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Testing a Local Inbreeding Hypothesis as a Cause of Observed Antler Characteristics in Managed Populations of White-Tailed Deer

Stephen L. Webb [1,*], Randy W. DeYoung [2], Stephen Demarais [3], Bronson K. Strickland [3] and Kenneth L. Gee [4]

[1] Noble Research Institute, LLC, Ardmore, OK 73401, USA
[2] Caesar Kleberg Wildlife Research Institute, Texas A&M University-Kingsville, Kingsville, TX 78363, USA; Randall.DeYoung@tamuk.edu
[3] Department of Wildlife, Fisheries, and Aquaculture, Mississippi State University, Mississippi, MS 39762, USA; steve.demarais@msstate.edu (S.D.); bronson.strickland@msstate.edu (B.K.S.)
[4] Oaks and Prairies Joint Venture, Gene Autry, OK 73401, USA; kennethlgee@gmail.com
* Correspondence: slwebb@noble.org; Tel.: +1-580-224-6443

Abstract: The increased use of antler restrictions by state game agencies has led to a focus on antlers by the hunting public, particularly the potential for an association between genetics and antler characteristics. We analyzed microsatellite data from 1231 male white-tailed deer (*Odocoileus virginianus*) from three states (Oklahoma, Mississippi, and Texas) within USA to determine if genetic relatedness, internal relatedness (IR), homozygosity weighted by locus (HL), or correlations among uniting gametes (F_{is}) influenced total antler points, antler score, non-typical points or antler malformations. Within each location, deer in the lower and upper quartile intervals for number of antler points and score were unrelated (95% CI included 0 or was <0) and relatively heterozygous for four measures of inbreeding. Antler score and points were positively influenced by age but negatively influenced by IR and HL, except for antler score in Mississippi. Relatedness, HL, IR and F_{is} did not differ between groups of deer with and without antler malformations. Perceived differences in antler quality do not appear to be affected by heterozygosity or a result of close inbreeding because we found deer were unrelated and measures of inbreeding and genome-wide heterozygosity were not correlated with antler characteristics.

Keywords: antlers; genetics; heterozygosity; inbreeding; microsatellite; *Odocoileus virginianus*; relatedness

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1. Introduction

Wildlife agencies play a large role in managing ungulate habitat and population demographics (e.g., age structure and sex ratio) through changes in harvest regulations. Harvest is regulated through season length, bag limits, and restrictions on sex and age classes. Recently, many states in the southeastern USA have adopted antler restrictions (ARs) [1] to balance male age structure and bring sex ratios closer to unity in white-tailed deer (*Odocoileus virginianus*). The increased use of ARs has led to a focus on antlers by the hunting public, particularly the potential for an association between genetics and antler characteristics. Antler size is correlated with age [2–4], whereby a male's first set of antlers is typically much smaller and the largest antlers are produced by prime-aged males (≥5.5 years). If carefully constructed, ARs shift a typically male-biased harvest from yearling males to older age classes by protecting all males with small antlers based on criteria such as number of antler points, spread between main beams, or a combination of both. For instance, Mississippi shifted the relative composition of males harvested from predominantly 1.5 years (59%) to 2.5 and older (83%) by protecting all males with <4 antler points [1].

Increased emphasis on the management and harvest of animals based on antler or horn characteristics has raised concerns over potential biological impacts. Antler regula-

tions in Mississippi reduced the antler sizes of cohorts in a variety of soil physiographic regions by differentially protecting smaller-antlered young males and allowing the harvest of larger-antlered young males [1]. Phenotype-based selective harvest practices for bighorn sheep (*Ovis canadensis*) may have decreased horn size and breeding values for horn size [5]. Harris et al. [6] highlighted the genetic consequences of hunting under certain circumstances because the population demographics altered by hunting (e.g., density, sex ratio, and age distribution) influenced population genetics.

Antler malformations are rare but tend to receive increased interest, raising questions about a potential genetic linkage. Deer with malformed antlers typically only have one side affected, whereby the length of the main beams and number of antler points are reduced on the affected side. A query of Mississippi's Deer Management Assistance Program (DMAP) [7] database over 12 years identified <0.4% (362 of 10,507) of deer exhibited abnormal antler development, defined as a $\geq$50% departure in main beam length from the opposite antler [8]. Thus, a small percentage of deer are receiving increased attention, perhaps due to an apparent increase in occurrence, as ARs result in increased encounter rates of males aged >1.5 years.

Several studies have found a genetic link between the heterozygosity of allozymes and morphometric traits in ungulates. Heterozygosity has been used as a measure of recent inbreeding because it is expected to be correlated inversely with inbreeding [9–11]. Deer with small antlers were more inbred, based on the heterozygosity of allozymes, than deer with larger antlers [12]. Individuals that were more heterozygous had greater antler sizes or points in white-tailed deer [13–16] and greater horn growth in bighorn sheep [17].

Antler traits such as points and mass are heritable [3,18–20]; thus, there is a genetic component to antler characteristics. Increased relatedness among deer with similar antler characteristics indicates that the antler trait is heritable [21]. It is possible to determine if a genetic link to observed antler characteristics exists by conducting genetic analyses of a group of deer within the population exhibiting unique antler traits (e.g., points and score) that are heritable or rare in occurrence (e.g., antler malformations).

More information is needed by wildlife agencies for dissemination to the general public on the influence of genetics on antler development and the cause of abnormal antler development. Antler characteristics provide a useful quantitative metric for genetic analysis because much is known about white-tailed deer antlers and their relationships with nutrition, stress, environment and age. Deciduous secondary sexual characteristics such as antlers demand high levels of nutrition to produce [22–24]. Antlers could be considered a handicap to produce [25] because only the most fit males should be able to afford to produce large antlers. Males with smaller antlers or antlers that fluctuate from bilateral symmetry may not be as able to cope with environmental stresses or physical damage [26]. Therefore, antlers may serve as a signal to the genetic quality of the individual during the breeding season [24,25,27,28].

We examined the link between antler characteristics and multi-locus heterozygosity as a measure of inbreeding. We used three types of antler traits from three diverse populations: (1) continuous traits, with a continuous range of values (e.g., antler score); (2) ordinal traits, with discrete, integral classes (e.g., antler points); and (3) nominal traits, whether present or absent (e.g., non-typical points). We used samples from an 8000 ha free-ranging population, a 1214 ha enclosed population, and a 3200 ha free-ranging population managed using ARs to test the local inbreeding hypothesis as an explanation for differences in antler points, score, and presence of non-typical points and rare occurrences of antler malformations. We focused on two levels of classification: (1) the effects of relatedness and inbreeding on groups of individuals with similar antler characteristics and (2) the effects of individual multi-locus heterozygosity on observed antler points and score. Our objectives were to determine whether (1) genetic relatedness and level of heterozygosity among a priori groups of male deer were different based on total antler points, antler score, non-typical points or antler malformations, and (2) an individual's number of antler points and score were related to heterozygosity. Most deer on all three study areas were unrelated

and relatively heterozygous; therefore, deer were unlikely to suffer from inbreeding (i.e., increased *r* and homozygosity) or reduced genetic diversity under the conditions described herein, which resulted in no detectable difference in antlers due to inbreeding.

2. Materials and Methods

2.1. Study Area

Three study areas were chosen to include a range of deer management practices across various habitat, ecological, ownership, and environmental contexts. The Noxubee National Wildlife Refuge (NNWR) is 19,425 ha located in northeastern Mississippi in the counties of Noxubee, Oktibbeha, and Winston, and is part of the interior flatwoods soil resource region [29]. NNWR is comprised of bottomland hardwoods, upland hardwoods, pines, wetlands, and herbaceous vegetation, and is considered a humid, subtropical climate. Public hunting is allowed on ~17,500-ha of the NNWR but sample collection was concentrated on ~3200 ha. Males and females were harvested at the same intensity (1 deer/85 ha) but most harvested males (70–80%) were $\leq$2.5 years of age [30].

The 1214 ha Wildlife Unit (NFWU), formerly owned and operated by The Samuel Roberts Noble Foundation, is 8.0 km south of Allen, Oklahoma in the Cross Timbers region [31]. The region is a transitional zone between the humid, subtropical climate in the eastern part of the state and the semi-arid region of western Oklahoma. A 2.5 m high-tensile electric fence containing 15 smooth wire strands was erected in 1992 to discourage human trespass and facilitate white-tailed deer management programs [32]. The NFWU is approximately 60% wooded and 40% open, with a high degree of interspersion [31]. Hunting of males was restricted beginning in 2000 due to ongoing, long-term genetic research projects. Harvest was moderate for females (1 deer/80 ha) and limited for males through 1999, most of which were $\geq$2.5 years (~1 male/500 ha) [30].

