

Winter 12-2012

THE IMPACT OF GENETIC BACKGROUND ON BODY TEMPERATURE REGULATION IN BEEF CATTLE DURING PERIODS OF HEAT AND COLD STRESS.

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THE IMPACT OF GENETIC BACKGROUND ON BODY TEMPERATURE
REGULATION IN BEEF CATTLE DURING PERIODS
OF HEAT AND COLD STRESS.

By

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A THESIS

Presented to the Faculty of
The Graduate College of the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Master of Science

Major: Animal Science

Under the Supervision of Professor Matthew L. Spangler

Lincoln, Nebraska

December, 2012

THE IMPACT OF GENETIC BACKGROUND ON BODY TEMPERATURE
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University of Nebraska, 2012

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Cattle are reared in environments that differ and vary greatly in climate, thus the ability to regulate body temperature across multiple environments is essential. However, inherent differences between animals do exist and can influence their response to extreme temperatures. The objectives of the current study were to model the impact of myostatin genotype (MG) on body temperature during heat and cold stress and conduct a genome-wide association study (GWAS) to better understand the genetic basis of body temperature regulation during extreme temperatures.

Crossbred steers and heifers (n= 239) with varying degrees of Piedmontese influence were fed in four groups over a two-year period, where groups 1 and 3 consisted of calf-fed steers and groups 2 and 4 consisted of yearling heifers. Prior to arrival, animals were genotyped to determine their MG as either homozygous normal (0-copy), heterozygous (1-copy), or homozygous for inactive myostatin (2-copy). Hourly Tympanic and Vaginal temperature ($^{\circ}\text{C}$) measurements were collected for steers and heifers, respectively, for 5 days during times of anticipated heat and cold stress. A GWAS was conducted for area under the curve using hourly body temperature observations for five days and during the maximal stress cycle to where body temperature equals zero.

A genotype-by-environment interaction was found between MG and trigonometric functions (sine + cosine), with 0 copy and 2 copy animals deviating the greatest from the

average body temperature of 38.6 °C during summer and winter conditions, respectively.

Moderately negative Genomic-EBV correlations were found between winter and summer stress events ($r_{\text{GEBV}} = -0.40$ to -0.50), although a small percentage of the top 5% 1 Mb windows were in common between winter and summer stress events.

Knowledge of how a genotype responds to environmental stress can aid in the management of cattle to ensure optimal performance. Genetic antagonisms between heat and cold stress can be circumvented using marker-assisted selection, which allows for improved selection for decreased heat and cold susceptibility.

Acknowledgements

I would like to express my sincere gratitude to Dr. Spangler for his guidance during my academic program and the discussions with him regarding beef cattle production and genetics. Through his teachings and guidance I have cultivated a strong desire and passion for the field of quantitative genetics and research in general. Dr. Spangler always challenged me to work through a problem I had on my research project, which led me to a deeper understanding of statistical modeling and programming. Furthermore, the discussions I had with him regarding livestock genetics has given me a great understanding of the current status of genetic improvement and where it may go in the future.

Also, I have thoroughly enjoyed the guidance and discussions I have had with Dr. Nielsen regarding the modeling aspects of my project and beef cattle genetics. I have been a teaching assistant for three of his classes, which has fostered a strong passion to teach and interact with the students. I would also like to thank Dr. Ciobanu for his assistance in the lab and working with GenSel. I have truly enjoyed learning lab techniques and being able to bridge the gap between quantitative and molecular genetics. I would also like to thank Dr. Kachman for aiding in my learning of statistical modeling and a deeper understanding of estimating variance components and programming in ASREML. Lastly, I would like to thank Dr. Kuehn for his guidance and support during my project.

Throughout this entire process my mom, dad and brother have given me great support and I couldn't ask for a better family. They have taught and showed me values to live by, which have always allowed me to excel at whatever I do. I really enjoyed my experience at UNL and couldn't ask for a better committee and group of faculty members to interact with.

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Introduction

Beef production is unique in that animals are kept in an extensive environment with minimal environmental modifications, unlike what is seen in dairy, swine and poultry production. Thus, beef cattle are reared in environments that differ greatly in temperature, humidity, and wind speed, which has forced cattle to be regionally adapted, thus creating sensitivity to environments that differ greatly from the adapted environment. This potentially decreases their production efficiency in un-adapted environments and usefulness across multiple regions or in international breeding programs (Hahn, 1999; Young, 1983). Consequently there has existed a long-term pursuit to develop breeds of cattle that can tolerate extremes in both directions while maintaining a high level of productivity and possessing superior carcass attributes (Scharf et al., 2010).

The objective of the current study was to model the impact of myostatin genotype (MG) on body temperature during periods of heat and cold stress. This will enable a general understanding of the degree to which the genetic background, here determined by the large effect myostatin mutation, interacts with the environment. Also, the animals were genotyped with the Illumina-BovineSNP50 panel in order to conduct a genome-wide association study (GWAS) to better understand the genetic basis of body temperature regulation during periods of heat and cold stress.

Suboptimal body temperature regulation during periods of extreme temperature events has deleterious effects on growth, feed efficiency, reproduction, and animal welfare (Hahn, 1999). Currently breeders mitigate the risks associated with heat or cold stress by using knowledge of breed strengths relative to heat or cold tolerance but direct selection of animals within breeds is currently not possible. The investigation of genetic components of environmental (temperature) tolerance or adaptation could allow for the development of novel

indicator traits that can aid in the selection for Economically Relevant Traits (ERT) such as fertility, disease resistance, and feed efficiency across varying environments. Alternatively, susceptibility to environmental stress may be decreased by identifying and selecting for animals within a population that have a greater genetic threshold for heat and/or cold extremes instead of relying on inherent average breed effects. Knowledge of genetic components of body temperature could also be used to improve the efficiency and fitness of animals through environmentally specific management decisions.

Literature Review

Modes of Heat Exchange from Animal to Environment

An animal must continuously interact with its thermal environment through heat exchange processes to remain near its set body temperature. A beef cow has an average body temperature ranging from 38.55 to 38.6°C, and a rise or fall of 1°C in body temperature in cattle is sufficient to produce detectable changes in a number of physiological processes (McDowell, 1972). To maintain this temperature in such narrow limits requires sensitive and immediate acting mechanisms. An animal is said to be in its thermoneutral zone when it is in a temperature range that requires the least thermoregulatory effort, and temperature regulation is achieved by nonevaporative physical processes alone (Hillman, 2009). The thermoneutral zone is bounded by a lower and upper critical temperature, which is dependent on the interaction between multiple environmental parameters (i.e. wind, humidity, ambient temperature). Once past the upper and lower critical temperature an animal is under heat or cold stress, respectively. When an animal is in its thermoneutral zone, the variance among animals in body temperature is small, and as the temperature exceeds the species thermoneutral zone, the variance increases due to differences among animals in their ability to cope with heat or cold stress (Hahn et al., 1990). These differences are manifested through a complex interaction between anatomical, physiological, and behavioral factors which are dependent on the life stage, nutrition, genetics, previous degree of heat or cold stress, and health of the animal creating a dynamic thermoneutral zone (McDowell, 1972; Hahn, 1999).

