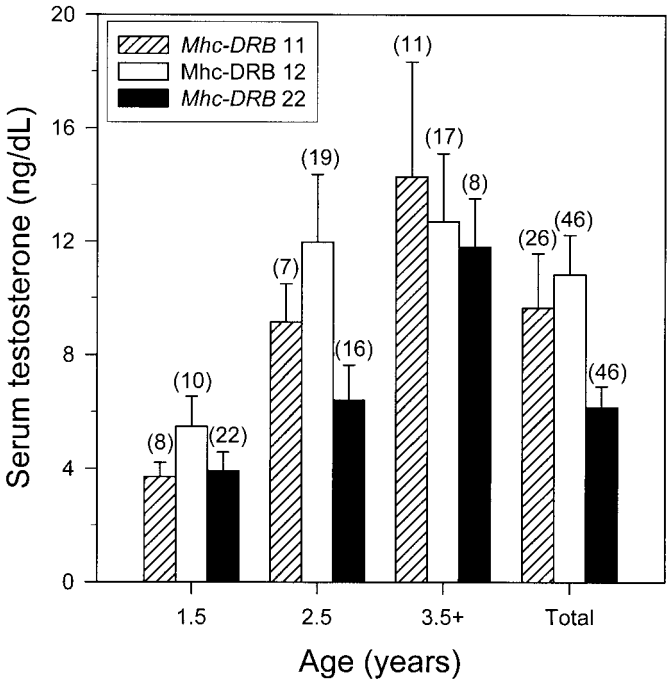

FIG. 1. Mean, age-specific levels of serum testosterone from male, white-tailed deer with different allelic composition at *Odvi-DRB*. Numbers in parentheses are sample sizes.

heterozygosity at select protein-coding loci is associated with increased development of antlers (Smith et al. 1983; Scribner et al. 1984, 1989; Scribner and Smith 1990) and growth of other tissues (Chesser and Smith 1987) in deer. Our observations provide the first evidence of an association between the MHC and antler development. An association has been observed between MHC genotype and spur length in pheasants (von Schantz et al. 1996, 1997), but additional insights into MHC-driven development of secondary sexual characters have yet to be reported.

According to the handicap principle (Zahavi 1975; Zahavi and Zahavi 1997), development of large antlers should be driven by genetic advantages that result in superior condition and thus serves as an honest signal of individual quality. Whereas degree of antler development represents a male's ability to allocate energy and nutrients to antler growth during the previous year, other morphological measures (e.g., body mass, skull length) are more representative of an individual's condition over the course of their life. We found that deer with alleles from both *Mhc-DRB* evolutionary lineages (type 12) had greater body mass and skull length than type 11 deer, suggesting that the MHC variability measured in these individuals may be an important determinant of fitness via its influence on nutritional condition and ultimately reproductive success. Previous work has indicated that body mass and antler size are important criteria for establishing dominance and maximizing mating success in cervids (Townsend and Bailey 1981; Clutton-Brock et al. 1982). Thus, male white-tailed deer with *Mhc-DRB* alleles from divergent evolutionary clades may maximize potential lifetime fitness because of greater resource allocation to body growth and antler development.

Although most models of the handicap principle propose testosterone-driven immunosuppression as an advertisement of genetic quality (Ligon et al. 1990; Folstad and Karter 1992), nutritional stress imposed by antler development may signal genetic quality as well. The importance of nutrition to antler development and body condition in deer is well documented (French et al. 1956; Ullrey 1983; Asleson et al. 1997), and it is probable that extreme nutrient requirements imposed by antler growth may compete for resources required by a functioning immune system (Lochmiller and Deerenberg 2000). It follows then that males that develop large antlers demonstrate strength and genetic quality to competitors by maintaining good health during a potentially stressful period of their life history. We posit that male deer with *Mhc-DRB* alleles from both evolutionary clades (type 12) are better able to cope with nutritional stresses associated with antler development (at least under the prevailing environmental conditions of this study) and to advertise this ability by maximizing antler development.

