THE GENOMICS OF ANTLER SIZE IN WHITETAIL DEER

An Undergraduate Research Scholars Thesis

by

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TABLE OF CONTENTS

ABSTRACT

The Genomics of Antler Size in Whitetail Deer

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The genotypic factors that affect antler size in whitetail deer are currently unknown. Environmental factors, such as nutrition, have been studied and have been shown to affect antler size, but the role of genomic variation is not yet understood. In this project, whole genome sequencing is being utilized to examine patterns of variation in whitetail deer individuals selected for below average, as well as significantly above average terminal antler size, from a closed breeding population. The loci found to be present with drastically different allele frequencies will be documented and functionally characterized. Any variation that is specifically associated with either the below average or the above average antler size population can be concluded to be the underlying genetic mechanisms resulting in the different phenotypes. Whitetail deer have great economic value in Texas, home of the largest deer breeding and hunting industry in the US and contribute 1.6 billion dollars annually[25]. There is potential for expanded use of whitetail deer antlers because of the unique properties of these structures. This research will allow us to identify genes and genomic variation that influence antler size in whitetail deer in order to explore the uses of antlers in research, in industry, and in clinical settings. Antlers are unique, regenerative organs and it is important to understand the

mechanisms underlying significant differences in antler growth rate and total antler size to find uses in science and medicine. There is great potential in using antlers as research models and this knowledge will improve our understanding of the control of phenotypic expression. Improving our knowledge of antlers would only improve our understanding of osteogenesis, musculoskeletal disorders, morphogenesis, positional memory of cells, and the endocrine system. Studying antlers is even relevant to cancer because of the low incidence tumors despite the high rates of cell division that are required to regenerate the antlers each year. The research, industrial, and medical uses of antlers are numerous, but efficient utilization can only be achieved after understanding the underlying, genotypic mechanisms.

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CHAPTER I

INTRODUCTION

Antlers are unique, regenerative organs that could be used to further research and science. Nutrition is an important component in antlerogenesis, but studies show that Cervidae genetics play a large role in the resulting antler phenotype. It is important to understand the genes behind the significant increases or decreases in antler growth in order to be able to properly utilize antlers in research and clinical use.

In order to find genes that control phenotypes, DNA extraction is the first step. The extracted DNA will then be sequenced so that the genetic information will be decoded and aligned to create a genome assembly for the organism. The genomes will be annotated using a reference genome gene model. Variation within the genomes will be compared to identify regions of significant F_{ST}, a summary of genetic differentiation between populations selected for small and large antlers.

The DNA of whitetail bucks was collected for sequencing in order to create a genomic assembly to evaluate for genes that could explain the differences in the antler phenotypes. Individuals with a score above 120 on the Boone and Crockett scale were selected for the high extreme group and individuals with a score below 90 on the Boone and Crockett scale were selected for the low extreme group. Individuals were also chosen based off the number of years their antlers were measured and the similarity of pedigrees to individuals already chosen for the study. The difference will allow the comparison of genomes to identify the genetic variation that could be the cause of phenotypic variation.

The Cervidae family is the only family that grows antlers. Antlers are different from horns grown by other families, such as the Bovidae [5,6,12]. The main difference between antlers and horns is that antlers are made of living, vascular tissue before the antlers ossify and harden and horns do not contain living tissue and are made of keratin [5,6,12]. Horns never regrow if they are removed, but antlers regrow and many species of deer shed and regrow antlers on an annual basis [5,6,12]. Antlers are bone protrusions from the cranium that grow as vascular, spongey tissue while covered in velvet, a special, hair-covered skin [5,6]. After the antler is fully formed, the antler tissue ossifies and loses vascular function. The deer rubs off the velvet to expose the hardened antlers that remain until they are shed after breeding season [5,6]. In all Cervid species except reindeer and caribou, only the males grow antlers [5,6].