Behavior changes, such as seeking shade or sheltering themselves from the wind, are the first mechanism to account for heat lost or gained (Hillman, 2009). If behavioral changes do not minimize the heat lost or gained, non-evaporative physical processes that involve the exchange of heat between an animal and its environment are used, which include conduction,

radiation, and convection (McDowell, 1972). Resistance to conductive (i.e. passage of heat energy from particle to particle) heat transfer is proportional to the temperature gradients within the animal and the outer extremities and environment (McDowell, 1972; Finch, 1986). The act of heat flow originating from the core and spreading to the skin is known as tissue conductance (Finch, 1986). As an animal increases in weight, its tissue conductance decreases linearly and it becomes more susceptible to heat stress while decreasing its susceptibility to cold stress (Finch, 1985). This is due to smaller sized animals having a larger surface area per unit of body weight making them lose heat more rapidly than larger animals (McDowell, 1972). During cold stress conditions the opposite occurs due to the animal wanting to retain its body heat, while the environment is absorbing it due to the differing temperature gradients. The animal accounts for this loss of heat by increasing its maintenance energy requirements in order to produce extra heat at a rate of 1% for each 1 °C reduction in effective temperature below its thermoneutral zone (Hicks, 2007). Newborns with reduced insulation and feed restricted animals are the most susceptible to cold stress, while adult ruminants on full feed with sufficient thermal insulation are cold hardy in dry, still conditions (Young, 1983).

The temperature gradient between the outer extremities and environment are influenced by the rate of air flow across the skin and physical properties of the animal coat (Finch, 1985). Olson et al. (2003), found evidence of a major gene affecting hair length in two South American heat tolerant *Bos Taurus* breeds, Senepol and Tuli. The gene referred to as “slick hair”, produces a very short and sleek coat which allows for increased heat loss. It has been shown that *Bos taurus* animals with darker hair coats have a warmer internal body temperature (Finch et al., 1984) and body surface temperature (Brown-Brandl et al., 2006) than their light colored counterparts. Arp et al. (1983), found similar results when comparing surface body temperature for black, red, and white hided animals with black and red hided being 11.6°C and

9.1°C warmer than white hided animals. During cold conditions a heavier hair coat will impede the arrival of cold air and warm air will remain, which allows for an animal to retain body heat.

The ability of an animal to internally direct heat outward or inward via conduction coupled with convection (i.e. heat exchange through a liquid or gas) is accomplished by vasodilation or vasoconstriction of blood vessels near the skin and lungs. The process of removing heat via the bloodstream becomes increasingly important as body heat rises due to a decreased core to skin gradient (McDowell, 1975). Furthermore, an increase in blood supply to the skin causes a concurrent increase in evaporative heat loss via sweating (Ingram et al., 1963).

An animal first exposed to an adverse environment reacts initially by activation or acceleration of non-evaporative processes to remain at thermal equilibrium, which involves short-term adaptive changes in behavior and physiology, such as seeking shade or increased peripheral blood flow during heat stress (Nienaber and Hahn, 2007). On a cellular level a short term response is referred to as thermotolerance and is defined as an organism's ability to survive an otherwise lethal heat stress from a prior heat exposure sufficient to cause the accumulation of heat shock proteins (HSPs) (Moseley, 1997). Expression of HSPs results in the repair of damaged proteins, anti-apoptotic effects via the chaperone pathway and apoptotic effects (Pirkkala et al., 2001).

If non-evaporative physical processes fail to keep an animal at thermal equilibrium, evaporative processes take over (Hahn, 1999). Resistance to evaporative heat transfer (i.e. vaporization of water from body surface and respiratory tract) is a function of the gradient through which the water vapors move (Finch, 1986). Evaporative heat transfer is not dependent on the temperature, which becomes important when the environment is warmer than the animal's body temperature and would result in the inward flow of heat from the environment to the animal (Davis et al., 2003). Water can be made available to the skin surface by simple

transudation through the superficial layer from the underlying tissues (i.e. insensible perspiration), activity of the sweat glands, or by external applications. Animal factors that affect the efficiency of evaporative heat loss from the skin surface are sweat gland density, function and morphology, hair coat density, length, and color and regulation of epidermal vascular supply (McDowell, 1972; Carvalho et al., 1995; Collier et al., 2008). A rise in respiratory heat loss through panting is one of the first physical signs of an animal experiencing heat stress (Nienaber and Hahn, 2007).

As a consequence of an animal's inability to regulate body temperature, inefficient measures commence that bring about a decrease in production. A decrease in feed efficiency often occurs due to more energy being used for thermoregulatory processes or to limit heat production during heat stress conditions. Also, a heat or cold stressed animal's immune system becomes suppressed and their cellular proteins lose their structure and function causing an increased susceptibility to sickness. These negative consequences cause a decrease in overall production efficiency due to energy being used for processes other than growth or immune regulation, which cause an animal to spend more days on feed. Lastly, cold or heat stress has deleterious effects on female and male fertility (Hahn, 1999).

After 2 to 4 days of heat or cold exposure depending on the individual animal and the degree of heat or cold exposure, mobilization of heat dissipation or retention functions (physiological coping) will have progressed to the point that acclimation is apparent (Hahn et al., 1990). Phenotypic acclimation is defined as the "within lifetime phenotypic response" to environmental stress and relies heavily on the endocrine system (Collier et al., 2008). An animal can attain heat or cold tolerance through previous generations of natural selection or within its lifetime by using alternative pathways that have variable penalties on productivity. The entire process of acclimation takes around 8 days and is dependent on the animal and degree of heat

or cold exposure. Once completed, the animal's body temperature fluctuates around a new set point (Hahn et al., 1990).

Behavioral and physiological processes aid in keeping an animal's body temperature near its set point, but under severe conditions, heat gain or loss is usually greater than an animal can remove or produce to equalize heat lost to heat gained. Due to this difference an animal stores extra or lacks enough body heat until the severity of the stress decreases. For example, an animal is aided by cooler nighttime temperatures during summer conditions or warmer daytime temperatures during winter conditions. These night and day low stress intervals during summer and winter conditions, respectively, allow for an animal to remove excess heat accumulated during the day or gain body heat which was lost during the night (Mader et al., 2006). In a study conducted by Lefcourt and Adams (1996, 1998), the authors found there to be a consistent circadian rhythm across all animals, and during cold or heat stress conditions, a disruption in the typical pattern occurs which affects the amplitude and mean of the diurnal curve. These effects create a diurnal rhythm, which infers one measurement may not be indicative of how an animal responds to extreme temperatures, but instead, a continuous measurement throughout the day is needed (Lefcourt and Adams, 1996; Lefcourt and Adams, 1998).

Historically, heat tolerant research has involved comparing the phenotypic differences within and between heat-tolerant *Bos indicus* cattle and heat-intolerant *Bos taurus* cattle in controlled or natural environments (Finch, 1985; Finch, 1986; Brown-Brandl et al., 2004; Thrift et al., 2004; Gaughan et al., 2009). Previous cold tolerance research focused on understanding the effects of adverse cold conditions on various production traits using cold tolerant *Bos taurus* cattle (Young, 1983; Hicks, 2007). Multiple indicator traits taken at a single time point or across multiple time points have been used to assess the ability of an animal to regulate body

temperature during periods of heat or cold stress. Examples include panting score, tympanic temperature, respiration rate (Gaughan et al., 2009), rectal temperature, sweating rate (Finch, 1986), radiotelemetry (Lefcourt and Adams, 1996; Lefcourt and Adams, 1998) and dry matter intake (DMI) (Young, 1983).