Contrary to our initial expectations, antler asymmetry was not associated with genetic characteristics. Fluctuating asymmetry is a measure of deviation from perfect bilateral symmetry in development of traits that are normally symmetrical (Van Valen 1962). Asymmetry is proposed to result from an individual's inability to adequately cope with environmental stress (Nilsson 1994). It has been suggested that degree of asymmetry could indicate levels of stress experienced by organisms (Leary and Allendorf 1989) and genetic quality of individuals in terms of ability to eliminate or cope with environmental stress (Møller 1990; Thornhill and Sauer 1992; Min 1997). Considering that males with alleles from both

TABLE 6. Parameter estimates for multiple regressions comparing age and abundance of select parasite species to mean antler score (dependent variable) of white-tailed deer from a population in southeastern Oklahoma.

Species	Parasite abundance (number/host)			Age (years)			Intercept			
	Est.	SE	*P*	Est.	SE	*P*	Est.	SE	*P*	R^2
Haemonchus contortus	−0.33	0.26	0.202	8.79	0.68	0.001	8.7	2.3	0.001	0.61
Apteragia odocoilei	−0.17	0.13	0.211	9.00	0.67	0.001	11.0	3.4	0.001	0.61
Ostertagia mossi	−0.20	0.19	0.287	8.81	0.86	0.001	9.9	3.5	0.006	0.59
O. dikmansi	−0.55	0.22	0.815	8.99	0.85	0.001	7.6	2.8	0.009	0.58
Total[1]	−0.22	0.11	0.050	8.96	0.66	0.001	13.3	3.5	0.001	0.61
Ticks[2]	0.08	0.04	0.033	9.31	0.72	0.001	4.6	2.1	0.032	0.66

[1] Total abomasal parasites.
[2] Index of ectoparasitic ticks measured by counting ticks within a specified area of the sternum.

evolutionary clades (type 12) had greater body mass and antler development than their counterparts, it follows that these individuals either were more efficient at obtaining food, had greater metabolic efficiency, or used some other mechanism to increase resource allocation to production. Nutritional stress has been shown to increase asymmetry in antlers of sika deer (*Cervus nippon*; Baccus and Welch 1982) and roe deer (*Capreolus capreolus*; Pelabon and van Breukelen 1998). However, any disparity in nutritional stress experienced by deer of this study with different *Mhc-DRB* characteristics was not expressed in antler asymmetry.

Circulating levels of testosterone, like antler development, also serve to advertise genetic quality during the breeding season through development of secondary sexual characters and modifications in behavior. Testosterone is important in stimulating reproductive behavior of most species during the breeding season, and the literature is replete with information documenting increases in displays and aggressive reproductive behaviors following testosterone supplementation (Ketterson and Nolan 1992). In white-tailed deer, testosterone has been found to be important for expression of rutting behaviors such as rubbing, scraping, and sparring and ultimately establishing dominance (Miller et al. 1987). Physical stresses resulting from such testosterone-mediated activity commonly result in elevated levels of mortality for male deer during and immediately following the breeding season (Clutton-Brock et al. 1982; Gavin et al. 1984; Ditchkoff 2000). Because of stresses placed on male deer during rut and the positive association that testosterone has on rutting behaviors, testosterone levels should be influenced by a deer's capacity to cope with stress. Ligon et al. (1990) stated that testosterone acts to channel physical condition into external advertisements of condition; thus, individuals in good condition should have the greatest levels of testosterone. Our data support this idea because type 12 deer had levels of serum testosterone that were up to 75% greater than type 11 and 22 deer.

The good-genes theory predicts that highly ornamented males should have the lowest parasite burdens because of genetic quality. We found that total abundance of abomasal nematodes was correlated negatively with antler size when controlling for age, suggesting that antlers can serve as an honest advertisement of quality. Although we did not detect significant relationships between abundances of specific species of nematodes and antler size, there tended to be negative relationships for all species. Mulvey and Aho (1993) found that heavy infections of liver flukes (*Fascioloides magna*) in white-tailed deer can reduce antler development, and Hibler and Adcock (1973) reported that disease can negatively influence antler development. Similar relationships have been reported for red jungle fowl (*Gallus gallus*), where males experimentally infected with an intestinal nematode had duller combs and hackle feathers and shorter combs and tail feathers than control males (Zuk et al. 1990a,b). Saino et al. (1995) found that ectoparasite infestation was correlated negatively with tail length in the barn swallow (*Hirundo rustica*), and Watve and Sukumar (1997) reported that tusk length in male Asian elephants (*Elaphus maximus*) was associated negatively with intestinal parasite load.