Antlers are used by male deer, or bucks, to attract females, or does, by displaying health and dominance [5,6]. More importantly, antlers are used as weapons against competing males in fights over does [7]. Scramble-competition polygyny, which favors competitive mate-searching over fighting and competing for females, is the practice of white-tailed deer [12]. Reproductive success comes at a cost for bucks, which are capital breeders meaning the energy for breeding comes from stored reserves [12]. The body mass changes, energetic output, and reproductive timing was measured for GPS radiocollared white-tailed bucks and prime age bucks, from 5.5 to 6.5 years exhibited the highest body mass loss, indicating highest reproductive effort [12]. One-third of the body mass loss of these bucks could be attributed to the rate of movement, which increased to as much as fourfold while searching for reproductive opportunities, but the majority is due to reduced food intake [12]. There have been instances where bucks have used antlers to ward off predators, but this can be determined to not be a primary function because the lack of antlers on does [7]. Larger antlers are seen to improve dominance and mating success, but there are negative

effects that cause an increase in the mortality rate of bucks during the rutting season [5]. These negative effects include heavier weight that decreases agility, the demineralization of skeletal bones, and the depletion of body fat to starvation levels as the antlers take energy to grow and the bucks forage minimally during mating season [5]. It has been found that only white-tailed bucks from the ages of 2.5 to 4.5 years experience greater antler allocation in result of better nutrition [19]. Antler mass, which decreases in variability over time, and body mass, which continues to be equally variable regardless of age or environment, showed a positive, proportional relationship and suggests body mass is a more accurate predictor of reproductive success [19].

Some Cervidae, such as the whitetail deer, *Odocoileus virginianus*, shed and regrow their antlers each year and are classified as temperate deer [5,6]. The antlers of *O. virginianus* will be regrown each year and will reach a larger size and have an increased number of points if there are similar or improved levels of nutrients available and environment stressors are similar or decreased. Whitetail deer antler size is evaluated by the Boone and Crockett Scoring System that evaluates number of points, the spread, the circumference, and the length of points and the main beam [3]. The current world record whitetail deer had 47 points and a Boone and Crockett score of 312 $^{3}/_{8}$ inches [24]. Antler size has been connected to nutrition, though research has shown the significance of the heritability of antler size [4]. Significant dietary factors are energy to preform antlerogenesis, protein as the major component in antlers, minerals calcium and phosphorus for ossification, vitamin D for in calcium absorption, and vitamin A, which can cause bone loss in excess [4].

The Boone and Crockett (B&C) Scoring System was first defined and implemented in 1932 and further refined in 1950 [3]. This system uses the number of points on each antler and measurements of tip to tip spread, greatest spread, inside spread of main beams, total lengths of

all abnormal points, lengths of main beam, lengths of normal points, and the circumferences of each antler measured to an eighth of an inch (Figure 1)[3]. We explore a potential alternative to this method using 3D scanning, which provides unbiased values for length, volume, and surface area.

