The influence of genetics on horn size in bighorn sheep

by

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Abstract

Headgear are cranial appendages usually seen in males of infraorder Pecora. This trait is used to determine hierarchy and gain access to mates. In bighorn sheep, males or rams have impressively large horns that are made of keratin and cancellous bone. These horns are used in yearly combats to determine access to females. The aim of this thesis was to better understand the role of genetics in horn size. This aim was addressed by reviewing the literature and analyzing horn data from National Bison Range in Montana, USA. I reviewed studies about intraspecific variation and functional genetics of headgear, which were then synthesized to make general conclusions and identify gaps in our knowledge of the genetics of headgear. The results of the literature review were antler and horn size are a heritable trait, heterozygosity-fitness correlations are hard to detect in both Cervids and Bovids, and several genes are associated with both antlers and horns. Based on the literature review, I was able to hypothesize that horn size is a heritable trait, horn size has a lot of genetic variation, and horn size should be affected by a heterozygosity-fitness correlation. These hypotheses were tested in my empirical study by using horn measurements for 114 rams and linear mixed effects models to test for the effects of genetic diversity on horn size. This study found that year 3 annuli length was highly heritable, heterozygosity explains some of the variation in horn size, and age or annuli number explains most of the variation found in horn size. At the end of the study, I found that horn size is a heritable trait. In addition, heterozygosity was a significant source of variation for horn length, horn mass, and annuli length on its own, but its role was diminished when age or annuli number was also in the model. Inbreeding coefficients on the other hand was an insignificant source of variation for any horn measurement looked at. This means a ram's yearly growth rate or age is a

better predictor of a ram's horn size than heterozygosity or inbreeding coefficients and there might be an HFC for longevity or survival. Both the literature review and the empirical study indicate that our understanding of intraspecific variation and the genetics of Pecoran headgear is limited. Some remaining questions are what is the quantitative genetic architecture of headgear size, do wild species have the same intraspecific variation or different intraspecific variation as domestic species, and does heterozygosity or the homozygosity evaluated by inbreeding coefficients affect horn size.

Preface

This thesis an original work by Miranda Reich. No part of this thesis had been previously published.

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I would like to thank, first and foremost, my advisor Dr. Dave Coltman for his support and guidance throughout my studies at University of Alberta. Without his commitment to this work and to me as a graduate student, this thesis would not have been possible. I would also like to thank the other member of my graduate committee, Dr. Corey Davis for all his insight he provided to this project. In addition, I would like to thank all the members of the Coltman lab for their assistance on this work. Finally, I would like to thank my family and friends for their encouragement and support as I pursued this thesis.

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Chapter 1: Introduction

Ruminantia is a large suborder within the order Artiodactyla with approximately 200 extant species. This suborder is divided into two infraorders, which are Pecora and Tragulina. The infraorder Tragulina has only one family, Tragulidae, while Pecora has five families, Bovidae, Cervidae, Giraffidae, Antilocapridae, and Moschidae (1, 2). All species within Pecora except for Moschidae have headgear, which is usually seen in males. Headgear evolved as weapons to be used in conflicts by males to gain access to mates. This trait is highly elaborate and sexually dimorphic as well as a sexually selected trait. Since headgear is a sexually selected trait, it is expected to have low additive genetic variation due to the fixation of advantageous alleles. However, there is quite a lot of intraspecific variation in headgear size as seen by antlers and horns having a heritable component. This is similar to what Prokuda and Roff found in their review of heritability of sexually selected traits and supports the lek paradox idea. In Prokuda and Roff's review, they found that heritabilities of sexually selected traits are not significantly different from those of nonsexually selected traits overall and suggests that sexually selected traits are subject to the same selection pressures as nonsexually selected traits (3). The lek paradox is the observation that persistent female choice for particular male trait values like antlers or horns should erode genetic variance in male traits and thereby remove the benefits of choice, but choice still persists (4). Since the variation in headgear can be related to sex, age, environment, and genetics, understanding the sources of this variation, why this variation persists, and the genetic architecture of sexually selected traits are all motivations to study Pecoran headgear.

Another motivation to study Pecoran headgear is to understand the implications of different management strategies. In domestic animals, researchers are mainly focused on determining what genes are associated with headgear to breed animals without headgear. As an example, three genes have been found to be associated with horns, which are RXFP2, HOXD, and MXT2. RXFP2 seems to be the gene that determines whether a male has horns or not (5, 6, 7, 8, 9). While the genes HOXD and MXT2 are associated with the polceraty trait or having multiple horns (10, 11). In wild species, both antlers and horns are prized as trophies and research has mostly focused on determining the impact of hunting on this trait. With cervids, hunters do select for antler size and could have a long-term negative impact on antler size (12, 13, 14). With

bovids, most studies on the effect of trophy hunting have been done on bighorn sheep (*Ovis canadensis*). All researchers have shown that hunting does have a negative impact on horn size over time (15, 16, 17, 18, 19, 20, 21). Since antler and horn size is a heritable trait, this could explain the impact of hunting (22, 23, 24, 25, 26, 27, 28, 29, 30, 31).

Further work needs to be done in wild species to fully understand the selective pressures, genetic architecture, and genetics of Pecoran headgear. At this moment, none of the genes associated with horns have been confirmed and most studies have been done at Ram Mountain, Alberta in bighorn sheep. Therefore, the purpose of this thesis is to review the intraspecific variation and functional genetics of headgear and analyze the genetic factors that influence horn size at National Bison Range. The literature review tells us that intraspecific variation of headgear size is heritable and is also affected by heterozygosity fitness correlations. The empirical study had the goal of testing whether intraspecific variation has a genetic basis. Both the literature review and the empirical study indicate that there is genetic variation and it partly explains the intraspecific variation found in headgear size. The literature review also suggests that many genes are known to play a role in headgear development and supports the idea that headgear has a complex, polygenic basis. Finally, this complex, polygenic basis helps explain the heritability of headgear size and headgear's response to selection and inbreeding.

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Chapter 2:
Intraspecific variation and genes associated with Pecoran headgear: A review

Abstract

The species within the infraorder Pecora have many fascinating features including cranial appendages called headgear. Headgear is usually seen in males only and is used to determine hierarchy and gain mating privileges in yearly intraspecific fights. Despite the vast amount of research that has been done on antlers and horns, there are still many gaps in our understanding of Pecoran headgear intraspecific variation and functional genetics. The purpose of this review is to highlight what is known about intraspecific variation and functional genetics of Pecoran headgear as well as what research still needs to be done. The conclusions about intraspecific variation are limited. For heritability, both antler and horn size are heritable traits. With heterozygosity-fitness correlations, they are hard to detect with antler size while they exist for horn size, but can be mediated by other factors. Overall, the research has been limited to a few species and this could limit our understanding of Pecoran headgear genetics. With Cervidae, we know that the expression of several genes is important during yearly antler regeneration including IHH, RUNX, and Nanog. In the genus *Ovis* of Bovidae, we know that the gene RXFP2 controls whether sheep have horns or not. One gap in our understanding is whether wild species in this infraorder have the same genes expressed in antlers or horns as domestic species. This gap could limit our understanding of the evolution of headgear traits and the impact of sexual selection and population dynamics on antler or horn size. Finally, I hypothesis that wild species in this infraorder do have the same genes expressed in antlers or horns as domestic species.

Introduction

The suborder Ruminantia is one of the four suborders within the order Artiodactyla and it is the largest group of ungulates with approximately 200 species. The six families within this suborder have traditionally been divided into two infraorders, Tragulina and Pecora (1, 2, Figure 1). In general, ruminants have fused cuboid and navicular bones in the tarsus, presence of an incisiform lower canine, reduction of the upper incisors, and a four-chamber stomach with Tragulina having a smaller rumen and less developed omasum than Pecora. In addition, Tragulina have an elongated astragulus with non-parallel sides while the Pecora have lost the trapezium and

possesses an astragalus with parallel sides (3). Finally, most males in Pecora have headgear that is used in yearly intraspecific fights by males to achieve social dominance status and gain mating opportunities.

The five families within Pecora are Moschidae, Antilocapridae, Giraffidae, Cervidae, and Bovidae. Moschidae is the only family where the males do not have headgear, instead both males and females have saberlike canines. The other defining characteristics of Moschidae are a closed metatarsal gully, a posterior tuberosity on the metatarsal, only one lacrimal orifice, and a gall bladder (3). In the early Miocene, Moschidae diverged from the Giraffidae and Antilocapridae at the same time as the Bovidae and Cervidae (1, Figure 1).

Antilocapridae has only one extant species (*Antilocapra americana*) and is endemic to North America. The distinguishing features of this family are hypsodont cheek teeth, elongated metapodials with loss of the side toes, and nondeciduous, unbranched cranial appendages covered by a deciduous forked keratin sheath that is shed every year called pronghorns (3, 4). Pronghorns have two centers of keratinization, are a direct outgrowth from the frontal bone and the structure is similar to horns (5).

Giraffidae has two genera and nine species that are defined by having elongated metapodials, complete loss of the side toes, a bilobed lower canine, and nondeciduous, unbranched, non-cartilaginous cranial appendages covered with skin called ossicones (3, 5). Ossicones arise as ossa cornua under the skin of the frontal and parietal region of the skull and they fuse to the frontal bones at sexual maturity (5, 6).

The family Cervidae has a near global distribution with approximately 20 genera, and 50 species (7, 8). All members of the family have two lacrimal orifices, lacrimal fossae, the first molar brachydont, and deciduous cranial appendages made of bone without a keratin cover called antlers (3). Antlers develop in the spring of an animal's second year of life as extensions from permanent bony frontal outgrowths known as pedicles and are shed and regrown every year (9). The development of antlers will be discussed further later in this review.

The largest family of ruminants are Bovidae. Bovidae contains approximately 140 species spilt across approximately 50 genera (10). Bovids can be found in Africa, Asia, Europe, and North America and the greatest diversity of bovids occurs in Africa. This family diverged from the cervids and giraffids in the early Miocene and all extant tribes were present by 7 million years ago (11, Figure 1). All male bovids have headgear made up of a sheath of keratin with a core of

cancellous bone called horns and females have horns as well in many species (12, 13, 14). The other distinguishing features are the third and fourth toes are combined into a common bone and the ulna and fibula are fused with the radius and tibia. Understanding the development of horns allows us to understand why horns seem to have a maximum size, and why do some individuals or lineages tend to grow the largest horns.

In Pecora, there are many gaps in our understanding of the genetics of headgear including what genes are associated with pronghorns and ossicones, how heritable are pronghorns and ossicones, what effect does the environment have on pronghorns and ossicones, do other cervids beyond red deer (*Cervus elaphus*), reindeer (*Rangifer tarandus*), and sika deer (*Cervus nippon*) have species specific gene associations, what genes are associated with horns in wild bovids, and what is the role of mothers on headgear size? For example, three studies have been done on the genetics of bighorn sheep (*Ovis canadensis*) horns. The results of these studies have not confirmed the previous conclusion that one gene controls horn type of sheep and sheep horn size (15, 16, 17). Therefore, the purpose of this review is to discuss the work that has been done to understand the genetics of headgear in cervids and bovids as well as to emphasize the areas where more research needs to be done. First, what is known about intraspecific variation will be investigated. Then the functional genetics of headgear size will be examined. Finally, we hope that this work is used as a resource to help inform future research and to identify candidate genes.

Intraspecific Variation

Intraspecific variation is the variation that occurs within a species and can be caused by genetic and environmental factors. One challenge of measuring it is separating plasticity and non-genetic sources from genetic sources of intraspecific variation. Understanding intraspecific variation is important because it can help predict the consequences of biodiversity loss. In regards to Pecorans, a better understanding of intraspecific variation helps us understand the genetics of headgear. The ways to overcome the challenges of measuring intraspecific variation are to estimate heritability or heterozygosity-fitness correlation (HFC). Heritability is a way to summarize the genetic basis of trait variation, but it is hard to measure in wild animals. Another way is to estimate intraspecific variation is an HFC, which is a way of identifying whether intraspecific variation is related to dominance genetic variance and affected by inbreeding. In addition, HFCs are a measure of the covariance between inbreeding and trait variation, but they

are typically inaccurate. HFCs are typically inaccurate because the correlation between a fitness trait and heterozygosity is weak, the markers used have null alleles, and very few markers are being used (18, 19). If there is heritability then it means there is additive genetic variance and multiple genes could be expressed during development that contribute to the variation in the trait. Finally, if there is an HFC than it means there is dominant genetic variance. In the next sections, I will discuss both concepts in detail as well as the studies that have been done in cervids and bovids.

Heritability

Heritability is a measure of the degree of variation in a phenotypic trait in a population that is due to additive genetic variation between individuals in a population. There are two types of heritability, which are broad sense and narrow sense. Broad sense heritability is the proportion of phenotypic variation due to genetic variation. Narrow sense heritability is the proportion of genetic variation that is due to additive genetic variation. Some ways to estimate heritability are to use an animal model, measure repeatability, use the breeder's equation, construct a genomic relationship matrix with a linear mixed model, or from a single parent regression. The underlying principle of all these methods is to compare the phenotypic similarity among individuals of known relationship to one another to quantify the genetic basis of trait variance. Heritability has been estimated in both cervids and bovids.

In cervids, heritability has been measured for several characteristics of antler size including mass, antler score, total number of points, main beam length, basal circumference, and inside spread. Antler score represents a sum of antler length, point lengths, circumferences, and inside spread usually measured in inches. In white-tailed deer, one study found that heritability was low (h^2=0.00-0.13) for incidence of spikes versus forks, number of points, maximum inside spread, total mass, beam length, and moderate (h^2=0.25) for beam circumference in 1.5-year-old males. With 2.5-year-old males, these measures of antler size were low to moderately heritable (h^2=0.08-0.39) (20). Another study in white-tailed deer found that characteristics of antler size were moderately to highly heritable (h^2=0.22-0.56) and 1.5-year-old antler size was only a moderate predictor of antler size at 3.5 years (21). The only other cervid that has been studied is a red deer population on Rum Island, Scotland. With red deer, antler mass of individuals 5 years or older was found to be heritable (h^2=0.35) (22). Based on these studies, antler size is heritable and

1.5-year-old antler size could predict antler size later in life for these populations. Also, these studies indicate that heritability can vary with age and environment.

In bovids, only bighorn sheep and Dall's sheep (*Ovis dalli dalli*) have had the heritability of horn size and body mass measured. With horn size and body mass, these two measurements have been shown to be heritable in the Ram Mountain, Alberta, Canada population of bighorn sheep (15, 23, 24, 25, 26). The other species that has had the heritability of horn size estimated was Dall's sheep. With Dall's sheep, horn length, base circumference, and horn volume were found to be moderately heritable and nongenetic effects like birth year and measurement year had a small but significant effect while permanent environmental effects were larger (27). Overall, for bovids, horn size is a heritable trait. In regards to marker-based estimates of heritability, there has been two studies using them in bovids. One study in bighorn sheep used 32 microsatellite loci and found that marker-based estimates to be imprecise and downwardly biased, which was disappointing to the researcher. In addition, marker-based inferences could be sufficient to detect broad trends in heritability among traits and when pedigrees cannot be generated the marker-based methods may provide conservative and useful estimates of quantitative genetic variation (24). The other study was done in thinhorn sheep using an OvineHD BeadChip, which has 606,606 markers originally designed for use in domestic sheep. This study did comment on the precision of marker-based estimates of heritability and indicates that maker-based estimates of heritability are accurate (27). These studies demonstrate that the number of markers could influence the precision of marker-based estimates of heritability. Therefore, marker-based estimates of heritability need to be studied more to determine the precision of them.

Finally, the effect of hunting as a selective pressure has been studied. In cervids, a study by Lukefahr and Jacobson does not support using yearling antler records as a criterion for selective breeding management or harvest schemes to alter the genetic quality of a white-tailed deer population because heritabilities for antler traits in yearling males were close to zero (20). This conclusion is supported by another study in white-tailed deer that found that hunter selection is unlikely to manipulate white-tailed deer antler characteristics at the population level because predicting future antler size is inefficient and the influence of parturition date and litter size on yearling antler is greater than the selection from hunting (21). For bighorn sheep, two studies have shown that hunting does exert selective pressure on horn size at Ram Mountain, Alberta, Canada (23, 26). The overall conclusions for the heritability of antler and horn size are it is

heritable, 1.5-year-old antler size could predict antler size later in life, the precision of marker-based estimates of heritability could be influenced by the number of markers, and the degree of heritability is variable. Finally, the heritability of headgear size can be estimated using both pedigree-based and marker-based estimates.

Heterozygosity-Fitness Correlations

HFCs are a way to describe the relationship between a fitness trait, which headgear size is being interpreted as, and heterozygosity. This correlation is one way to understand the sources of intraspecific variation as well as the underlying genetics of antlers or horns. However, HFCs are really a misnomer because most of the time the trait being studied is not fitness related. They have been used to detect inbreeding depression based on the assumption that genome-wide heterozygosity is a good proxy for inbreeding (28). The relationship between a fitness trait and heterozygosity could be from one of three hypotheses. First, HFCs could be due to the direct effect hypothesis. This hypothesis states that multilocus HFCs might result from selection acting directly on the marker loci and was important for early studies of HFCs (19; 29). Next is the local effect hypothesis. The local effect hypothesis states that apparent heterozygote advantage at markers is a result of heterozygosity at closely linked fitness loci. This hypothesis does require strong linkage disequilibrium, which is expected in recently bottlenecked and expanded populations (29). Finally, the general effect hypothesis states that apparent heterozygote advantage at markers is a result of effects of homozygosity at genome-wide distributed fitness loci. This hypothesis requires identity disequilibrium, which is generated by partial inbreeding (29). Finally, identity disequilibrium is the covariance in heterozygosity among markers within individuals, which should reflect identity by descent (19, 30). This correlation has been studied in cervids and bovids.

With cervids, HFCs are hard to detect and may be population specific. In Iberian red deer (*Cervus elaphus hispanicus*), Perez-Gonzalez et al found that males with very small antlers had lower levels of heterozygosity than those with larger antlers. The authors found heterozygosity at the marker set they used was weakly correlated with inbreeding and antler size in Iberian red deer, which could be explained by the local effect hypothesis. This reveals an expected but not previously detected association between low heterozygosity and reduced antler size (31). The other species that has been studied for HFCs in this family is white-tailed deer (*Odocoileus*

virginianus) but the conclusions in white-tailed deer do not support each other. One study from a white-tailed deer population from the Department of Energy's Savannah River found that main-beam diameter and length of antlers varied significantly among deer with different levels of heterozygosity and deer with higher levels of heterozygosity had greater asymptotic antler size, body mass, and fat levels (32). On the other hand, an isolated population of white-tailed deer in Finland did not have an association between heterozygosity and age-corrected antler score or with the development of antlers (33). Basically, in cervids, HFCs are hard to detect and may be population specific because of different environmental or physiological conditions for these species. The other reason an HFC might be population specific is a population may be isolated and experiencing the effects of inbreeding depression.

With bovids, HFCs have been detected in bighorn sheep and Alpine ibex (*Capra ibex*) and seem to indicate that horn growth is higher in more heterozygous bovids. In a bighorn sheep population from the Rocky Mountains in and near Wyoming, horn growth was significantly higher in more heterozygous than less heterozygous rams for years 6, 7, and 8 of life. Also, more heterozygous rams had 13% higher horn volume than less heterozygous rams by the end of year 8 (34). Finally, two studies in Alpine ibex from the Italian Alps found that standardized multilocus heterozygosity was positively related to body mass or horn growth, had a negative relationship with faecal egg counts of nematodes, and was age specific (35, 36). Basically, horn growth is higher in more heterozygous bovids and this relationship could be family wide not just species specific. In addition, the relationship between horn growth and heterozygosity can be mediated by other traits like age or faecal counts of nematode eggs.

Overall, Pecorans do not have a clear relationship between a headgear trait and heterozygosity, which could be due to different environmental or physiological conditions for each Pecoran species. In cervids, HFCs are hard to detect. On the other hand, HFCs exist for horn growth for bovids, but they can be mediated by other traits like age and faecal counts of nematode eggs. The fact that HFCs are hard to detect has also been described by Slate et al. In their study on Coopworth sheep, Slate et al found that multilocus heterozygosity was weakly correlated with inbreeding coefficients and heterozygosity was not positively correlated between markers more often than expected by chance. In addition, inbreeding coefficients calculated from markers, not multilocus heterozygosity, detected evidence of inbreeding depression for morphological traits (37). Finally, multilocus heterozygosity does not always provide a robust

estimate of genome-wide heterozygosity and may reflect heterozygosity at linked loci (30, 37). This is because genetic markers are a snapshot of an animal's genome and many markers are needed to get an accurate estimate of heterozygosity. Therefore, all the studies on HFCs indicate that HFCs should be used cautiously and in conjunction with studying inbreeding coefficients until heterozygosity can be accurately estimated from genomic data. In addition, the studies on the heritability of headgear size indicate that it is heritable and marker-based estimates of heritability could be influenced by the number of markers. Finally, the studies done on both heritability and HFCs suggest that the genetic architecture of headgear could be complex with many genes involved.

Functional Genetics

The aim of functional genetics is to define properties and functions of genes and gene variation. Understanding the functional genetics of headgear traits could provide insight into the evolution of these traits and why they have been maintained in this infraorder. In cervids, several genes have been found to play a role in the development of antlers and this means antlers are a polygenic trait. With bovids, a few genes have been found that determine horn size or type. Since both antlers and horns are polygenic, this polygenicity indicates that there is a lot of variation in the headgear trait and inbreeding and heritability could have an effect on this trait. In the next sections, the genes that are associated with both antlers and horns will be talked about and the research that has been done up to now will be explored.

Cervidae

In the family Cervidae, antlers are made up of bone and are regrown every year. Yearly antler regeneration is a spectacular example of stem-cell based regeneration. Therefore, it has been extensively studied to further our understanding of stem-cell regeneration for human health applications. Antler regeneration begins with casting of the previous year antlers, which is caused by a decrease of testosterone after the rutting season (9). After casting, the pedicle forms a scab and osteoclasts reabsorbs any bone on the pedicle. Within hours, the epidermis starts to migrate across the exposed surface of the pedicle bone, which is followed by some bone growth to restore the portion of the pedicle that was lost. By nine to ten days after casting, epithelialization has been completed and a number of growth zones can be distinguished in the pedicle. Below the

epidermis is a zone of proliferating mesenchymal cells called the mesenchyme and this zone contains a type of mesenchymal cells called chondrocytes (7, 38). Proximally to the zone of proliferating mesenchymal cells is where bone formation takes place (7). At the same time as bone formation, the integument is being regenerated. The integument in cervids is called velvet and it surrounds the antler shaft. During the first month after casting, antlers grow slowly, but during the next two months antler growth is very rapid and is the fastest growth described in the kingdom Mammalia (7). Antler growth is terminated by an increase in circulating testosterone that causes full mineralization of antler bone and shedding of the velvet. This termination exposes the bare bone of the antler, which is referred to as 'hard antlers' (9). At the end of the rutting season, testosterone levels drop off and the process of antler regeneration begins again. All of the research that has been done on antler regeneration and the genes associated with antlers has been done in the genera *Cervus* and *Rangifer* because these genera have species that are farmed.