Contrary to our intestinal helminth data, ectoparasitic ticks were correlated positively with antler size. Previous work has demonstrated that density of ectoparasites increases as host body size increases in African ungulates (Mooring and Mundy 1996; Gallivan and Horak 1997). We also detected a positive association between density of ectoparasitic ticks and body mass. This positive relationship between antler size and parasite density may be an artifact of body size and results from colinearity of body mass and antler development.

We anticipated that testosterone levels would influence parasite loads positively because of the immunosuppressive properties of androgenic hormones (Grossman 1985; Folstad et al. 1989; Saino et al. 1995), but the timing of our sampling may have precluded detection of any potential relationships. Testosterone levels begin to rise during August and September and peak during October and November (Bubenik 1983) in white-tailed deer, the period during which we collected samples. If there is a lag in parasite response to rising systemic testosterone, then we may have collected our samples before testosterone-parasite relationships were detectable.

Males possessing *Odvi-DRB* alleles from different lineages tended to have greater antler development and body size than deer with two alleles from the same lineage, suggesting an indirect association between the MHC and intrasexual advertisement. Additionally, the MHC was associated with testosterone levels during the breeding season, which are related positively to antler development and breeding effort. High testosterone levels are indicative of deer that are in good condition and can cope with stresses imposed by extensive breeding activity and subsequent pathogenic challenges. Parasite burdens tended to be lower among large-antlered deer, providing support for the hypothesis that antlers are an honest signal of genetic quality.

It is not our intention to suggest that MHC loci code for antler development or body size, but rather that general characteristics of the MHC may be evident in phenotypic expression of adult, male white-tailed deer. We have briefly described pathways by which the MHC could influence physical characteristics or hormone levels in white-tailed deer to illustrate indirect effects that the MHC may have on development of secondary sexual characters and ultimately intrasexual advertisement. We thereby have created a model that supports essential components of the handicap principle, although several caveats regarding this study must be mentioned.

As discussed by Edwards and Hedrick (1998), interpreting patterns of selection on the MHC is difficult for a number of reasons. First, the MHC is a tightly linked gene complex consisting of class I, II, and III loci, and it is not clear how these loci interact. We cannot discount the possibility of influences of other linked or epistatic loci on *Odvi-DRB* alleles, overlap in the peptide repertoires of individual MHC alleles, or importance of loci outside the MHC to parasite resistance. We must also note that our grouping of alleles into two evolutionary lineages and then considering only individuals containing alleles from both lineages as heterozygotes masks many true heterozygotes. Although we realize this is a potentially confounding factor in our study, it was not possible to perform statistical analyses to address the objectives of the study without this evolutionary classification. Previous studies documenting strong associations between MHC loci

and parasite resistance (Kaufman and Wallny 1996) and between MHC loci and sexually selected traits (von Schantz et al. 1996) have used molecular techniques (restriction fragment length polymorphisms) with even less resolution, which by their very nature fail to reveal much of the variation present and mask the effect of many heterozygote individuals.

Finally, although it is not yet clear how variation in the *Mhc-DRB* would provide an immunological advantage, Paterson et al. (1998) detected a significant association between MHC variation, juvenile survival, and parasite resistance in a large, unmanaged population of Soay sheep *(Ovis aries)*. Paterson et al. (1998) suggested that different MHC alleles may exhibit different associations with parasites at various stages during life, possibly reflecting the complex interactions between helminth parasites and the vertebrate immune system. They further stated that this complex interaction could lead to heterozygous individuals demonstrating greatest overall fitness.

Given these caveats, it is interesting to note that calculations of expected and observed frequencies of MHC genotypes among male deer in this population showed a significant deficiency of MHC homozygotes relative to Hardy-Weinberg expectations. The calculated selective advantage of heterozygotes over homozygotes (Edwards and Hedrick 1998) of 0.122 can be interpreted as suggesting that allelic diversity at the *Odvi-DRB* exon 2 locus is being maintained by some form of heterozygote advantage. Alternatively, this selective differential may be due to correlation of MHC genotype and antler size, and the deficiency of MHC homozygotes may be caused by disassortative MHC-based mating preferences, as has been reported for mice (*Mus* spp.; Potts et al. 1991; Potts and Wakeland 1993; Eklund 1997) and humans (*Homo sapiens*; Wedekind et al. 1995; Wedekind and Furi 1997) and suggested for ring-necked pheasants (*Phasianus colchicus*; von Schantz et al. 1996).