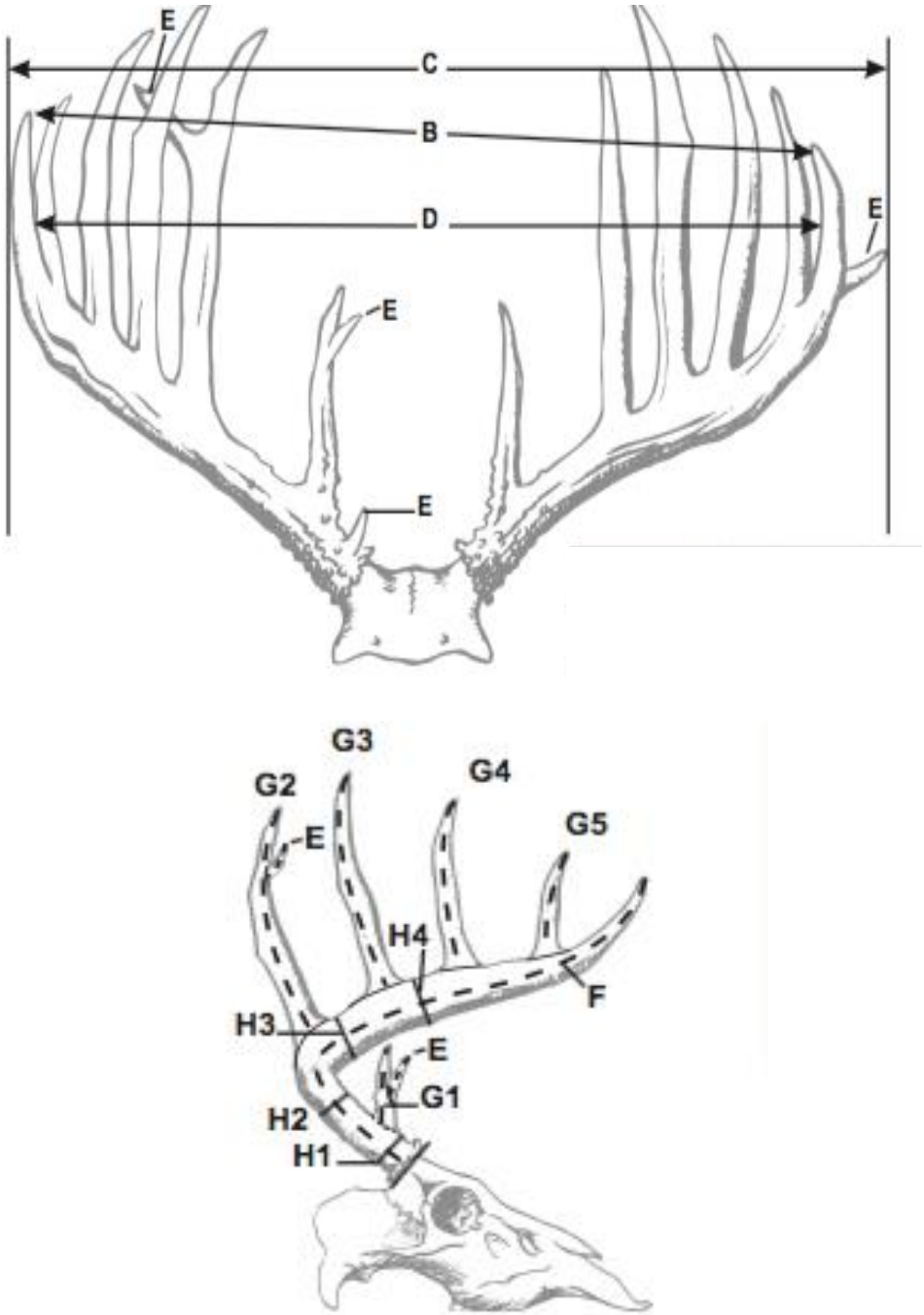

Figure 1: Diagrams of Boone and Crockett scoring www.boone-crockett.org

Antlers have been recognized to be a genetically regulated phenotype and antler size has been found to be inheritable. The heritability of antler size has been shown by a study that compared the progeny of one superior buck to those of eight inferior bucks who had spikes, or two unforked antlers, as yearlings [4]. The F1 offspring of the spike buck sires had a much high percentage of spike offspring than the F1 generation of the superior buck [4]. The does produced in the F1 generation were bred back to their respective sires and the produced F2 progeny showed an increase in the percentage of spike offspring produced by the spike bucks while the superior buck produced no spike offspring [4]. This study is further supported by a whitetail deer breeding operation that was used to estimate the heritability of birth weight, body weight, antler points, main beam length, antler spread, basal circumference, and antler weight [24]. Respectively, the values were calculated to be 0.00-0.17, 0.58, 0.56, 0.22-0.56, 0.47-0.70, 0.03-0.43, 0.80-0.89, and 0.71-0.86, making antler spread the most variable and least heritable and basal circumference the least variable and most heritable [31].

Research of sika deer identified the PEDF, pigment epithelium-derived factor, gene and the CDKN1C, cyclin-dependent kinase inhibitor 1C, gene to be two regulatory genes involved in antlerogenesis [23]. In a study on the genetics of velvet antler size in sika deer, the genomes were sequenced of 50 deer with heavy velvet antler and 50 deer with light velvet antler [16]. The sequence genomes were then used to find a single polymorphism, or SNP, site on the OAS2 exon region and another SNP on the ALYREF/THOC4 exon region within 94 genetic variations linked to velvet antler weight [16]. A follow up to the previous study of sika deer velvet antler genetics used transcriptome sequencing and microRNA sequencing to examine proliferating cells at the tip of the velvet antler, where more blood vessels are located [17]. Highly expressed genes

and microRNAs were found that had strong correlation with the physiology and growth characteristics of the velvet antlers [17]. 14 genes and 6 microRNAs were found to be candidates for DNA indicators for velvet antler weight after analyzing mRNA and microRNA of the velvet antler to show the regulation of the development of the components of antlers [17].