The Laureles Division of the King Ranch is located 4 km east of Kingsville in Kleberg County, Texas. The 103,691 ha division contains no deer-proof fences. Sampling was conducted on approximately 8000 ha. The division is characterized as a semi-arid, mixed shrub rangeland dominated by mesquite (*Prosopis glandulosa*) and huisache (*Acacia farnesiana*) [33]. Females were harvested at ~1 female/300 ha and males at ~1 male/250 ha.

2.2. Sample Collection

Deer on NNWR were sampled by harvest or special collection by the Mississippi Department of Wildlife, Fisheries, and Parks (MDWFP) or NNWR personnel from 1999 to 2001. Muscle or tissue samples were collected from all harvested deer because check-in of harvested deer was mandatory. During spring special collections, adult females were collected as part of the MDWFP's population health assessment monitoring program. Fetuses obtained from females provided known dam/offspring relationships. Muscle and tissue samples were frozen and stored at -20 °C. We used predictive equations developed by Strickland et al. [34] to calculate gross Boone and Crockett score (hereafter score) [35] from available antler measurements (i.e., number of antler points, inside spread, main beam lengths, and basal circumferences). Score is a means of assessing total antler size and is a composite index to antler length and mass. In 1995, Mississippi initiated a statewide AR that only allowed the harvest of males with $\geq$4 antler points [1]. There was the potential that males harvested in Mississippi were not a random sample due to the imposed ARs, whereas capture of deer in Oklahoma and Texas allowed for a more representative sample of deer in the study area.

Deer in Oklahoma were captured using a drop-net [36,37] baited with corn from January to April of 1991 to 2005. We sedated deer using Xylazine (3–6 mg/kg, Phoenix Scientific, St. Joseph, MO, USA) or a Telazol®-Xylazine mixture (4.4 mg/kg Telazol®, Fort Dodge Animal Health, Fort Dodge, IA, USA, plus 2.2 mg/kg xylazine) and used yohimbine (Abbott Laboratories, North Chicago, IL, USA) at 0.125 mg/kg or tolazine (Lloyd Laboratories, Shenandoah, Iowa) at 0.4 mg/kg as an antagonist to the xylazine. Blood (20 mL/deer) was obtained from captured deer and preserved in 0.5M EDTA and

stored at 4 °C. Tissue samples were taken from all harvested deer that had not previously been captured, and samples were stored at −20 °C. Fetuses were collected from harvested does to provide known dam/offspring relationships. Shed antlers also provided additional DNA samples from males not harvested or captured. Antlers were measured and scored according to Boone and Crockett scoring standards [35]. However, we excluded inside spread from the final score because antlers were removed from deer at time of capture and later scored; therefore, we could not determine inside spread.

We captured deer in Texas using a helicopter and net-gun [38] during September to October from 1999 to 2005. Tissue samples were removed from captured deer via ear punch whereas muscle samples were collected on all harvested deer along with fetuses of harvested females, which provided known dam/offspring relationships. Tissue samples were frozen and stored at −20 °C until DNA could be extracted. Antlers were measured and scored at time of capture or harvest according to Boone and Crockett scoring standards [35].

2.3. DNA Isolation and Extraction

DNA was isolated from whole blood as described by DeYoung et al. [39]. Tissue samples were sectioned (~0.5 cm^3) and scored with a razor blade to increase surface area. We isolated DNA using Qiagen® DNeasy® tissue kits (Qiagen® Genomics Inc., Bothell, WA, USA). Supplier recommendations were followed except that tissue lysis was performed with 30 µL Proteinase K (20 mg/mL) with overnight incubation. DNA was also extracted from bones and antlers from the NFWU as described by DeYoung [30].

2.4. DNA Amplification and Separation

We used 17 microsatellite markers (INRA011, Cervid1, ILSTS011, BovPRL, N, Q, K, BL25, BM6438, O, BM848, R, BM6506, P, BM4208, OarFCB, and D) evaluated by DeYoung et al. [39] from a 21 locus cervid microsatellite panel [40]. Deer with <10 scored loci were excluded from analyses. This was to help assure that if any differences are observed they are the product of actual signal, and not the result of using different numbers of markers for some individuals. Extracted DNA was amplified via polymerase chain reaction (PCR) on a PE Gene Amp® 9600 thermocycler (Applied Biosystems Inc., Foster City, CA, USA) using fluorescent tagged primers in single and multiplexed reactions. For a complete description of reaction conditions and primers see Anderson et al. [40]. PCR products were mixed together from 2–4 reactions (~3 µL from each reaction) and 1 µL of this mixture was applied to a denaturing formamide and size standard mix (GeneScan™ 500 ROX™; Applied Biosystems Inc.). PCR product and denatured mixes were loaded onto an ABI Prism® 3130 Genetic Analyzer (Applied Biosystems Inc.) for separation and detection. DNA was analyzed and alleles were assigned using GeneMapper® software (Applied Biosystems Inc.).

2.5. Data Analysis

We assigned deer to a priori antler groups based on similar antler characteristics to determine whether deer with similar antlers shared common ancestors or showed similar levels of heterozygosity. We determined study area and age-specific quartiles (i.e., lower and upper 25%) for antler points and score (Table 1). Deer with the largest antlers (i.e., number of points and score) in the upper 25% quartile and deer with the smallest antlers in the lower 25% quartile (Figure A1) were compared. Deer also were placed into 1 of 2 groups based on presence or absence of non-typical points because a smaller proportion of the population grows these extra points. Deer were further classified into groups: deer without non-typical points, and deer with ≥2 non-typical points. Each male deer only entered the dataset once even if multiple years of data were available. If multiple years of data were available, we classified deer based on the number of years with the most frequent non-typical classification. For example, we classified a deer with 1 year of no non-typical points, 1 year of 1 non-typical point, and 2 years with ≥2 non-typical points as a deer with ≥2 non-typical points. Last, deer were grouped depending on whether they

exhibited normal or malformed antlers (Figure A2). Malformed antlers were classified based on a >50% departure in main beam length between the left and right antlers or a departure of >60% between number of antler points on each antler. We used data from Oklahoma and Texas because data on antler points from Mississippi were unavailable and no deer exhibited >50% departure between left and right main beams. Similar to designations by Rachlow et al. [41], we classified deer with normal antlers as those deer with antlers that conformed to the shape and orientation representative of the species [35]. Deer with malformed antlers typically had one side of the antler pair affected. The antler on the malformed side was smaller and contained fewer antler points in comparison to the normal antler on the opposite side. Antler deformities may occur because of pedicle injury (Figure A3), so when these were observed, these deer and antler sets were not included into the genetic analysis. Antler malformations also may occur because of bodily injury, but we did not have a way of documenting this potential cause of antler malformation.

Table 1. Age-specific lower and upper antler point and score (cm) quartiles used in antler group comparisons of white-tailed deer (*Odocoileus virginianus*) from Mississippi (1999–2001), Oklahoma (1991–2005) and Texas (1999–2005).

		Mississippi [1]		**Oklahoma** [2]		**Texas** [3]	
Metric	**Age**	**Lower**	**Upper**	**Lower**	**Upper**	**Lower**	**Upper**
Score	1	≤104.1	≥137.2	≤78.7	≥142.2	≤76.2	≥137.2
	2	≤188.0	≥233.7	≤167.6	≥256.5	≤180.3	≥238.8
	3	≤195.6	≥292.1	≤236.2	≥281.9	≤254.0	≥309.9
Points	1	≤4	≥6	≤3	≥8	≤2	≥6
	2	≤5	≥8	≤7	≥9	≤6	≥9
	3	≤6	≥8	≤8	≥10	≤7	≥10

[1] Predictive equation used to calculate antler score [33]. [2] Inside spread was excluded from final antler score. [3] Antler score followed Boone and Crockett Club guidelines [34].

Deer age was estimated using tooth replacement and wear techniques [42] on all three study areas. Deer that were not of known age at time of harvest or capture (i.e., fawn or yearling) were conservatively placed into an age class [43]. We analyzed data for 1-, 2- and 3-year-old males due to limited sample sizes for deer ≥4 years of age for antler point and score analyses. We used all ages for documenting relatedness of the sexes and for group comparisons of typical versus non-typical antler points.