Genus *Cervus*

Most of the research on what genes are associated with antlers have been done in either red deer or sika deer. In the mesenchyme, the genes melatonin (MLT), melatonin receptor I (MT1), insulin-like growth factor I (IGF-1), microRNA-18a (miR-18a), Sprouty homolog 1, insulin receptor substrate 1 and 2 (IRS 1/2), Runt-related transcription factor 1(RUNX1), and Sox9 are all expressed. A master transcription factor in the chondrocyte lineage and regulator of chondrocyte differentiation is Sox9. Sox9 will activate the genes Col2a1, ACAN, Col9a1, Col11a1, Hapln1, Sox6, and Wwp2, which act downstream of it to increase antler growth (38, Table 1). Another gene expressed in this cell type is IGF-1. IGF-1 could promote the proliferation and differentiation of these cells and acting downstream of it is another possible regulator, IRS1/2. IRS1/2 may regulate chondrocyte differentiation through RUNX1 and the crosstalk between IGF-1 and RUNX1 (39, Table 1). In addition, Yang et al found that MLT promotes mesenchymal cell proliferation, which is mediated by MT1 in sika deer. The signaling of these two genes promotes the expression of pro-proliferation genes and improves the expression of IGF-1 whereas the expression of cyclin-dependent kinase inhibitor and apoptosis-related genes were down-regulated (40, Table 1). Another study in sika deer found that the 3'-UTR of IGF-1 contains miR-18a target sites that are complementary to the mature miR-18a sequence. As IGF-1 promote the growth and differentiation of mesenchyme cells, miR-18a may

regulate the expression of IGF-1 and influence antler growth (41). In red deer antlers, IGF-1 and Trk tyrosine kinase receptor (TrkB) along with genes from the fibroblast growth factor (FGF) and Wnt signaling pathways have been shown to be expressed in the mesenchyme. One gene, Sprouty homolog 1, may restrain the effects of FGF/epidermal growth factor stimuli (42).

At same time as the mesenchyme is being regenerated, the velvet is also being regenerated. The only gene that has been shown to be expressed in the velvet is neurotrophin-3 (43). Unfortunately, no other studies have been done in other cervids to confirm this result or determine what other genes are expressed in the antler velvet. Towards the end of antler growth, chondrogenesis and ossification take place. Osteoblast cells act as a scaffold for where the bone should be placed and where collagen family genes, extracellular matrix proteins, adhesion molecules, matrix metalloproteinases, and signaling molecules are expressed (38). During ossification, several genes are highly expressed like bone morphogenetic protein 1 (Bmp-1), fibroblast growth factor receptor 3, and RUNX2. All genes either cleave substrates, induce bone and cartilage development, induce apoptosis in hypertrophic chondrocytes, or modulate the replacement of cartilage with bone (44, Table 1). One possible regulator of ossification is IGF-1 since it stimulates the proliferation of antlerogenic cells in all four ossification stages (7, Table 1). One gene associated with different cartilage tissues and possibly important is Trk tyrosine kinase receptor (TrkB). TrkB has two isoforms and the full-length isoform along with brain-derived neurotrophic factor (BDNF) are highly expressed in perichondrium, periosteum, and bone tissues. On the other hand, the truncated isoform was up-regulated in osteocartilaginous compartments and found more in the velvet and inner vascular dermis where it can inhibit BDNF signaling (45).

Since antler regeneration is a complex process, some genes are expressed in all tissues and stages of antler regeneration like Indian Hedgehog (IHH), transforming growth factor β1 (TGF-β1), and Nanog. IHH is highly expressed in chondrocytes, which is induced by IGF-1, and it acts as an inhibitor of chondrocyte differentiation as seen by a decrease in its expression whenever IRS1/2 is expressed (38, 39, Table 1). In addition, IHH is a regulator of antler ossification, chondrocyte proliferation, and osteoblast differentiation (45, 46, Table 1). With TGF-β1, it has been found to be expressed in the mesenchyme and cartilage and associated with chondrocyte, osteoblast, ossification, and the velvet (46, 47, Table 1). When this gene is knocked out, the Bmp signaling pathway may to take over this gene's role (38, Table 1). Nanog is expressed in antler stem cells (ASC) and the RNA expressed by ASCs is a pseudogenized form

of Nanog (Nanog-ps). In addition, Nanog enhances the proliferation of antlerogenic periosteal cells and antler tip reserve mesenchymal cells. When the sequence of Nanog-ps is compared with bovine Nanog, Wang et al found that two nucleotides after position 391 were missing, which causes a frame shift mutation and changed the expression of Nanog-ps in deer antlers. Nanog-ps expression is related to the degree of differentiation in stem cells during development and found in the nucleus of the ASCs, which is different from the expression of Nanog (48, Table 1).

Since most of the research on what genes are associated with antlers have been done in the tip where active antler growth occurs, Gu et al decided to study what genes are expressed in the different regions of the red deer antler by using the base of the antler as a standard to compare the tip, upper section of the antler, and mid-section of the antler to. They found when tip was compared with the base 84 genes were up regulated and 132 genes were down regulated, when the upper section was compared with the base 39 genes were up regulated and 72 genes were down regulated, and when the mid-section was compared with the base eight genes were up regulated and 31 genes were down regulated. Some of the up-regulated genes in the tip versus base comparison or the mid versus base comparison were connective tissue growth factor, heat shock factor binding protein 1, retinoic acid receptor responder 1, and splicing factor 3b. Some of the down-regulated genes in the tip versus base comparison, the upper versus base, or the mid versus base comparison were TGF β receptor II, IGF binding protein 7, WNT inhibitory factor 1, transmembrane 9 superfamily, RUNX1, and Retinoic acid receptor responder. An interesting result of this study was that each gene related to antler growth processes may have multiple roles. For example, genes involved in mediating the effects of retinoic acid were up-regulated in the mid-section versus the base comparison while they were down-regulated in the tip versus base comparison (49).

Genus *Rangifer*

There has been only one study done in this genus which looked at the gene expression differences in male and female antlers of reindeer. Zhai et al found that alpha 3 (VI) collagen gene (COL6A3) is differentially expressed in the mesenchyme of female and male reindeer. This gene forms a microfibrillar network associated with structural integrity and biomechanical properties and it contains several CpG dinucleotides in the consensus sequences for the binding of transcription factors. The methylation level of these sites in female antler mesenchyme is

significantly higher than male antler mesenchyme, which leads to a lower expression of COL6A3 in females. This could be a regulatory mechanism of antler growth in females since females have smaller antlers than males (50).

In the family Cervidae, more work should be done to understand the similarities and differences in what genes are associated with antlers in different species beyond red deer, sika deer, and reindeer. With red deer and sika deer, we know that eight major genes are associated with antlers. In addition, most of the research up to now has been focused on understanding antler regeneration as a way to progress stem-cell based regeneration research for human medicine. This focus assumes that all species in Cervidae have the same genes associated with antler regeneration. However, we know that reindeer has differential gene expression for a gene associated with antler regeneration. Therefore, we need to change our research focus to other species especially those of conservation concern because they could have unique gene expression of known genes or unique genes associated with antler regeneration.

Bovidae

In the family Bovidae, horns are made up of a sheath of keratin with a core of cancellous bone (12, 13, 14). The keratin molecules are held together by hydrogen-bonding and disulfide bridges, which gives horns rigidity and contributes to the insolubility of keratin (13). This rigidity and durability allow the horns to survive an animal's lifetime since horns are not shed and regrown annually like antlers or pronghorns. The main alternative phenotypes are scurs and polled. Scurs are small horn-like structures that are not attached to the skull whereas polled is being hornless, which is a desirable trait in domestic species. Polled is a desirable trait in domestic species because it reduces the harm the animals do to themselves, other animals, and the people caring for them. The species with most research done on what genes are associated with horns are found in the genera *Ovis*, *Bos*, and *Capra* where all the domestic species are found.

Genus *Ovis*

In sheep, three main genes have been found to be associated with horns. The most well studied gene is relaxin/insulin-like family peptide receptor 2 (RXFP2). The other two genes, the homeobox D gene cluster (HOXD) and metaxin 2 (MXT2), have been shown to be associated

with the phenomena of having multiple horns also known as polycerarty. Unfortunately, whether RXFP2, HOXD, and MXT2 are associated with bighorn sheep horns have not been well studied.

First, RXFP2 is an important gene that is necessary for proper testicular descent and bone mass. Expression of this gene is positively correlated with blood concentration of testosterone in mice (51). Mutations of this gene are associated with impaired testicular descent, reduced bone mass density, and osteoporosis in mice and humans (51, 52, 53). The expression of RXFP2 in sheep happens only in horn tissue and has been demonstrated to control horn type and size by several researchers (15, 16, 17, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71). Early work focused on trying to determine what gene(s) controlled horn morphology in sheep. At the same time as this research, researchers were trying to identify selection sweeps and what single nucleotide polymorphisms (SNPs) underlie different horn types. More recently research has moved towards identifying how does the polled phenotype happen and whether RXFP2 is associated with bighorn sheep horns.

The first study to determine where the horn locus is located was done in Merino sheep (*Ovis aries*). Montgomery et al examined the genetic markers from sheep chromosome OAR1 by creating large half-sib families to test whether the horn locus is the same locus as the polled locus in cattle. They found no evidence of linkage to markers from this region, but they did find linkage between the horn locus and markers from OAR10 (57). Pickering et al built upon the work of Montgomery et al by screening Montgomery et al's large half-sib families with microsatellite markers to localize a region of interest. Pickering et al was able to localize the region of interest to a 50kb region on OAR10 (58). At the same time as Pickering et al's work, another group was trying to fine map the genomic location of the horn locus in the Australian Merino sheep population. A linkage disequilibrium (LD) analysis of horn data mapped this locus to a SNP located at position 29.38Mb on Chromosome 10, which is close to RXFP2 and the eukaryotic translation elongation factor1 delta pseudogene3 genes (61). This same locus was mapped to about a 7.4cM interval on chromosome 10 in Soay sheep by Johnston et al. They also located a quantitative trait locus (QTL) spanning a 34cM interval with a peak centered near horn locus that explained the majority of the genetic variation for horn length and base circumference in normal-horned males (59). As an expansion of this work, Johnston et al did a genome-wide association study (GWAS) to determine the loci affecting discrete variation in horn type and QTL affecting quantitative variation in horn size. They found a single candidate gene, RXFP2, and

confirmed that the horn locus not only controls whether males has normal horns or not, but is also a QTL with major contribution to heritable variation in horn size in normal-horned males (60). Finally, Liu et al investigated the behavior of LD in Chinese Merino sheep using the Illumina Ovine SNP50 BeadChip. Overall, they found 46,062 SNPs distributed across all of the autosomal chromosomes and that LD was distinct for each population. Finally, they did detect the RXFP2 gene on Chromosome 10 and state that this was consistent with the breeding of hornless Merino sheep (68).

At the same time as researchers were determining where the horn locus is located, some researchers were looking at selection sweeps and the SNPs that underlie the different horn types. With New Zealand Arapawa sheep (*Ovis aries*), Pickering et al were investigating the population history and patterns of genetic diversity present in an isolated population of this breed. They found selective sweeps on Chromosomes 1, 2, 9, 10, and X including the region around RXFP2. This result suggested to them that there was natural selection for horns once sheep were introduced to Arapawa Island since the breeds used in the comparison were polled breeds (62). Kijas et al genotyped 49,034 SNPs in 74 sheep breeds with 10 polled breeds to understand the consequences of both domestication and selection. They found 31 regions with selection signals and these regions contained genes for coat pigmentation, skeletal morphology, body size, growth, and reproduction. A selection signal was detected on Chromosome 10 with the highest ranked SNP near RXFP2, which provides evidence that this gene has been under selection in polled breeds (63). This same selective signal has been found in Dall's sheep; European, Chinese, Brazilian, African, Caribbean, and Middle Eastern sheep breeds (65, 66, 67, 69, 71). In addition, certain SNPs in RXFP2 have been found to be associated with horn type. Wang et al used a candidate gene re-sequencing approach in Tan and Suffolk sheep breeds to find polymorphisms that could account for distinct horn phenotypes. One variant p.375, which was a coding variant located in a conserved region, was found to be a candidate variant underlying the appearance of horns in sheep (64). On the other hand, Pan et al found eight RXFP2-linked SNPs that seem to be associated with the spiral and horizontal extension horn type of the semi-feral Prairie Tibetan and Oula sheep breeds. These eight RXFP2-linked SNPs included two protein-altering SNPs and two intronic SNPs (70).

More recently research has moved towards identifying how does the polled phenotype happen? Wiedemar and Drogemullar amplified a 4'-kb region of the 3'-end of RXFP2 in horned

and polled sheep from seven Swiss sheep breeds. They found a 1.8-kb insertion in the 3'-UTR of RXFP2 in polled sheep that adds a potential antisense RNA sequence and is similar to a sequence on BTA12 (54). This same insertion was found in an additional thirteen sheep breeds and seems to be not the only cause of polledness, especially in sheep breeds with variable horn status (55, 56).

All the previous research on genetics of RXFP2 has been done on domestic breeds. The only wild species that has been studied in this regard is bighorn sheep. The first study was done by Poissant et al and they did a genome-wide scan for horn dimension and body mass QTLs using a microsatellite genetic linkage map. They found a QTL on chromosome 10 near the horn locus (16). This QTL is most likely RXFP2 based on previous work on mapping the horn locus. Next, Kardos et al. did whole-genome resequencing to explore patterns of genome wide diversity and to search for signatures of selection as indicted by regions with very low genetic variation relative to the genome wide mean. They found a signature of selection on Chromosome 10 located directly over RXFP2 that arose due to selection on horn size (17). Finally, Miller et al did a GWAS on horn length, horn base circumference, and body mass using the Ovine Infinum® HD SNP BeadChip to examine the genetic architecture of complex quantitative traits in bighorn sheep. With horn morphology, they did not find suggestive association between horn morphology and the region surrounding RXFP2 on chromosome 10 or the suggestive QTL for horn volume in this same region. Miller et al did state that they did not have sufficient marker coverage to adequately test for association in the horns region due to the number of samples used and the use of a cross species SNP chip (15). The work that has been done until now suggests that a functional RXFP2 gene is likely required for the expression of normal horns in sheep and mutations in the gene can affect horn size in Soay sheep. In bighorn sheep, there is evidence for a selective sweep and the failure to find associated polymorphisms in this region suggest that there are not mutations that have major effect on horn size in RXFP2. This further suggests that all bighorns have the allele for large horns. From other species in genus *Ovis*, we know that RXFP2 is required for horns which means any intraspecific variation may be caused by many genes of small effect.

Besides RXFP2, the association of HOXD and MXT2 with polyceraty has been studied. Polyceraty is having multiple horns as seen in four-horned Chinese sheep breeds. The HOXD gene cluster belongs to the homeobox family of genes that encode a highly conserved family of

transcription factors that specify differences in morphogenesis in all multicellular organisms (72, 73, 74, 75, 76, 77). MTX2 is a protein that is located on the cytosolic face of the mitochondrial outer membrane and works with metaxin 1 to import mitochondrial preproteins into mammalian mitochondria (78). Several GWASs have been done in multi-horned sheep breeds. All of these studies have shown that there is a region spanning positions 126-134 Mb on Chromosome 2 associated with the polyceraty trait. This region contains the HOXD gene cluster and the MXT2 gene (79, 80, 81, 82). In addition, He et al tried to determine if the previously found 1.8kb insertion associated with polled sheep existed in four-horned Chinese breeds. Unfortunately, they failed to verify the association with polledness in these sheep breeds (80). Overall for genus *Ovis*, there are three genes demonstrably associated with horns and limited research on wild species that needs to be expanded.

Genus *Bos*

Bos is a large genus that has many important species to humans like cattle and yak. Most of the work in this genus focused on determining what genes are associated with polledness and where are the genes located in the genome. Early work focused on determining where the POLLED locus is located in the cow genome using microsatellites. Georges et al showed linkage between two microsatellite markers and the POLLED locus and mapped this linkage group to the centromeric region of chromosome 1 (83). The location of the POLLED locus was confirmed in the Charolais breed, a *Bos indicus* x *Bos taurus* cross, the Simmental breed, and the Pinzgauer breed (84, 85, 86). Brenneman et al's work provides evidence that the POLLED locus is proximal to the centromere, which is important for fine mapping the location of this trait on chromosome 1 (85). In addition, Schmutz et al suggests that the genes collagen 6A1 and 6A2 are associated with the polled trait in cattle (84).

After the POLLED locus was determined to be on chromosome 1, research turned to fine mapping the location of the locus and what SNPs or genes are in the region. In Nelore cattle, a GWAS was done to identify the genomic region causing polledness. The researchers found a 3.11 Mb centromeric region on chromosome 1, which contains 28 protein-coding genes associated with polledness (87). However, earlier work by Drogemuller et al fine mapped the polled trait in six German cattle breeds to a 1-Mb region on chromosome 1 that contains thirteen genes (88). This work helped researchers to start identifying possible causal SNPs and candidate genes. Two

different case-control association mapping studies narrowed down the location of the polled trait and started to identify the genes associated with polledness in cattle. The first case-control association mapping study was done with eleven different cattle breeds using genome-wide SNP genotyping with the Illumina BovineSNP50 BeadChip. The researchers found a significant signal on the centromeric end of chromosome 1 and the most likely location of the POLLED locus was a 381-kb region that was homozygous in all polled animals. This region has one core haplotype with nine neighboring SNPs. In nine breeds, the region has three protein coding genes and two pseudogenes (89). A second study genotyped 31 European cattle breeds using the Illumina BovineSNP50 BeadChip. They found a single complex 202-bp insertion-deletion event with perfect association with polledness in European cattle breeds except for the Holstein-Friesian breed. In the Holstein-Friesian breed, they found five candidate variants that segregate as a 260-kb haplotype that is perfectly associated with polledness. This haplotype does not recombine or interfere with the 202-bp insertion-deletion (90). Rothammer et al refined the 260-kb haplotype found in the Holstein-Friesian breed to an 80-kb duplication called P_{80kbID} as the most likely casual mutation for polledness in this breed. This mutation is not in any known coding region (91). Finally, Wiedemar et al confirmed the presence of two different polled alleles in 14 different cattle breeds using high-density SNP genotyping. In addition, they found that the genes FOXL2 and RXFP2 were down-regulated in polled horn buds whereas C1H21orf62, a protein coding gene of unknown function, to be up-regulated in polled horn buds. The fact that FOXL2 and RXFP2 are involved in the development of horns in goats and sheep suggested to the researchers that these two genes may have the same function in cattle (92).

Another chromosome closely associated with a particular horn type is bovine chromosome 19. This chromosome has been shown to be associated with scurs. In the Charolais, Angus, Limousin, Simmental, Hereford, and Belgian Blue cattle breeds, the genes closest to the scurs locus are ALOX12 and MFAP4, which could be associated with the scurs locus (93). However, a later GWAS in the Simmental breed found a SNP closer to the scurs locus than the genes ALOX12 and MFAP4 are (94). Therefore, the gene(s) associated with scurs in cattle is still unknown.

The other species that has been studied in the genus *Bos* is yak (*Bos grunniens*). Yak display a similar genetic pattern as cattle in regards to what chromosome is associated with polledness. In Datong domestic yak, a haplotype analysis shows that a 147-kb segment on

chromosome 1 is the most likely location of the POLL mutation. This region contains the genes C1H21orf62, GCFC1, and SYNJ1 (95). Another GWAS in Datong domestic yaks found that the POLLED locus in a 200-kb region on Chromosome 1 that contains the genes SYNJ1, PAXB1, and C1H21orf62 (96). Based on these two studies, the genes most likely associated with polledness are SYNJ1 and C1H21orf62. During horn bud development of polled horns, 29 proteins were up-regulated and 71 proteins were down-regulated. The top down-regulated proteins were keratin type II cytoskeletal 6B, keratin type I cytoskeletal 17, collagen alpha-1 chain, and desomoplakin while the top up-regulated proteins were serine/threonine-protein kinase and polypeptide N-acetylgalactosaminyltransferase 16 (97). For genus *Bos*, chromosome 1 is associated with the POLLED locus and the genes FOXL2 and RXFP2 are most likely to be involved in the development of cattle horns. In addition, Chromosome 19 is associated with the scurs phenotype and yak have a similar genetic pattern as cattle in regards to what chromosome is associated with polledness. Finally, the research in this genus needs to be expanded to other wild species beyond yak to confirm what chromosomes are associated with cattle horns.

Genus *Capra*

In the genus *Capra*, most of the studies have focused on understanding the causes of the polled/intersex syndrome (PIS). PIS is a phenomenon in goats (*Capra aegargrus*) that links the polled trait to the intersex syndrome. This linkage was first discovered when comparative mapping of the goat and cow chromosome 1 showed that PIS was linked to four microsatellites at the distal end of goat chromosome 1 (98). Additional work found a 11.7 kb deletion on chromosome 1 near the genes PISRT1 and FOXL2, which contains a critical region of repetitive sequences and affects the transcription of these genes, as the cause of PIS in European goats (99). This same region was identified as being associated with PIS in non-European goats. However, the inheritance of PIS is more complex once a haplotype analysis was done. The haplotype analysis revealed that some animals have a horn haplotype and are not homozygous for poll. There are three possible explanations for this observation. First, a historic recombination event occurred to generate a haplotype that appears to associate with horns but carries the 11-kb PIS deletion. Second, a karyotypic chimerism that is unrelated to the PIS locus exists. Third, the presence of a different sized deletion that causes PIS in these animals. The authors think the second and third explanation are mostly likely explanations for why some animals have a horn

haplotype and are not homozygous for poll, but requires future analyses to determine which one. Finally, this study does indicate that the basis for the intersex condition is more complex than the inheritance of two 11-kb PIS deletions carrying chromosomes (100). For genus *Capra*, the research on their horns needs to be expanded to wild species, determining what genes are associated with horns, what genes are associated with the scurs phenotype, and determining whether this genus has the scurs phenotype.

In the family Bovidae, all the research on the genes associated with horns has been done on domestic species. The only gene that seems to have documented effects on horn development across the family is RXFP2. In sheep, this gene does have different variants as seen in Soay sheep that affect horn development and are associated with horn size in rams. In cattle, RXFP2 is expressed in the female reproductive system and maybe involved in horn development (92, 101, 102). With goats, the gene is instead expressed in the cauda epididymis and vas deferens of the male reproductive system (103). The research into RXFP2's importance in horn development is still being studied in bighorn sheep. Finally, the alleles of RXFP2 may play a co-operative role with other genes in a polygenic model based on previous studies done on heritability and HFCs, which suggest that the genetic architecture of horns could be complex with many genes involved.