Whitetail deer have great economic value. Texas has the largest deer breeding and hunting industry of any state in the US, contributing 1.6 billion dollars annually [25]. Whitetail deer comprise 75% of Texas deer breeding operations and increased antler size is the focus of modern hunters and thus what many operations select for in farmed deer and managed herds [25]. Antler point restriction (APR) is a deer herd management technique that increases the proportion of older male white-tailed deer [13]. Surveys of hunters in Missouri after the implementation of APR showed there was no effect on how respondents viewed deer population trends, but satisfaction had correlation to areas of the state where there were moderate to heavy deer population densities and an excess of harvest rate opportunities [13]. Antlers are used as decoration or to make furniture, while some companies are using the antler velvet to make protein powder.

The research potential of antlers is vast because of the many unique properties. The most exceptional characteristic of antlers is that they are the only organ that completely regenerates. This annual regeneration can allow researchers to study morphogenesis, positional memory, and cancer because of the increased rate of cell replication [5].

Deer antler regeneration can also be used as a model to study scarless wound healing. The proteomes of the stem cells used for antler growth and regeneration and the cells obtained from facial periosteum, or the membrane that covers the outside of the facial bones, have been compared [10]. The epithelial-mesenchymal transition process, in which epithelial cells gain migratory properties through loss of cell adhesion and polarity, has been indicated to be an

important part of initiating antler regeneration and cell mobility has been shown to be highly regulated during this process, further supporting antlers as models for positional movement and cell migration [10].

Pilose antler extracts have been used in rat models for Parkinson's disease, a neurodegenerative disease most commonly found worldwide, and shown to inhibit 6-OHDA-induced neuronal cell death and tyrosine hydroxylase positive neuronal cells in the substantia nigra pars compacta [20]. Increased amounts of dopamine, 3,4-Dihydroxyphenylacetic acid, and 5-hydroxytryptamine were found after treatment with pilose antler extract while decreasing levels of glutamate and GABA [20]. 11 components of the pilose antler extracts have been found through proteomic analysis to possibly have neuropharmacological effects [20]. This is important because current treatments only mitigate motor dysfunction through dopamine therapy and there are no treatments to reverses neuronal cell death in the substantia nigra pars compacta, which leads to the largest loss of dopamine in Parkinson's disease [20]. A treatment that targeted this part of the disease could stop and reverse Parkinson's disease progression.

Antlers can provide more information on osteogenesis, osteoporosis, and the influence of hormones and nerves in bone growth [5]. The very specific stages of the reproductive cycle in these bucks can also help study the endocrine system [5].The identification of the genes that influence antlerogenesis can help further research and be used for medical and industrial uses. Finding these genes in whitetail deer can help improve the understanding of gene models and further genetic research of more beneficial genes in other species or understand genetic diseases in humans. Whitetail deer farming could be greatly improved by having the knowledge of the underlying genotypes that produce trophy bucks for avid hunters. These genes could be key for

understanding different bone diseases, such as osteoporosis, by learning how whitetail deer genes redirect minerals from the skeletal system for use in antlerogenesis.

CHAPTER II

METHODS

Genome Sequencing Methods

Blood samples were collected and antlers were removed during annual health checks of deer removed by management personnel annually from 1988-2008. This population has been under artificial selection for large and small antler size for 4-8 generations. The Boone and Crockett scores of these bucks and their corresponding age were recorded (Figure 2).