We calculated genetic relatedness (r) as a means of detecting the presence of close relationships among groups of deer with similar antler characteristics. We used Queller and Goodnight's [44] regression method to estimate relatedness. The relatedness estimator uses population allele frequencies to estimate the proportion of alleles between 2 individuals that are identical by descent. Variability of r estimates is due to weighting of r by the frequency of shared alleles, with rare shared alleles being weighted more heavily than shared common alleles [44].

Pairwise comparisons of r among deer were restricted to within antler characteristic and group. When group comparisons were conducted, we used allele frequencies from the whole sample as the reference population. Mean r and 95% confidence intervals were computed by jackknifing over loci and compared between groups within antler characteristics. We used spatial pattern analysis of genetic diversity (SPAGeDi) 1.2 [45] for calculating r.

We calculated internal relatedness (IR) as a measure of heterozygosity. Internal relatedness, similar to Queller and Goodnight's [44] measure of relatedness between individuals or groups, is a measure based on allele sharing whereby the frequency of each allele counts towards the final score where shared rare alleles are weighted more than common alleles [46]. Internal relatedness values are approximately normally distributed and centered around mean zero [46], similar to relatedness values. Values near zero suggest individuals were born to unrelated parents, while negative values indicate higher heterozygosity and

positive values suggest higher homozygosity or inbreeding [46]. We calculated IR using a macro written in visual basic code for Microsoft Excel [47].

We calculated homozygosity weighted by locus (HL) using IRmacroN4. Homozygosity is estimated by weighing the contribution of each locus to the index score rather than the contribution of each allele, which gives more weight to more informative loci (i.e., more alleles/locus and more evenly frequent) [48]. This is important when few microsatellite markers are used and when the markers differ in allelic diversities [48]. This measure varies between 0 and 1; 0 when all loci are heterozygous and 1 when all loci are homozygous.

Last, we calculated *F*-statistics [49] as a way to describe genetic population structure in diploid organisms. F_{is} was defined by Wright [49] as the correlation between homologous alleles within samples with reference to the local population. We used Weir and Cockerham's [50] method of calculating F_{is} in SPAGeDi because their method weights F_{is} from each sample by its sample size to take into account unequal sample sizes. More homozygous individuals will be positive (maximum = 1) indicating positive correlations among uniting gametes due to inbreeding, whereas more heterozygous individuals will be negative (minimum = −1) under Hardy−Weinberg equilibrium (HWE) [51].

We calculated sex-specific estimates of *r* as a reference of the underlying *r* of each of the three study populations. Group *r* was estimated for females and males separately and then together. We plotted sex- and population-specific multilocus pairwise genetic coefficients (i.e., *r*) for later use in qualitative comparisons with antler group distributions. For plotting purposes, we divided *r* into 20 equidistant segments in units of 0.1 from −1 to 1.

Each year, on the three study areas, females were harvested and fetuses collected and genotyped, which provided known sibling pairs. We estimated an average within-group (i.e., fetuses within dam) *r* of sibling pairs. Theoretically, the expected *r* of full siblings should be 0.5 and 0.25 for half siblings, which may result from multiple paternities [52,53]. Distributions of pairwise *r* estimates also were plotted for reference.

2.6. Statistical Analysis

We calculated study area- and age-specific means and corresponding 95% CI for *r*, IR, HL, and F_{is} for each antler characteristic (i.e., antler points, score, non-typical antler points and antler malformations) to determine whether there was a difference between antler groups (i.e., lower and upper quartiles and presence or absence of non-typical points and antler malformations). We tested the relationship between antler points and score (dependent variables) and IR and HL (explanatory variables) using general linear mixed models (GLMM) with deer identification, year and study area as random intercepts. Using deer identification as a random intercept enabled us to account for multiple measurements taken on the same individual in different years. Year and study area were also modeled as random intercepts, which took into consideration random environmental variation from year to year and from study area to study area. Age was included as a covariate to control for differences in age-specific antler size. We examined plots of residuals and normal probability plots to ascertain whether data were normally distributed. We conducted all statistical analyses using SAS 9.2 (SAS Institute, Cary, NC, USA). We concluded statistical significance for $p \leq 0.05$. For all GLMM, we used a degrees of freedom adjustment developed by Kenward and Roger [54]. The Kenward−Roger option accounts for unbalanced data, multiple random effects, and any model with correlated errors [55].

3. Results

We genotyped 1231 deer; 259 from Mississippi, 529 from Oklahoma, and 443 from Texas. Previous research from the same study populations revealed no significant linkage disequilibrium or deviations from HWE [56].

3.1. Relatedness

Females and males, and both sexes combined, in all three study areas had a mean *r* near zero (i.e., 95% CI included zero or was <0; Table 2), indicating deer were unrelated. Distributions of pairwise *r* estimates were normally distributed around $\bar{x} = 0$ for females and males on all three study areas (Figure 1a–c). No more than 7.4% of pairwise relatedness estimates exceeded 0.25 for any sex in any population (range: 3.9–7.4%).

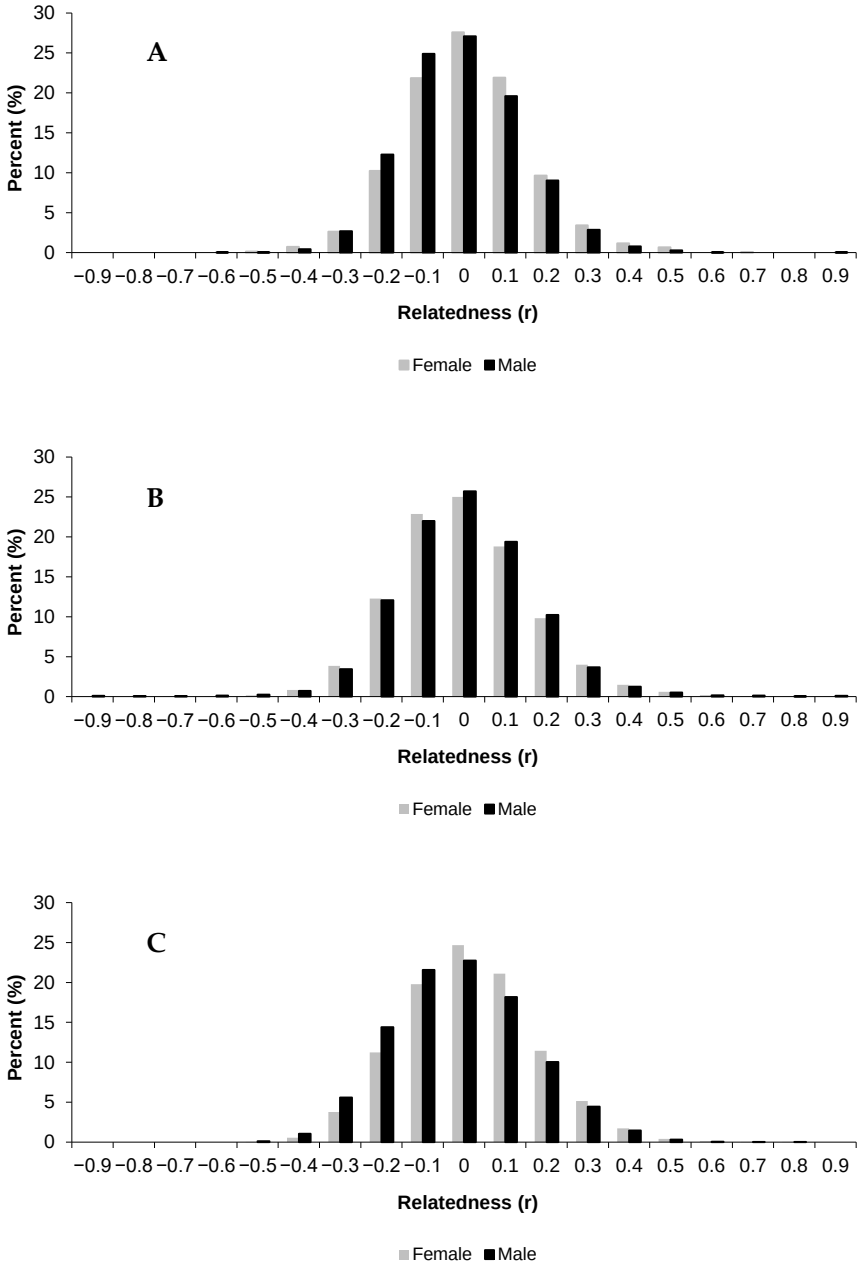

Figure 1. Distribution of pairwise relatedness (*r*) estimates of female and male white-tailed deer (*Odocoileus virginianus*) from Mississippi (**A**) 1999–2001, Oklahoma (**B**) 1991–2005 and Texas (**C**) 1999–2005.