Tympanic temperature recording is a relatively sensitive non-invasive technique that uses a device that can be placed in an animal for an extended period of time. The tympanic membrane is near the hypothalamus, which is vital to the regulation of immunological and endocrine functions, has a central role in regulating feed intake, and is associated with the maintenance of body temperature. Tympanic temperature readings have been shown to be more reflective of an animal's actual core body temperature and are unaffected by the fluctuations in fill in the lower gut in comparison to rectal temperature or a rumen temperature bolus (Guidry et al., 1966; Hahn et al., 1990). Another valid internal temperature measurement is vaginal temperature. In studies by McGee et al. (2008) and Bergen and Kennedy (2000), the authors found the phenotypic correlation between vaginal and tympanic temperature to be 0.83 and 0.77, respectively. These two processes provide a thorough examination of an animal's ability to cope with stress across differing environments and to decipher the differences across animals in their coping mechanisms.

McGee et al. (2008) used hourly tympanic and vaginal body temperature measurements to further understand the effects of maximum 48-hour Temperature-Humidity Index (THI) values on the diurnal cycle seen in cattle. To smooth out the diurnal cycle, McGee et al. (2008) used a trigonometric function $(\beta_1 \sin(2\pi i/S_m) + \beta_2 \cos(2\pi i/S_m))$, where "i" is the particular hour of the observation and "S_m" denotes the length of the periodicity for the mth cycle (Fuller, 1976). McGee et al. (2008) found that as the environmental temperature increased, so did the amplitude and mean of the diurnal cycle and body temperature variance.

Internal body temperature measurements can be used to indicate the degree of heat or cold stress that an animal is experiencing, and selection or management decision can be made based on the results. The results can then be used to select for decreased susceptibility to heat and/or cold stress. Selection for decreased susceptibility would broaden the temperature threshold for a population, which in turn would reduce the occurrence of deleterious effects during heat or cold stress conditions. A simulated selection scheme by Nardone and Valentini (2000), compared selection for heat tolerance within a high milking breed and milk production within a highly adapted breed. The authors found that selection for heat tolerance within the high milking breed was more efficient due to the adapted breed needing several generations (30 plus) to reach comparable levels of milk production to the high production breed.

A continuous internal body temperature measurement is difficult and expensive to measure, thus identifying and using genetic variants for selection and management purposes is highly applicable. Furthermore, the same methodology used by McGee et al. (2008) can be used to predict the effects of a specific genotype on body temperature during periods of heat or cold stress. Knowledge of a gene having variable effects on the phenotype depending on the environment would be beneficial for cattle feeders to implement management strategies based on the genotype of the individual or group. Additionally, breeders can select for genotypes that have increased levels of fitness given the predicted production environment of their customers' or own location.

Measurements used as Predictors for Heat Stress and Cold Stress

Multiple factors influence the amount of heat lost or gained in a certain environment, with one of these being the external conditions. An example would be the cumulative effects of ambient temperature, relative humidity (RH, %), solar radiation (RAD, Kcal/-m²) and wind speed (WSPD, km/hour). Over the years multiple combinations of these effects have been used to

create an index value that takes into account multiple external factors in order to accurately predict the heat load for a specified time period. Temperature indices allow for the implementation of management steps to improve the performance, health, and well-being of the animals during times of adverse conditions.

One of the first individuals to try to quantify the predicted heat load was Thom (1959). At the time it was called the “Discomfort Index” (DI) and was a measure designed for human discomfort. Since then, the DI has been adapted and renamed the temperature humidity index (THI) and derivations have been introduced for use in domesticated animal populations. The THI has been widely used as an indicator of thermal stress in livestock for the past forty years. Other THI derivations have been developed using dry bulb temperature in combination with wet bulb temperature, relative humidity, or dew point (Gaugnan et al., 2008).

Dry-bulb temperature (T_{DB} , C°) quantifies the air (ambient) temperature while disregarding the temperature due to radiation and moisture. Wet bulb temperature (T_{WB} , C°), relative to dry bulb temperature, is a measurement of the amount of moisture in the air (Thom, 1959). RH is a measure of how much moisture is present compared to how much moisture the air could hold at that temperature (Shelton, 2008).

THI formulae:

- $THI_1 = [0.8 \times T_{DB}] + [(RH / 100) \times (T_{DB} - 14.4)] + 46.4$. (Mader *et al.*, 2006)
- $THI_2 = 0.72 * (T_{DB} + T_{WB}) + 40.6$ (NRC, 1971)

A common method of quantifying THI values is to arrange them into a table to serve as a benchmark to assess the predicted heat severity, referred to as the Livestock Weather Safety Index (Mader et al., 2006). The THI formulae previously outlined can be effectively used as an indicator of an animal’s susceptibility to heat stress, but there are some drawbacks to the formula. Two major drawbacks are the inability of the model to account for the effects of WSPD

and RAD. The RAD can significantly affect an animal by increasing the skin temperature and disrupting conductive heat transfer, while WSPD can alter convective cooling. Work done by Mader et al., (2006) used panting score to determine the adjustments to THI for WSPD and RAD. The adjusted THI using hourly weather conditions is: $[4.51 + \text{THI} - (1.992 * \text{WSPD}) + (0.0068 * \text{RAD})]$. At elevated WSPD, THI values can be reduced by greater than 10 units compared with the case when no adjustments were made and elevated RAD can increase THI by approximately 5 units compared with low RAD (Mader et al., 2006).

The THI is an index based on environmental conditions and does not account for animal characteristics such as breed, coat color, management practices, or the cumulative effect of heat load and natural cooling (Gaughan et al., 2008). Examples of management practices that affect an animal's heat load include access to shade and water temperature. To account for these shortcomings Gaughan et al., (2008) developed a heat load index (HLI) based on panting score, respiration rate and tympanic temperature that included adjustments for the index temperature at which an animal will experience heat stress based on the breed of the animal, coat color, and management practices. Another model was developed called the Accumulated Heat Load (AHL) and it incorporates time and animal heat load or the amount of time the animal is exposed to an HLI above its threshold. This addition is important because it indicates when an animal is unable to dissipate heat based on the HLI model. Individual animals can be further adjusted based on percentage influence of *Bos indicus* or *Bos taurus*, coat color, number of days on feed, and management practices including depth of manure pack, drinking water temperature, and the degree of shade in the pens (Gaughan et al., 2008).

For cold conditions there has been an index designed by Sipple and Passle (1945) called the Wind Chill Index (WCI) that accounts for the combined effects of T_{DB} and WSPD in bare skinned animals. In 2001 the WCI was improved with a biologically based formula which is:

$$\text{WCI} = 13.112 + (0.6215 * T_{\text{DB}}) - (11.37 * \text{WSPD}^{0.16}) + (0.3965 * T_{\text{DB}}) * \text{WSPD}^{0.16} \text{ (Tew, 2002).}$$

The indexes discussed previously are geared toward either summer or winter conditions, but not the combination of the two. Mader et al. (2010), derived an index termed Comprehensive Climate Index (CCI) that is designed for extremes in either direction and adjusts ambient temperature for the combined effects of RH, WSPD, and RAD. The equation is based on hourly environmental conditions within the range of -30 °C to 45°C and accompanied with thresholds for cold and heat stress dependent on the susceptibility of the animal(s) in question.