Conclusion

In the infraorder Pecora, headgear have evolved as weapons to determine hierarchy and gain mating privileges in yearly interspecific fights. Most of the previous research on the intraspecific variation of the trait and what genes are associated with this trait has been done in the families Cervidae and Bovidae, specifically their domestic species. The previous research suggests on intraspecific variation suggests that headgear size is heritable and therefore has an additive genetic basis. Also, HFCs have been detected in some species and suggest genetic effects due to directional dominance and the potential for inbreeding effects. In addition, a number of genes have been identified that play a role in headgear development. However, it is not clear whether their significance is conserved across taxa. The previous research does suggest that horn development is genetically complex and is also likely to be influenced by many genes. The genes identified in this review could serve as a list of candidate genes for future studies and the polymorphisms related to these genes could affect intraspecific variation. There is some

evidence for genes of large effect on intraspecific variation in some species that specify discrete variation like polled, scurs, and polyceraty. Based on the amount of variation in horns and the list of genes associated with horns, horns could be a polygenic trait. The goal of the next chapter is to begin to answer questions about intraspecific variation of bighorn sheep horns including how does heterozygosity or inbreeding coefficients effect horn size, what are the genetic sources of variation that influence horn size, and are there any nongenetic factors that influence horn size.

Figures and Tables

Table 1: Summary of the function of the most important genes or gene families in regenerating antler tissue

Gene	Abbreviation	Function	Reference (s)
Indian Hedgehog	IHH	Regulator of ossification and inhibitor of chondrocyte differentiation	38, 44, 46
Runt-related transcription factors	RUNX	Expressed during osteoblast and chondrocyte differentiation	39, 44
insulin-like growth factor I	IGF-1	Promotes the proliferation and differentiation of antler chondrocytes, and induces the expression of IHH	7, 39, 40
Nanog	N/A	Maintains stem cell pluripotency and pseudogene expression matches the differentiation degree of the antler stem cells	48
transforming growth factor β1	TGF-β1	Crucial regulatory factor in antler cartilage cells, and localized in skin, perichondrium, mesenchyme, and differentiated chondrocyte	46, 47

Sox9	N/A	Master transcription factor in the chondrocyte lineage; controls rapid antler growth and regeneration	38
insulin receptor substrate 1 and 2	IRS1/2	Regulates chondrocyte differentiation, inhibits differentiation of antler chondrocytes through IHH, and has an important role in crosstalk between IGF1 and RUNX1	39
bone morphogenetic proteins	Bmp	Induces bone and cartilage development	44

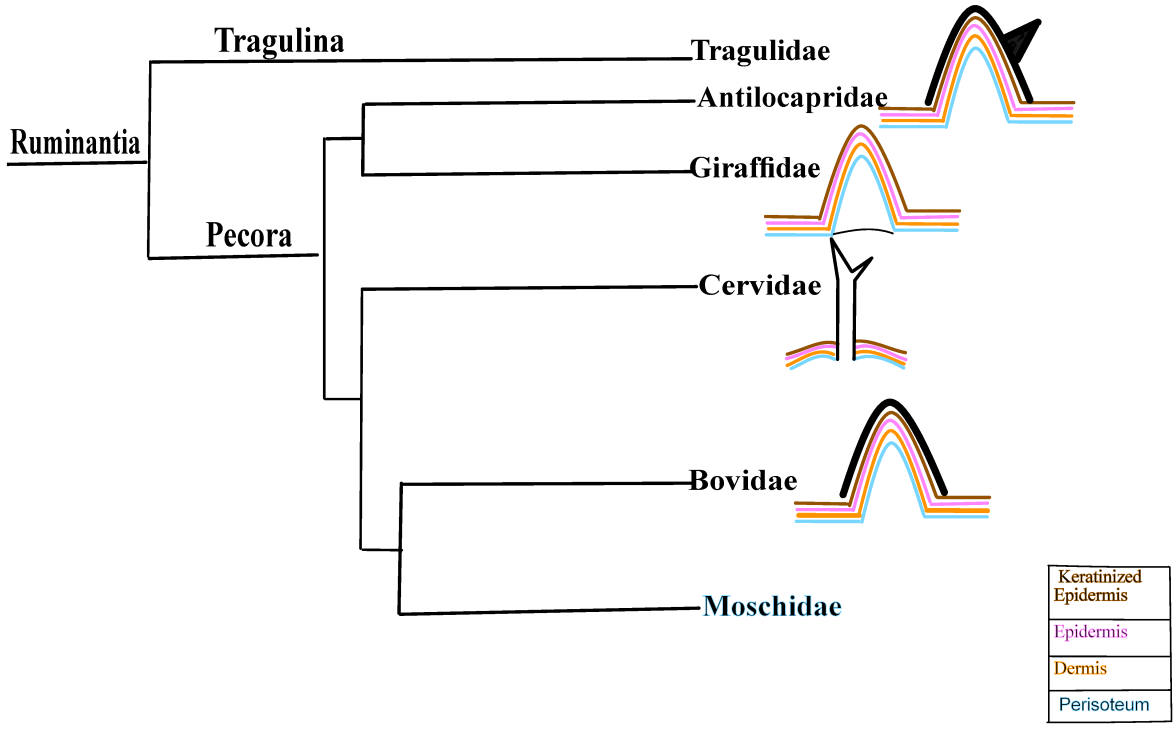

Figure 1: Phylogenetic tree of infraorder Pecora. Reproduced from Hassanin and Douzery, 2003 and Fernandez and Vrba, 2005 with schematic diagrams of the different headgear found in the infraorder reproduced from Davis et al, 2011.

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Chapter 3:
The genetic factors that influence bighorn sheep ram horn size

Abstract

Bighorn sheep rams are known for their impressively large horns that are used in yearly intrasexual contests to determine dominance and access to females. This trait has been studied at Ram Mountain, Alberta to understand the genetic architecture of, and the selective pressure of hunting, on horn size. In addition, horn size has been shown to be heritable in other populations of wild sheep. The purpose of this study was to identify genetic variation for horn size in a non-hunted population, National Bison Range. The genetic variation of horn size was identified by analyzing horn measurements for 114 rams to estimate heritability and using linear mixed effects modeling to test for HFC/inbreeding effects on horn size. This study found that horn length, horn mass, and annuli length to be heritable with year 3 annuli length being highly heritable. Also, heterozygosity on its own was a significant contributor to horn size, but it had a diminished role when age or annuli number was included in the model. In the end, the study confirmed the conclusion that horn size is heritable and indicates that age is more important than genetics in determining horn size.

Introduction

Horns are impressively large structure made of a sheath of keratin with a core of cancellous bone that are grown throughout an animal's lifetime (1, 2, 3). In bighorn sheep, whose range extends from Alaska to northern Mexico, horns are found in both males, or rams, and females, or ewes (4, 5). Rams use horns in intrasexual contests to determine dominance (6, 7). In addition to being weapons, horns can be used as a shield and a shock absorber during these fights (3, 8). On the other hand, ewes have smaller horns than rams, which are used to protect young. Ram horns are a fascinating feature that has been studied by researchers and early growth has been shown to affect reproductive success (9, 10).

The purpose of this study was to identify or characterize the genetic influences on horn size of a non-hunted population at National Bison Range (NBR). This was done by estimating heritability and testing for an HFC/inbreeding effect on horn size. The heritability estimation tests for and measures additive genetic variance while the HFC/inbreeding effect test provides

evidence for dominance genetic variance for horns. NBR is a National Wildlife Refuge located in northwestern Montana, USA. Bighorn sheep were established by transplant of four rams and eight ewes from Banff National Park in 1922. Two of the rams were offspring of transplanted ewes and most ewes were carrying fetuses conceived in Banff during the autumn of 1921. Individual monitoring started in 1979 with genetic sampling beginning in 1988. From 1922 until 1985, this population remained isolated by distance and human development. Beginning in 1985, NBR experienced a 'genetic rescue' via intentional translocation of fifteen individuals from neighboring populations to prevent local extinction after years of isolation and inbreeding. Since the introduction, there has been an increase in both census size and genetic diversity (11, 12). At NBR, the mating tactics of bighorn sheep and the use of them have been the main focus of study (7, 8, 13, 14). This makes NBR a good location to study the genetics of horns since it is a non-hunted population and the genetics of horn size has not been studied yet.

Since horn size is heritable in other populations and evidently polygenic as seen in the previous chapter, horn size is expected to be heritable in the NBR bighorn sheep population. Due to the genetic rescue at NBR, NBR is admixed and expected to be genetically diverse. Therefore, it is expected to have high additive genetic variance and heritability for horn size. In addition, NBR has never been hunted and this may contribute to having a higher genetic variance for horns than hunted populations like Ram Mountain, Alberta. Based on NBR's history of inbreeding, if an HFC or inbreeding effects are detected then it would most likely be due to the general effect.

Methods

National Bison Range Background

NBR is a 7500-hectare National Wildlife Refuge located in northwestern Montana, USA. The terrain is Rocky Mountain foothill that supports vegetation composed primarily of bunchgrass-dominated grasslands, patches of coniferous forest, and stingers of riparian shrub and woodland. Bighorn sheep were established by transplant of four rams and eight ewes from Banff National Park in 1922. Individual monitoring at this location started in 1979, with genetic sampling beginning in 1988. From 1922 until 1985 this population remained isolated by distance and human development. Beginning in 1985, NBR experienced a 'genetic rescue' via intentional translocation of fifteen individuals from neighboring populations to prevent local extinction after years of isolation and inbreeding. Prior to the introduction, census size and growth rate had been

steadily declining. Following the supplementation there has been an increase in both census size and genetic diversity (11, 12).

Horn Measurements

114 rams had their base circumference, horn length, horn mass, the circumference of each annulus, and annulus length measured once post-mortem. The base circumference of a horn is measured by wrapping a measuring tape around the widest part of the horn that is closest to the skull. Then horn length was measured along the outside edge of the horn while the length of each annulus is the length between each increment. Horns grow from the base of the skull and annuli are counted from the tip of the horn. So, the annulus closest to skull is the newest one to be grown and is similar to a growth ring of a tree. The circumference of each annulus was measured similarly to the base circumference of the horn. Since yearly horn growth slows down as a ram ages, only annulus length until age eight were analyzed. Finally, all horns were weighed to get horn mass. For all analyses in this thesis, only horn length, horn mass, and annuli lengths were analyzed because these horn measurements are the main measurements of horn growth. Since NBR is isolated, observed heterozygosity (H_o), standardized H_o, and inbreeding coefficients were studied to determine whether they have an effect on a ram's horn measurements. Observed and standardized H_o was calculated from the microsatellite genotypes of 93 rams while inbreeding coefficients was calculated directly from the pedigree that was constructed from parentage analyses run on microsatellite genotyped sheep using CERVUS (15). The pedigree is incomplete with nine generations of information that starts in 1980.

Microsatellite Genotyping

Rams were microsatellite genotyped at 28 loci following lab protocols (11, 16). First DNA was extracted from all rams using Qiagen DNeasy 96 Blood and Tissue kits, following their protocol for DNA extraction. Then PCR master mixes were made for the 25 loci that are end labeled and separate PCR master mixes were made for the three loci that are M-13 labeled. Afterwards the loci were multiplexed into seven groups of two to four loci each with six loci run as solos with group specific dilutions and PCR programs (11, 16, Table 1). Finally, genotypes were typed using GeneMapper Version 4.1 (17). After microsatellite genotyping was completed, the genotypes were checked for errors by identifying mismatched alleles and null alleles. Finally,

summary statistics were generated using GenAlEx Version 6.503 (18, 19). The average p-value for the average F_{IS} was calculated using Fisher's rule and the degrees of freedom for this summary statistic was determined by multiplying the number of microsatellite loci by two. Then a parentage analysis was run in CERVUS Version 2.0 to build the pedigree using the 95% confidence interval and the microsatellite loci to determine parentage (15, 20). The likelihoods and critical values for the delta test statistic for the parentage analysis were computed from year-specific data using rams 1.5 years and older as potential fathers while assuming a 0.01 genotype error rate and using 10,000 simulated paternity tests to establish critical values for the delta statistic (16).

Observed and Standardized H_o calculation

Observed H_o for each ram was calculated as the fraction of heterozygous loci typed for each individual. The expected H_o for each ram was calculated as the average observed H_o for each locus that ram was typed at. Finally, standardized H_o was calculated by dividing each ram's observed H_o by his expected observed H_o. This calculation accounts for bias due to missing genotype data.

Data Exploration

Data exploration was completed to get a sense of variation in the horn measurements, observed H_o, standardized H_o, and inbreeding coefficients as well as identify any outlier measurements that could create biases in the analyses. Summary statistics for the horn measurements, observed H_o, and standardized H_o were generated in Excel Version 16.50 using the Analysis Tool Pak. Correlation coefficients were calculated by using the "PEARSON" function in Excel between the horn measurements, observed H_o, standardized H_o, inbreeding coefficients, and age. Then the horn measurements, observed Ho, standardized H_o, inbreeding coefficients, age, and birthyear were plotted using the packages ggplot2 Version 3.3.3 and patchwork Version 1.1.1 in R Version, 1.3.959 (21, 22, 23). Histograms were made for each horn measurement to identify any outlier data points (Appendix A). Then scatter plots were made to help inform the modeling (Appendix A). Finally, any outlier horn measurements and rams who died at two years old were removed before modeling was done. The outlier horn measurements were identified as any measurement far from most of the measurements and causing that horn

measurement not to have a normal distribution. Rams who died at two years old were removed because their horn lengths were creating skewness in the distribution for horn length.

Mixed Effects and Repeated Measures Modeling Setup

After data exploration was completed, modeling was done in R using the lme4 Version 1.1-26 and lmerTest Version 3.1-3 packages (24, 25). The package "lme4" was used to build and run linear mixed effect models while the "lmerTest" package was used to get p-values for the models built in "lme4". For all modeling, csv versions of Excel spreadsheets were read into R and checked for any errors. Then the horn measurements, genetic parameters, and non-genetic parameters were made into their own variables. The horn measurements examined for linear mixed effects models were horn length, horn mass, and annuli lengths. The genetic parameters examined were observed H_o, standardized H_o, and inbreeding coefficients. The non-genetic parameters examined were age, annuli number, birthyear, and ID. Maternal effects were not examined in this thesis, which is why maternal ID was not included as a non-genetic parameter. Age is the age at which a ram died and all rams that died at 15, 16, or 17 years old were pooled together as one variable called 15. An annulus represents growth at a particular age corresponding to the annulus number and the annulus number was only used in the repeated measures modeling. Birthyear is just the year that a ram was born in. Finally, ID is the alphanumeric identification for each ram. All of the non-genetic parameters were fitted as factors. Since the rams have variable depth in the pedigree and this makes it hard to make conclusions about the effect of inbreeding on horn size or to compare the effect of H_o to the effect of inbreeding, the data was subset to just the rams with all four grandparents in the pedigree as the final data preparation step. The rams with all four grandparents in the pedigree did have variation in inbreeding coefficients, but g-statistics were not calculated. Finally, annuli lengths were modeled as individual measurements and as one variable using repeated measures modeling.

Non-genetic Modeling

To determine the best structure for the genetic models, non-genetic models were built first for all horn measurements. This modelling determines what non-genetic parameters has the most influence on horn size at NBR. Non-genetic models were fitted between a horn measurement and age, birthyear, or both age and birthyear as fixed effects as well as the interaction between

predictors. The other models looked at had age or birthyear as a random effect while the other non-genetic variables were a fixed effect. After determining the best model structure for the individual horn measurements, annuli lengths were modeled and included ID as a random effect in addition to age, birthyear, and annuli number. The best non-genetic model structure for both the horn measurements and annuli lengths were determined by examining both the biological significance and lowest Akaike Information Criterion (AIC) of the model (Appendix C).

Heritability Calculation

After non-genetic modeling was completed, heritability was estimated using an animal model by first adding any rams or founders that do not appear in the animal column of the NBR pedigree using the Spyder Python Version 3.3.6 wrapper (26, Appendix B). Then the pedigree with added founders was read into R and sorted using the "orderPed" function from the package MasterBayes Version 2.57 (27, Appendix B). After the pedigree was prepared, the MCMCglmm Version 2.32 package was used to set priors, build models, and calculate heritability. The prior distribution of variances had an inverse-Gamma distribution with a nu= 0.002 and a V= 1 (28, Appendix B). Then each horn measurement had heritability estimated using the MCMCglmm function and the model structure with a horn measurement as the response variable, age (as a factor) as the only fixed effect and animal as a random effect. With the repeated measures model, ID was added as an additional random effect and the prior distribution had an additional g-structure included. All models used the Gaussian family and run with 100,000 iterations, 10,000 iterations dropped at the beginning, and 10 iterations stored in memory. Afterwards, narrow sense heritability was calculated by dividing the additive genetic variance (V_A) by the sum of V_A plus the residual variance (Appendix B).

Mixed Effects and Repeated Measures Modeling

Once heritability was estimated for each horn measurement, genetic modeling was done next. The genetic modeling used the best non-genetic model for each horn measurement with observed H_o, standardized H_o, and inbreeding coefficients as the genetic parameters to be fitted. The modeling was first done using restricted maximum likelihood (REML) to begin determining the effect of the genetic and non-genetic parameters on horn size (Appendix C). Then models were estimated by maximum likelihood and compared using ANOVAs to determine if there was

a significant difference between the model where age is added to the genetic parameter or interacting with the genetic parameter (Appendix C). Finally, individual horn measurements were modeled using general linear models (GLM) with the genetic parameters and age as fixed effects. With the GLMs, the family used was Gaussian with the identity link.

Finally, annuli lengths were modeled as repeated measures modeling using the best non-genetic model structure with observed H_o, standardized H_o, and inbreeding coefficients as the genetic parameters to be fitted. The modeling was first done using REML and then maximum likelihood was used. The models for REML were compared using AIC and the models for maximum likelihood were compared using ANOVAs (Appendix C).

Results

Microsatellite genotyping

After checking the microsatellite genotypes for errors, there was a large amount of missing data for locus BMC1222. In addition, FCB 128 is monomorphic. So, this locus was removed from the final set of microsatellites used in this thesis. The F_{IS} values for the loci ranged between -0.11 and 0.09 and none of the F_{IS} values were significant (Table 1, Figure 1). The observed H_o for the loci ranged between 0.000 and 0.821, which was higher than the expected H_o for most loci (Table 1). The pedigree is nine generations deep that starts in 1980 with 449 paternal links, 437 maternal links, and 78 founders. This pedigree also has 39 rams with all four grandparents in it.

Data Exploration

The distributions of horn measurements are bell shaped (Figure 2). Horn mass increased with horn length in a non-linear fashion (Figure S1). When the annuli lengths are plotted against each other, each annulus length predicts at least one other annulus length (Figures S2, S3, S4, S5, S6, and S7).

For observed and standardized H_o, the mean, median, and mode are all close to each other and they are highly correlated with each other (Table 2 and Figure 3). Since observed and standardized H_o are highly correlated with each other, only the results for observed H_o are being reported in this thesis. When observed H_o and each horn measurement are plotted against each other, observed H_o does predict horn length and horn mass, and somewhat predicts year 2

annulus length, year 3 annulus length, year 4 annulus length, year 5 annulus length, year 6 annulus length, year 7 annulus length, and year 8 annulus length. Only the relationship between observed H_o and horn length or horn mass was significant (Figure 4). When inbreeding coefficients for the rams with all four grandparents in the pedigree and observed H_o are plotted against each other, there is no obvious pattern or trends (Figure 5).

The last part of data exploration was looking at the relationship between age and either the horn measurements or genetic parameters to try to help inform the modeling. There is a positive trend between age and horn length, horn mass, or observed H_o, there is no obvious trend between age and the annuli lengths, and a negative relationship between age and inbreeding coefficients (Figure 6).

Non-genetic Modeling

The variance in each horn measurement explained by age ranged between 0.12 and 0.72 with age being a significant source of variation in horn length (p-value < 0.001), horn mass (p-value < 0.001), and year 2 annulus length (p-value < 0.001). Year 2 annulus length is the annulus formed in the second year of a ram's life, which is before rams have reached maximum horn size. Therefore, hunting starting in the second year of life can be a selective force against having large horns. For the repeated measures models, the variance in annuli lengths that was explained by ID was 0.36 with a p-value < 0.001. Finally, the variance in annuli lengths that was explained by annuli number was 0.90 with a p-value< 0.001.

Heritability Calculation

Horn measurements exhibited variable degrees of heritable variation with year 3 annulus length having the highest degree of heritable variation while year 4 annulus length having the lowest degree of heritable variation. In addition, horn length, horn mass, year 2 annulus length, year 7 annulus length, and year 8 annulus length having a moderate degree of heritable variation (Table 3).

Mixed Effects and Repeated Measures Modeling

When observed H_o was modeled on its own, the variance in each horn measurement explained by it ranged between 0.00 and 0.1 with H_o being a significant source of variation in

horn length (p-value< 0.001), horn mass (p-value< 0.001), and annuli lengths as one variable (p-value< 0.001). However, when observed H_o was fitted with the best nongenetic structure, observed Ho was not a significant source of variation in any of the horn measurements (Table 4). When inbreeding coefficients were modeled on their own, the variance in each horn measurement explained by it ranged between 0.00 and 0.1 with inbreeding coefficients not being a significant source of variation for any horn measurement. When inbreeding coefficients were fitted with the best non-genetic structure, the ANOVAs show that age or annuli number was the significant source of variation in the model and inbreeding coefficient's role was insignificant (Table 5). The lack of significance for inbreeding coefficients in the modeling may be due to the genetic rescue being over 30 years ago, which is long enough to undo the effects of inbreeding.

Discussion

After estimating heritability using an animal model, all measures of horn size are heritable at NBR with year 3 annulus length being highly heritable. Other studies in bighorn sheep at Ram Mountain, Alberta, Canada used horn length and horn base circumference as estimates of horn size with the estimated heritability of horn length ranging between 0.15 and 0.69. In addition, the estimated heritability of horn base circumference was between 0.23 and 0.68. (29, 30, 31, 32, 33). For horn length, the reported estimates of heritability in this thesis were close to the values reported by Coltman, Coltman et al, and Pigeon et al. All of these studies used the animal model to estimate heritability (31, 32, 33). This indicates that the animal model is a good option to estimate heritability. However, the heritability estimates reported here should be interpreted with caution because of their large credible interval and using an incomplete pedigree. Finally, heritability estimates for both horn mass and annuli lengths were reported here for the first time.