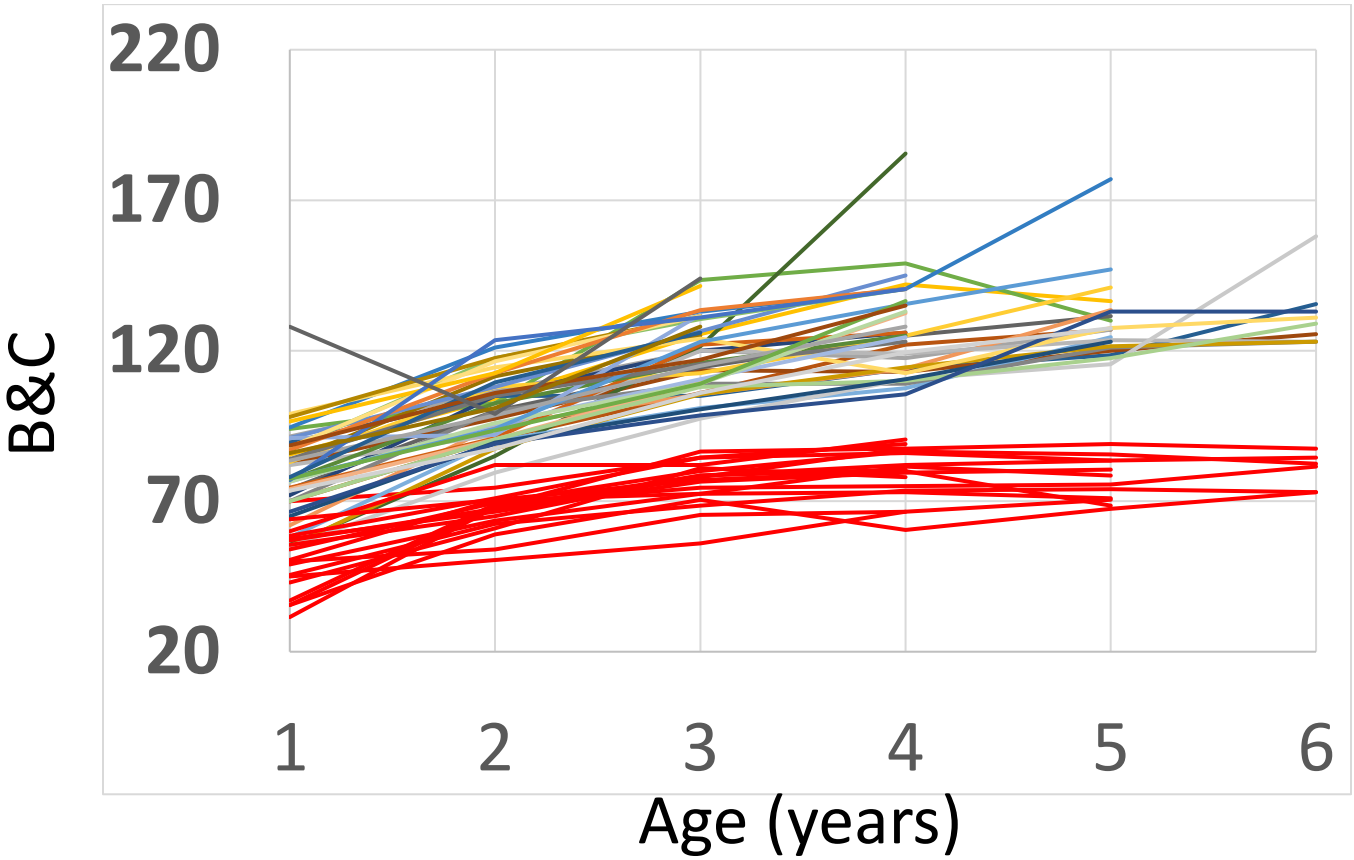

Figure 2: Annual Boone and Crockett measurements from a subset of our sampled population used to select individuals for genome sequencing. Red indicates candidates for “small antler” pools and the remainder for “large antler”

The first analysis was performed using DNA sequences from 20 individuals identified based on the maximum B&C scores and by choosing individuals with the most varied sire and dam combinations. From these individuals, equimolar DNA was combined into four pools of five unbarcoded individuals each, representing some of the extreme B&C scores from our initial measurements (Figure 3). Paired-end Illumina 150 base pair whole genome sequencing was obtained targeting 20x coverage for each pool. Sequence reads from the four pools of five individuals was aligned to the *Odocoileus virginianus* genome (GCA_002102435.1) using BWA[20]. Single nucleotide variants were called using CRISP[18] Weir's weighted F_{ST} was calculated using Popoolation2 in sliding windows of 25kb, with a step size of 1kb for the 10 largest B&C scores vs 10 smallest. F_{ST} values were z-transformed to identify outliers.