Genetic parameters for body temperature and relationship to other production traits:

Animal variation has been shown to exist for body temperature regulation during periods of temperature related environmental stress in beef cattle (Burrow, 2001; Da Silva et al, 1973; Mackinnon et al., 1991; Turner, 1982; Turner, 1984), dairy cattle (Dikmen et al., 2012; Ravagnolo and Misztal, 2000; Ravagnolo and Misztal, 2002; Seath, 1947) and pigs (Zumbach et al., 2008). The heritability of various indicators of body temperature regulation during periods of heat stress has been heavily studied while minimal research has been conducted during cold stress conditions. Burrow (2001) estimated the heritability of repeated measurements of log transformed rectal temperature to be 0.17 on a composite breed of tropical cattle when ambient temperatures exceeded 30°C. In the same study a favorable genetic and phenotypic relationship was found between rectal temperature and period weights (-0.11 to -0.26 and -0.05 to -0.13, respectively) and period weight gains (-0.12 to -0.49 and -0.06 to -0.08, respectively). Low to moderate favorable genetic relationships between rectal temperatures and pregnancy status of the first 3 parities (-0.16) and days to calving once the bull entered (0.16) have been shown to exist (Burrow, 2001). Turner (1984 and 1982) estimated the heritability of repeated measurements of log transformed rectal temperature to be of 0.33 and 0.25 on *Bos indicus*, *Bos taurus* and crossbred lines when the daily maximum ambient temperature was approximately

30°C. A strong favorable genetic correlation (-0.76) between log transformed rectal temperature and fertility, measured as success or failure in producing a calf at term has been shown to exist (Turner, 1982). Da Silva et al. (1973) estimated the heritability for the tropically adapted Canchin breed to be 0.11 (± 0.16) and 0.44 (± 0.27) for initial and increase in rectal temperature during a heat stress event. Mackinnon et al. (1991) estimated the heritability of a single rectal temperature measurement to be 0.19 for *Bos indicus* and *Bos taurus* lines when the daily maximum ambient temperature was approximately 30°C. Dikman et al. (2012) estimated the heritability of a single rectal temperature measurement in Holstein cows to be 0.17 (± 0.13). Seath (1947) estimated the heritability for repeated measurements of rectal temperature in Holstein cows to be 0.151 and 0.309 for the years of 1944 and 1945, respectively. Seath et al. (1947) estimated the repeatability for repeated measurements of rectal temperature in Holstein cows to be 0.152 and 0.385, respectively.

An alternative strategy instead of relying on body temperature measurements is to use a test-day model (i.e. conception status at day 90, milk production at day 90 etc.) with random regressions on a heat stress function (Misztal, 1999; Ravagnolo and Misztal, 2000; Ravagnolo and Misztal, 2002). Ravagnolo and Misztal (2000) utilized the test-day model for milk production across varying degrees of heat stress in dairy cattle and estimated the additive variance to be zero during periods of no heat stress, but increased as the heat stress increased in severity. In the same study the additive genetic variance for heat stress was as large as the additive genetic variance for milk production at a THI of 86.

From these studies it has been established that there is a genetic component to the ability of an animal to regulate its body temperature (h^2 of 0.11 to 0.44) through the use of various indicator traits. The genetic correlation between components of body temperature

regulation and various production traits were favorable, suggesting measures of body temperature could serve as useful indicators to improve various economically relevant traits.

Use of genomics in the improvement of quantitative traits in beef cattle:

The traits of importance in domestic animals are for the most part quantitative or complex in nature. The classical model of quantitative traits states the phenotypic value is controlled by an infinite number of genes each with an infinitesimal small effect as well as by non-genetic or environmental factors (Fisher, 1930). Under this model it is nearly impossible to establish the genotypes of all loci that affect a trait, instead a prediction of the total additive effect of all the genes an animal carries is calculated, referred to as an estimated breeding value (EBV). Traditionally, genetic value predictions have been based on the use of dense phenotypes containing the animals and relatives with prior knowledge of the heritability for the particular trait. This approach has been effective and tremendous genetic and phenotypic gains have occurred for a number of economically relevant traits. This reliance on dense recording of phenotypes is limiting for traits that are sex specific (milk yield), measured late in life (longevity), expensive to measure (e.g. methane production, disease resistance, etc.), can only be measured after harvest (meat quality), or have a low heritability (fertility) (Dekkers and Hospital, 2001). In order to increase the accuracy of selection for these traits based on traditional selection schemes requires progeny or sib-testing practices, which increases generation interval. For these particular traits, accuracy of selection can be increased and generation interval decreased through the use of genomic information to supplement traditional information, which in turn will increase the annual rate of genetic change (Meuwissen et al., 2001). Because the ability to regulate body temperature during hot and cold conditions is difficult and expensive to measure, it lends itself to a genomics approach. Genomics can be used to locate genomic regions within a population that make an animal less sensitive to heat or cold extremes and then select

individuals based on the marker(s) identified. Furthermore, identification of genetic markers that make an animal less sensitive to heat or cold extremes in thermally tolerant breeds allows for improved introgression of the marker into thermally intolerant breeds (Dekkers and Hospital, 2002; Ravagnolo and Misztal, 2002).

The approach of locating genes or markers that affect production traits was first attempted by Sax et al. (1923) for bean weight. The author demonstrated that the effect of an individual locus on bean weight could be isolated by a series of well thought out crosses. After this experiment, limited experiments were conducted on Quantitative Trait Loci (QTL) mapping in part because at the time there was a lack of abundant segregating genetic markers available for livestock species (Weller, 2009). A QTL is the estimated position of a marker that contributes to variation in a trait. A quantitative trait is one that depends on the cumulative effects of many genes and the environment and can vary among individuals over a given range to produce a continuous phenotype (Goddard and Hayes, 2009). The available markers at the time were morphological markers, blood groups and protein polymorphisms with the use of Southern blotting as the preferred method for genotyping (Neimann-Sorensen et al., 1961; Larsen, 1971; Southern, 1975). During this time linkage studies involving production traits with the previously mentioned markers were undertaken, but complete genome analysis was not possible due to the limited coverage of the available markers (Weller, 2009).

Grodzicker et al. (1975) introduced a new type of marker at the DNA-level referred to as restriction fragment length polymorphisms (RFLP), in the Adenovirus for temperature sensitivity. RFLP were more abundantly spaced across the genome, which prompted the creation of a sparse genome-wide map in multiple livestock species. Before this breakthrough, prior knowledge of the gene of interest that caused the phenotype and multiple polymorphisms spread throughout the proposed gene were needed in order to elucidate the causative

mutation. This technique made things cumbersome due to the limited knowledge of the genome at the time (Botstein, 1980).

From this sparse map, linkage analysis within full-sib or half-sib families was performed to identify variants associated with a trait of interest and selection could be practiced for the advantageous allele of the identified variant (Weller, 2009). Linkage analysis is based on the use of family data due to family members having a higher than expected level of sharing of genetic material near the gene that influences a phenotype (Feingold, 2001; Botstein, 1980). Linkage analysis locates the area of interest by testing if a marker and the trait or disease of interest show a correlated transmission within a pedigree starting from the common ancestor (Lander et al., 1994). Due to family members having a higher degree of sharing of genetic material, isolation of the causative mutation is not needed, instead just a marker that is sufficiently close and linked to the causative mutation. If the marker is linked with the causative mutation (within a family) it will show a high correlation with the phenotype even though it is not the true cause of the phenotype and selection can be practiced on the linked marker. Linkage analysis in domesticated animals increased in the 1990's by the identification of abundant highly polymorphic microsatellite markers across the genome and the application of polymerase chain reaction (PCR) to amplify any particular short sequence (Risch, 2000; Weller, 2009; Weber, 1989).