Table 2. Relatedness (*r*) of females, males, sibling pairs, and both sexes combined in white-tailed deer (*Odocoileus virginianus*) from Mississippi (1999–2001), Oklahoma (1991–2005) and Texas (1999–2005).

				95% CI	
Study Area	**Group**	**N**	**Mean (SE)**	**Lower**	**Upper**
Mississippi	Both	197	−0.005 (0.001)	−0.007	−0.003
	Female	53	0.005 (0.008)	−0.012	0.022
	Male	144	−0.008 (0.004)	−0.011	0.022
	Siblings	62	0.415 (0.024)	0.367	0.463
Oklahoma	Both	495	−0.002 (0.003)	−0.007	0.003
	Female	266	−0.003 (0.002)	−0.008	0.002
	Male	229	−0.001 (0.006)	−0.012	0.01
	Siblings	34	0.405 (0.04)	0.324	0.486
Texas	Both	330	−0.006 (0.003)	−0.011	0.000
	Female	132	0.013 (0.011)	−0.008	0.034
	Male	198	−0.012 (0.007)	−0.026	0.002
	Siblings	113	0.439 (0.022)	0.395	0.483

We detected higher levels of *r* by using known sibling pairs from all three study areas. The mean *r* was 0.415 (0.024), 0.405 (0.04), and 0.439 (0.022) for sibling pairs in Mississippi, Oklahoma, and Texas, respectively (Table 2), which is between the theoretical values of half siblings (0.25) and full siblings (0.50).

Most sibling pairs in Mississippi were related between 0.25 and 0.5, with $r = 0.5$ having the highest frequency (Figure 2). In Oklahoma, the highest frequency of pairwise comparisons occurred at around 0.4 (25%), followed by 0.2 (15%), 0.3 (15%) and 0.5 (15%; Figure 2). In total, 57% and 23% of siblings were related around 0.5 and 0.25, respectively, in Texas (Figure 2). In Mississippi, Oklahoma and Texas, only 6%, 5% and 2% of pairs were estimated to be unrelated, respectively (Figure 2).

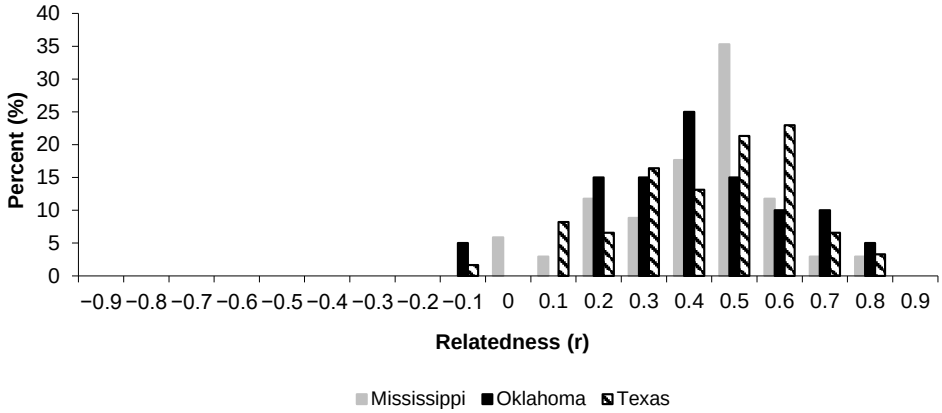

Figure 2. Distribution of pairwise relatedness (*r*) estimates of known sibling pairs of white-tailed deer (*Odocoileus virginianus*) from Mississippi (1999–2001), Oklahoma (1991–2005) and Texas (1999–2005).

All antler groupings (i.e., antler points and score, non-typical points and antler malformations) were unrelated regardless of age or study area because the 95% CI values included zero or were <0 (Table A1). The confidence intervals of all antler group comparisons overlapped; therefore, there was no difference between antler groups (Table A1). We compared distributions of antler groupings (i.e., antler points and score and non-typical points) to known sibling pair distributions and sex-specific distributions as a reference. Distributions of pairwise *r* were normally distributed around $\bar{x} = 0$ and did not show bimodal or skewed distributions.

3.2. Internal Relatedness

The mean IRs of all males were 0.034 (0.015 SE), 0.056 (0.013 SE) and 0.087 (0.013 SE) for the Mississippi, Oklahoma and Texas populations, respectively. The mean IR of antler groups ranged from 0.003 to 0.131. Most (29 of 44; 66%) antler group IR scores indicated deer within antler groups were unrelated because 95% CI included zero (Table A1). The remaining 15 antler groups tended to be slightly more homozygous because the 95% CI values were >0 (Table A1). However, IR did not differ between groups of deer with the most and fewest points, highest and lowest scores, and the presence and absence of non-typical points and antler malformations, as revealed by comparisons of 95% CI (Table A1).

GLMMs revealed the minimal influence of IR on antler points ($F_{1,354} = 2.56$, $p = 0.11$) or score ($F_{1,343} = 2.36$, $p = 0.126$). However, we did observe a qualitative negative trend between IR and antler points and score—as IR increased, antler points ($\beta = -0.97 \pm 0.61$ SE) and score ($\beta = -23.13 \pm 15.07$ SE) decreased. The internal relatedness scores of deer from Oklahoma (n = 7) and Texas (n = 5) with antler malformations were below (3), above (6) and within (3) the 95% CI of IR scores for deer with normal antlers from their corresponding population (Table 3).

Table 3. Individual internal relatedness (IR) and homozygosity weighted by locus (HL) scores of white-tailed deer (*Odocoileus virginianus*) with antler malformations from Oklahoma (1991–2005) and Texas (1999–2005). Sign indicates whether individual deer score was below (<), above (>) or within (=) the 95% CI calculated for deer with normal antlers from its corresponding population (cf. Table A1).

		Internal Relatedness (IR)		Homozygosity (HL)	
Study Area	**Age**	**Score**	**Sign**	**Score**	**Sign**
Oklahoma	3	−0.022	<	0.199	<
	2	0.207	>	0.403	>
	2	0.135	>	0.341	>
	1	0.258	>	0.447	>
	1	0.068	=	0.274	<
	1	0.040	=	0.285	=
	3	0.147	>	0.353	>
Texas	4	−0.032	<	0.181	<
	2	0.404	>	0.526	>
	2	0.027	<	0.246	<
	6	0.167	>	0.318	>
	8	0.099	=	0.347	>

3.3. Homozygosity Weighted by Locus

The mean HL values for all males in the Mississippi, Oklahoma and Texas populations were 0.26 (0.012 SE), 0.298 (0.01 SE) and 0.294 (0.009 SE), respectively. The homozygosity estimates for all group comparisons ranged from 0.23 to 0.349 (Table A1). Subtracting HL from 1 gives an estimate of heterozygosity. Therefore, population and antler group heterozygosity ranged from 0.702 to 0.74 and 0.651 to 0.77, respectively. Thus, it appears as though all populations and groups of deer were relatively heterozygous. Additionally, there was no difference between groups of deer with the most and fewest points, highest and lowest scores, and the presence and absence of non-typical points and antler malformations (Table A1).

Similar to IR, HL negatively influenced antler points ($\beta = -1.72 \pm 0.8$ SE; $F_{1,357} = 4.64$, $p = 0.032$) and score ($\beta = -37.79 \pm 19.99$ SE; $F_{1,344} = 3.57$, $p = 0.06$)—both decreased with increasing homozygosity. Homozygosity scores of deer from Oklahoma (n = 7) and Texas (n = 5) with antler malformations were below (4), above (7) and within (1) the 95% CI of IR scores for deer with normal antlers from their corresponding population (Table 3).

3.4. F-statistics

F_{is} was used to indicate a heterozygote deficit and values ranged from 0.019 to 0.204 across all antler groups. There was a positive correlation between homologous alleles (i.e., more homozygous or heterozygote deficit), as indicated by positive F_{is} values (95% CI > 0) in 11 of 44 groups (25%; Table A1). Groups of deer with fewer antler points and lower antler scores (4 of 18) tended to be more homozygous due to inbreeding as well as deer without malformed antlers (Table A1). Other groups of deer that tended to be more homozygous included two-year-old deer with larger antler scores from Texas and groups with and without non-typical points from Oklahoma and Texas (Table A1). Despite 11 groups being more homozygous than expected, their 95% CI overlapped with the corresponding antler group comparison (Table A1). Therefore, inbreeding, as indicated by positive F_{is} values, may not be associated strongly with observed differences in antler characteristics.