From the results of the mixed effect modeling, H_o on its own does play a role in determining horn size. However, that role is diminished when age or annuli number is in the model as well. This may be due to the relationship between H_o and age. In addition, there is a suggestive relationship between inbreeding and age. Both of these observations suggest that there might be an HFC for longevity or survival, which creates the apparent relationship with horn length. This is most likely a remaining signal from the genetic rescue that was done at NBR. Both studies that have been done on this rescue found that individuals with more introduced alleles had higher reproductive success and longevity (11, 12). The results of this thesis indicates that an

update on the consequences of the genetic rescues at NBR may be required to further understand the relationship between horn length and longevity or survival.

A secondary conclusion is age or annuli number has a greater influence on a ram's horn size than H_o or inbreeding coefficients. This result makes sense because older rams tend to have more time to grow horns and is similar to what is seen in Alpine ibex. In Alpine ibex, Von Hardenberg et al found that the effect of multilocus heterozygosity on horn growth was age-specific and the slope of the correlation between multilocus heterozygosity and yearly horn growth changed from negative to positive as rams age (34). From the results in this thesis, the relationship between horn length and age was positive while the relationship between annuli lengths and annuli number was negative. This is most likely from the fact that most horn growth occurs early during a ram's life.

Another secondary conclusion is that observed H_o does not appear to be a good proxy for inbreeding coefficients at NBR. This is similar to Slate et al's study in Coopworth sheep that found multilocus heterozygosity was weakly correlated with inbreeding coefficients and inbreeding coefficients, not multilocus heterozygosity, detected evidence of inbreeding depression for morphological traits (35). Other researchers have also found that HFCs difficult to detect and do recommend using traits closely related to fitness, large sample sizes, and interpret test statistics cautiously in order to successfully detect a HFC (36, 37, 38). For this thesis, I was not able to determine that H_o was a good proxy for inbreeding coefficients because my sample size and marker set was small. With the full dataset, there were 114 rams with horn measurements estimated while the inbreeding subset dataset had 39 rams with horn measurements estimated. This makes it difficult to make big sweeping conclusions about the effects of age, annuli number, or genetics on horn size. Future studies in the NBR bighorn sheep population should focus on confirming the existence of the HFC for longevity found in this thesis, the existence of HFCs for horn size found in previous studies, how large of a role RXFP2 plays in determining a ram's horn size, and what is the role of H_o and inbreeding coefficients on a ram's horn size.

Figures and Tables:

Table 1: The summary statistics for each locus genotyped at NBR for 93 rams analyzed in this thesis. The first column has the name of each locus used with the PCR group of the locus and how the locus is labeled in parentheses. The second column is the number of alleles for each locus, the third column is observed H_o for each locus, the fourth column is the expected H_o for each locus, the fifth column is the F_{IS} for each locus with p-values in parentheses, and the last column is the amount of missing data for each locus. The last row is the average values for the second through sixth columns. With the average P-value, it is less than the critical chi-square value for 56 degrees of freedom, which is insignificant.

Locus name	Number of Alleles	Observed H_o	Expected H_o	F_{IS}	Missing Data
MAF33 (Group 1)	4	0.60	0.59	-0.02 (0.18)	1
MAF36 (Group 1)	5	0.71	0.64	-0.11 (0.88)	4
FCB11 (Group 1)	3	0.59	0.59	0.00 (0.98)	30
FCB304 (Group 1)	5	0.70	0.70	0.00 (0.27)	0
OarCP20 (Group 1)	5	0.69	0.65	-0.07 (0.76)	21
MAF48 (Group 2)	4	0.32	0.33	0.04 (0.86)	0
ADCY1AP (Group 2)	3	0.38	0.38	0.02 (0.36)	5
MAF65 (Group 3)	9	0.82	0.79	-0.04 (0.78)	9
OarHH47 (Group 3)	4	0.61	0.67	0.09 (0.48)	1
OarAE16 (Group 3)	7	0.75	0.80	0.06 (0.84)	18

MAF209 (Group 4)	6	0.72	0.72	0.00 (0.94)	10
OarFCB266 (Group 4)	7	0.77	0.76	-0.02 (0.55)	11
OarHH62 (Group 4)	8	0.82	0.77	-0.07 (0.81)	8
OarFCB226 (Group 4)	5	0.81	0.72	-0.13 (0.29)	2
BM848 (Group 5)	4	0.56	0.54	-0.04 (0.86)	7
JMP29 (Group 5)	6	0.70	0.74	0.05 (0.30)	6
TGLA387(Group 6)	9	0.75	0.74	-0.01 (0.62)	1
BM4505 (Group 6)	5	0.60	0.62	0.04 (0.96)	5
BM1225 (Group 6)	7	0.80	0.76	-0.06 (0.43)	2
MAF92 (Group 7)	3	0.47	0.49	0.04 (0.86)	0
BM4630 (Group 7)	7	0.85	0.83	-0.02 (0.44)	0
BL25 (Group 7)	4	0.60	0.60	0.06 (0.36)	1
BM1818 (Group 8)	7	0.75	0.79	0.05 (0.50)	1
TGLA126 (Group 8)	6	0.78	0.73	-0.07 (0.65)	0
MCMA54i (M-13 labeled; Group 9)	7	0.79	0.78	-0.02 (0.90)	30

MCMA54ii (M-13 labeled; Group 9)	5	0.66	0.67	0.01 (0.94)	47
EPCDV21 (M-13 labeled; Group 9)	8	0.72	0.77	0.06 (0.39)	33
BMC1222	3	0.37	0.36	-0.03 (0.45)	58
Average	6	0.67	0.66	-0.01 (31.33)	11

Table 2: The summary statistics for observed and standardized H_o studied with the NBR rams. The standard error of the mean is seen next to the mean in parentheses.

	Mean	Median	Mode	Standard Deviation	Kurtosis	Skewness
Observed H_o	0.66 (0.01)	0.68	0.70	0.11	-0.44	-0.15
Standardized H_o	1.00 (0.02)	1.01	1.05	0.15	-0.43	-0.09

Table 3: Heritability estimates for horn measurements studied at NBR. The credible interval for the heritability estimates and additive genetic variance is seen in parentheses next to the value. The abbreviation V_A stands for additive genetic variance.

Horn Measurement	Heritability	V_A	Effective Sample Size
Horn Length	0.49 (0.00-1.00)	21.92 (0.00-53.21)	51.73
Horn Mass	0.48 (0.00-0.96)	0.27 (0.00-0.62)	173.96
Year 2 Growth	0.36 (0.00-1.00)	6.91 (0.02-21.51)	12.88
Year 3 Growth	0.65 (0.00-1.00)	4.29 (0.00-8.66)	36.33
Year 4 Growth	0.08 (0.00-0.36)	0.45 (0.00-1.66)	77.32
Year 5 Growth	0.19 (0.00-0.96)	1.06 (0.00-3.93)	53.04
Year 6 Growth	0.19 (0.00-0.84)	1.05 (0.00-3.19)	68.59
Year 7 Growth	0.37 (0.00-1.00)	0.87 (0.00-2.60)	35.09
Year 8 Growth	0.50 (0.00-1.00)	1.02 (0.00-2.38)	30.75
Annuli Lengths	0.15 (0.00-0.26)	1.05 (0.12-1.99)	75.17

Table 4: The results of the ANOVA tests done after modeling observed H_o using the model structure horn mesurement~heterozygosity + age + (1|birthyear) or annuli lengths~heterozygosity + annuli number + (1|id). The abbreviation D.F. stands for degrees of freedom.

Horn Measurement	Term	Sum of Squares	F-value	D.F.	P-value
Horn Length	H_o	101.30	3.25	82.05	0.08
	Age	7094.70	18.97	38.31	<0.001
Horn Mass	H_o	0.09	0.47	65.24	0.50
	Age	17.52	7.79	63.19	<0.001
Year 2 Growth	H_o	7.08	1.43	35.14	0.24
	Age	59.36	1.50	26.26	0.21
Year 3 Growth	H_o	5.92	1.35	79.30	0.25
	Age	51.55	0.98	57.89	0.48
Year 4 Growth	H_o	4.44	1.03	76	0.31
	Age	53.00	1.12	76	0.36
Year 5 Growth	H_o	0.14	0.05	66	0.83
	Age	63.67	2.32	66	0.02
Year 6 Growth	H_o	5.84	3.99	38.97	0.05
	Age	30.01	2.28	41.88	0.03
Year 7 Growth	H_o	1.93	1.37	44	0.25
	Age	21.86	1.95	44	0.08

Year 8 Growth	H_o	0.40	0.44	22.28	0.51
	Age	4.70	0.75	20.70	0.63
Annuli Lengths	H_o	7.30	1.56	88.54	0.21
	Annuli Number	18969.50	676.86	346.76	<0.001

Table 5: The results of the ANOVA tests done after modeling inbreeding coefficients using the model structure horn mesurement~inbreeding + age + (1|birthyear) or annuli lengths~inbreeding + annuli number + (1|id). The abbreviation D.F. stands for degrees of freedom.

Horn Measurement	Term	Sum of Squares	F-value	D.F.	P-value
Horn Length	Inbreeding	0.20	0.01	37	0.92
	Age	3911.40	20.97	37	<0.001
Horn Mass	Inbreeding	2.00e-03	0.02	34	0.90
	Age	55.73	52.73	34	<0.001
Year 2 Growth	Inbreeding	1.48	0.29	21	0.60
	Age	50.10	1.93	21	0.13
Year 3 Growth	Inbreeding	10.85	2.73	36	0.11
	Age	86.59	2.43	36	0.03
Year 4 Growth	Inbreeding	2.79	0.74	30	0.40
	Age	32.45	1.08	30	0.40

Year 5 Growth	Inbreeding	3.67	2.12	26	0.16
	Age	29.00	2.40	26	0.05
Year 6 Growth	Inbreeding	1.08	2.26	20	0.15
	Age	38.26	13.41	20	<0.001
Year 7 Growth	Inbreeding	1.21	3.49	16	0.08
	Age	9.51	5.50	16	<0.001
Year 8 Growth	Inbreeding	0.43	0.88	14	0.36
	Age	7.56	3.89	14	0.02
Annuli Lengths	Inbreeding	0.00	2.90e-03	38.38	0.96
	Annuli Number	8470.10	390.41	135.99	<0.001

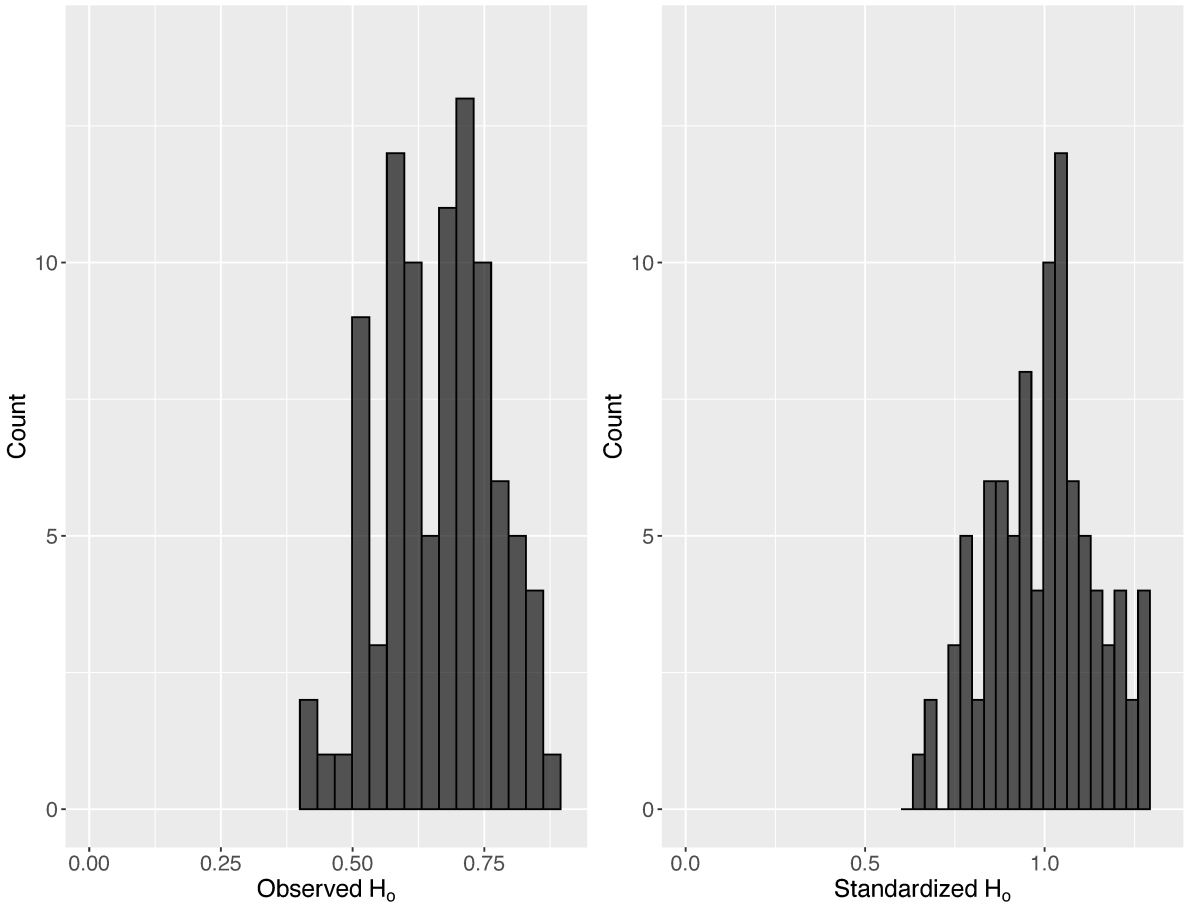

Figure 1: Distribution of observed and standardized H_o for 114 rams genotyped at NBR using 29 microsatellites loci. The abbreviation H_o stands for heterozygosity.

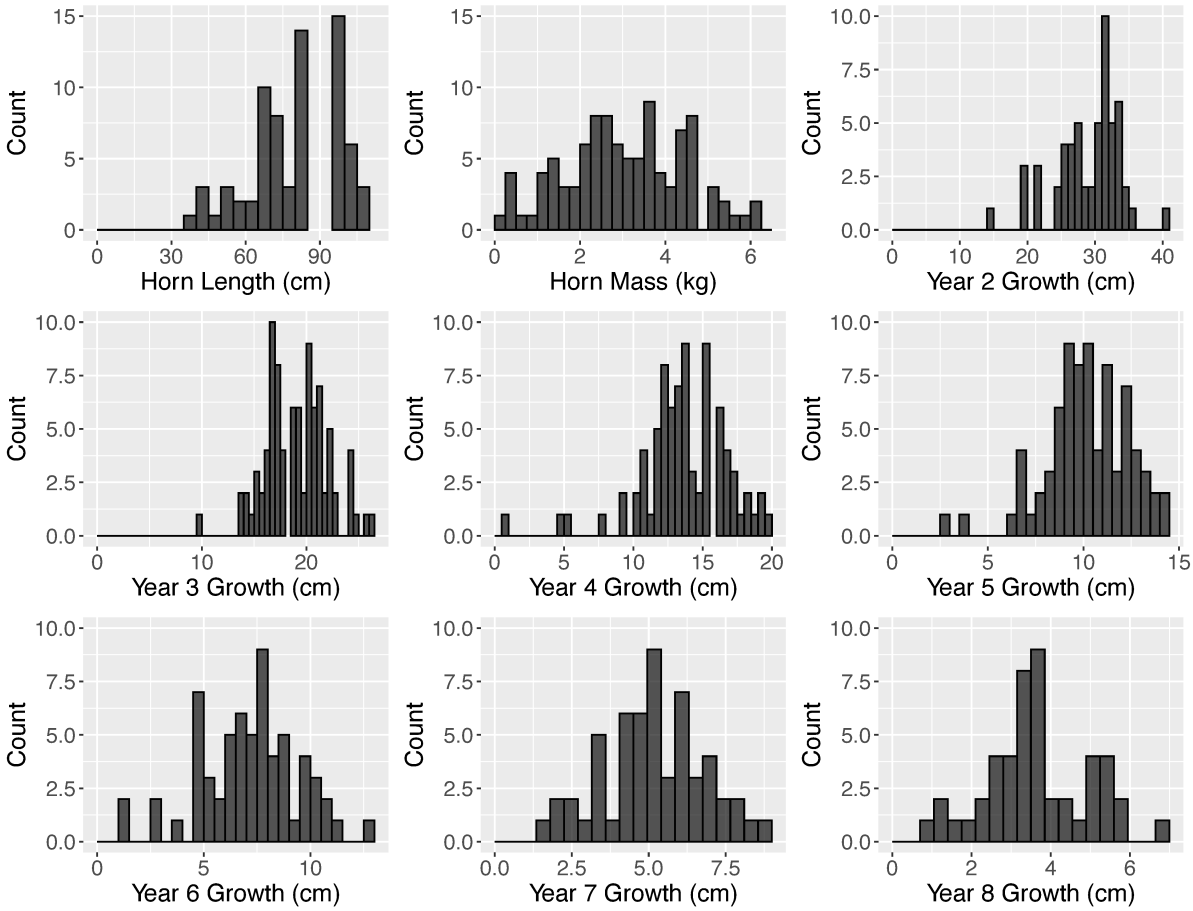

Figure 2: Distribution of horn measurements analyzed at NBR for 114 rams.

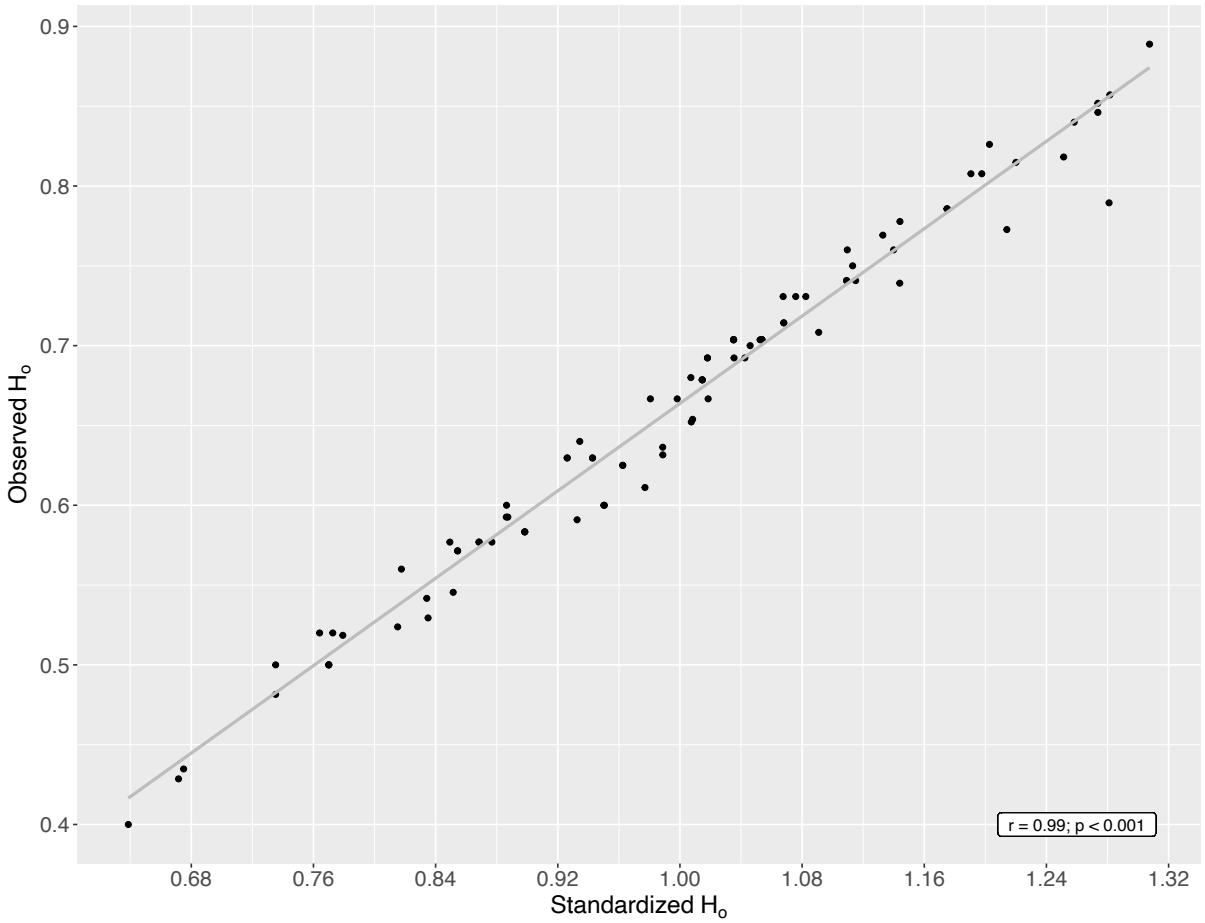

Figure 3: A scatter plot of the relationship between standardized and observed H_o. Standardized and observed H_o have a high correlation coefficient and are good substitutes for each other. The abbreviations in this plot are H_o for heterozygosity, "r" for Pearson's correlation coefficient, and "p" for p-value.