Undoubtedly, additional work is needed to improve our understanding of genetic advertisement through phenotypic expression. Specifically, we should focus on improving our understanding of how the MHC affects condition so that we can better interpret the indirect manner in which the MHC influences intrasexual advertisement. Moreover, additional loci need to be examined to determine how representative any one locus is of the entire MHC. Such studies will help to improve our understanding of MHC variability, which currently is a topic at the forefront of evolutionary ecology.

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Literature Cited

Alexander, J., and W. H. Stimson. 1988. Sex hormones and the course of parasitic infection. Parasitol. Today 4:189–193.

Andersson, M. 1994. Sexual selection. Princeton Univ. Press, Princeton, NJ.

Asleson, M. A., E. C. Hellgren, and L. W. Varner. 1997. Effects of seasonal protein restriction on antlerogenesis and body-mass in adult, male white-tailed deer. J. Wildl. Manage. 61: 1098–1107.

Baccus, J. T., and R. D. Welch. 1982. Asymmetry in the antler structure of sika deer from the Edwards Plateau of Texas. Pp. 211–221 *in* R. D. Brown, ed. Antler development in Cervidae. Caesar Kleberg Wildlife Research Institute, Kingsville, TX.

Bubenik, G. A. 1983. The endocrine regulation of the antler cycle. Pp. 73–107 *in* R. D. Brown, ed. Antler development in Cervidae. Caesar Kleberg Wildlife Research Institute, Kingsville, TX.

Chesser, R. K., and M. H. Smith. 1987. Relationships of genetic variation to growth and reproduction in white-tailed deer. Pp. 168–177 *in* C. M. Wemmer, ed. Biology and management of the Cervidae. Smithsonian Institution Press, Washington, D.C.

Clutton-Brock, T. H., F. E. Guinness, and S. D. Albon. 1982. Red deer: behavior and ecology of two sexes. Univ. Chicago Press, Chicago.

Collins, S. 1993. Is there only one type of male handicap? Proc. R. Soc. Lond. B 252:193–197.

Davidson, J. 1985. Avian spurs. J. Zool., Lond. 206:353–366.

Ditchkoff, S. S. 2000. Honest advertisement in male white-tailed deer: evidence for the handicap hypothesis of mate selection. Ph.D. diss., Oklahoma State University, Stillwater, OK.

Ditchkoff, S. S., E. R. Welch Jr., W. R. Starry, W. C. Dinkines, R. E. Masters, and R. L. Lochmiller. 1997. Quality deer management at the McAlester Army Ammunition Plant: a unique approach. Pp. 389– 399 *in* Proceedings of the annual conference of the Southeastern Association of Fish and Wildlife Agencies. Vol. 51.

Ditchkoff, S. S., E. R. Welch Jr.,and R. L. Lochmiller. 2000. Using cast antler characteristics to profile quality of white-tailed deer *Odocoileus virginianus* populations. Wildl. Biol. 6:23–28.

Doherty, P. C., and R. Zinkernagel. 1975. Enhanced immunologic surveillance in mice heterozygous at the H-2 gene complex. Nature 256:50–52.

Edwards, S. V., and P. W. Hedrick. 1998. Evolution and ecology of MHC molecules: from genomics to sexual selection. Trends Evol. Ecol. 13:305–311.

Eklund, A. 1997. The major histocompatibility complex and mating preferences in wild house mice (*Mus domesticus*). Behavi. Ecol. 8:630–634.

Folstad, I., and A. J. Karter. 1992. Parasites, bright males, and the immunocompetence handicap. Am. Nat. 139:603–622.

Folstad, I., A. C. Nilssen, O. Halvorsen, and J. Andersen. 1989. Why do male reindeer (*Rangifer t. tarandus*) have higher abundances of second and third instar larvae of *Hypoderma tarandi* than females? Oikos 55:87–92.

French, C. E., L. E. McEwen, N. D. Magruder, R. H. Ingram, and R. W. Swift. 1956. Nutrient requirements for growth and antler development in the white-tailed deer. J. Wildl. Manage. 20: 221–232.

Gallivan, G. J., and I. G. Horak. 1997. Body-size and habitat as determinants of tick infestations of wild ungulates in South Africa. S. Afr. J. Wildl. Res. 27:63–70.