4. Discussion

Most deer in all three study areas were unrelated and relatively heterozygous. We detected relationships at the full or half sibling level (i.e., 0.5 and 0.25, respectively) from known sibling pairs. Therefore, if any a priori antler groupings were being affected by level of *r*, it is likely we would have been able to detect it at the half sibling level. All three measures of heterozygosity (i.e., IR, HL, and F_{is}) revealed that deer, regardless of antler group, were relatively heterozygous. Studies of red deer (*Cervus elaphus*) [11] and elk (*C. canadensis*) [21] also revealed that deer were relatively heterozygous and close inbreeding was rare. However, it is not always inbreeding that would be detected using individual heterozygosity indices because inbreeding is expected to be rare in most populations, especially in polygynous species such as white-tailed deer. In addition, tests of heterozygosity may reveal heterozygosity effects through linkage with areas experiencing selection [57]. For example, von Hardenberg et al. [58] acknowledged that heterozygosity–fitness correlations for horn growth in Alpine ibex (*Capra ibex*) may have been due to linkage disequilibrium and not inbreeding. However, we did not test for linkage disequilibrium by assessing single-locus effects.

There could, however, be some individuals that did have higher levels of inbreeding in our study. For example, the incidence of antler malformations could reflect rare cases of close inbreeding because the number of cases was relatively low. For example, severe inbreeding may have led to increased fluctuating asymmetry (i.e., antler malformations) of sika deer (*Cervus nippon*) antlers from a small (39 ha) enclosed population, which was started from six founding individuals [26]. As a group, deer with antler malformations in our study were not more related or inbred than the group of deer with normal antlers. Our findings are similar to those of Hicks and Rachlow [21], who found that elk with malformed antlers were not more related or inbred than elk with normal antlers. Overall, our data also corroborate that there is minimal evidence for a strong genetic basis of inheritance of antler malformations in randomly mating populations [41].

Environmental or other factors likely played a role in observed antler size and malformations in white-tailed deer. Maternal effects (e.g., non-heritable facets of condition, environment, year, and behavior) are more prominent in 1.5-year-old males, and to a lesser extent in 2.5-year-old males [3]. We found that 3 of 12 and 4 of 12 individuals with antler malformations were 1.5 and 2.5 years of age, respectively. Therefore, antler configuration may have been more related to maternal or environmental factors, and not to heritable genetic effects. The remaining five deer were older, and thus could have sustained a body or pedicle injury predisposing them to developing malformed antlers. Rachlow et al. [41] found that malformed antlers, in every case, were associated with pedicles deformed in size or orientation. Observations of deer with antler malformations from Mississippi, not included in our study, may also have been due to pedicle deformation or injury (Figure A3). We were unable to determine whether malformed antlers from Oklahoma and Texas were due to deformed pedicles or body injury because deer were classified as malformed from antler measurements, photographs and removed antler sets.

It has long been recognized that age influences antler size [2–4]. Genetic factors, such as heterozygosity or inbreeding, may also contribute to antler size [3,59]. When testing for effects of heterozygosity on antler size, we accounted for age-specific antler size. There was not a strong linear relationship between IR and individual antler points or score, although a qualitative negative trend was observed. However, HL was more strongly related to both antler measures and revealed the same negative trend. Our findings of a negative trend between heterozygosity and antler size were in the expected direction, albeit weak. These negative trends are similar to previous research, which found a genetic link between the heterozygosity of allozymes and morphometric traits in ungulates. Deer with small antlers were more inbred, based on the heterozygosity of allozymes, than deer with larger antlers [12]. Individuals that were more heterozygous had greater antler sizes or points in white-tailed deer [13–16] and greater horn growth in bighorn sheep [17].

Factors such as hunting [6,60,61], population size, and confinement to fenced enclosures may [62] or may not [63] affect the genetic characteristics of the population. Increased homozygosity (i.e., inbreeding) may occur when populations are relatively small and isolated [10,64]. Therefore, our enclosed population in Oklahoma may have shown signs of increased inbreeding due to reduced gene flow or mating between close relatives. However, the enclosed population showed similar levels of heterozygosity to large free-ranging populations from Mississippi and Texas for several reasons [63]. First, the HTEF surrounding the Oklahoma property was not a complete barrier to deer movements [32,65]. Second, GPS collar data [32] revealed deer could enter and leave the study area. Last, the HTEF surrounds a relatively large area, has not been installed very long in terms of deer generations, and encloses a population of deer consisting of several hundred individuals.

Selective harvest has also been implicated in influencing the genetic properties of hunted populations [5,6,60–62]. The horn size of bighorn sheep rams decreased due to intense harvest pressure of rams with large horns [5]. In Mississippi, a four-point AR resulted in a shift to fewer antler points of deer at 3.5 years of age [1]. If antler quality were related to genetic variability (i.e., heterozygosity), and selective harvest influences genetic variability, then we might expect heterozygosity to differ between Mississippi and our two other populations. Based on these data, all populations had similar levels of neutral genetic diversity. Therefore, AR may not result in changes in population level genetic diversity because antler size and heterozygosity were not strongly related. Other factors, such as environment, may exert a greater influence on antler morphology [19]. In addition, antlers may not be the true object of selection [19], which would result in no change of genetic diversity from selective harvesting.

5. Conclusions

The ability to detect inbreeding from a modest number of microsatellite loci is limited, but based on these data there was no detectable difference in antlers due to inbreeding. Therefore, deer were unlikely to suffer from inbreeding (i.e., increased *r* and homozygosity) or reduced genetic diversity under the conditions described here, and in DeYoung et al. [56] and Latch et al. [63]. Our two free-ranging populations were large and allowed movement of individuals into and out of the population. Successful reproduction of dispersing individuals will result in populations of deer being less subdivided genetically. Even the enclosure was not a complete barrier to deer movement in the Oklahoma population [32,65], likely allowing for some level of gene flow among populations. Selective harvest programs, under the inbreeding hypothesis, will likely not have an effect on reducing future genetic diversity, particularly if these traits are influenced by other factors such as injury, nutritional deficiencies, or environmental conditions. There does not appear to be a strong inherited genetic basis for the occurrence of similar antler traits within groups [21], because deer were not inbred and deer with similar antler traits did not share common ancestors. Therefore, selective harvest programs, or the use of antler point restrictions, should focus on removing individuals to maintain proper densities and age structures, which may improve mean cohort antler size at maturity, and not to change the genetics of the population [66].

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Data Availability Statement: The data used herein may be made available upon reasonable requests to the Noble Research Institute, LLC by contacting the corresponding author (S.L.W.).

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Appendix A

Table and images to accompany results and findings presented herein.

Table A1. Relatedness (*r*), internal relatedness (IR), homozygosity weighted by locus (HL), and F_{is} among male white-tailed deer (*Odocoileus virginianus*) within age class for lower and upper antler point and score (cm) quartiles and presence (≥2) or absence of non-typical points and antler malformations from Mississippi (1999–2001), Oklahoma (1991–2005) and Texas (1999–2005).