Linkage analysis narrows the predicted location of the QTL, but the region identified often contains multiple genes spread across many mega-bases of DNA, which limits the use of the marker information in animal breeding programs (Goddard and Hayes, 2009). In order to fine-map a region associated with the trait of interest, a positional cloning or a positional candidate gene approach using linkage dis-equilibrium (LD) mapping techniques is undertaken (Cardon and Bell, 2001; Andersson and Georges, 2004). LD is the nonrandom relationship

between alleles present at two or more loci and mainly reflects the recombination history in a population for a specific haplotype (Conner and Hartl, 2004). The degree of LD surrounding the causative mutation is dependent on multiple factors including regional variability in recombination patterns, effective population size, mutation age, and population admixture (Botstein and Risch, 2003; Cardon and Bell, 2001). LD mapping approaches are based on saturating the location with variants and as the markers get further away from the QTL the amount of LD decreases. In a positional candidate approach specific genes or variants are examined on the basis of their relation to the phenotype. In contrast, a positional cloning approach, selected markers are evaluated based purely on the proximity to the estimated location of a QTL (Cardon and Bell, 2001). In either approach, having or producing the physical map surrounding the proposed position is critical. Before the advent of information derived from dense sequencing of multiple organisms, radiation hybrid and clone based mapping using large-insert yeast artificial chromosomes or bacterial artificial chromosome libraries were used to obtain a physical map of the area of interest (Botstein and Risch, 2003).

It was soon realized that linkage analysis was not as efficient for finding causative mutations for complex or multifactorial phenotypes compared to simple or monogenic traits. A major drawback of this approach was that the QTL was mapped imprecisely and the linkage phase varied between families. LD is population specific inferring that a marker may be in LD with the causative mutation in one population, but not in another population. Due to this specificity, the linkage phase between a marker and QTL had to be determined within each family before the marker could be used for selection (Goddard et al., 2010). Furthermore, quantitative traits are controlled by many genes and consequently the benefit from MAS is limited by the proportion of variance explained by the marker (Meuwissen et al., 1996). Also, once you found a QTL it was difficult to fine map the prospective area due to the limited

knowledge of the location of genes in domesticated animal genomes, a limited number of known variants to saturate the area with, and the proposed region may have multiple genes (Andersson and Georges, 2004).

The landscape of this arena changed with the discovery of a large number of single nucleotide polymorphisms (SNP) derived from sequencing multiple livestock species, HapMap studies, and reduced representation library sequencing (Fan et al., 2010). The sequencing of multiple livestock species allowed for the position of genes across the genome to be known which created the infrastructure to allow fine mapping to be more efficient. The discovery of a large number of SNP also prompted the creation of high-throughput genotyping platforms of varying sizes (i.e 384, 50k, 770k in cattle) that evenly covered the entire genome (Fan et al., 2010). Since the advent of the BovineSNP50 (54,609 SNP; Illumina Inc., San Diego, CA) and BovineHD (e.g. 770,000 SNP; Illumina Inc., San Diego, CA), genome-wide associations studies (GWAS) have become possible. These GWAS are performed by genotyping a subset of a population that is phenotyped for the trait of interest. By having both genomic and phenotypic information on a subset of animals, it is then possible to determine SNP effects and select the most informative SNP to build low-density assays or locate genomic regions that are associated with the trait of interest. The association of a QTL is detected by it being in LD with a nearby marker on a population-wide level. Cryptic associations may be caused by relationship between individuals or an admixed population (Goddard and Hayes, 2009). Interest was instigated by Risch and Merikangas (1996), when they noted that GWAS have far greater power than linkage analysis to detect genetic variants with small or moderate phenotypic effects. Also the number of genetic variants used could be reduced by taking advantage of LD across the genome, which is more extensive in domesticated animals in comparison to humans (Meuwissen et al., 2001).

If the marker(s) are in sufficient LD with QTL an estimate of QTL effects can be predicted from a linear combination of the marker effects in LD with the respective QTL. Summation of marker effects for all QTL affecting a trait results in an estimated breeding value derived from genomic markers, referred to as a molecular breeding value (MBV). This can be represented as $\widehat{MBV}_j = \sum_{i=1}^N M_{ij} \hat{b}_i$, where \hat{b}_i is the estimated effect of the i th marker due to its LD with one or more QTL, m_{ij} is the genotype of the j th individual at the i th marker and N is the number of markers (Goddard et al., 2010). In this case the focus is on inference of genetic value, rather than detection of QTL. Therefore the main challenge is relating phenotype to SNP genotypes (thousands of possibly highly confounded/correlated covariates), to polygenic additive genetic values, and to other nuisance effects (i.e. sex, age, year) simultaneously (Gianola et al., 2006).

An estimate of marker effects can be derived via least-squares analyses, but many effects have to be estimated (e.g. 50,000+ for the 50k bovine chip) simultaneously from a small number of records (e.g. 1,000-2,000), which leads to insufficient degrees of freedom to estimate the effects simultaneously (Lande and Thompson, 1990). Also, a large number of markers in the regression model produce co-linearity among the markers, causing unstable least-squares estimates (Whittaker et al., 2000). An alternative is to use model selection to reduce the number of markers in the model based on some predetermined criteria to keep or remove markers. However this approach leads to over-predicting the markers with the largest effects, and if an effect falls just below some threshold value, it is entirely removed from the model (Meuwissen et al., 2001; Xu, 2002).

Whittaker et al. (2000), proposed an alternative to least-squares model selection approaches, using ridge regression. Ridge regression assumes that marker effects are independent and normally distributed. The fixed marker effects are estimated simultaneously and all markers are uniformly shrunk toward zero, with the degree of shrinkage determined by

lambda (λ). Lambda is chosen by the researcher and is used to reduce the co-linearity between markers. The inclusion of a shrinkage parameter allows for markers with close-to-zero effects to be shrunk to zero (Whittaker et al., 2000).

If the markers are used to calculate the relationship between individuals instead of using the pedigree it is referred to as genomic-BLUP (GBLUP) (VanRaden, 2008). This can be represented by an animal model of the form, $y = Wf + Zg + e$, where y is a vector of phenotypic values, f is a vector of fixed effects and g is a vector of breeding values and W and Z are incidence matrices relating effects (i.e. markers or contemporary group effects) to individual records. In the traditional BLUP model the relationship matrix (A matrix) or the proportion of the genome that two individuals share, is estimated from the pedigree using the expected average relationship value. The expected average relationship is derived assuming alleles are identical by descent (IBD), indicating that they descend from the same ancestor derived from a base population (Nejati-Javaremi et al., 1997). In contrast, the A matrix in GBLUP is estimated from the markers and is derived from alleles being identical by state (IBS), thus estimating the realized proportion of the genome that two individuals share (Goddard et al., 2011; Hayes et al., 2009). Thousands of SNP (i.e. 10,000 +) are used in GBLUP and ridge regression, which implies that all markers have small effects, which is similar to the classical model of quantitative traits (Meuwissen et al., 2001; Goddard et al., 2010).

An alternative is to take a Bayesian approach, which assumes that each marker effect is sampled from a normal distribution with mean zero and marker specific variance of σ^2 . Thus the variance across markers varies and the marker effects are shrunk in a non-linear fashion (Meuwissen et al., 2001; Xu, 2002). In the Bayesian framework, we treat everything as random variables classified into observables and unobservables. The observables include the phenotypic trait for each individual along with its marker genotypes. The unobservables include the marker

effects and the variance for each marker effect (Xu, 2002). From Bayes theorem, this can be represented as $f(\theta | \text{data}) \propto f(\text{data} | \theta) * f(\theta)$, where θ represents the unobservable marker effects and their respective variances and data represents the phenotypes and marker genotypes, $f(\theta)$, is the prior distribution of θ , which reflects the relative uncertainty about the possible values of θ before the data are realized and $f(\text{data} | \theta)$ is the likelihood function, which represents the contribution of the data to knowledge about θ (Gianola and Fernando, 1986; Blasco, 2001). The posterior distribution, $f(\theta | \text{data})$, is generated from combining information from the prior distribution and the likelihood function. From the posterior distribution, Monte Carlo Markov Chain (MCMC) simulation are used to draw samples from the posterior, and from this estimates of the marker effects and variances are obtained (Xu, 2002). As the number of observations increase, Bayesian learning allows for the prior to receive less weighting and the likelihood dominates the posterior distribution (Gianola and Fernando, 1986; Gianola et al., 2009).