Gavin, T. A., L. H. Suring, P. A. Vohs Jr.,and E. C. Meslow. 1984. Population characteristics, spatial organization, and natural mortality in the Columbian white-tailed deer. Wildl. Monogr. 91: 1–41.

Geist, V. 1981. Behavior: adaptive strategies in mule deer. Pp. 157–224 *in* O. C. Wallmo, ed. Mule and black-ailed deer of North America. Univ. of Nebraska Press, Lincoln.

Goss, R. J. 1983. Deer antlers: regeneration, function, and evolution. Academic Press, New York.
Gould, S. J. 1974. The origin and function of ''bizarre'' structures: antler size and skull size in the ''Irish elk'' *Megaloceros giganteus*. Evolution 28:221–231.
Grossman, C. J. 1984. Regulation of the immune system by sex steroids. Endocr. Rev. 5:435–455.
———. 1985. Interactions between the gonadal steroids and the immune system. Science 227:257–261.
Hamilton, W. D., and M. Zuk. 1982. Heritable true fitness and bright birds: a role for parasites? Science 218:384–387.
Hibler, C. P., and J. L. Adcock. 1973. Elaeophorosis. Pp. 263–278 *in* J. W. Davis and R. C. Anderson, eds. Parasitic diseases of wild mammals. Iowa State Univ. Press, Ames.
Hillgarth, N. 1990. Parasites and female choice in the ring-necked pheasant. Am. Zool. 30:227–233.
Howard, J. C. 1991. Disease and evolution. Nature 352:565–567.
Hughes, A. L., and M. Nei. 1988. Pattern of nucleotide substitution at major histocompatibility complex class I loci reveals overdominant selection. Nature 335:167–170.
Kaufman, J., and H. J. Wallny. 1996. Chicken MHC molecules, disease resistance and the evolutionary origin of birds. Curr. Top. Microbiol. Immunol. 212:129–141.
Kennedy, C. E. J., J. A. Endler, S. L. Poynton, and H. McMinn. 1987. Parasite load predicts mate choice in guppies. Behav. Ecol. Sociobiol. 21:291–295.
Ketterson, E. D., and V. Nolan Jr. 1992. Hormones and life histories: an integrative approach. Am. Nat. 140:S33–S62.
Kitchener, A. 1987. Fighting behaviour of the extinct Irish elk. Mod. Geol. 11:1–28.
Klein, J. 1986. Natural history of the major histocompatibility complex. John Wiley and Sons, New York.
Kubat, G., A. A. Kocan, and W. R. Davidson. 1980. Identification of abomasal parasites of white-tailed deer. Wildlife Disease Association, Ames, IA.
Leary, R. F., and F. W. Allendorf. 1989. Fluctuating asymmetry as an indicator of stress: implications for conservation biology. Trends Ecol. Evol. 4:214–217.
Ligon, J. D., R. Thornhill, M. Zuk, and K. Johnson. 1990. Male-male competition, ornamentation, and the role of testosterone in sexual selection in red jungle fowl. Anim. Behav. 40:367–373.
Lochmiller, R. L. 1996. Immunocompetence and animal population regulation. Oikos 76:594–602.
Lochmiller, R. L., and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88:87–98.
Longmire, J. L., M. Maltbie, and R. J. Baker. 1997. Use of ''lysis buffer'' in DNA isolation and its implications for museum collections. Occas. Pap Mus. Tex. Tech Univ. 163:1–3.
Lotem, A. 1993. Secondary sexual ornaments as signals: the handicap approach and three potential problems. Etologia 3:209–218.
Margolis, L., G. W. Esch, J. C. Holmes, A. M. Kuris, and G. A. Schad. 1982. The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). J. Parasitol. 68:131–133.
Maynard Smith, J. 1985. Mini review: sexual selection, handicaps and true fitness. J. Theor. Biol. 115:1–8.
———. 1991. Theories of sexual selection. Trends Ecol. Evol. 6: 146–151.
Meister, W. 1956. Changes in biological structure of the long bones of white-tailed deer during the growth of antlers. Anat. Rec. 124:709–721.
Mikko, S., and L. Andersson. 1995. Low major histocompatibility complex class II diversity in European and North American moose. Proc. Natl. Acad. Sci. USA 92:4259–4263.
Mikko, S., H. A. Lewin, and L. Andersson. 1997. A phylogenetic analysis of cattle *DRB3* alleles with a deletion of codon 65. Immunogenetics 47:23–29.
Miller, K. V., R. L. Marchinton, K. J. Forand, and K. L. Johansen. 1987. Dominance, testosterone levels, and scraping activity in a captive herd of white-tailed deer. J. Mammal. 68:812–817.