					Relatedness	**Internal Relatedness**	**Homozygosity**	F_{is}
Study Area	**Age**	**Trait**	**Class**	**N**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**
Mississippi	1	Score	Lower	10	−0.013 (−0.098, 0.072)	0.064 (−0.03, 0.158)	0.299 (0.224, 0.374)	0.062 (−0.056, 0.18)
			Upper	11	−0.055 (−0.087, −0.022)	0.059 (−0.027, 0.145)	0.286 (0.214, 0.358)	0.073 (−0.043, 0.189)
		Point	Lower	22	−0.022 (−0.050, 0.006)	0.02 (−0.07, 0.111)	0.25 (0.182, 0.319)	0.034 (−0.041, 0.109)
			Upper	11	−0.04 (−0.077, −0.002)	0.069 (−0.025, 0.164)	0.288 (0.209, 0.366)	0.068 (−0.023, 0.159)
	2	Score	Lower	8	−0.048 (−0.106, 0.010)	0.031 (−0.104, 0.166)	0.27 (0.165, 0.376)	0.054 (−0.071, 0.17)
			Upper	9	−0.063 (−0.101, −0.025)	0.105 (0.014, 0.196)	0.303 (0.219, 0.386)	0.115 (−0.003, 0.233)
		Point	Lower	6	−0.038 (−0.098, 0.021)	0.099 (−0.066, 0.264)	0.308 (0.157, 0.458)	0.103 (−0.08, 0.286)
			Upper	12	−0.054 (−0.095, −0.013)	0.016 (−0.046, 0.078)	0.23 (0.186, 0.274)	0.024 (−0.046, 0.094)
	3	Score	Lower	8	−0.04 (−0.086, 0.007)	0.014 (−0.116, 0.145)	0.249 (0.143, 0.355)	0.025 (−0.072, 0.122)
			Upper	8	0.001 (−0.110, 0.113)	0.017 (−0.109, 0.144)	0.259 (0.164, 0.354)	0.02 (−0.103, 0.143)
		Point	Lower	12	−0.04 (−0.118, 0.038)	0.026 (−0.066, 0.118)	0.266 (0.195, 0.337)	0.04 (−0.028, 0.108)
			Upper	13	−0.047 (−0.071, −0.023)	0.007 (−0.092, 0.106)	0.242 (0.168, 0.315)	0.02 (−0.072, 0.112)
Oklahoma	1	Score	Lower	15	−0.007 (−0.033, 0.019)	0.106 (0.023, 0.189)	0.341 (0.276, 0.407)	0.102 (−0.02, 0.224)
			Upper	15	−0.027 (−0.070, 0.017)	0.01 (−0.083, 0.103)	0.256 (0.188, 0.324)	0.023 (−0.043, 0.089)
		Point	Lower	19	−0.006 (−0.036, 0.024)	0.112 (0.042, 0.182)	0.349 (0.29, 0.409)	0.113 (0.014, 0.212)
	1	Point	Upper	15	−0.013 (−0.048, 0.021)	0.022 (−0.076, 0.12)	0.263 (0.194, 0.331)	0.019 (−0.078, 0.116)
	2	Score	Lower	11	−0.068 (−0.115, −0.020)	0.059 (−0.031, 0.149)	0.315 (0.248, 0.382)	0.071 (−0.076, 0.218)
			Upper	12	−0.003 (−0.056, 0.050)	0.032 (−0.076, 0.141)	0.274 (0.186, 0.361)	0.029 (−0.061, 0.119)
		Point	Lower	15	−0.048 (−0.074, −0.022)	0.122 (0.052, 0.192)	0.345 (0.299, 0.392)	0.129 (0.024, 0.234)
			Upper	17	−0.03 (−0.063, 0.011)	0.019 (−0.065, 0.104)	0.262 (0.193, 0.331)	0.034 (−0.038, 0.106)

Table A1. *Cont.*

					Relatedness	Internal Relatedness	Homozygosity	F_{is}
Study Area	**Age**	**Trait**	**Class**	**N**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**
	3	Score	Lower	8	−0.027 (−0.067, 0.012)	0.069 (−0.047, 0.184)	0.311 (0.229, 0.392)	0.073 (−0.045, 0.191)
			Upper	8	−0.04 (−0.096, 0.015)	0.027 (−0.107, 0.161)	0.271 (0.159, 0.382)	0.042 (−0.072, 0.156)
		Point	Lower	16	−0.03 (−0.058, −0.003)	0.057 (−0.007, 0.12)	0.304 (0.253, 0.355)	0.064 (−0.015, 0.143)
			Upper	10	−0.045 (−0.084, −0.005)	0.057 (−0.056, 0.17)	0.296 (0.198, 0.393)	0.067 (−0.057, 0.191)
	N/A	NT [1]	Absent	81	−0.01 (−0.028, 0.009)	0.08 (0.048, 0.112)	0.31 (0.286, 0.334)	0.086 (0.034, 0.138)
			Present	19	0.006 (−0.035, 0.047)	0.083 (0.009, 0.156)	0.308 (0.245, 0.37)	0.072 (0.011, 0.133)
		Abn [2]	Yes	7	−0.025 (−0.111, 0.060)	0.119 (0.029, 0.209)	0.329 (0.251, 0.406)	0.13 (−0.009, 0.269)
			No	109	−0.007 (−0.018, 0.004)	0.065 (0.037, 0.093)	0.298 (0.276, 0.319)	0.068 (0.02, 0.116)
Texas	1	Score	Lower	12	−0.005 (−0.059, 0.049)	0.113 (0.014, 0.212)	0.327 (0.253, 0.401)	0.14 (0.021, 0.259)
			Upper	12	−0.035 (−0.091, 0.020)	0.003 (−0.085, 0.091)	0.232 (0.173, 0.292)	0.06 (−0.041, 0.161)
		Point	Lower	13	−0.015 (−0.061, 0.031)	0.073 (−0.029, 0.174)	0.297 (0.218, 0.376)	0.106 (−0.001, 0.213)
			Upper	12	−0.029 (−0.092, 0.035)	0.016 (−0.065, 0.098)	0.248 (0.191, 0.304)	0.056 (−0.072, 0.184)
	2	Score	Lower	15	0.011 (−0.046, 0.067)	0.11 (0.026, 0.195)	0.32 (0.252, 0.387)	0.128 (−0.003, 0.259)
			Upper	15	−0.039 (−0.064, −0.014)	0.117 (0.047, 0.186)	0.3 (0.251, 0.349)	0.142 (0.022, 0.262)
		Point	Lower	13	−0.01 (−0.050, 0.029)	0.131 (0.043, 0.218)	0.334 (0.269, 0.399)	0.156 (0.01, 0.302)
			Upper	11	−0.061 (−0.095, −0.027)	0.045 (−0.044, 0.134)	0.247 (0.184, 0.309)	0.04 (−0.071, 0.151)
	3	Score	Lower	9	−0.027 (−0.123, 0.069)	0.049 (−0.084, 0.183)	0.261 (0.168, 0.354)	0.064 (−0.049, 0.177)
			Upper	9	−0.005 (−0.098, 0.088)	0.06 (−0.093, 0.214)	0.283 (0.179, 0.387)	0.099 (−0.03, 0.228)
		Point	Lower	4	−0.105 (−0.243, 0.034)	0.083 (−0.045, 0.211)	0.279 (0.141, 0.417)	0.117 (−0.144, 0.378)
			Upper	10	0.004 (−0.077, 0.085)	0.098 (−0.01, 0.206)	0.3 (0.228, 0.372)	0.099 (−0.034, 0.232)
	N/A	NT [1]	Absent	134	−0.013 (−0.025, −0.001)	0.077 (0.049, 0.105)	0.287 (0.266, 0.307)	0.093 (0.032, 0.154)
			Present	22	0.019 (−0.021, 0.059)	0.111 (0.058, 0.164)	0.311 (0.272, 0.351)	0.099 (0.009, 0.189)
	N/A	Abn [2]	Yes	5	−0.063 (−0.138, 0.011)	0.101 (−0.068, 0.279)	0.3 (0.163, 0.437)	0.204 (−0.06, 0.468)
			No	239	−0.008 (−0.016, 0.000)	0.082 (0.062, 0.102)	0.29 (0.276, 0.305)	0.094 (0.039, 0.149)

[1] Non-typical points. [2] Abnormal antlers.

Figure A1. Male white-tailed deer (*Odocoileus virginianus*) exhibiting lower range of antler points for adult deer (visually estimated $\geq$2.5 years). Photograph by Stephen L. Webb.

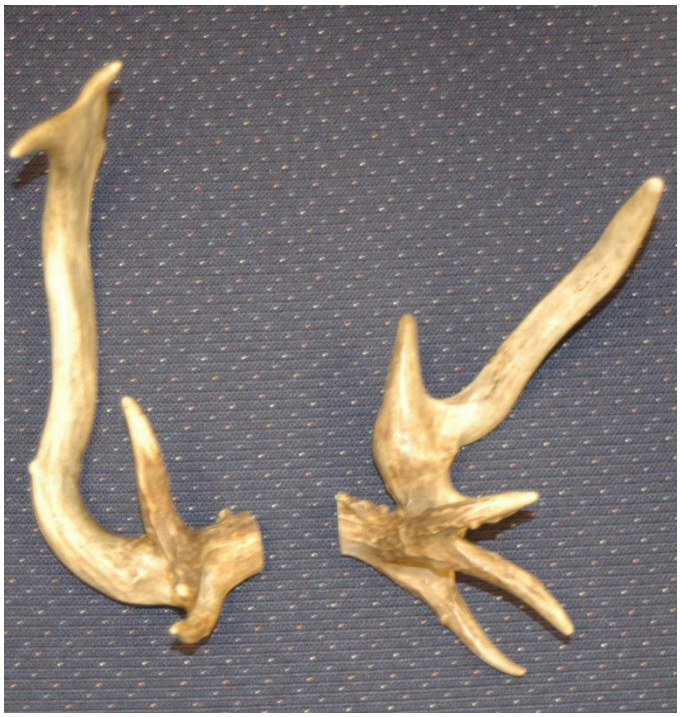

Figure A2. Antlers of a male white-tailed deer (*Odocoileus virginianus*) displaying malformed antlers in the absence of pedicle damage. Photograph by Stephen L. Webb.