Meuwissen et al. (2001) proposed two Bayesian hierarchical models, referred to as Bayes A and B. In Bayes A the prior distribution of each marker effect, given some marker-specific uncertainty variance is assumed to be normal with a null mean and dispersion parameter σ^2 . The variance associated with the effect of each marker is a scaled inverse chi-squared distribution, $\text{chi}^{-2}(v, S)$, where v is the number of degrees of freedom and S is a scale parameter (Meuwissen et al., 2001). This distribution might reflect the true situation of some variants having moderate to large effects, while most variants have small or no effect on the trait of interest (Goddard et al., 2010). Bayes A assumes that all markers have a non-zero effect, which may not be the case since the genotyping assays are saturated with thousands of markers (Goddard, et al. 2010). An alternative would be Bayes B, which assumes a proportion of the markers have no effect, represented as π . The markers that have an effect, $1 - \pi$, follow a scaled

inverted chi-square distribution that is similar to Bayes A. The π value, or proportion of markers that do not have an effect, is set by the researcher based on the genetic architecture of the trait of interest. One of the drawbacks of Bayes A and B is the inherent heavy weight of the prior information, and thus the inability of the MCMC chain to progress far from the prior information or starting values. Thus the prior will always have an effect on the amount of shrinkage toward zero of marker effects and the degree of shrinkage is dependent on the scale parameter (Gianola et al., 2009). An alternative Bayesian model is to assume a single effect variance that is common to all marker effects (Bayes C), instead of locus specific variances as in Bayes A and B (Habier et al., 2011). The prior distribution and the assumption of a proportion of the markers having no effect (π) in Bayes C, is similar to Bayes B. An extension of Bayes C is to treat π as an unknown and estimate it from the data, instead of a value chosen by the researcher (Bayes C π) (Habier et al., 2011). The difficulty is attaining a large enough sample size to estimate π from the data. Also, iterations of the MCMC chain in small data sets exchange a higher number of SNP compared to larger data sets, thus SNP with low effect will have a greater chance of being shrunk closer to zero (Habier et al., 2011). Habier et al. (2011) showed that Bayes C π allows for Bayesian learning to occur as the procedure is less influenced by the scale parameter when data are of sufficient size.

Linear combinations of marker effects have been used to estimate MBV and have allowed for the integration of genomic data into traditional EBV estimates. MBV combined with available phenotypic information is advantageous because the inclusion of the phenotypic information allows for the improvement of QTL not explained by the markers on the SNP array (Dekkers, 2007). Currently there are four methods being used to combine genomic data with phenotypic data. The first one includes integrating MBV as a correlated trait to the phenotypic trait of interest, which is similar to the way ultrasound information is currently being integrated

into carcass EVB (Kachman, 2008; MacNeil et al., 2010). As the genetic correlation increases between the MBV and trait of interest so does the accuracy whereby lower accuracy animals benefit more than higher accuracy animals. The second method would be to augment the numerator relationship matrix to include both a genomic and pedigree based relationship matrix (Goddard et al., 2011; Hayes et al., 2009). The third method includes computing independent values, both EVB via the traditional BLUP method and MBV via summation of marker effects and combining them using selection index theory and is referred to as “blending”. The weighting of the EBV and MBV is then based on their respective proportion of genetic variance explained (Lande and Thompson, 1990; Dekkers, 2007). The final method is to incorporate the MBV as external information into traditional genetic evaluations, much like incorporating external breed information into another breed association’s genetic evaluation. This method uses a Bayesian framework and the degree that an MBV impacts an individual’s EBV is dependent on the MBV accuracy. This method was first introduced to provide EBV information on F1 bulls whose parents were from different breeds (Quaas et al., 2001).

Molecular breeding values for traits where phenotypes are collected on a regular basis (i.e. birth, weaning and yearling weight) have been integrated into National Cattle Evaluation (NCE) for some breeds with others rapidly working towards this end. The challenge lies in the development and implementation of genomic selection (GS) for traits where the phenotype is not measured on a regular basis. Unfortunately, many of these traits (fertility, feed efficiency, adaptation, disease susceptibility) are of paramount importance to the beef industry. Genomic information used to enhance traditional NCE will become more important in the future to aid in developing selection tools for novel traits as those listed above where phenotypic data is sparse at best (National Beef Cattle Evaluation Consortium, 2012).

This technology can also be transferred to aid in the management of cattle. This is known as Marker-Assisted Management (MAM) and it consists of using the results of DNA-marker tests to predict phenotypic performance of the animal being tested in a certain environment or management practice. From the marker scores, feedlots would pay premiums for feeder cattle that are most likely to achieve specific endpoints given their specific production environment (Van Eenennaam et al., 2012). This allows cattle feeders to more efficiently optimize carcass endpoints (i.e. target backfat, weight or quality grade) by deciding how long to feed or whether to use growth-promoting technologies on a group of animal's based on genomic information. Another viable option for MAM is to optimize individual animal fitness by placing animals in an environment that matches up with their upper and lower threshold temperature. Marker-Assisted Management allows improved feedlot efficiency by placing animals in a location and feeding them at a specific time of year based on their temperature threshold, which results in faster growth rate and increased feed efficiency due to less energy being used for thermoregulatory processes.

Economically Relevant Traits and Physiological Indicator Traits:

Multiple EPD computed in NCE today do not directly affect profit, but are correlated with traits that affect profit. As an example, birth weight and scrotal circumference are measured not because a producer gets more or less money for the weight of his cattle at birth or the scrotal circumference of his bulls, rather these traits are used to indicate the genetic merit of an animal for another trait, in this case calving ease and daughter age at puberty for birth weight and scrotal circumference, respectively (Golden et al., 2000). Traits that are directly associated with a specific cost of production or an income stream are called economically relevant traits (ERT) (Golden et al., 2000). Examples of ERT include heifer pregnancy rate, sale weight, or cow maintenance feed requirement. The importance of indicator traits (IT) to predict

the genetic merit of ERT is realized for ERT that are unobservable, difficult to obtain/identify a phenotype, expensive to measure, or have low heritability. Important characteristics of IT are the ease of collection and their cost-effectiveness. Also, the genetic correlation between the IT and ERT multiplied by the accuracy of phenotypic selection (i.e. $\sqrt{h^2}$) on the IT should be greater than the accuracy of phenotypic selection on the ERT, unless phenotypic selection on the ERT is not possible or very expensive and difficult to measure (Falconer and Mackay, 1996). The efficacy of selection is improved by the increase in accuracy for the ERT, which in turn increases the rate of genetic improvement (Golden et al., 2000).

An additional approach would be to use physiological indicator traits (PIT) or traits that are expected to be closely related to physiological processes that are components of the trait of interest (Thallman, 2008). This approach takes advantage of the fact that genes related to the physiological process have genetic polymorphisms that affect the ERT and selection for these will in turn positively impact the trait of interest. Potential PIT could be processes that are associated with body temperature regulation (i.e. Heat Shock Proteins, hormone levels, etc.), disease resistance (i.e. immunological blood factors, etc.), and feed efficiency (hormone levels, enzyme levels, etc.). Another benefit of developing genomic selection tools for PIT is that they could be measured with less error as compared to complex phenotypes such as feed efficiency or fertility, potentially allowing for genomic predictors of high accuracy for PIT.