Min, S. E. 1997. Variation in sexually dimorphic traits of male pronghorns. J. Mammal. 78:31–47.
Møller, A. P. 1990. Fluctuating asymmetry in male sexual ornaments may reliably reveal male quality. Anim. Behav. 40: 1185–1187.
———. 1992. Patterns of fluctuating asymmetry in weapons: evidence for reliable signaling of quality in beetle horns and bird spurs. Proc. R. Soc. Lond. B 248:199–206.
Mooring, M. S., and P. J. Mundy. 1996. Factors influencing host selection by yellow-billed oxpeckers at Matobo National-Park, Zimbabwe. Afr. J. Ecol. 34:177–188.
Morris, J. M., and G. A. Bubenik. 1982. The effects of androgens on the development of antler bone. Pp. 123–141 *in* R. D. Brown, ed. Antler development in Cervidae. Caesar Kleberg Wildlife Research Institute, Kingsville, TX.
Mulvey, M., and J. M. Aho. 1993. Parasitism and mate competition: liver flukes in white-tailed deer. Oikos 66:187–192.
Nesbitt, W. H., and P. L. Wright. 1981. Records of North American big game. The Boone and Crockett Club, Alexandria, VA.
Nilsson, J. A. 1994. Energetic stress and degree of fluctuating asymmetry: implications for a long-lasting signal. Evol. Ecol. 8: 248–255.
Norris, K. 1993. Heritable variation in a plumage indicator of viability in male great tits *Parus major*. Nature 362:537–539.
Orita, M., H. Iwahana, H. Kanazawa, K. Hayashi, and T. Sekiya. 1989. Detection of polymorphisms of human DNA by gel electrophoresis as single-stranded conformation plymorphisms. Proc. Natl. Acad. Sci. USA 86:2766–2770.
Paterson, S., K. Wilson, and J. M. Pemberton. 1998. Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population (*Ovis aries* L.). Proc. Natl. Acad. Sci. USA 95: 3714–3719.
Pelabon, C., and L. van Breukelen. 1998. Asymmetry in antler size in roe deer (*Capreolus capreolus*): an index of individual and population conditions. Oecologia 116:1–8.
Petrie, M. 1994. Improved growth and survival of offspring of peacocks with more elaborate trains. Nature 371:598–599.
Potts, W. K., and E. K. Wakeland. 1993. Evolution of MHC genetic diversity: a tale of incest, pestilence and sexual preference. Trends Genet. 9:408–412.
Potts, W. K., C. J. Manning, and E. K. Wakeland. 1991. Mating patterns in seminatural populations of mice influenced by MHC genotype. Nature 352:619–621.
Prestwood, A. K., F. A. Hayes, J. H. Eve, and J. F. Smith. 1973. Abomasal helminths of white-tailed deer in southeastern United States, Texas, and the Virgin Islands. J. Am. Vet. Med. Assoc. 163:556–561.
Saino, N., A. P. Møller, and A. M. Bolzern. 1995. Testosterone effects on the immune system and parasitic infestations in the barn swallow (*Hirundo rustica*): an experimental test of the immunocompetence hypothesis. Behav. Ecol. 6:397–404.
Sams, M. G., R. L. Lochmiller, C. W. Qualls Jr., D. M. Leslie Jr.,and M. E. Payton. 1996. Physiological correlates of neonatal mortality in a overpopulated herd of white-tailed deer. J. Mammal. 77:179–190.
Scribner, K. T., and M. H. Smith. 1990. Genetic variability and antler development. Pp. 460–473 *in* G. A. Bubenik and A. B. Bubenik, eds. Horns, pronghorns, and antlers. Springer-Verlag, New York.
Scribner, K. T., M. H. Smith, and P. E. Johns. 1984. Age, condition, and genetic effects on incidence of spiked bucks. Pp. 23–32 Proceedings of the annual conference of the Southeastern Association of Fish and Wildlife Agencies. Vol. 38.
———. 1989. Environmental and genetic components of antler growth in white-tailed deer. J. Mammal. 70:284–291.
Severinghaus, C. W. 1949. Tooth development and wear as criteria for age in white-tailed deer. J. Wildl. Manage. 13:195–216.
Sigurdardottir, S., C. Borsch, K. Gustaffson, and L. Andersson. 1991. Cloning and sequence analysis of 14 *DRB* alleles of the bovine major histocompatibility complex by using the polymerase chain reaction. Anim. Genet. 22:199–209.
Smith, M. H., R. K. Chesser, E. G. Cothran, and P. E. Johns. 1983. Genetic variability and antler growth in a natural population of white-tailed deer. Pp. 365–388 *in* R. D. Brown, ed. Antler de-