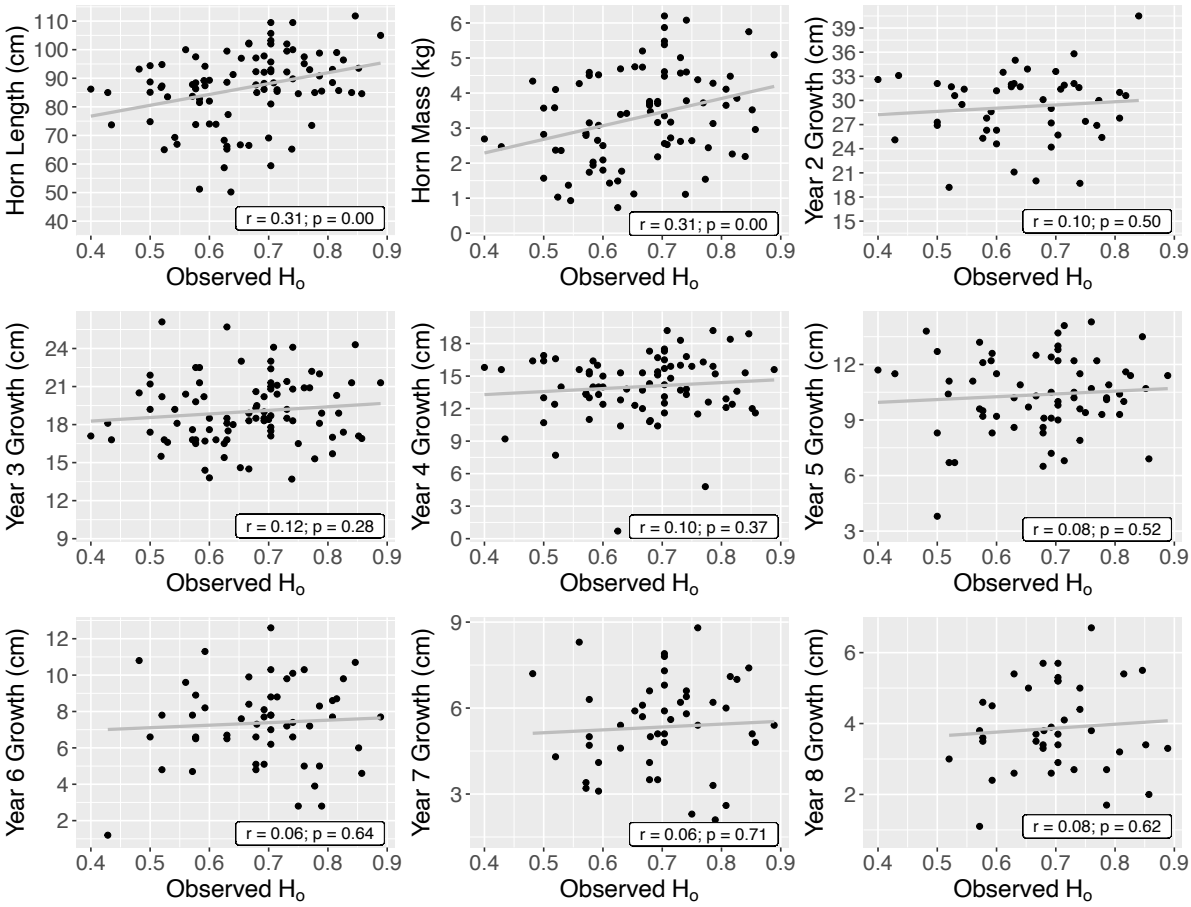

Figure 4: Scatter plots of the relationship between H_o and each horn measurement. The abbreviations in this plot are H_o for heterozygosity, "r" for Pearson's correlation coefficient, and "p" for p-values.

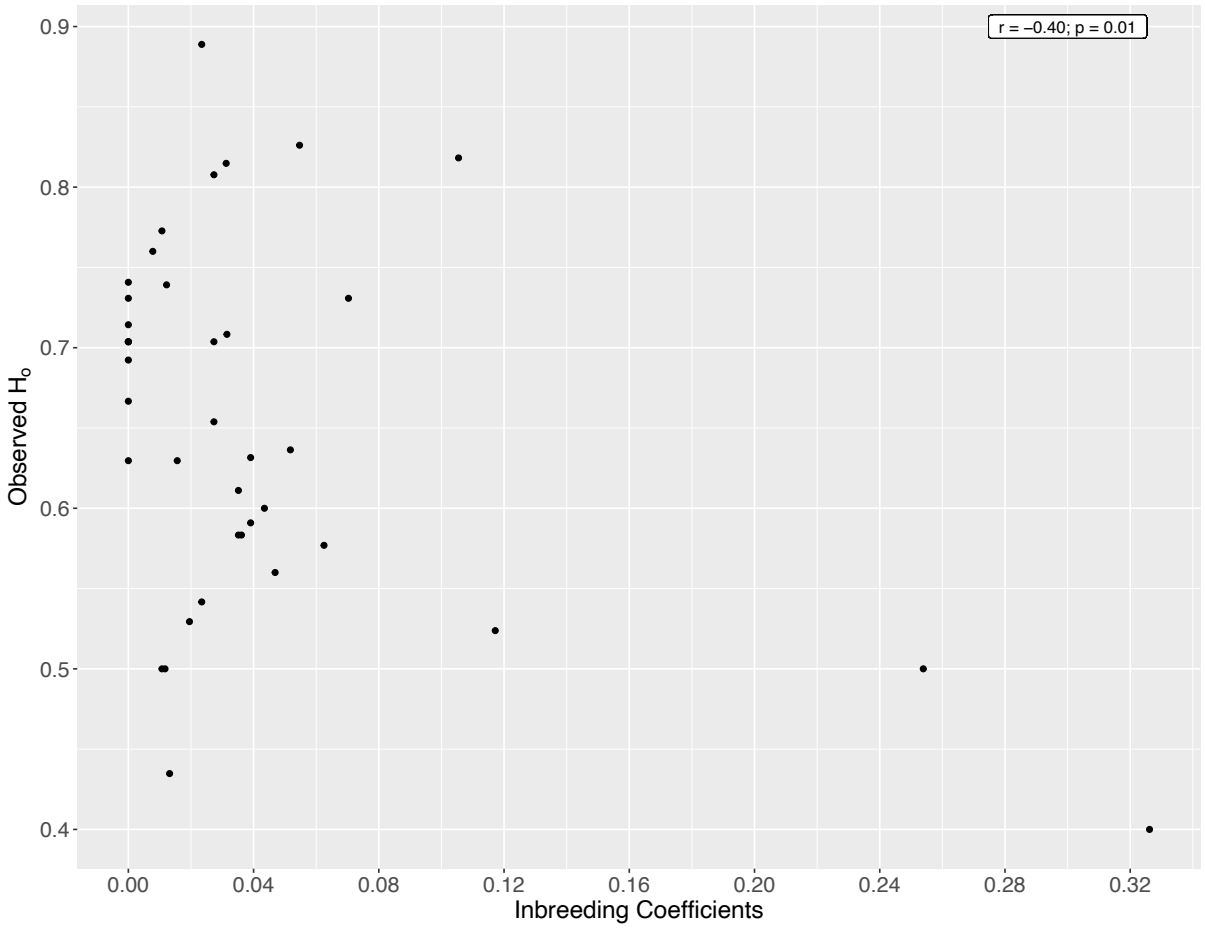

Figure 5: Scatter plot of the relationship between inbreeding coefficients and observed H_o. The abbreviations in this plot are H_o for heterozygosity, "r" for Pearson's correlation coefficient, and "p" for p-value.

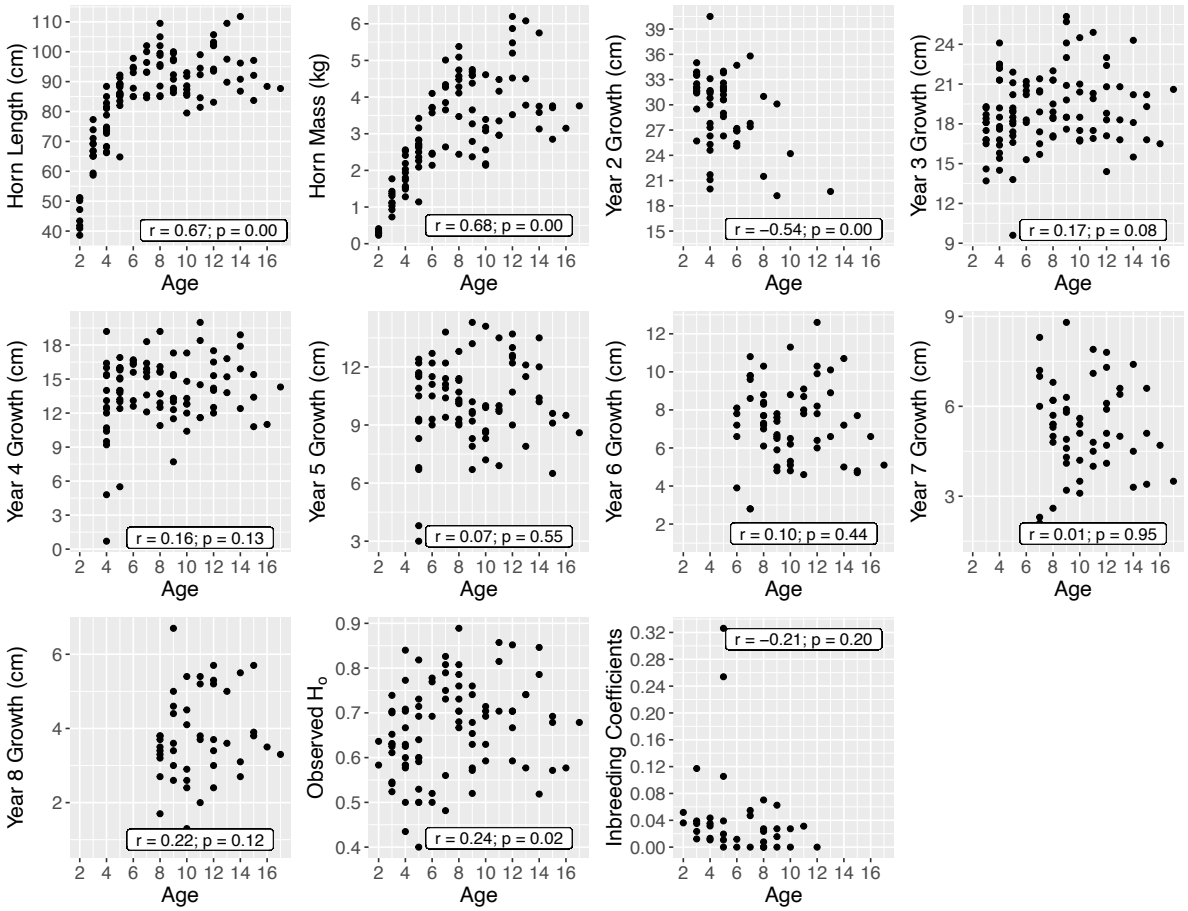

Figure 6: Scatter plots of the relationship between age and each horn measurement as well as observed H_o and inbreeding coefficients. The abbreviations in this plot are H_o for heterozygosity, "r" for Pearson's correlation coefficient, and "p" for p-values.

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Chapter 4: Conclusions

Headgear are cranial appendages usually seen in males of the infraorder Pecora, which are used to determine hierarchy and gain access to mates. The aim of this thesis was to better understand the role of genetics in horn size. In Chapter 2, I reviewed the intraspecific variation and functional genetics of headgear in Pecorans. Then this resource was used in Chapter 3 to try to address questions around intraspecific variation of horn size in bighorn sheep.

In Chapter 2, I did a literature review of headgear size in Pecorans to examine the role of intraspecific variation of headgear size and the functional genetics of headgear. With intraspecific variation, I was able to conclude that antler and horn size is heritable and HFCs are hard to detect in both cervids and bovids. For functional genetics, antlers and horns are both polygenic with horns appearing to have species specific genes. In addition, the gene RXFP2 has different functions in each genus of Bovidae. This review served as a resource to identify the current gaps in knowledge, which was used in Chapter 3 to start addressing the questions about the intraspecific variation of horn size in bighorn sheep.

In Chapter 3, I examined the role that genetics play in determining a ram's horn size. My study did confirm that horn size is heritable and for the first-time estimated heritability for horn mass and annuli lengths. In addition, heterozygosity does influence a ram's horn size, but that role is diminished when age or annuli number is added to the model. This indicates as rams age that later variation in horn size is more non-genetic. However, inbreeding coefficients were not a significant genetic factor for determining horn size. This indicates that there is an HFC between horn length and longevity or survival. This HFC exists even though horn growth is continuous over a ram's lifetime and indicates that a ram's age or annuli number has a bigger role in determining horn size than any of the genetic factors. One limitation of this study was sample size. Therefore, a next step is to increase the sample size then analyze the role of heterozygosity and inbreeding coefficients again. Some of the additional research questions still left to answer in Pecorans are what genetic changes occurred to cause the evolution of saber-like teeth in Moschidae, what is the quantitative genetic architecture of headgear size, do wild species have the same intraspecific variation as domestic species, how does gene expression during development play a role in intraspecific variation of Pecorans, and does heterozygosity or inbreeding coefficients effect headgear size.