Figure A3. Male white-tailed deer (*Odocoileus virginianus*) exhibiting damage to pedicle of right antler. Photograph by Stephen L. Webb.

References

1. Demarais, S.; Strickland, B.K.; Castle, L.E. Antler regulation effects on white-tailed deer on Mississippi public hunting areas. *Proc. Southeast. Assoc. Fish Wildl. Agen.* **2005**, *59*, 1–9.
2. Jacobson, H.A. Age and quality relationships. In *Quality Whitetails: The Why and How of Quality Deer Management*; Miller, K.V., Marchinton, R.L., Eds.; Stackpole Books: Mechanicsburg, PA, USA, 1995; pp. 103–111.
3. Lukefahr, S.D.; Jacobson, H.A. Variance component analysis and heritability of antler traits in white-tailed deer. *J. Wildl. Manag.* **1998**, *62*, 262–268. [CrossRef]
4. Strickland, B.K.; Demarais, S. Age and regional differences in antlers and mass of white-tailed deer. *J. Wildl. Manag.* **2000**, *64*, 903–911. [CrossRef]
5. Coltman, D.W.; O'Donoghue, P.; Jorgenson, J.T.; Hogg, J.T.; Strobeck, C.; Festa-Bianchet, M. Undesirable evolutionary consequences of trophy hunting. *Nature* **2003**, *26*, 655–658. [CrossRef] [PubMed]
6. Harris, R.B.; Wall, W.A.; Allendorf, F.W. Genetic consequences of hunting: What do we know and what should we do? *Wildl. Soc. Bull.* **2002**, *30*, 634–643.
7. Guynn, D.C., Jr.; Mott, S.P.; Cotton, W.D.; Jacobson, H.A. Cooperative management of white-tailed deer on private lands in Mississippi. *Wildl. Soc. Bull.* **1983**, *11*, 211–214.
8. Strickland, B.K. (Mississippi State University, Mississippi State, MS, USA). Personal communication, 2009.
9. Falconer, D.S.; Mackay, T.F.C. *Introduction to Quantitative Genetics*, 4th ed.; Longman Group Ltd.: Essex, UK, 1996.
10. Coltman, D.W.; Bowen, W.D.; Wright, J.M. Birth weight and neonatal survival of harbour seal pups are positively correlated with genetic variation measured by microsatellites. *Proc. R. Soc. Lond. B* **1998**, *265*, 803–809. [CrossRef] [PubMed]
11. Coulson, T.N.; Pemberton, J.M.; Albon, S.D.; Beaumont, M.; Marshall, T.C.; Slate, J.; Guinness, F.E.; Clutton-Brock, T.H. Microsatellites reveal heterosis in red deer. *Proc. R. Soc. Lond. B* **1998**, *265*, 489–495. [CrossRef] [PubMed]
12. Smith, M.H.; Hillestad, H.O.; Manlove, M.N.; Marchinton, R.L. Use of population genetics data for the management of fish and wildlife populations. *Trans. N. Am. Wildl. Nat. Resour. Conf.* **1976**, *41*, 119–133.
13. Smith, M.H.; Chesser, R.K.; Cothran, E.G.; Johns, P.E. Genetic Variability and Antler Growth in a Natural Population of White-tailed Deer. In *Antler Development in Cervidae*; Brown, R.D., Ed.; Caesar Kleberg Wildlife Research Institute: Kingsville, TX, USA, 1983; pp. 365–387.
14. Scribner, K.T.; Smith, M.H.; Johns, P.E. Age, condition, and genetic effects on incidence of spike bucks. *Proc. Southeast. Assoc. Fish Wildl. Agen.* **1984**, *38*, 23–32.
15. Scribner, K.T.; Smith, M.H. Genetic variability and antler development. In *Horns, Pronghorns, and Antlers: Ecology, Morphology, Physiology, and Social Significance*; Bubenik, G.A., Bubenik, A.B., Eds.; Springer: New York, NY, USA, 1990; pp. 460–473.
16. Smith, M.H.; Scribner, K.T.; Johns, P.E.; Rhodes, O.E., Jr. Genetics and antler development. In *Global Trends in Wildlife Management*; Bobek, B., Perzanowski, K., Regelin, W., Eds.; Swiat Press: Krakow, Warszawa, 1991; pp. 323–326.

17. Fitzsimmons, N.N.; Buskirk, S.W.; Smith, M.H. Population history, genetic variability, and horn growth in bighorn sheep. *Conserv. Biol.* **1995**, *9*, 314–323. [CrossRef]
18. Williams, J.D.; Krueger, W.F.; Harmel, D.H. Heritabilities for antler characteristics and body weight in yearling white-tailed deer. *Heredity* **1994**, *73*, 78–83. [CrossRef] [PubMed]
19. Kruuk, L.E.B.; Slate, J.; Pemberton, J.M.; Brotherstone, S.; Guinness, F.; Clutton-Brock, T. Antler size in red deer: Heritability and selection but no evolution. *Evolution* **2002**, *56*, 1683–1695. [CrossRef] [PubMed]
20. Michel, E.S.; Demarais, S.; Strickland, B.K.; Smith, T.; Dacus, C.M. Antler characteristics are highly heritable but influenced by maternal factors. *J. Wildl. Manag.* **2016**, *80*, 1420–1426. [CrossRef]
21. Hicks, J.F.; Rachlow, J.L. Is there a genetic basis for antler and pedicle malformations in reintroduced elk in northern Arizona? *Southwest Nat.* **2006**, *51*, 276–282. [CrossRef]
22. French, C.E.; McEwen, L.C.; Magruder, N.D.; Ingram, R.H.; Swift, R.W. Nutrient requirements for growth and antler development in the white-tailed deer. *J. Wildl. Manag.* **1956**, *20*, 221–232. [CrossRef]
23. Ullrey, D.E. Nutrition and antler development in white-tailed deer. In *Antler Development in Cervidae*; Brown, R.D., Ed.; Caesar Kleberg Wildlife Research Institute: Kingsville, TX, USA, 1983; pp. 49–59.
24. Ditchkoff, S.S.; Lochmiller, R.L.; Masters, R.E.; Starry, W.R.; Leslie, D.M., Jr. Does fluctuating asymmetry of antlers in white-tailed deer (*Odocoileus virginianus*) follow patterns predicted for sexually selected traits? *Proc. R. Soc. Lond. B* **2001**, *268*, 891–898. [CrossRef] [PubMed]
25. Zahavi, A. Mate selection: A selection for a handicap. *J. Theor. Biol.* **1975**, *53*, 205–214. [CrossRef]
26. Baccus, J.T.; Welch, R.D. Asymmetry in the Antler Structure of Sika Deer from the Edwards Plateau of Texas. In *Antler Development in Cervidae*; Brown, R.D., Ed.; Caesar Kleberg Wildlife Research Institute: Kingsville, TX, USA, 1983; pp. 211–221.
27. Gould, S.J. The origin and function of "bizarre" structures: Antler size and skull size in the "Irish elk," *Megaloceros giganteus*. *Evolution* **1974**, *28*, 191–220.
28. Morina, D.L.; Demarais, S.; Strickland, B.K.; Larson, J.E. While males fight, females choose: Male phenotypic quality informs female mate choice in mammals. *Anim. Behav.* **2018**, *138*, 69–74. [CrossRef]
29. Pettry, D.E. *Soil Resources Areas of Mississippi, Information Sheet 1278*; Mississippi Agricultural and Forestry Experiment Station, Mississippi State University: Starkville, MS, USA, 1977.
30. DeYoung, R.W. Effects of Social and Population Characteristics on the Reproductive Success of Male White-Tailed Deer. Ph.D. Thesis, Mississippi State University, Starkville, MS, USA, 2004.
31. Gee, K.L.; Porter, M.D.; Demarais, S.; Bryant, F.C.; Van Vreede, G. *White-Tailed Deer: Their Foods and Management in the Cross Timbers*, 2nd ed.; The Samuel Roberts Noble Foundation: Ardmore, OK, USA, 1994.
32. Webb, S.L.; Gee, K.L.; Demarais, S.; Strickland, B.K.; DeYoung, R.W. Efficacy of a 15-strand high tensile electric fence to control white-tailed deer movements. *Wildl. Biol. Pract.* **2009**, *5*, 45–57. [CrossRef]
33. McCoy, J.E.; Hewitt, D.G.; Bryant, F.C. Dispersal by yearling male white-tailed deer and implications for management. *J. Wildl. Manag.* **2005**, *69*, 366–376. [CrossRef]
34. Strickland, B.K.; Jones, P.D.; Demarais, S.; Dacus, C.M.; Dillard, J.R.; Jacobson, H.A. Estimating Boone and Crockett scores for white-tailed deer from simple antler measurements. *Wildl. Soc. Bull.* **2013**, *37*, 458–463. [CrossRef]
35. Boone and Crockett Club. *Measuring and Scoring North American Big Game Trophies*, 2nd ed.; Boone and Crockett Club: Missoula, MT, USA, 1997.
36. Ramsey, C.W. A drop-net deer trap. *J. Wildl. Manag.* **1968**, *32*, 187–190. [CrossRef]
37. Gee, K.L.; Holman, J.H.; Demarais, S. A man-power efficient drop-net system for capturing white-tailed deer. *Proc. Annu. Southeast Deer Study Group* **1999**, *22*, 31.
38. Webb, S.L.; Lewis, J.S.; Hewitt, D.G.; Hellickson, M.W.; Bryant, F.C. Assessing the helicopter and net-gun as a capture technique for white-tailed deer. *J. Wildl. Manag.* **2008**, *72*, 310–314. [CrossRef]
39. DeYoung, R.W.; Demarais, S.; Honeycutt, R.L.; Gonzales, R.A.; Gee, K.L.; Anderson, J.D. Evaluation of a DNA microsatellite panel useful for genetic exclusion studies in white-tailed deer. *Wildl. Soc. Bull.* **2003**, *31*, 220–232.
40. Anderson, J.D.; Honeycutt, R.L.; Gonzales, R.A.; Gee, K.L.; Skow, L.C.; Gallagher, R.L.; Honeycutt, D.A.; DeYoung, R.W. Development of microsatellite DNA markers for the automated genetic characterization of white-tailed deer populations. *J. Wildl. Manag.* **2002**, *66*, 67–74. [CrossRef]
41. Rachlow, J.L.; Lee, R.M.; Riley, R.K. Abnormal antlers and pedicles on Rocky Mountain elk in northern Arizona. *Southwest Nat.* **2003**, *48*, 147–153. [CrossRef]
42. Severinghaus, C.W. Tooth development and wear as criteria of age in white-tailed deer. *J. Wildl. Manag.* **1949**, *13*, 195–216. [CrossRef]
43. Gee, K.L.; Holman, J.H.; Causey, M.K.; Rossi, A.N.; Armstrong, J.B. Aging white-tailed deer by tooth replacement and wear: A critical evaluation of a time-honored technique. *Wildl. Soc. Bull.* **2002**, *30*, 387–393.
44. Queller, D.C.; Goodnight, K.F. Estimating relatedness using genetic markers. *Evolution* **1989**, *43*, 258–275. [CrossRef]
45. Hardy, O.J.; Vekemans, X. SPAGeDi: A versatile computer program to analyse spatial genetic structure at the individual or population levels. *Mol. Ecol. Notes* **2002**, *2*, 618–620. [CrossRef]
46. Amos, W.; Wilmer, J.W.; Fullard, K.; Burg, T.M.; Croxall, J.P.; Bloch, D.; Coulson, T. The influence of parental relatedness on reproductive success. *Proc. R. Soc. Lond. B* **2001**, *268*, 2021–2027. [CrossRef] [PubMed]