velopment in Cervidae. Caesar Kleberg Wildlife Research Institute, Kingsville, TX.
Swarbick, P. A., F.-W. Schwaiger, J. T. Epplen, G. S. Buchan, J. F. T. Griffith, and A. M. Crawford. 1995. Cloning and sequencing of expressed *DRB* genes of the red deer (*Cervus elaphus*) Mhc. Immunogenetics 42:1–9.
Thornhill, R., and K. P. Sauer. 1992. Genetic sire effects on the fighting ability of sons and daughters and mating success of sons in the scorpionfly (*Panorpa vulgaris*). Anim. Behav. 43:255–264.
Townsend, T. W., and E. D. Bailey. 1981. Effects of age, sex and weight on social rank in penned white-tailed deer. Am. Midl. Nat. 106:92–101.
Ullrey, D. E. 1983. Nutrition and antler development in white-tailed deer. Pp. 49–60 *in* R. D. Brown, ed. Antler development in Cervidae. Caesar Kleberg Wildlife Research Institute, Kingsville, TX.
Van Den Bussche, R. A., S. R. Hoofer, and R. L. Lochmiller. 1999. Characterization of *Mhc-DRB* allelic diversity in white-tailed deer (*Odocoileus virginianus*) provides insight into *Mhc-DRB* allelic evolution in Cervidae. Immunogenetics 49:429–437.
Van Valen, L. 1962. A study of fluctuating asymmetry. Evolution 16:125–142.
von Schantz, T., G. Göransson, G. Andersson, I. Fröberg, M. Grahn, A. Helgeé, and H. Witzell. 1989. Female choice selects for a viability-based male trait in pheasants. Nature 337:166–169.
von Schantz, T., M. Grahn, and G. Göransson. 1994. Intersexual selection and reproductive success in the pheasant *Phasianus colchicus.* Am. Nat. 144:510–527.
von Schantz, T., H. Wittzell, G. Göransson, M. Grahn, and K. Persson. 1996. MHC genotype and male ornamentation: genetic evidence for the Hamilton-Zuk model. Proc. R. Soc. Lond. B 263:265–271.
von Schantz, T., H. Wittzell, G. Göransson, and M. Grahn. 1997. Mate choice, male condition-dependent ornamentation and MHC in the pheasant. Hereditas 127:133–140.
Watve, M. G., and R. Sukumar. 1997. Asian elephants with longer tusks have lower parasite loads. Curr. Sci. 72:885–889.
Wedekind, C. T., and S. Furi. 1997. Body odour preferences in men and women: do they aim for specific MHC combinations or simply heterozygosity? Proc. R. Soc. Lond. Ser. B 264: 1471–1479.
Wedekind, C., T. Seebeck, F. Bettens, and A. J. Paepke. 1995. MHC-dependent mate preferences in humans. Proc. R. Soc. Lond. Ser. B 260:245–249.
Zahavi, A. 1975. Mate selection: a selection for a handicap. J. Theor. Biol. 53:205–214.
———. 1977. Reliability in communication systems and the evolution of altruism. Pp. 253–259 *in* B. Stonehouse and C. M. Perrins, eds. Evolutionary ecology. Macmillan Press, London.
Zahavi, A., and A. Zahavi. 1997. The handicap principle: a missing piece of Darwin's puzzle. Oxford Univ. Press, Oxford, U.K.
Zuk, M., K. Johnson, R. Thornhill, and J. D. Ligon. 1990a. Parasites and male ornaments in free-ranging and captive red jungle fowl. Behaviour 114:232–248.
Zuk, M., R. Thornhill, J. D. Ligon, and K. Johnson. 1990b. Parasites and mate choice in red jungle fowl. Am. Zool. 30:235–244.

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