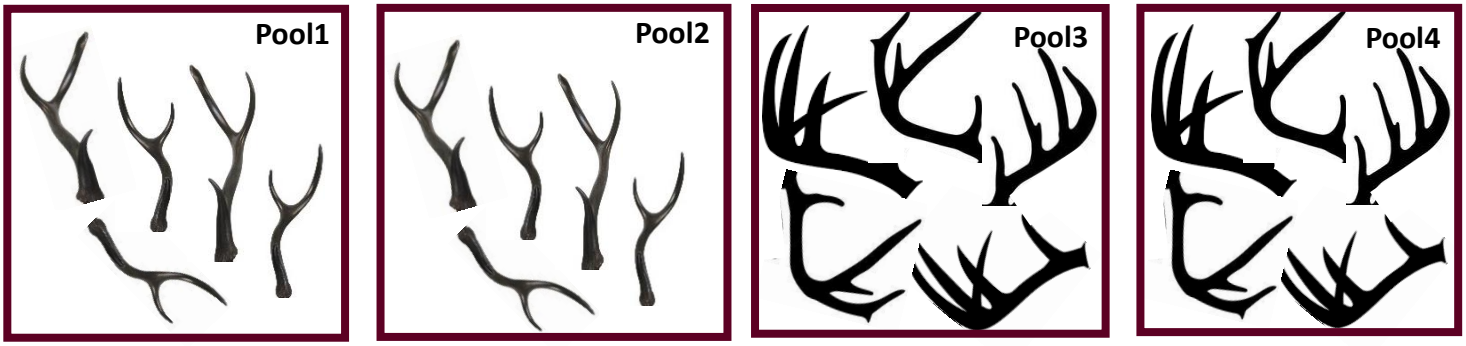

Figure 3: Pooling strategy for whole genome sequencing consists of four pools of 5 individuals each: Two 'small antler', and two 'large antler' pools by B&C.

The DNA necessary for whole genome sequencing was obtained by taking a sample of each individual's antler and using demineralization, purification, and extraction techniques to attain the desired product. The samples were obtained by using a high-speed drill to bore a small hole into the center of the shaft of the antler and the ground antler was collected.

200 mg of the osseous material from each individual was demineralized in 5.00 mL Centricon tubes with 2.5 mL demineralization buffer (0.5 M EDTA: 1 mg Proteinase K; 0.5% SDS). The solution was vortexed for one minute and then incubated at 42°C with gentle shaking for 48 hours.

DNA was extracted using standard phenol: chloroform: isoamyl alcohol methods. After 48 hours, 0.20 mL of Proteinase K was added to the solution, and the solution was vortexed and incubated for another 30 minutes. 2.50 mL of phenol: chloroform: isoamyl alcohol solution and 7.50 mL of TLE solution was added to the demineralized antler solution and then vortexed for one minute. Four gel separation tubes were centrifuged at 1300 rpm for one minute and then 1.60 mL of the vortexed solution was added to each gel tube. The gel tubes and solution were centrifuged at 1300 rpm for five minutes. The top layer of solution above the gel was then collected in a 15.0 mL centricon tube and the gel tubes were reused for the same individual to collect the remainder of the DNA within the solution. To the DNA collected, $1/10^{th}$ the volume of NaOH was added and twice the amount of 100% alcohol was added to a 15.0 mL centricon tube. The solution was vortexed for one minute and then distributed equally among 2.00 mL centrifuge tubes. The divided solution was then stored for 24 hours in a freezer at 4°C.