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Appendix A: Code for the Histograms and Comparison Plots made in R

```
#Load the required packages to make and combine plots
library("ggplot2")
library("patchwork")

#Read in the Horn Measurement sheet
horn_measurements <-read.csv("Horn_Measurements.csv", header = T)

#Check to make sure the data was read in properly
horn_measurements

#Make all of the Horn Measurements into their own variables
heterozygosity <-horn_measurements$Heterozygosity
sh <-horn_measurements$Std_Heterozygosity
horn_length <-horn_measurements$Longest_Horn_Length
horn_mass <-horn_measurements$Heaviest_Horn_Mass
two_growth <-horn_measurements$year_Two_growth
third_growth <-horn_measurements$year_Three_growth
fourth_growth <-horn_measurements$year_Four_growth
fifth_growth <-horn_measurements$year_Five_growth
sixth_growth <-horn_measurements$year_Six_growth
seventh_growth <-horn_measurements$year_Seven_growth
eighth_growth <- horn_measurements$year_Eight_growth

#Read in the inbreeding coefficient subset spreadsheet
inbreeding_subset <-read.csv("comparison_graphdata.csv", header = T)

#Check to make sure the data was read in properly
inbreeding_subset
```

```
#Make the important Inbreeding Subset measurements into their own variables
inbreedingB <- inbreeding_subset$Inbreeding_Coefficient
heterozygosityB <- inbreeding_subset$Heterozygosity
shB <- inbreeding_subset$Std_Heterozygosity
ageB <- inbreeding_subset$Age_at_Death

#Make histograms for each of the horn measurements
p1 <- ggplot(horn_measurements, aes(x=horn_length)) + geom_histogram(breaks=seq(0,112,
by=5), col="black", fill="black", alpha=0.65) + labs(x="Horn Length (cm)", y="Count") +
xlim(c(0,112)) + ylim(c(0,15)) + theme(axis.text = element_text(size = 14)) + theme(axis.title =
element_text(size = 16))
p2 <- ggplot(horn_measurements, aes(x=horn_mass)) + geom_histogram(breaks=seq(0,6.50,
by=0.25), col="black", fill="black", alpha=0.65) + labs(x="Horn Mass (kg)", y="Count") +
xlim(c(0,6.50)) + ylim(c(0,15)) + theme(axis.text = element_text(size = 14)) + theme(axis.title =
element_text(size = 16))
p3 <- ggplot(horn_measurements, aes(x=two_growth)) + geom_histogram(breaks=seq(0,41,
by=1), col="black", fill="black", alpha=0.65) + labs(x= "Year 2 Growth (cm)", y="Count") +
xlim(c(0,41)) + ylim(c(0,10)) + theme(axis.text = element_text(size = 14)) + theme(axis.title =
element_text(size = 16))
p4 <- ggplot(horn_measurements, aes(x=third_growth)) + geom_histogram(breaks=seq(0, 26.50,
by=0.5), col="black", fill="black", alpha=0.65) + labs(x="Year 3 Growth (cm)", y="Count") +
xlim(c(0,26.50)) + ylim(c(0,10)) + theme(axis.text = element_text(size = 14)) + theme(axis.title =
element_text(size = 16))
p5 <- ggplot(horn_measurements, aes(x=fourth_growth)) + geom_histogram(breaks=seq(0,20,
by=0.5), col="black", fill="black", alpha=0.65) + labs(x="Year 4 Growth (cm)", y="Count") +
xlim(c(0,20)) + ylim(c(0,10)) + theme(axis.text = element_text(size = 14)) + theme(axis.title =
element_text(size = 16))
p6 <- ggplot(horn_measurements, aes(x=fifth_growth)) + geom_histogram(breaks=seq(0,14.50,
by=0.5), col="black", fill="black", alpha=0.65) + labs(x="Year 5 Growth (cm)", y="Count") +
xlim(c(0,14.50)) + ylim(c(0,10)) + theme(axis.text = element_text(size = 14)) + theme(axis.title =
element_text(size = 16))
```

```
p7 <- ggplot(horn_measurements, aes(x=sixth_growth)) + geom_histogram(breaks=seq(0,13,
by=0.5), col="black", fill="black", alpha=0.65) + labs(x="Year 6 Growth (cm)", y="Count") +
xlim(c(0,13)) + ylim(c(0,10)) + theme(axis.text = element_text(size = 14)) + theme(axis.title =
element_text(size = 16))
p8 <- ggplot(horn_measurements, aes(x=seventh_growth)) + geom_histogram(breaks=seq(0,9,
by=0.45), col="black", fill="black", alpha=0.65) + labs(x="Year 7 Growth (cm)", y="Count") +
xlim(c(0,9)) + ylim(c(0,10)) + theme(axis.text = element_text(size = 14)) + theme(axis.title =
element_text(size = 16))
p9 <- ggplot(horn_measurements, aes(x=eighth_growth)) + geom_histogram(breaks=seq(0,7,
by=0.35), col="black", fill="black", alpha=0.65) + labs(x="Year 8 Growth (cm)", y="Count") +
xlim(c(0,7)) + ylim(c(0,10)) + theme(axis.text = element_text(size = 14)) + theme(axis.title =
element_text(size = 16))
p10 <- ggplot(horn_measurements, aes(x=heterozygosity)) +
geom_histogram(breaks=seq(0.4,0.9, by=0.033), col="black", fill="black", alpha=0.65) + labs(x=
expression(Observed~H[o]), y="Count") + xlim(c(0,0.9)) + ylim(c(0,14)) + theme(axis.text =
element_text(size = 14)) + theme(axis.title = element_text(size = 16))
p11 <- ggplot(horn_measurements, aes(x=sh)) + geom_histogram(breaks=seq(0.6,1.32,
by=0.033), col="black", fill="black", alpha=0.65) + labs(x= expression(Standardized~H[o]),
y="Count") + xlim(c(0,1.32)) + ylim(c(0,14)) + theme(axis.text = element_text(size = 14)) +
theme(axis.title = element_text(size = 16))

#Combine the plots together by logical groups
p13 <- wrap_plots(p10, p11)
p15 <- wrap_plots(p1, p2, p3, p4, p5, p6, p7, p8, p9)

#Make comparison plots between each horn measurement and heterozygosity
p1 <- ggplot(horn_measurements, aes(x=heterozygosity, y=horn_length)) + geom_point() +
geom_smooth(method=lm, se=FALSE, color="gray") + geom_label(label=" r = 0.31; p = 0.00 ",
x= 0.77, y= 41, label.padding= unit(0.25,"lines"), label.size= 0.11, color= "black") +
scale_x_continuous(expression(Observed~H[o]), breaks=seq(0,0.9,0.1)) +
```

```
scale_y_continuous(name="Horn Length (cm)", breaks=seq(0,112,10)) + theme(axis.text =
element_text(size = 14)) + theme(axis.title = element_text(size = 16))
p2 <- ggplot(horn_measurements, aes(x=heterozygosity, y=horn_mass)) + geom_point() +
geom_smooth(method=lm, se=FALSE, color="gray") + geom_label(label=" r = 0.31; p = 0.75 ",
x= 0.77, y= 0.27, label.padding= unit(0.25,"lines"), label.size= 0.11, color= "black")  +
scale_x_continuous(expression(Observed~H[o]), breaks=seq(0,0.9,0.1)) +
scale_y_continuous(name="Horn Mass (kg)", breaks=seq(0,7,1)) + theme(axis.text =
element_text(size = 14)) + theme(axis.title = element_text(size = 16))
p3 <- ggplot(horn_measurements, aes(x=heterozygosity, y=two_growth)) + geom_point() +
geom_smooth(method=lm, se=FALSE, color="gray") + geom_label(label=" r = 0.10; p = 0.50 ",
x= 0.77, y= 15, label.padding= unit(0.25,"lines"), label.size= 0.11, color= "black") +
scale_x_continuous(expression(Observed~H[o]), breaks=seq(0, 0.9,0.1)) +
scale_y_continuous(name="Year 2 Growth (cm)", breaks=seq(0,42,3)) + theme(axis.text =
element_text(size = 14)) + theme(axis.title = element_text(size = 16))
p4 <- ggplot(horn_measurements, aes(x=heterozygosity, y=third_growth)) + geom_point() +
geom_smooth(method=lm, se=FALSE, color="gray") + geom_label(label=" r = 0.12; p = 0.28 ",
x= 0.77, y= 10, label.padding= unit(0.25,"lines"), label.size= 0.11, color= "black") +
scale_x_continuous(expression(Observed~H[o]), breaks=seq(0,0.9,0.1)) +
scale_y_continuous(name="Year 3 Growth (cm)", breaks=seq(0,27,3)) + theme(axis.text =
element_text(size = 14)) + theme(axis.title = element_text(size = 16))
p5 <- ggplot(horn_measurements, aes(x=heterozygosity, y=fourth_growth)) + geom_point() +
geom_smooth(method=lm, se=FALSE, color="gray") + geom_label(label=" r = 0.10; p = 0.37 ",
x= 0.77, y= 1.10, label.padding= unit(0.25,"lines"), label.size= 0.11, color= "black") +
scale_x_continuous(expression(Observed~H[o]), breaks=seq(0,0.9,0.1)) +
scale_y_continuous(name="Year 4 Growth (cm)", breaks=seq(0,21,3)) + theme(axis.text =
element_text(size = 14)) + theme(axis.title = element_text(size = 16))
p6 <- ggplot(horn_measurements, aes(x=heterozygosity, y=fifth_growth)) + geom_point() +
geom_smooth(method=lm, se=FALSE, color="gray") + geom_label(label=" r = 0.08; p = 0.52 ",
x= 0.77, y= 3.10, label.padding= unit(0.25,"lines"), label.size= 0.11, color= "black")  +
scale_x_continuous(expression(Observed~H[o]), breaks=seq(0,0.9,0.1)) +
```

```
scale_y_continuous(name="Year 5 Growth (cm)", breaks=seq(0,15,3)) + theme(axis.text =
element_text(size = 14)) + theme(axis.title = element_text(size = 16))
p7 <- ggplot(horn_measurements, aes(x=heterozygosity, y=sixth_growth)) + geom_point() +
geom_smooth(method=lm, se=FALSE, color="gray") + geom_label(label=" r = 0.06; p = 0.64 ",
x= 0.77, y= 1.30, label.padding= unit(0.25,"lines"), label.size= 0.11, color= "black") +
scale_x_continuous(expression(Observed~H[o]), breaks=seq(0,0.9,0.1)) +
scale_y_continuous(name="Year 6 Growth (cm)", breaks=seq(0,14,2)) + theme(axis.text =
element_text(size = 14)) + theme(axis.title = element_text(size = 16))
p8 <- ggplot(horn_measurements, aes(x=heterozygosity, y=seventh_growth)) + geom_point() +
geom_smooth(method=lm, se=FALSE, color="gray") + geom_label(label=" r = 0.06; p = 0.71 ",
x= 0.77, y= 1.60, label.padding= unit(0.25,"lines"), label.size= 0.11, color= "black") +
scale_x_continuous(expression(Observed~H[o]), breaks=seq(0,0.9,0.1)) +
scale_y_continuous(name="Year 7 Growth (cm)", breaks=seq(0,9,3)) + theme(axis.text =
element_text(size = 14)) + theme(axis.title = element_text(size = 16))
p9 <- ggplot(horn_measurements, aes(x=heterozygosity, y=eighth_growth)) + geom_point() +
geom_smooth(method=lm, se=FALSE, color="gray") + geom_label(label=" r = 0.08; p = 0.62 ",
x= 0.77, y= 1.10, label.padding= unit(0.25,"lines"), label.size= 0.11, color= "black") +
scale_x_continuous(expression(Observed~H[o]), breaks=seq(0,0.9,0.1)) +
scale_y_continuous(name="Year 8 Growth (cm)", breaks=seq(0,8,2)) + theme(axis.text =
element_text(size = 14)) + theme(axis.title = element_text(size = 16))

#Combine those plots together
p10 <- wrap_plots(p1, p2, p3, p4, p5, p6, p7, p8, p9)
p10

#Make comparison plots between horn length and horn mass
p21 <- ggplot(horn_measurements, aes(x=horn_mass, y=horn_length)) + geom_point() +
geom_label(label=" r = 0.91; p < 0.001 ", x= 6.00, y= 40, label.padding= unit(0.25,"lines"),
label.size= 0.11, color= "black") + scale_x_continuous(name="Horn Mass (kg)",
breaks=seq(0,7,1)) + scale_y_continuous(name="Horn Length (cm)", breaks=seq(0,112,10)) +
theme(axis.text = element_text(size = 14)) + theme(axis.title = element_text(size = 16))
```

```
p21

#Make comparison plots between each annulus length
p24 <- ggplot(horn_measurements, aes(x=two_growth, y=third_growth)) + geom_point() +
geom_label(label=" r = -0.15; p = 0.28 ", x= 33, y= 26, label.padding= unit(0.25,"lines"),
label.size= 0.11, color= "black")  + geom_smooth(method=lm, se=FALSE, color="gray") +
scale_x_continuous(name="Year 2 Growth (cm)", breaks=seq(0,42,3)) +
scale_y_continuous(name="Year 3 Growth (cm)", breaks=seq(0,27,3)) + theme(axis.text =
element_text(size = 14)) + theme(axis.title = element_text(size = 16))
p25 <- ggplot(horn_measurements, aes(x=two_growth, y=fourth_growth)) + geom_point() +
geom_label(label=" r = -0.04; p = 0.80 ", x= 33, y= 20.25, label.padding= unit(0.25,"lines"),
label.size= 0.11, color= "black") + geom_smooth(method=lm, se=FALSE, color="gray") +
scale_x_continuous(name="Year 2 Growth (cm)", breaks=seq(0,42,3)) +
scale_y_continuous(name="Year 4 Growth (cm)", breaks=seq(0,21,3)) + theme(axis.text =
element_text(size = 14)) + theme(axis.title = element_text(size = 16))
p26 <- ggplot(horn_measurements, aes(x=two_growth, y=fifth_growth)) + geom_point() +
geom_label(label=" r = -0.18; p = 0.35 ", x= 33, y= 14.25, label.padding= unit(0.25,"lines"),
label.size= 0.11, color= "black") + geom_smooth(method=lm, se=FALSE, color="gray") +
scale_x_continuous(name="Year 2 Growth (cm)", breaks=seq(0,42,3)) +
scale_y_continuous(name="Year 5 Growth (cm)", breaks=seq(0,15,3)) + theme(axis.text =
element_text(size = 14)) + theme(axis.title = element_text(size = 16))
p27 <- ggplot(horn_measurements, aes(x=two_growth, y=sixth_growth)) + geom_point() +
geom_label(label="  r = -0.07; p = 0.81 ", x= 33, y= 12.5, label.padding= unit(0.25,"lines"),
label.size= 0.11, color= "black") + scale_x_continuous(name="Year 2 Growth (cm)",
breaks=seq(0,42,3)) + scale_y_continuous(name="Year 6 Growth (cm)", breaks=seq(0,14,2)) +
theme(axis.text = element_text(size = 14)) + theme(axis.title = element_text(size = 16))
p28 <- ggplot(horn_measurements, aes(x=two_growth, y=seventh_growth)) + geom_point() +
geom_label(label=" r = -0.61; p = 0.06 ", x= 33, y= 8.75, label.padding= unit(0.25,"lines"),
label.size= 0.11, color= "black") + scale_x_continuous(name="Year 2 Growth (cm)",
breaks=seq(0,45,5)) + scale_y_continuous(name="Year 7 Growth (cm)", breaks=seq(0,9,3)) +
theme(axis.text = element_text(size = 14)) + theme(axis.title = element_text(size = 16))
```

```
p29 <- ggplot(horn_measurements, aes(x=two_growth, y=eighth_growth)) + geom_point() +
geom_label(label=" r = 0.00; p = 0.99 ", x= 33, y= 6.75, label.padding= unit(0.25,"lines"),
label.size= 0.11, color= "black") + scale_x_continuous(name="Year 2 Growth (cm)",
breaks=seq(0,45,5)) + scale_y_continuous(name="Year 8 Growth (cm)", breaks=seq(0,8,2)) +
theme(axis.text = element_text(size = 14)) + theme(axis.title = element_text(size = 16))

p30 <- wrap_plots(p24, p25, p26, p27, p28, p29)
p30

#Repeat the above for all annuli lengths after year 2 Growth

#Make comparison plots between birth year and each horn measurement
p73 <- ggplot(horn_measurements, aes(x=birth_year, y=horn_length)) + geom_point() +
geom_label(label=" r = -0.34; p = 0.00 ", x= 1990, y= 41, label.padding= unit(0.25,"lines"),
label.size= 0.11, color= "black") + labs(x="Birth Year") + scale_y_continuous(name="Horn
Length (cm)", breaks=seq(0,112,10)) + theme(axis.text = element_text(size = 10)) +
theme(axis.title = element_text(size = 14))
p74 <- ggplot(horn_measurements, aes(x=birth_year, y=horn_mass)) + geom_point() +
geom_label(label=" r = -0.25; p = 0.02 ", x= 1990, y= 0.35, label.padding= unit(0.25,"lines"),
label.size= 0.11, color= "black") + labs(x="Birth Year") + scale_y_continuous(name="Horn
Mass (kg)", breaks=seq(0,7,1)) + theme(axis.text = element_text(size = 10)) + theme(axis.title =
element_text(size = 14))
p75 <- ggplot(horn_measurements, aes(x=birth_year, y=two_growth)) + geom_point() +
geom_label(label=" r = 0.38; p = 0.01 ", x= 1990, y= 16, label.padding= unit(0.25,"lines"),
label.size= 0.11, color= "black") + labs(x="Birth Year") + scale_y_continuous(name="Year 2
Growth (cm)", breaks=seq(0,42,3)) + theme(axis.text = element_text(size = 10)) +
theme(axis.title = element_text(size = 14))
p76 <- ggplot(horn_measurements, aes(x=birth_year, y=third_growth)) + geom_point() +
geom_label(label=" r = -0.26; p = 0.01 ", x= 1990, y= 10, label.padding= unit(0.25,"lines"),
label.size= 0.11, color= "black") + labs(x="Birth Year") + scale_y_continuous(name="Year 3
```

```
Growth (cm)", breaks=seq(0,27,3)) + theme(axis.text = element_text(size = 10)) +
theme(axis.title = element_text(size = 14))
p77 <- ggplot(horn_measurements, aes(x=birth_year, y=fourth_growth)) + geom_point() +
geom_label(label=" r = -0.16; p = 0.13 ", x= 1990, y= 1.15, label.padding= unit(0.25,"lines"),
label.size= 0.11, color= "black") + labs(x="Birth Year") + scale_y_continuous(name="Year 4
Growth (cm)", breaks=seq(0,21,3)) + theme(axis.text = element_text(size = 10)) +
theme(axis.title = element_text(size = 14))
p78 <- ggplot(horn_measurements, aes(x=birth_year, y=fifth_growth)) + geom_point() +
geom_label(label=" r = -0.03; p = 0.83 ", x= 1990, y= 3.5, label.padding= unit(0.25,"lines"),
label.size= 0.11, color= "black") + labs(x="Birth Year") + scale_y_continuous(name="Year 5
Growth (cm)", breaks=seq(0,15,3)) + theme(axis.text = element_text(size = 10)) +
theme(axis.title = element_text(size = 14))
p79 <- ggplot(horn_measurements, aes(x=birth_year, y=sixth_growth)) + geom_point() +
geom_label(label=" r = 0.00; p = 0.97 ", x= 1990, y= 1.5, label.padding= unit(0.25,"lines"),
label.size= 0.11, color= "black")  + labs(x="Birth Year") + scale_y_continuous(name="Year 6
Growth (cm)", breaks=seq(0,14,2)) + theme(axis.text = element_text(size = 10)) +
theme(axis.title = element_text(size = 14))
p80 <- ggplot(horn_measurements, aes(x=birth_year, y=seventh_growth)) + geom_point() +
geom_label(label=" r = 0.23; p = 0.08 ", x= 1990, y= 1.75, label.padding= unit(0.25,"lines"),
label.size= 0.11, color= "black") + labs(x="Birth Year") + scale_y_continuous(name="Year 7
Growth (cm)", breaks=seq(0,9,3)) + theme(axis.text = element_text(size = 10)) + theme(axis.title
= element_text(size = 14))
p81 <- ggplot(horn_measurements, aes(x=birth_year, y=eighth_growth)) + geom_point() +
geom_label(label=" r = 0.22; p = 0.16 ", x= 1990, y= 1.0, label.padding= unit(0.25,"lines"),
label.size= 0.11, color= "black") + labs(x="Birth Year") + scale_y_continuous(name="Year 8
Growth (cm)", breaks=seq(0,8,2)) + theme(axis.text = element_text(size = 10)) + theme(axis.title
= element_text(size = 14))
birthyear_plot <- ggplot(horn_measurements, aes(x=birth_year, y=heterozygosity)) +
geom_point() + geom_label(label=" r = -0.20; p = 0.06 ", x= 1990, y= 0.41, label.padding=
unit(0.25,"lines"), label.size= 0.11, color= "black") + labs(x="Birth Year") +
```

```
scale_y_continuous(expression(Observed~H[o]), breaks=seq(0,0.9,0.1)) + theme(axis.text =
element_text(size = 10)) + theme(axis.title = element_text(size = 14))
birthyear_plot

#Combine those plots together
p102 <- wrap_plots(p73, p74, p75, p76, p77, p78, p79, p80, p81, birthyear_plot)
p102

#Make comparison plots between age and each horn measurement
p83 <- ggplot(horn_measurements, aes(x=age, y=horn_length)) + geom_point() +
geom_label(label=" r = 0.67; p = 0.00 ", x= 12, y= 40.25, label.padding= unit(0.20,"lines"),
label.size= 0.10, color= "black") + scale_x_continuous(name="Age", breaks=seq(0,18,2)) +
scale_y_continuous(name="Horn Length (cm)", breaks=seq(0,112,10)) + theme(axis.text =
element_text(size = 12)) + theme(axis.title = element_text(size = 14))
p84 <- ggplot(horn_measurements, aes(x=age, y=horn_mass)) + geom_point() +
geom_label(label=" r = 0.68; p = 0.00 ", x= 11, y= 0.50, label.padding= unit(0.20,"lines"),
label.size= 0.10, color= "black") + scale_x_continuous(name="Age", breaks=seq(0,18,2)) +
scale_y_continuous(name="Horn Mass (kg)", breaks=seq(0,7,1)) + theme(axis.text =
element_text(size = 12)) + theme(axis.title = element_text(size = 14))
p85 <- ggplot(horn_measurements, aes(x=age, y=two_growth)) + geom_point() +
geom_label(label=" r = -0.54; p = 0.00 ", x= 11, y= 15.25, label.padding= unit(0.20,"lines"),
label.size= 0.10, color= "black") + scale_x_continuous(name="Age", breaks=seq(0,18,2)) +
scale_y_continuous(name="Year 2 Growth (cm)", breaks=seq(0,42,3)) + theme(axis.text =
element_text(size = 12)) + theme(axis.title = element_text(size = 14))
p86 <- ggplot(horn_measurements, aes(x=age, y=third_growth)) + geom_point() +
geom_label(label=" r = 0.17; p = 0.08 ", x= 11, y= 10, label.padding= unit(0.20,"lines"),
label.size= 0.10, color= "black") + scale_x_continuous(name="Age", breaks=seq(0,18,2)) +
scale_y_continuous(name="Year 3 Growth (cm)", breaks=seq(0,27,3)) + theme(axis.text =
element_text(size = 12)) + theme(axis.title = element_text(size = 14))
p87 <- ggplot(horn_measurements, aes(x=age, y=fourth_growth)) + geom_point() +
geom_label(label=" r = 0.16; p = 0.13 ", x= 11, y= 1.0, label.padding= unit(0.20,"lines"),
```

```
label.size= 0.10, color= "black") + scale_x_continuous(name="Age", breaks=seq(0,18,2)) +
scale_y_continuous(name="Year 4 Growth (cm)", breaks=seq(0,21,3)) + theme(axis.text =
element_text(size = 12)) + theme(axis.title = element_text(size = 14))
p88 <- ggplot(horn_measurements, aes(x=age, y=fifth_growth)) + geom_point() +
geom_label(label=" r = 0.07; p = 0.55 ", x= 11, y= 3.25, label.padding= unit(0.20,"lines"),
label.size= 0.10, color= "black") + scale_x_continuous(name="Age", breaks=seq(0,18,2)) +
scale_y_continuous(name="Year 5 Growth (cm)", breaks=seq(0,15,3)) + theme(axis.text =
element_text(size = 12)) + theme(axis.title = element_text(size = 14))
p89 <- ggplot(horn_measurements, aes(x=age, y=sixth_growth)) + geom_point() +
geom_label(label=" r = 0.10; p = 0.44 ", x= 11, y= 1.5, label.padding= unit(0.20,"lines"),
label.size= 0.10, color= "black") + scale_x_continuous(name="Age", breaks=seq(0,18,2)) +
scale_y_continuous(name="Year 6 Growth (cm)", breaks=seq(0,14,2)) + theme(axis.text =
element_text(size = 12)) + theme(axis.title = element_text(size = 14))
p90 <- ggplot(horn_measurements, aes(x=age, y=seventh_growth)) + geom_point() +
geom_label(label=" r = 0.01; p = 0.95 ", x= 11, y= 1.75, label.padding= unit(0.20,"lines"),
label.size= 0.10, color= "black") + scale_x_continuous(name="Age", breaks=seq(0,18,2)) +
scale_y_continuous(name="Year 7 Growth (cm)", breaks=seq(0,9,3)) + theme(axis.text =
element_text(size = 12)) + theme(axis.title = element_text(size = 14))
p91 <- ggplot(horn_measurements, aes(x=age, y=eighth_growth)) + geom_point() +
geom_label(label=" r = 0.22; p = 0.12 ", x= 11, y= 1.0, label.padding= unit(0.20,"lines"),
label.size= 0.10, color= "black") + scale_x_continuous(name="Age", breaks=seq(0,18,2)) +
scale_y_continuous(name="Year 8 Growth (cm)", breaks=seq(0,8,2)) + theme(axis.text =
element_text(size = 12)) + theme(axis.title = element_text(size = 14))
p95 <- ggplot(horn_measurements, aes(x=age, y=heterozygosity)) + geom_point() +
geom_label(label=" r = 0.24; p = 0.02 ", x= 11, y= 0.42, label.padding= unit(0.20,"lines"),
label.size= 0.10, color= "black") + scale_x_continuous(name="Age", breaks=seq(0,18,2)) +
scale_y_continuous(expression(Observed~H[o]), breaks=seq(0,0.9,0.1)) + theme(axis.text =
element_text(size = 12)) + theme(axis.title = element_text(size = 14))
p97 <- ggplot(inbreeding_subset, aes(x=ageB, y=inbreedingB)) + geom_point() +
geom_label(label=" r = -0.21; p = 0.20 ", x= 11, y= 0.31, label.padding= unit(0.20,"lines"),
label.size= 0.10, color= "black") + scale_x_continuous(name="Age", breaks=seq(0,16,2)) +
```

```
scale_y_continuous(name="Inbreeding Coefficients", breaks=seq(0,0.36,0.04)) + theme(axis.text
= element_text(size = 12)) + theme(axis.title = element_text(size = 14))

#Combine those plots together
p101 <- wrap_plots(p83, p84, p85, p86, p87, p88, p89, p90, p91, p95, p97)
p101

#Make comparison plots between observed and standardized heterozygosity
p94 <- ggplot(horn_measurements, aes(x=sh, y=heterozygosity)) + geom_point() +
geom_label(label=" r = 0.99; p < 0.001 ", x= 1.26, y= 0.40, label.padding= unit(0.25,"lines"),
label.size= 0.11, color= "black")  + geom_smooth(method=lm, se=FALSE, color="gray") +
scale_x_continuous(expression(Standardized~H[o]), breaks=seq(0.6,1.32,0.08)) +
scale_y_continuous(expression(Observed~H[o]), breaks=seq(0,0.9,0.1)) + theme(axis.text =
element_text(size = 14)) + theme(axis.title = element_text(size = 16))
p94

#Make comparison plots between inbreeding and observed heterozygosity
p98 <- ggplot(inbreeding_subset, aes(x=inbreedingB, y=heterozygosityB)) + geom_point() +
geom_label(label=" r = -0.40; p = 0.01 ", x= 0.30, y= 0.90, label.padding= unit(0.25,"lines"),
label.size= 0.11, color= "black") + scale_x_continuous(name="Inbreeding Coefficients",
breaks=seq(0,0.36,0.04)) + scale_y_continuous(expression(Observed~H[o]),
breaks=seq(0,0.9,0.1)) + theme(axis.text = element_text(size = 14)) + theme(axis.title =
element_text(size = 16))
p98
```

Appendix B: Heritability Calculation Code

```
#Add rams without microsatellite genotypes and founders to the pedigree using Spyder Python wrapper
#Open file and read it in
inFile = open("NBRpedigree.txt", "r")
inLines = inFile.readlines()
inFile.close()
outLines = inLines

#Empty arrays to hold data
a = []  # Animal
p1 = [] # Parent 1
p2 = [] # Parent 2
fa = [] # Founders added

#Loop over Animals and put them into individual arrays.
for i in range(1,len(inLines)):
   aLine = inLines[i]
   [c1, c2, c3] = aLine.split('\t')
   a.append(c1)
   p1.append(c2)
   p2.append(c3)
# next i

#Loop over Animals. Select the Sire and Dam for each.
#If the Sire/Dam are not NA, then get the index number of the Sire/Dam.
#If the index does not exist, then add the Sire/Dam to the list as:
#Sire/Dam ID {tab} NA {tab} NA

for i in range(len(a)):
```

```
    q1 = p1[i].rstrip()
    if ((q1 in a) or (q1 == "NA") or (q1 in fa)) == 0:
        # q1 (i.e. this parent) needs to be added as a founder
        fa.append(q1)
        q1f = q1 + "\tNA\tNA\n"
        outLines.insert(1,q1f)
    # endif
    q2 = p2[i].rstrip()
    if ((q2 in a) or (q2 == "NA") or (q2 in fa)) == 0:
        # q2 (i.e. this parent) needs to be added as a founder
        fa.append(q2)
        q2f = q2 + "\tNA\tNA\n"
        outLines.insert(1,q2f)
    # endif
# next i

#Write the output to a file.
outFile = open("NBRpedigree_with_Founders.txt", "w")
outFile.writelines(outLines)
outFile.close()

#Switch to R and load the packages needed to sort the pedigree and calculate heritability
library("MCMCglmm")
library("MasterBayes")

#Read in the Horn Measurement sheet
horn_measurements <-read.csv("Horn_MeasurementsC.csv", header = T)

#Check to make sure the data was read in properly
horn_measurements
```

```
#Read in the pedigree
rampedigree_temp <- read.table("NBRpedigree_with_Founders.txt", header = T)

#Check to make sure the data was read in properly
rampedigree_temp

#Sort the pedigree
ram_pedigree <- orderPed(rampedigree_temp)
ram_pedigree

#Make all of the relevant Horn Measurements into their own variables
age <- horn_measurements$age
animal <- horn_measurements$animal
hornlength <- horn_measurements$hornlength
hornmass <- horn_measurements$hornmass
yearTwoGrowth <- horn_measurements$yearTwoGrowth
yearThreeGrowth <- horn_measurements$yearThreeGrowth
yearFourGrowth <- horn_measurements$yearFourGrowth
yearFiveGrowth <- horn_measurements$yearFiveGrowth
yearSixGrowth <- horn_measurements$yearSixGrowth
yearSevenGrowth <- horn_measurements$yearSevenGrowth
yearEightGrowth <- horn_measurements$yearEightGrowth

#Change age to a factor
class(age)
horn_measurements$age <- as.factor(age)
class(horn_measurements$age)
age <- horn_measurements$age
class(age)
```

```
#Read in the Annuli Lengths sheet
annuli_lengths <-read.csv("Annuli_length_wideB.csv", header = T)

#Check to make sure the data was read in properly
annuli_lengths

#Make all of the Annuli Lengths measurements into their own variables
annuliNumber <- annuli_lengths$annuliNumber
id <- annuli_lengths$id
animal <- annuli_lengths$animal
annuliLength <-annuli_lengths$annuliLength

#Change annuli number to a factor
class(annuliNumber)
annuli_lengths$annuliNumber <- as.factor(annuliNumber)
class(annuli_lengths$annuliNumber)
annuliNumber <- annuli_lengths$annuliNumber
class(annuliNumber)

#Set the priors for the models
prior <- list(R = list(V=1, nu=0.002), G = list(G1 = list(V=1, nu=0.002)))

#Make a MCMCglmm model for horn length
length_model <- MCMCglmm(hornlength~age, random = ~animal, family = "gaussian", prior =
prior, pedigree = ram_pedigree, data = horn_measurements, nitt = 100000, burnin = 10000, thin =
10)
plot(length_model$Sol)
plot(length_model$VCV)
effectiveSize(length_model$Sol)
effectiveSize(length_model$VCV)
summary(length_model$VCV)
```

```
HPDinterval(length_model$VCV)
summary(length_model)

#Calculate heritability for horn length
herit_length <- length_model$VCV[, "animal"]/(length_model$VCV[, "animal"] +
length_model$VCV[, "units"])
effectiveSize(herit_length)
mean(herit_length)
HPDinterval(herit_length)

#Make a MCMCglmm model for horn mass
mass_model <- MCMCglmm(hornmass~age, random = ~animal, family = "gaussian", prior =
prior, pedigree = ram_pedigree, data = horn_measurements, nitt = 100000, burnin = 10000, thin =
10)
plot(mass_model$Sol)
plot(mass_model$VCV)
effectiveSize(mass_model$Sol)
effectiveSize(mass_model$VCV)
summary(mass_model$VCV)
HPDinterval(mass_model$VCV)
summary(mass_model)

#Calculate heritability for horn mass
herit_mass <- mass_model$VCV[, "animal"]/(mass_model$VCV[, "animal"] +
mass_model$VCV[, "units"])
effectiveSize(herit_mass)
mean(herit_mass)
HPDinterval(herit_mass)
```

```
#Make a MCMCglmm model for Year 2 Growth
yearTwo_model <- MCMCglmm(yearTwoGrowth~age, random = ~animal, family = "gaussian",
prior = prior, pedigree = ram_pedigree, data = horn_measurements, nitt = 100000, burnin =
10000, thin = 10)
plot(yearTwo_model$Sol)
plot(yearTwo_model$VCV)
effectiveSize(yearTwo_model$Sol)
effectiveSize(yearTwo_model$VCV)
summary(yearTwo_model$VCV)
HPDinterval(yearTwo_model$VCV)
summary(yearTwo_model)

#Calculate heritability for Year 2 Growth
herit_yearTwo <- yearTwo_model$VCV[, "animal"]/(yearTwo_model$VCV[, "animal"] +
yearTwo_model$VCV[, "units"])
effectiveSize(herit_yearTwo)
mean(herit_yearTwo)
HPDinterval(herit_yearTwo)

#Make a MCMCglmm model for other annuli lengths using Year 2 Growth model as a guide

#Set the priors for the annuli lengths model
prior <- list(R = list(V=1, nu=0.002), G = list(G1 = list(V=1, nu=0.002), G2 = list(V=1,
nu=0.002)))

#Make a MCMCglmm model for annuli lengths with annuli number as an additional fixed effect
annuli_model <- MCMCglmm(annuliLength~annuliNumber, random = ~animal + id, family =
"gaussian", prior = prior, pedigree = ram_pedigree, data = annuli_lengths, nitt = 100000, burnin =
10000, thin = 10)
plot(annuli_model$Sol)
plot(annuli_model$VCV)
```

```
effectiveSize(annuli_model$Sol)
effectiveSize(annuli_model$VCV)
summary(annuli_model$VCV)
HPDinterval(annuli_model$VCV)
summary(annuli_model)

#Calculate heritability for annuli length with annuli number as an additional fixed effect
herit_annuli <- annuli_model$VCV[, "animal"]/(annuli_model$VCV[, "animal"] +
annuli_model$VCV[, "id"] + annuli_model$VCV[, "units"])
effectiveSize(herit_annuli)
mean(herit_annuli)
HPDinterval(herit_annuli)
```