In order for MBV to be estimated accurately, thousands of phenotypes need to be collected and the resulting prediction equations will need to be re-estimated periodically with newly genotyped animals that are closely related to the targeted population. Thus, continuous body temperature measurements via tympanic or vaginal may not be an optimal phenotype, which makes PIT a possible approach to assessing how an animal responds to heat or cold stress in a production setting. For example Scharf et al. (2010), found that prolactin, cholesterol and

creatinine could serve as physiological markers to predict how an animal is coping during heat stress conditions.

Myostatin Mutation

Multiple breed specific mutations within the myostatin gene have been shown to give rise to varying degrees of increased musculature in cattle (Grobet et al., 1997; Kambadur et al. 1997; McPherron & Lee 1997; Grobet et al., 1998; Marchitelli et al., 2003). The protein product produced by the myostatin gene is a member of the transforming growth factor β (TGF- β) and its primary function is a negative regulator of myogenesis. The TGF- β family encompasses a large group of secreted growth and differentiation factors that play important roles in regulating development and tissue homeostasis. The protein sequence belonging to this family are all comprised of a putative signal sequence for secretion, a putative RXXR proteolytic processing site, which is followed by a region containing the conserved C-terminal cysteine residues (McPherron and Lee, 1997).

The well-characterized “double muscling” phenotype is caused by multiple breed specific mutations within the myostatin gene producing an inactive myostatin protein product. The different breed specific mutations within the myostatin gene give rise to varying degrees of increased musculature, such as the less severe F94L mutation that does not cause complete inactivation of the protein product (Grobet et al., 1998). The increased musculature primarily results from an increase in the number of muscle fibers (hyperplasia). An animal with two copies of the mutation has two times the number of muscle fibers as compared to a normal animal, while an animal with one copy (i.e. heterozygote) displays a lesser degree of extreme muscling and tends to be phenotypically similar to a normal animal (Kambadur et al., 1997). The causative Piedmontese-specific mutation was localized to the myostatin gene through a linkage study and a mice comparative mapping approach (Charlier et al., 1995; Kambadur et al., 1997). The

expression pattern was elucidated for the protein product of normal and double muscled animals and it was found that there was not a difference in protein expression (Kambadur et al., 1997). In the same study, Kambadur et al. (1997) found a mutation, which causes a non-synonymous amino acid change (Cysteine to Tyrosine) in the conserved C-terminal cysteine residue of the protein product. The C-terminal repeated cysteine residues are important in the folding of the final protein product and due to the amino acid change the spatial configuration of the final protein product is disrupted, which leads to reduced biological activity (Lee, 2004).

An animal with two copies of the mutation yields an extremely lean and heavily muscled carcass which has advantages for production, but the individuals are more susceptible to dystocia which has hindered its introgression in multiple breeds. A more efficient approach to using breeds segregating alleles producing the double muscling phenotype is to produce offspring with one copy because they will have heavier weaning weight and increased percentage lean while minimizing calving difficulty (Casas, 2004). Table 1 depicts the phenotypic differences for multiple production traits by number of copies of the inactive myostatin allele for Piedmontese cross-bred animals in a study conducted by Short *et al.* (2002).

Summary

Suboptimal body temperature regulation has been shown to have negative effects on efficiency of production including growth, feed efficiency, reproduction, and animal welfare (Hahn, 1997). The diversity between breeds in their ability to cope with heat or cold stress and the deleterious effects of suboptimal body temperature regulation on multiple economic production traits suggest that inherent differences in body temperature regulation could serve as useful indicator traits to improve the adaptation of animals and efficiency of beef production. Decreased sensitivity to thermal stress events allows for high levels of production to be sustained in the midst of extreme stress events.

The response of animals during times of extreme temperature stress events can be used as an indicator trait to improve ERT across varying environments. PIT associated with body temperature regulation, including blood hormones, can be used as an indicator trait in tandem with others in order to estimate the genetic value of an individual for a complex ERT that is a combination of multiple production traits. Knowledge of an animal's genetic threshold paves the way for the implementation of cold or heat stress management practices. Based on an animal's genetic makeup, it could be determined that they would excel if placed on feed in a given region during a specific time of year.

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Table 1. Phenotypic differences for multiple production traits based of the genotype¹ of Peidmontese cross-bred animals.²

	Zero Copies	One Copy	Two Copies
Birth Weight (kg.)	35.7	37.0	40.1
Percent with Dystocia:			
Heifers (%)	12.9	43.1	49.6
Cows (%)	0.8	0.1	7.9
Weaning Weight (kg.)	174	173	166
Live Slaughter Weight (kg.)	464	465	458
Carcass Slaughter Weight (kg.)	269	278	291
Dressing Percent (%)	57.9	59.7	63.2
Longissimus muscle area, cm ²	74.3	86.4	109
Fat depth, mm	6.3	5.6	2.6
Yield Grade	2.13	1.51	0.10
Liver Weight (kg.)	5.13	5.00	4.42

¹ Genotype refers to the myostatin genotype as either homozygous normal (0 copy), heterozygous (1 copy), or homozygous for inactive myostatin (2 copy).

² Short, R. E., M. D. MacNeil, M. D. Grosz, D. E. Gerrard and E. E. Grings. 2002. Pleiotropic effects in Hereford, Limousin, and Piedmontese F2 crossbred calves of genes controlling muscularity including the Piedmontese myostatin allele. *J. Anim. Sci.* 80:1-11.

Chapter 1

The effect of Myostatin genotype on body temperature during extreme temperature events.

Abstract

Extreme heat and cold events can create deleterious physiological changes in cattle as they attempt to cope with temperature related stress. The genetic background of animals can influence their response to these events. The objective of the current study was to model the impact of myostatin genotype (MG) on body temperature during periods of heat and cold stress. Two groups of crossbred heifers and steers were placed in a feedlot over two summers and two winters. Prior to arrival, animals were genotyped to confirm MG as either homozygous normal (0 copy, n=84), heterozygous (1 copy, n=96), or homozygous for inactive myostatin (2 copy, n=59). Hourly tympanic and vaginal temperature ($^{\circ}\text{C}$) measurements were collected for steers and heifers, respectively, for 5 d during times of anticipated heat and cold stress. Mean (\pm SD) ambient temperature ($^{\circ}\text{C}$) for summer and winter stress events were 24.4 (\pm 4.64) and -1.80 (\pm 11.71), respectively. A trigonometric function (sine + cosine) was used to describe the diurnal cyclical pattern. Hourly body temperature was analyzed within a season, and fixed effects included MG, group, trigonometric functions nested within group and interaction of MG with trigonometric functions nested within group; random effects were animal and residual (Model 1). A combined analysis of season and group was also investigated with the inclusion of season as a main effect and the nesting of effects within both group and season (Model 2). In both models, the residual was fitted using an autoregressive covariance structure. A three-way interaction of MG, season and trigonometric function periodicities of 24 hr ($P < 0.001$) and 12 hr ($P = 0.015$) were significant for Model 2. For MG, an additive estimate of 0.10 $^{\circ}\text{C}$ ($P = 0.003$) and dominance estimate of -0.12 $^{\circ}\text{C}$ ($P < 0.001$) were significant during summer stress events. The additive estimate of 0.10 $^{\circ}\text{C}$ ($P < 0.001$) was significant and dominance estimate of 0.054 $^{\circ}\text{C}$ ($P =$

0.182) was not significant during winter stress events. Least-squares means for 0-copy animals were significantly ($P < 0.001$) warmer than 1- or 2-copy animals during summer stress events, and during winter conditions 2-copy animals had a significantly ($P < 0.01$) lower body temperature than 0- or 1-copy animals. The repeatability of hourly body temperature measurement for Model 2 was 0.27. The current study illustrated that a genotype by environment interaction exists for MG during periods of heat and cold stress.