47. Amos, W. IR Macron3. Available online: http://www.zoo.cam.ac.uk/system/files/documents/IRmacroN4.doc (accessed on 27 August 2008).
48. Aparicio, J.M.; Ortego, J.; Cordero, P.J. What should we weight to estimate heterozygosity, alleles or loci? *Mol. Ecol.* **2006**, *15*, 4659–4665. [CrossRef] [PubMed]
49. Wright, S. The genetical structure of populations. *Ann. Eugen.* **1951**, *15*, 323–354. [CrossRef] [PubMed]
50. Weir, B.S.; Cockerham, C.C. Estimating *F*-statistics for the analysis of population structure. *Evolution* **1984**, *38*, 1358–1370. [PubMed]
51. Avise, J.C. *Molecular Markers, Natural History, and Evolution*, 2nd ed.; Sinauer Associates, Inc.: Sunderland, MA, USA, 2004.
52. DeYoung, R.W.; Demarais, S.; Gonzales, R.A.; Honeycutt, R.L.; Gee, K.L. Multiple paternity in white-tailed deer (*Odocoileus virginianus*) revealed by DNA microsatellites. *J. Mammal.* **2002**, *83*, 884–892. [CrossRef]
53. Sorin, A.B. Paternity assignment for white-tailed deer (*Odocoileus virginianus*): Mating across age classes and multiple paternity. *J. Mammal.* **2004**, *85*, 356–362. [CrossRef]
54. Kenward, M.G.; Roger, J.H. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* **1997**, *53*, 983–997. [CrossRef]
55. Littell, R.C.; Milliken, G.A.; Stroup, W.W.; Wolfinger, R.D.; Schabenberger, O. *SAS® for Mixed Models*, 2nd ed.; SAS Institute, Inc.: Cary, NC, USA, 2006.
56. DeYoung, R.W.; Demarais, S.; Gee, K.L.; Honeycutt, R.L.; Hellickson, M.W.; Gonzales, R.A. Molecular evaluation of the white-tailed deer (*Odocoileus virginianus*) mating system. *J. Mammal.* **2009**, *90*, 946–953. [CrossRef]
57. Balloux, F.; Amos, W.; Coulson, T.N. Does heterozygosity estimate inbreeding in real populations? *Mol. Ecol.* **2004**, *13*, 3021–3031. [CrossRef] [PubMed]
58. Von Hardenberg, A.; Bassano, B.; Festa-Bianchet, M.; Luikart, G.; Lanfranchi, P.; Coltman, D. Age-dependent genetic effects on a secondary sexual trait in male Alpine ibex, *Capra ibex*. *Mol. Ecol.* **2007**, *16*, 1969–1980. [CrossRef] [PubMed]
59. Scribner, K.T.; Smith, M.H.; Jones, P.E. Environmental and genetic components of antler growth in white-tailed deer. *J. Mammal.* **1989**, *70*, 284–291. [CrossRef]
60. Hartl, G.B.; Klein, F.; Willing, R.; Aapollonio, M.; Lang, G. Allozymes and the genetics of antler development in red deer (*Cervus elaphus*). *J. Zool.* **1995**, *237*, 83–100. [CrossRef]
61. Hartl, G.B.; Zachos, F.; Nadlinger, K. Genetic diversity in European red deer (*Cervus elaphus* L.): Anthropogenic influences on natural populations. *Comptes Rendus Biol.* **2003**, *326*, 37–42. [CrossRef]
62. Ryman, N.; Baccus, R.; Reuterwall, C.; Smith, M.H. Effective population size, generation interval, and potential loss of genetic variability in game species under different hunting regimes. *Oikos* **1981**, *36*, 257–266. [CrossRef]
63. Latch, E.K.; Gee, K.L.; Webb, S.L.; Honeycutt, R.L.; DeYoung, R.W.; Gonzales, R.A.; Demarais, S.; Toby, R. Genetic consequences of fence confinement in a population of white-tailed deer. *Diversity* **2021**, accepted.
64. Zachos, F.E.; Althoff, C.; Steynitz, Y.V.; Eckert, I.; Hartl, G.B. Genetic analysis of an isolated red deer (*Cervus elaphus*) population showing signs of inbreeding depression. *Eur. J. Wildl. Res.* **2007**, *53*, 61–67. [CrossRef]
65. Webb, S.L.; Gee, K.L.; Wang, G. Survival and fidelity of an enclosed white-tailed deer population using capture–recapture-reporting data. *Pop. Ecol.* **2010**, *52*, 81–88. [CrossRef]
66. Webb, S.L.; Demarais, S.; Strickland, B.K.; DeYoung, R.W.; Kinghorn, B.P.; Gee, K.L. Effects of selective harvest on antler size in white-tailed deer: A modeling approach. *J. Wildl. Manag.* **2012**, *76*, 48–56. [CrossRef]