Appendix C: Linear Mixed Effects and Repeated Measures Modeling Code

```
#Load the required packages to do mixed effects models
library("lme4")
library("lmerTest")
library("emmeans")
library("dplyr")
library("broom.mixed")

#Read in the Horn Measurement sheet
horn_measurements <-read.csv("Horn_MeasurementsB.csv", header = T)

#Check to make sure the data was read in properly
horn_measurements

#Make all of the Horn Measurements into their own variables
heterozygosity <-horn_measurements$Heterozygosity
sh <-horn_measurements$Std_Heterozygosity
inbreeding <- horn_measurements$Inbreeding_Coefficient
birthyear <- horn_measurements$Birth_Year
age <- horn_measurements$Age_at_Death
hornlength <- horn_measurements$Longest_Horn_Length
hornmass <- horn_measurements$Heaviest_Horn_Mass
yearTwoGrowth <- horn_measurements$year_Two_growth
yearThreeGrowth <- horn_measurements$year_Three_growth
yearFourGrowth <- horn_measurements$year_Four_growth
yearFiveGrowth <- horn_measurements$year_Five_growth
yearSixGrowth <- horn_measurements$year_Six_growth
yearSevenGrowth <- horn_measurements$year_Seven_growth
yearEightGrowth <- horn_measurements$year_Eight_growth
```

```
#Find out the class of the age and birthyear variables. Then change them to a factor as needed
class(age)
class(birthyear)
ageB <- as.factor(age)
birthyearB <- as.factor(birthyear)

#Read in the inbreeding coefficient subset spreadsheet
inbreeding_subset <-read.csv("Inbreeding_Subset.csv", header = T)

#Check to make sure the data was read in properly
inbreeding_subset

#Make the important Inbreeding Subset measurements into their own variables
inbreedingB <- inbreeding_subset$Inbreeding_Coefficient
heterozygosityB <- inbreeding_subset$Heterozygosity
shB <- inbreeding_subset$Std_Heterozygosity
birthyearC <- inbreeding_subset$Birth_Year
ageC <- inbreeding_subset$Age_at_Death
hornlengthB <- inbreeding_subset$Longest_Horn_Length
hornmassB <- inbreeding_subset$Heaviest_Horn_Mass
yearTwoGrowthB <- inbreeding_subset$year_Two_growth
yearThreeGrowthB <- inbreeding_subset$year_Three_growth
yearFourGrowthB <- inbreeding_subset$year_Four_growth
yearFiveGrowthB <- inbreeding_subset$year_Five_growth
yearSixGrowthB <- inbreeding_subset$year_Six_growth
yearSevenGrowthB <- inbreeding_subset$year_Seven_growth
yearEightGrowthB <- inbreeding_subset$year_Eight_growth

#Change age and birthyear to factors
ageD <- as.factor(ageC)
birthyearD <- as.factor(birthyearC)
```

```
#Make nongenetic models for horn length and birthyear and/or age
model1 <- lm(hornlength ~ ageB, horn_measurements)
summary(model1)
AIC(model1)
model1_sum <- tidy(model1)

model2 <- lm(hornlength ~ birthyearB, horn_measurements)
summary(model2)
AIC(model2)
model2_sum <- tidy(model2)

model3 <- lm(hornlength ~ ageB + birthyearB, horn_measurements)
summary(model3)
AIC(model3)
model3_sum <- tidy(model3)

model4 <- lmer(hornlength~ageB + (1|birthyearB), horn_measurements)
summary(model4)
AIC(model4)
model4_sum <- tidy(model4)

model5 <- lmer(hornlength~birthyearB + (1|ageB), horn_measurements)
summary(model5)
AIC(model5)
model5_sum <- tidy(model5)

model6 <- lm(hornlength ~ ageB * birthyearB, horn_measurements)
summary(model6)
AIC(model6)
model6_sum <- tidy(model6)
```

```
#Make models for horn length and observed heterozygosity starting with no random effects and building up
model7 <- lm(hornlength ~ heterozygosity, horn_measurements)
summary(model7)
AIC(model7)
model7_sum <- tidy(model7)

model8 <- lmer(hornlength ~ heterozygosity + ageB + (1|birthyearB), horn_measurements)
summary(model8)
AIC(model8)
model8_sum <- tidy(model8)

model9 <- lmer(hornlength ~ heterozygosity * ageB + (1|birthyearB), horn_measurements)
summary(model9)
AIC(model9)
model9_sum <- tidy(model9)

#Inbreeding Coefficients models using the inbreeding subset data
model19 <- lm(hornlengthB ~ inbreedingB, inbreeding_subset)
summary(model19)
AIC(model19)
model19_sum <- tidy(model19)

model20 <- lmer(hornlengthB ~ inbreedingB + ageD + (1|birthyearD), inbreeding_subset)
summary(model20)
AIC(model20)
model20_sum <- tidy(model20)

model21 <- lmer(hornlengthB ~ inbreedingB * ageD + (1|birthyearD), inbreeding_subset)
summary(model21)
AIC(model21)
```

```
model21_sum <- tidy(model21)

#Repeat the above with horn mass and each genetic parameter starting with nongenetic models
#Make nongenetic models for horn mass and birthyear and/or age
model22 <- lm(hornmass ~ ageB, horn_measurements)
summary(model22)
AIC(model22)
model22_sum <- tidy(model22)

model23 <- lm(hornmass ~ birthyearB, horn_measurements)
summary(model23)
AIC(model23)
model23_sum <- tidy(model23)

model24 <- lm(hornmass ~ ageB + birthyearB, horn_measurements)
summary(model24)
AIC(model24)
model24_sum <- tidy(model24)

model25 <- lmer(hornmass~ageB + (1|birthyearB), horn_measurements)
summary(model25)
AIC(model25)
model25_sum <- tidy(model25)

model26 <- lmer(hornmass~birthyearB + (1|ageB), horn_measurements)
summary(model26)
AIC(model26)
model26_sum <- tidy(model26)

model27 <- lm(hornmass ~ ageB * birthyearB, horn_measurements)
summary(model27)
```

```
AIC(model27)
model27_sum <- tidy(model27)

#Make models for horn mass and observed heterozygosity starting with no random effects and building up
model28 <- lm(hornmass ~ heterozygosity, horn_measurements)
summary(model28)
AIC(model28)
model28_sum <- tidy(model28)

model29 <- lmer(hornmass ~ heterozygosity + ageB + (1|birthyearB), horn_measurements)
summary(model29)
AIC(model29)
model29_sum <- tidy(model29)
model30 <- lmer(hornmass ~ heterozygosity * ageB + (1|birthyearB), horn_measurements)
summary(model30)
AIC(model30)
model30_sum <- tidy(model30)

#Repeat the above modeling with the inbreeding subset data for horn mass to model inbreeding coefficients

#Repeat the above with year 2 Growth and each genetic parameter starting with nongenetic models
#Make nongenetic models for year 2 Growth and birthyear and/or age
model43 <- lm(yearTwoGrowth ~ ageB, horn_measurements)
summary(model43)
AIC(model43)
model43_sum <- tidy(model43)

model44 <- lm(yearTwoGrowth ~ birthyearB, horn_measurements)
```

```
summary(model44)
AIC(model44)
model44_sum <- tidy(model44)

model45 <- lm(yearTwoGrowth ~ ageB + birthyearB, horn_measurements)
summary(model45)
AIC(model45)
model45_sum <- tidy(model45)

model46 <- lmer(yearTwoGrowth~ ageB + (1|birthyearB), horn_measurements)
summary(model46)
AIC(model46)
model46_sum <- tidy(model46)

model47 <- lmer(yearTwoGrowth~ birthyearB + (1|ageB), horn_measurements)
summary(model47)
AIC(model47)
model47_sum <- tidy(model47)

model48 <- lm(yearTwoGrowth ~ ageB * birthyearB, horn_measurements)
summary(model48)
AIC(model48)
model48_sum <- tidy(model48)

#Make models for year 2 Growth and observed heterozygosity
model49 <- lm(yearTwoGrowth ~ heterozygosity, horn_measurements)
summary(model49)
AIC(model49)
model49_sum <- tidy(model49)
```

```
model50 <- lmer(yearTwoGrowth ~ heterozygosity + ageB + (1|birthyearB),
horn_measurements)
summary(model50)
AIC(model50)
model50_sum <- tidy(model50)

model51 <- lmer(yearTwoGrowth ~ heterozygosity * ageB + (1|birthyearB),
horn_measurements)
summary(model51)
AIC(model51)
model51_sum <- tidy(model51)

#Repeat the above modeling with the inbreeding subset data for year 2 Growth to model
inbreeding coefficients

#Repeat the above code with each annuli lengths after year 2 Growth and each genetic parameter
starting with the nongenetic models

#Repeat the above expect make the models be maximum likelihood and do ANOVAs for the
variables and to compare the models
#Make models for horn length and observed heterozygosity starting with no random effects and
building up
model1 <- lm(hornlength ~ heterozygosity, horn_measurements)
summary(model1)
AIC(model1)
model1_sum <- tidy(model1)

model2 <- lmer(hornlength ~ heterozygosity + ageB + (1|birthyearB), horn_measurements,
REML=FALSE)
summary(model2)
model2_sum <- tidy(model2)
```

```
model3 <- lmer(hornlength ~ heterozygosity * ageB + (1|birthyearB), horn_measurements,
REML=FALSE)
summary(model3)
model3_sum <- tidy(model3)

anova1 <- anova(model2, model3)
summary(anova1)
anova(model1)
anova(model2)
aov1 <- aov(hornlength~ageB, horn_measurements)
aov1
summary(aov1)
aov2 <- aov(hornlength~birthyearB, horn_measurements)
summary(aov2)

#Repeat the above modeling with inbreeding coefficients models using the inbreeding subset
dataset

#Make models for horn mass and observed heterozygosity starting with no random effects and
building up
model16 <- lm(hornmass ~ heterozygosity, horn_measurements)
summary(model16)
AIC(model16)
model16_sum <- tidy(model16)

model17 <- lmer(hornmass ~ heterozygosity + ageB + (1|birthyearB), horn_measurements,
REML=FALSE)
summary(model17)
model17_sum <- tidy(model17)
```

```
model18 <- lmer(hornmass ~ heterozygosity * ageB + (1|birthyearB), horn_measurements,
REML=FALSE)
summary(model18)
model18_sum <- tidy(model18)

anova(model17, model18)
anova(model16)
anova(model17)
aov5 <- aov(hornmass~ageB, horn_measurements)
summary(aov5)
aov6 <- aov(hornmass~birthyearB, horn_measurements)
summary(aov6)

#Repeat the above to model inbreeding coefficients using the inbreeding subset dataset

#Make models for year 2 Growth and observed heterozygosity
model31 <- lm(yearTwoGrowth ~ heterozygosity, horn_measurements)
summary(model31)
AIC(model31)
model31_sum <- tidy(model31)

model32 <- lmer(yearTwoGrowth ~ heterozygosity + ageB + (1|birthyearB),
horn_measurements, REML=FALSE)
summary(model32)
model32_sum <- tidy(model32)

model33 <- lmer(yearTwoGrowth ~ heterozygosity * ageB + (1|birthyearB), horn_measurements,
REML=FALSE)
summary(model33)
model33_sum <- tidy(model33)
```

```
anova(model32, model33)
anova(model31)
anova(model32)
aov9 <- aov(yearTwoGrowth~ageB, horn_measurements)
summary(aov9)
aov10 <- aov(yearTwoGrowth~birthyearB, horn_measurements)
summary(aov10)

#Repeat the above modeling with the inbreeding subset data for year 2 Growth to model the
inbreeding coefficients

#Repeat the above to make models for all annuli lengths after year 2 Growth

#Then combine each annuli lengths into one variable to do Repeated Measures modeling
#Load the required packages to do mixed effects models
library("lme4")
library("lmerTest")
library("emmeans")
library("dplyr")
library("broom.mixed")

#Read in the Annuli Lengths sheet
annuli_lengths <-read.csv("Annuli_length_wide.csv", header = T)

#Check to make sure the data was read in properly
annuli_lengths

#Make all of the Annuli Length measurements into their own variables
heterozygosity <-annuli_lengths$Heterozygosity
sh <-annuli_lengths$Std_Heterozygosity
inbreeding <- annuli_lengths$Inbreeding_Coefficient
```

```
annuliNumber <- annuli_lengths$Annuli_Number
birthyear <- annuli_lengths$Birth_Year
age <- annuli_lengths$Age_at_Death
id <- annuli_lengths$Ram_ID
annuliLength <-annuli_lengths$Annuli_Lengths
hornlength <- annuli_lengths$Longest_Horn_Length
hornmass <- annuli_lengths$Heaviest_Horn_Mass

#Change age and birthyear to a factor
ageB <- as.factor(age)
class(annuliNumber)
annuliNumberB <- as.factor(annuliNumber)
birthyearB <- as.factor(birthyear)
class(id)

#Read in the Inbreeding Subset wide sheet
inbreeding_wide <-read.csv("Inbreeding_subset_wide.csv", header = T)

#Check to make sure the data was read in properly
inbreeding_wide

#Make all of the Annuli Length measurements into their own variables
heterozygosityC <-inbreeding_wide$Heterozygosity
shC <-inbreeding_wide$Std_Heterozygosity
inbreedingC <- inbreeding_wide$Inbreeding_Coefficient
annuliNumberC <- inbreeding_wide$Annuli_Number
ageC <- inbreeding_wide$Age_at_Death
idB <- inbreeding_wide$Ram_ID
annuliLengthB <-inbreeding_wide$Annuli_Lengths
hornlengthB <- inbreeding_wide$Longest_Horn_Length
hornmassB <- inbreeding_wide$Heaviest_Horn_Mass
```

```
#Change annuli number to a factor
annuliNumberD <- as.factor(annuliNumberC)

#Make nongenetic models for annuli lengths and birthyear and/or age
model1 <- lmer(annuliLength ~ ageB + (1|id), annuli_lengths)
summary(model1)
AIC(model1)
model1_sum <- tidy(model1)

model2 <- lmer(annuliLength ~ birthyearB + (1|id), annuli_lengths)
summary(model2)
AIC(model2)
model2_sum <- tidy(model2)

model3 <- lmer(annuliLength ~ ageB + birthyearB + (1|id), annuli_lengths)
summary(model3)
AIC(model3)
model3_sum <- tidy(model3)

model4 <- lmer(annuliLength~ageB + (1|birthyearB) + (1|id), annuli_lengths)
summary(model4)
AIC(model4)
model4_sum <- tidy(model4)

model5 <- lmer(annuliLength~ageB + id + (1|birthyearB), annuli_lengths)
summary(model5)
AIC(model5)
model5_sum <- tidy(model5)

model6 <- lm(annuliLength~ageB + birthyearB + id, annuli_lengths)
summary(model6)
```

```
AIC(model6)
model6_sum <- tidy(model6)

model7 <- lmer(annuliLength~birthyearB + (1|ageB) + (1|id), annuli_lengths)
summary(model7)
AIC(model7)
model7_sum <- tidy(model7)

model8 <- lmer(annuliLength~birthyearB + id + (1|ageB), annuli_lengths)
summary(model8)
AIC(model8)
model8_sum <- tidy(model8)

model9 <- lmer(annuliLength ~ ageB * birthyearB + (1|id), annuli_lengths)
summary(model9)
AIC(model9)
model9_sum <- tidy(model9)

model10 <- lmer(annuliLength ~ annuliNumberB + (1|id), annuli_lengths)
summary(model10)
AIC(model10)
model10_sum <- tidy(model10)

#Make models for annuli lengths and observed heterozygosity starting with no random effects
and building up
model11 <- lmer(annuliLength ~ heterozygosity + (1|id), annuli_lengths)
summary(model11)
AIC(model11)
model11_sum <- tidy(model11)
model12 <- lmer(annuliLength ~ heterozygosity + annuliNumberB + (1|id), annuli_lengths)
summary(model12)
```

```
AIC(model12)
model12_sum <- tidy(model12)

model13 <- lmer(annuliLength ~ heterozygosity * annuliNumberB + (1|id), annuli_lengths)
summary(model13)
AIC(model13)
model13_sum <- tidy(model13)

#Repeat above with the inbreeding subset wide dataset to model the inbreeding coefficients

#Repeat the above code to do the modeling using maximum likelihood and to do ANOVAs
#Make models for annuli lengths and observed heterozygosity starting with no random effects
and building up
model1 <- lmer(annuliLength ~ heterozygosity + (1|id), annuli_lengths, REML=FALSE)
summary(model1)
model1_sum <- tidy(model1)

model2 <- lmer(annuliLength ~ heterozygosity + annuliNumberB + (1|id), annuli_lengths,
REML=FALSE)
summary(model2)
model2_sum <- tidy(model2)

model3 <- lmer(annuliLength ~ heterozygosity * annuliNumberB + (1|id), annuli_lengths,
REML=FALSE)
summary(model3)
model3_sum <- tidy(model3)

anova(model2, model3)
anova(model1, model2)
anova(model1)
anova(model2)
```

```
aov1 <- aov(annuliLength~annuliNumberB, annuli_lengths)
summary(aov1)
aov2 <- aov(annuliLength~ageB, annuli_lengths)
summary(aov2)
aov5 <- aov(annuliLength~id, annuli_lengths)
summary(aov5)
aov6 <- aov(annuliLength ~ heterozygosity, annuli_lengths)
summary(aov6)

#Repeat above with the inbreeding subset wide dataset to model inbreeding coefficients
```

Appendix D: Supplemental Figures

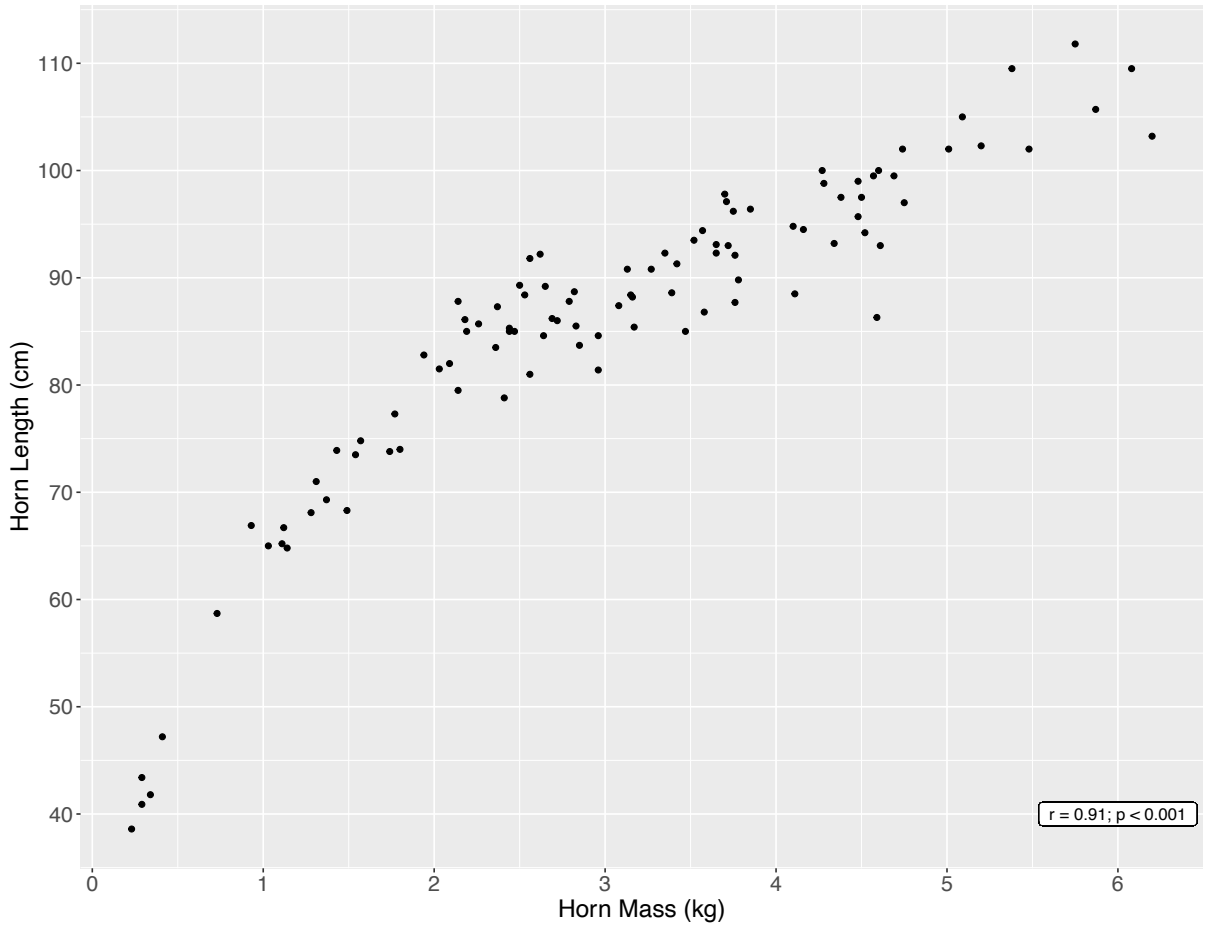

Figure S1: A scatterplot showing the relationship between horn length and mass. The abbreviations in this plot are "r" for Pearson's correlation coefficient and "p" for p-value.