The samples were then centrifuged at 1600 rpm at 4°C for 30 minutes. The supernatant was decanted and then 0.30 mL of 70% alcohol was added to the remaining pellets in each tube, vortexed for 30 seconds, and then centrifuged at 1600 rpm at 4°C for 15 minutes. The

supernatant was disposed of and then 0.30 mL of 80% alcohol was added to the remaining pellet in each tube, vortexed for 30 seconds, and then centrifuged at 1600 rpm at 4°C for 15 minutes. This 80% alcohol wash was repeated, but no more alcohol solution was added and the pellets were allowed to dry. Once the pellets were dry, then 0.50 mL of TLE solution was added to each pellet in each centrifuge tube and vortexed to resuspend.

The vortexed solutions of the same individual were then added to one 50.0 mL Centricon tube with a filter and spun at 2000 rpm for 5 minutes. 3.00 mL of distilled water was added to each Centricon tube and then centrifuged again at 2000 rpm for 5 minutes. This wash was repeated once more. 0.30 mL of TLE solution was added to the collected filtrate in the middle compartment of the Centricon tube and then pipetted out into a 2.00 mL centrifuge tube. The solution was then evaluated using a Nanodrop to ensure presence and required amount of DNA. Quantification via Qbit and fragment analysis with Bioanalyzer showed high quality, with fragment sizes averaging 20kb, and quantity, with a minimum of 5 µg. Samples of each individual were sent out for whole genome sequencing and barcoding, with individuals of abnormally large antlers being pooled together and individuals of abnormally small antlers being pooled together.

3D Scanning Methods

3D scanning was performed using a commercial handheld scanner in full-color mode, and images were processed on a Dell Inspiron laptop using Solidworks. 3D scanning of antlers proved to be an effective way of measuring antler size. 3D scans were used to be able to find surface area and volume of the antlers to 0.01 mm^3 and 0.01 mm^2, respectively (Fig. 3).

CHAPTER III

RESULTS

Genome Sequencing Results

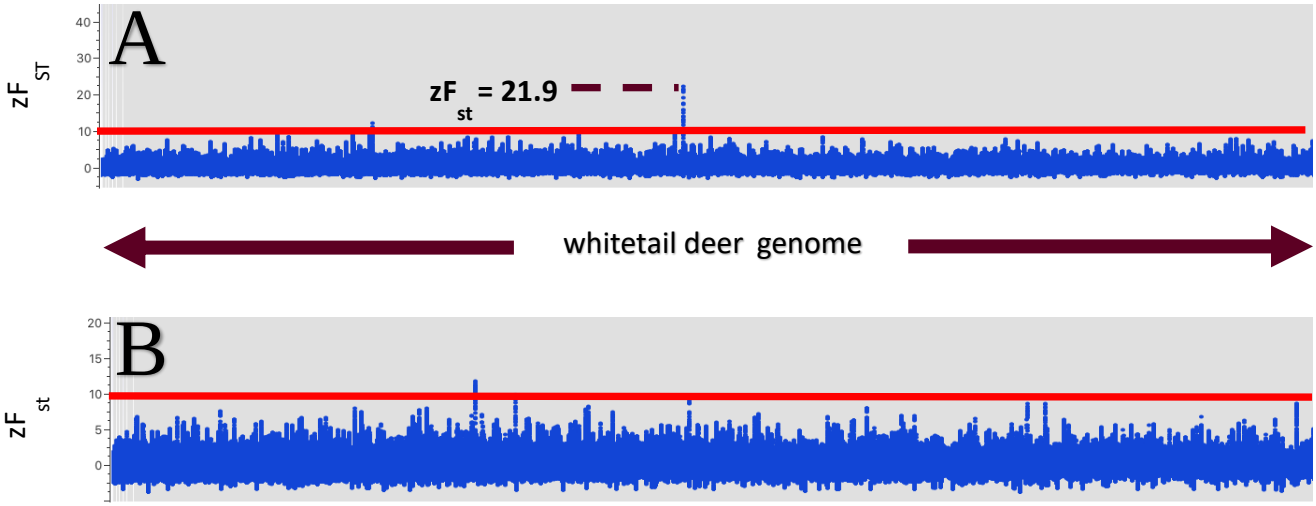

Figure 4: zF_{ST} values from sliding-window comparison of pooled sequence data for two selected populations: (A) 10 with small antlers, having a B&C score between 47 and 76.5, and (B) 10 with large antlers having a maximum B&C between 117.5 and 126.5.

There is an area of the genome with a zF_{st} of 12 and significant area of divergence within the genome of the small antler population with a zF_{st} score of 21.9 (Fig. 4A). There is also an area of the genome of the large population pool with a zF_{st} score of 13 (Fig. 4B).

3D Scanning Results

Figure 5: Examples of 3D scanning results. A) An abnormally large, palmated whitetail deer antler [courtesy of Dr. James Derr]. B) Left and right antlers from individual 98-94 with a maximum Boone and Crockett score of 132.5 at 4 years.

The 3D images produced by scanning are highly detailed and accurate to the antlers they represent (Fig. 5).

Table 1: Comparison of Max B&C with 3D scanning data: surface area and volume. R denotes right antler, L left antler. Highlights indicate samples with identical B&C, but divergent 3D scanning measurement.

Antler ID	Max BC	L Volume (mm3)	L Surface Area (mm2)	L V/SA	R Volume	R Surface Area	R V/SA
00-47 year 2006	129	511524.14	89171.74	5.74	546844.92	95142.19	5.75
00-70 year 2003	132	510894.29	91892.46	5.56	352532.90	63338.58	5.57
03-55 year 2008	147	869967.82	133826.28	6.50	925228.42	147938.68	6.25
04-42 year 2008	140.5	653285.77	105149.99	6.21	804645.18	128585.94	6.26
93-47 year 1996	185.5	337533.21	61102.51	5.52	364508.92	63624.55	5.73
94-07 year 2000	123.5	445617.02	78571.94	5.67	445436.18	77874.10	5.72
94-18 year 1999	156	593890.02	99829.47	5.95	322353.87	53347.95	6.04
94-58 year 1999	133.5	545227.84	90154.77	6.05	574965.87	93271.46	6.16
94-88 year 1999	124.5	357831.24	67395.02	5.31	324373.24	61652.77	5.26
96-17 year 2000	126	427933.81	79376.83	5.39	437900.64	80182.59	5.46
96-24 year 2001	131.5	541924.83	83469.22	6.49	605285.44	90843.29	6.66
96-38 year 2003	133	592190.45	90775.10	6.52	630121.08	97735.18	6.45
97-09 year 2001	125	617490.91	102255.48	6.04	661519.71	105743.93	6.26
97-88 year 2001	123	499174.18	82906.65	6.02	477334.23	78926.75	6.05
98-03 year 2001	125	383492.27	66201.57	5.79	410505.39	71248.36	5.76

3D scanning of antlers can be used to obtain the volume and the surface area of the antlers to the nearest 0.01 mm^3 and 0.01 mm^2, respectively. This is a viable alternative to traditional scoring methods and shows how the Boone and Crockett method can be inaccurate (Table 1). Antler ID 00-47 and 00-70 show one of these inaccuracies because 00-47 has a smaller Max BC than 00-70, but both the right and left antlers of 00-47 have larger volumes than the antlers of 00-70 and the R surface area of 00-47 is larger than that of 00-70 (Table 1).

CHAPTER IV

CONCLUSION

There are multiple areas of divergence between the genomes of 10 individuals with large antlers and 10 with extremely small antlers. These regions indicate high selection response within the population because such defined divergence was obtained over a short timeframe. This is not indicative of the entire whitetail deer species.

The zF_{st} scores indicate that the region with the most significant divergence is within the small antlered population.

A larger cohort of 60 individuals has been sequenced with individual barcodes and is being combined with these data for a more comprehensive view of this population. We expect much higher resolution for regions under selection for antler size to further explore the genomics of antler size within whitetail deer. Further comparison with separate populations bred for large antlers exclusively could confirm our findings.

The results of 3D scanning show how individuals with the same maximum B&C score have vastly different volumes and surface areas. 3D scanning will continue to be used as an alternative to traditional antler scoring methods. We are generating an algorithm that will incorporate the metrics obtained from scans to automate measurement and increase accuracy.

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