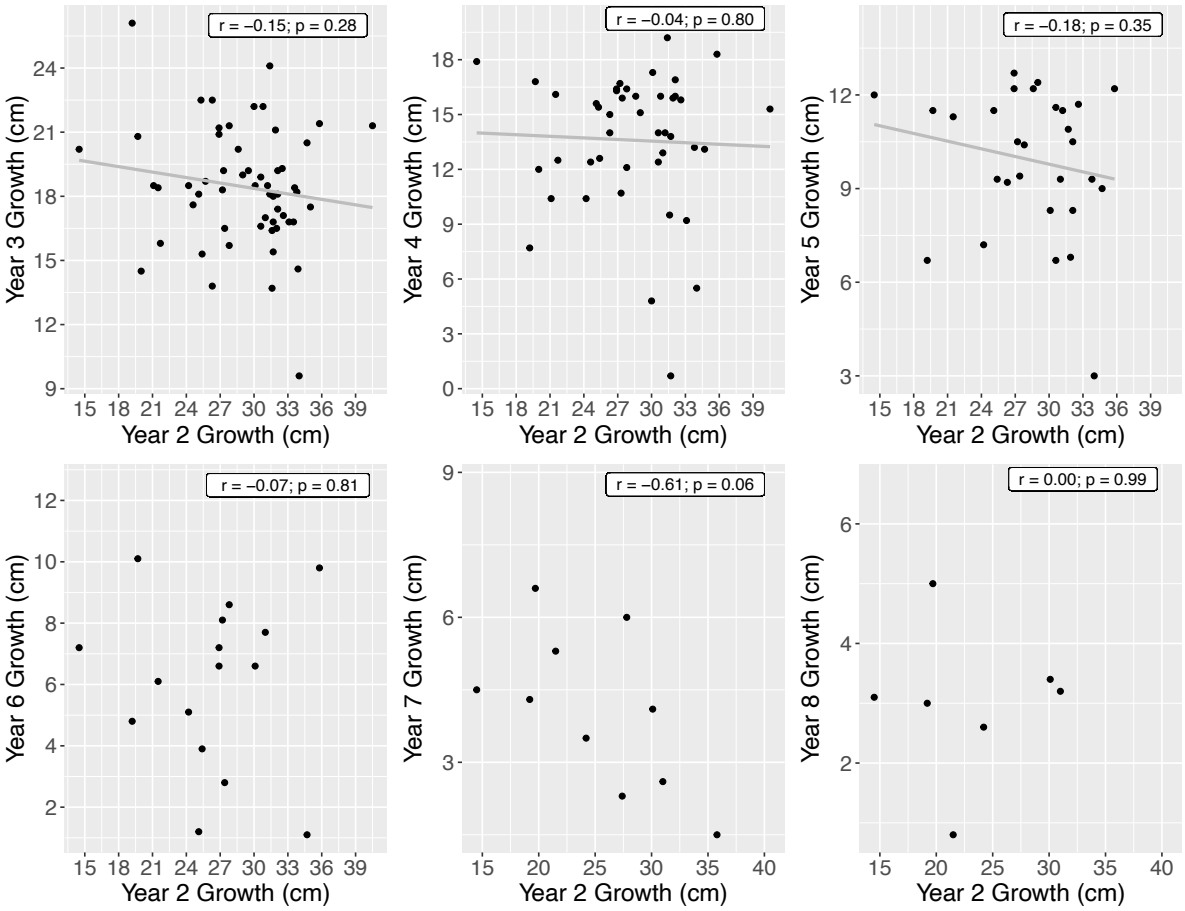

Figure S2: Scatter plots showing the relationship between year 2 growth and each subsequent year until year 8. The abbreviations in this plot are "r" for Pearson's correlation coefficient and "p" for p-values.

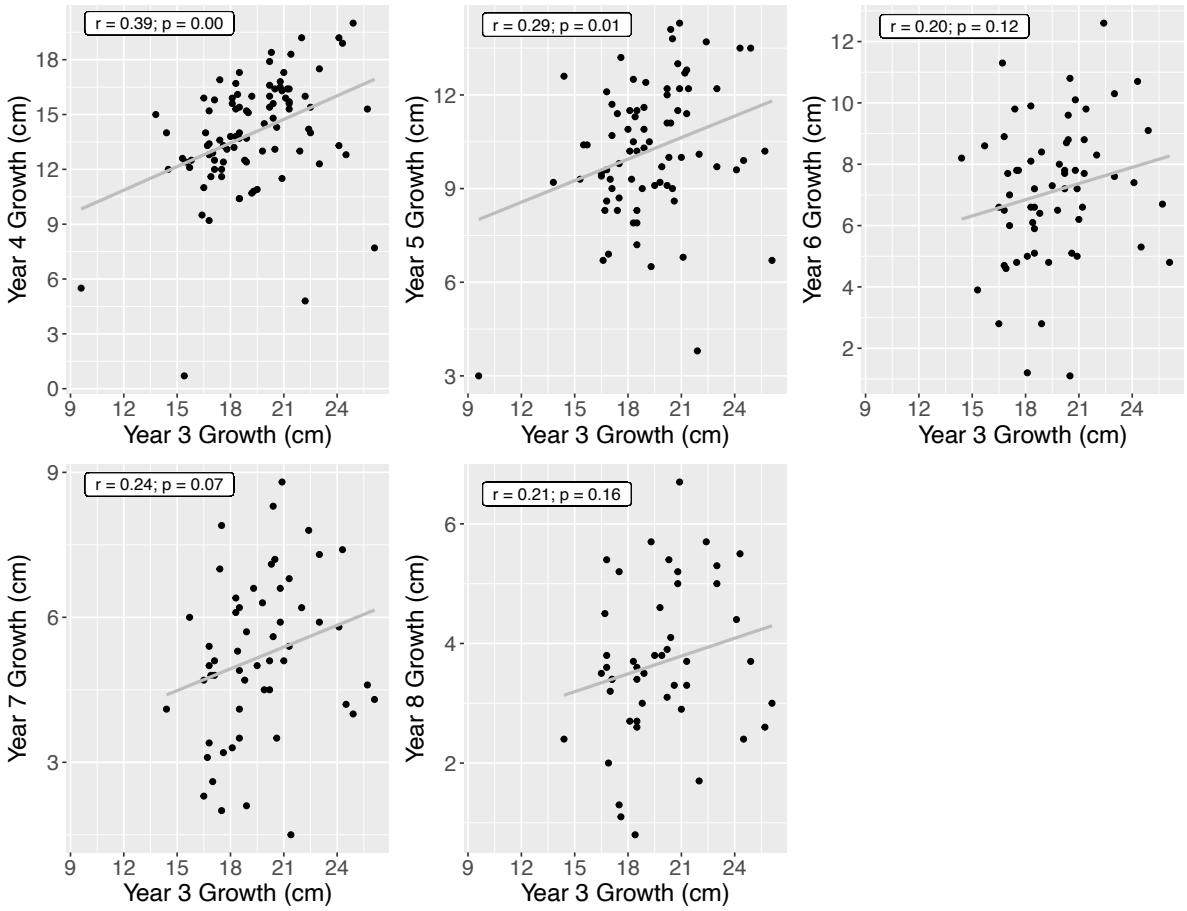

Figure S3: Scatter plots showing the relationship between year 3 growth and each subsequent year until year 8. The abbreviations in this plot are "r" for Pearson's correlation coefficient and "p" for p-values.

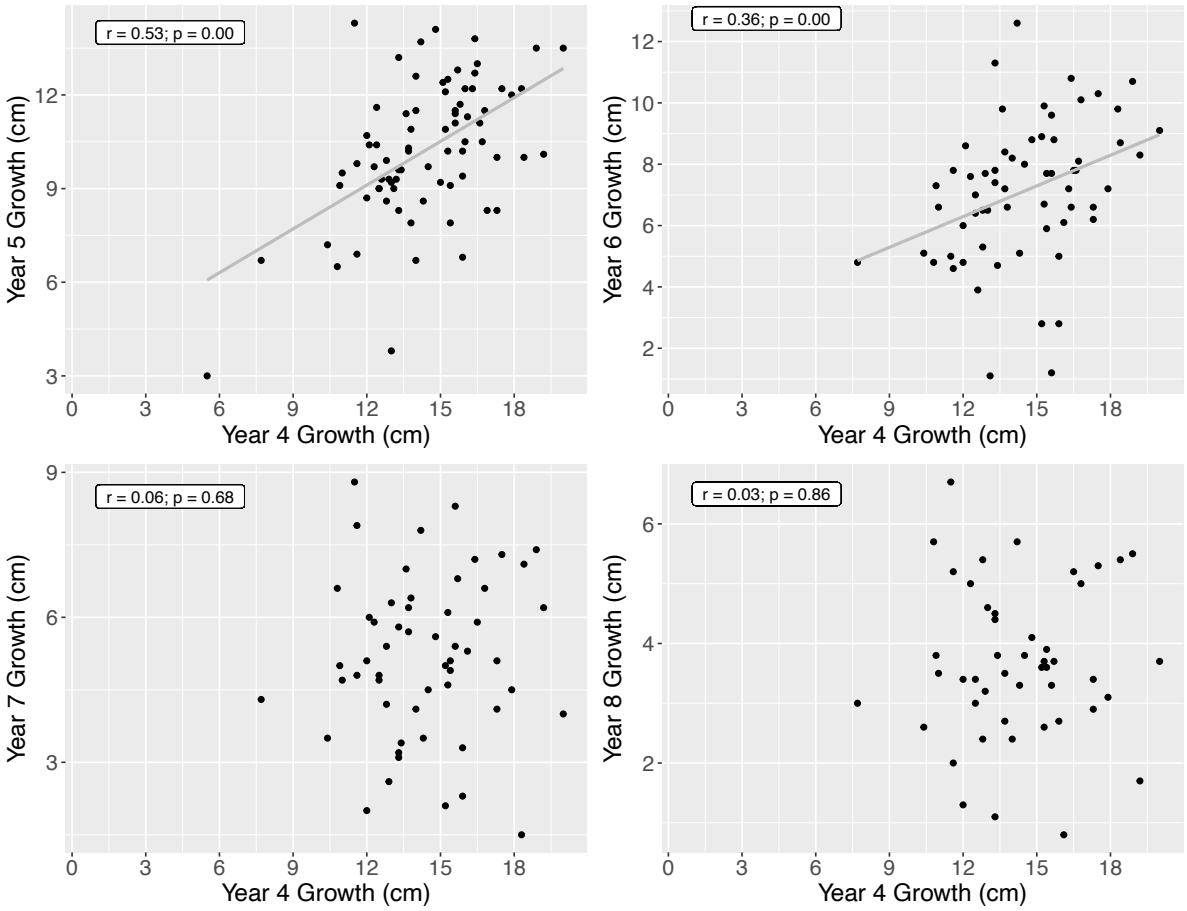

Figure S4: Scatter plots showing the relationship between year 4 growth and each subsequent year until year 8. The abbreviations in this plot are "r" for Pearson's correlation coefficient and "p" for p-values.

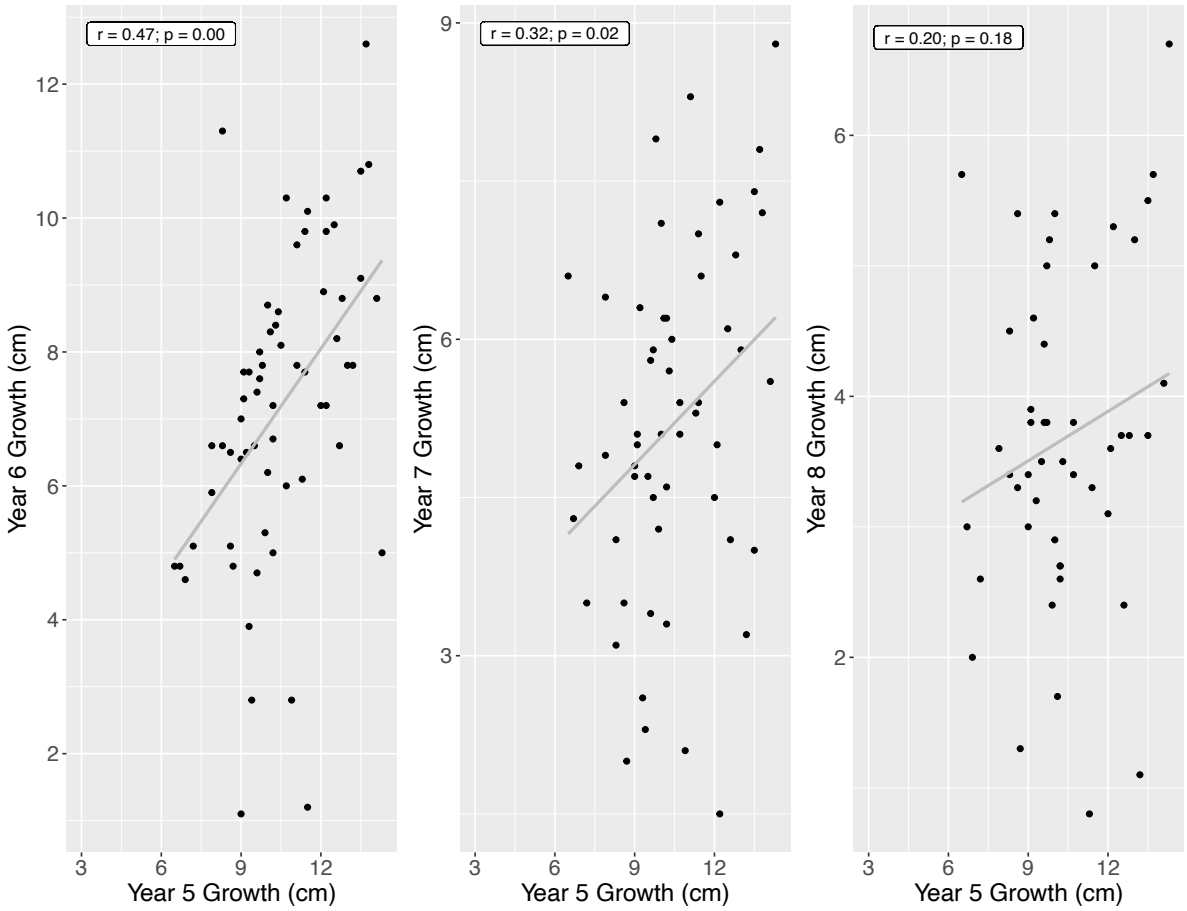

Figure S5: Scatter plots showing the relationship between year 5 growth and each subsequent year until year 8. The abbreviations in this plot are "r" for Pearson's correlation coefficient and "p" for p-values.

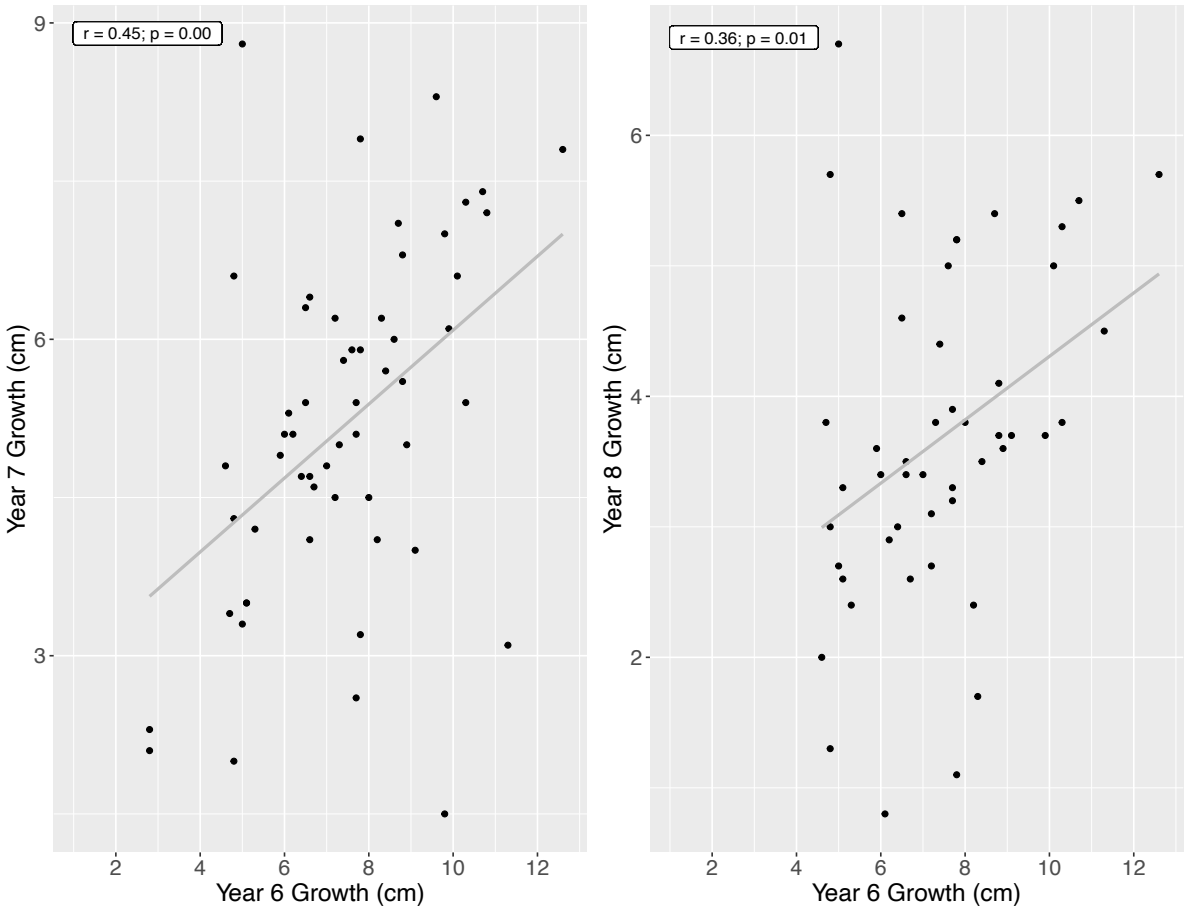

Figure S6: Scatter plots showing the relationship between year 6 growth and each subsequent year until year 8. The abbreviations in this plot are "r" for Pearson's correlation coefficient and "p" for p-values.

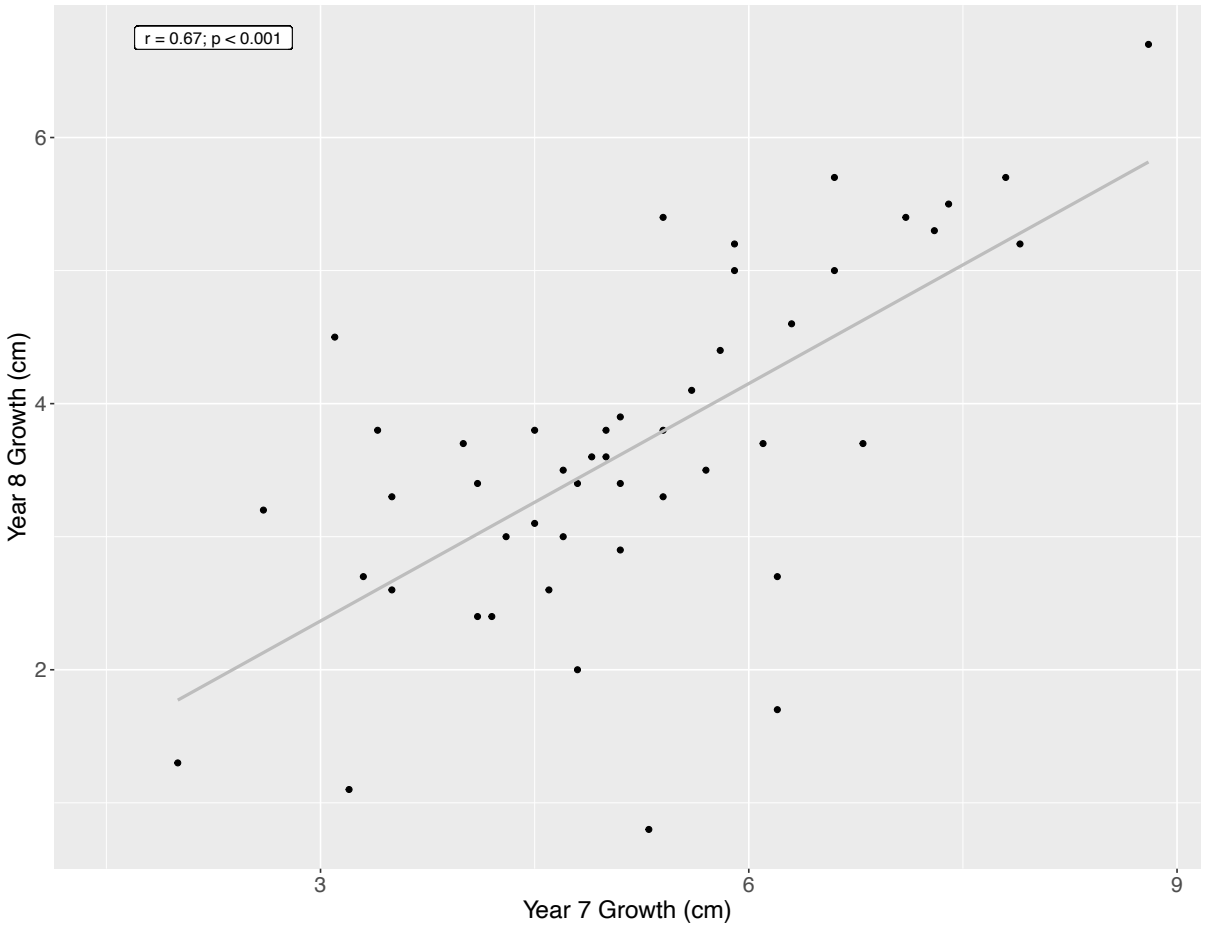

Figure S7: A scatter plot showing the relationship between year 7 growth and year 8 growth. The abbreviations in this plot are "r" for Pearson's correlation coefficient and "p" for p-value.