Key Words: beef cattle, body temperature, genotype-by-environment interaction, myostatin

Introduction

Beef production is unique in that animals are managed in extensive production systems with minimal environmental modifications, making body temperature regulation an essential component to maintaining overall animal efficiency (Hahn, 1999; Young, 1983). To mitigate these risks, producers currently use knowledge of breed strengths relative to heat or cold tolerance to determine which breed(s) will perform best in a particular environment. An alternative strategy is to differentiate animals within a population based on their inherent differences for body temperature regulation. The mean body temperature of cattle is 38.6 °C (McDowell, 1972). Indicators of core body temperature from the mean include tympanic (Davis et al., 2003) or vaginal measurements (McGee et al., 2008). Animal variation has been shown to exist for body temperature regulation during periods of external temperature related stress in beef cattle (Burrow, 2001; Da Silva et al., 1973; Turner, 1982, 1984) and dairy cattle (Dikmen et al., 2012; Ravagnolo and Misztal, 2000, 2002).

Knowing that some genetic backgrounds, or large effect mutations, interact with their environment differently is extremely beneficial as these genotype by environment interactions could inform management decisions at multiple levels throughout the production chain. One such large effect mutation is myostatin which produces an inactive myostatin protein product

causing the well characterized “double muscling” phenotype (Kambadur et al., 1997). An animal with two copies of the inactive myostatin allele yields an extremely lean and heavily muscled carcass, while an animal with one copy displays some increased leanness and muscularity, but is similar to a conventional animal (Short et al., 2002; Casas et al., 2004). The objective of the current study was to model the impact of myostatin on body temperature during periods of heat and cold stress.

Materials and Methods

Experimental Design

Crossbred steers and heifers (n= 239) with varying degrees of Piedmontese influence were placed in a Calan gate facility at the Agricultural Research and Development Center (ARDC) feedlot facility near Mead, NE. The project was approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. Animals were genotyped prior to arrival to determine their myostatin genotype (MG) as either homozygous normal (0-copy, n=84), heterozygous (1-copy, n=96), or homozygous for inactive myostatin (2-copy, n=59). Cattle were fed in four groups over a 2-yr period where groups 1 and 3 consisted of calf-fed steers and groups 2 and 4 consisted of yearling heifers. The steer groups were on feed from Dec. 16, 2009 to June 22, 2010 (S1) and Dec. 23, 2010 to June 22, 2011 (S2). The heifer groups were on feed from July 28, 2010 to Nov. 28, 2010 (H1) and July 28, 2011 to Dec 2, 2011 (H2).

Animals had *ad libitum* access to water and were fed a diet that met or exceeded NRC requirements. The finishing ration for H1 and S1 included wet distillers grain with solubles, a 1:1 blend of high moisture and dry rolled corn, grass hay and supplement at 35, 52, 8, and 5 percent of the diet, respectively. The finishing ration for H2 and S2 included modified distillers grain with solubles, sweet bran, a 1:1 blend of high moisture and dry rolled corn, grass hay and supplement at 20, 20, 48, 8, and 4 percent of the diet, respectively. Animals were on an all-natural program

and were not implanted nor fed growth-promoting additives. Ultrasonic rump fat, rib fat, ribeye area, intramuscular fat percentage and live BW were recorded monthly. Individual feed bunks were filled each day and refusals were calculated on average every 6 d with a range of 1 to 9 d. A feeding period is described as the time between two successive feed refusal collections. Cattle were harvested as a group based on average body weight and external fat.

During anticipated times of heat and cold stress, hourly body temperature recording devices were placed for a minimum of 5 d inside the ear canal (tympanic) for steers or intravaginally for heifers. Body temperature was recorded using the micro-T software (Nexsens Technology, Beavercreek, OH) along with the DS1921H ibutton data loggers with a resolution of 0.0625 °C (Maxim Integrated Products, Inc., Sunnyvale, CA). Loggers were individually entered into a database and programmed to begin recording at a specified time. The tympanic temperature protocol included placing each logger in the finger of a latex glove and tying the logger off with the remaining portion discarded. It was then placed in the ear as far as possible along with a stress ball to pack the logger inside the ear, in order to seal the logger from the external environment. Vet wrap was wrapped around the ear to hold the data logger and stress ball in place and then athletic tape was used to secure everything for the duration of the recording period. Vaginal temperature protocol used the same data logger and software device as was used for tympanic temperature. A blank (i.e. did not contain hormones) controlled internal drug release (CIDR) was modified by cutting out the center silicone section to allow for the placement of the data logger. The data logger was then sealed in the CIDR using silicone sealant and inserted into the vagina using a CIDR applicator. A subset of heifers (n=8) had both tympanic and vaginal body temperature recorded and a correlation of 0.98 was estimated between the two. Tympanic temperature averaged 0.163 °C greater than vaginal temperature. To account for this, steer body temperature measurements were adjusted down by 0.163. In a

study by Bergen and Kennedy (2000), the authors found a high phenotypic correlation (0.77; $P < 0.05$) between vaginal and tympanic temperature. The average (\pm SD) age, ultrasonic rump and rib fat, weight, and dry matter intake (DMI) along with the number of days on feed prior to the recorded stress event by group are in Tables 1 and 2 for heat and cold stress events, respectively.

Ambient temperature (T_a , °C), relative humidity (RH, %), wind speed (WS, km/h), and solar radiation (SR, kcal m²) were taken hourly at ARDC using an automated weather station. ARDC is located at 41° 14' N latitude and 96° 48' W longitude, with a mean elevation of 353 m above sea level. These parameters were used to compute a Comprehensive Climate Index (CCI) that is effective for winter and summer conditions (Mader et al., 2010). The animals were housed in a partially enclosed cement floor barn with a flush system. The open side of the barn faced the south and led to a small dirt floored pen. Due to the type of housing, the effects of the environmental parameters may not be as severe as animals on pasture or pens without access to shade or wind protection. The average (\pm SD) environmental parameters and hourly animal body temperatures along with the number of animals in the analysis and dates of the stress period by group are in Tables 3 and 4 for heat and cold stress events, respectively. Animals were removed from the analysis for summer (n=14) and winter (n=13) stress events due to missing hourly body temperature observations. Additional steers (n=5) were removed from the analysis for winter stress events due to body temperature observations not following a cyclical pattern similar to other animals in the group, likely due to data logger malfunctions.

Statistical Analysis

Hourly body temperature was analyzed using a trigonometric function (sine + cosine). The trigonometric function consisted of multiple regressions of the vector of animal temperatures on sine ($2\pi a/S_m$) and cosine ($2\pi a/S_m$), where a was the particular hour of a day

(i.e. 1 to 24) and S_m denotes the length of the periodicity (Fuller, 1976). In the current study only hourly (1 to 24) periodicities were investigated. Best fit trigonometric function periodicities were determined within each season by including all periodicities nested within group, MG, group and the interaction of MG and group as fixed effects and animal as a random effect. Trigonometric function periodicities that were retained were significant ($P < 0.05$) and had a large impact on decreasing the residual variance. Trigonometric function periodicities of 24 (24H) and 12 (12H) hour were retained for both stress events.

To account for the inherent covariance structure between hourly body temperatures, the residual was fitted with an autoregressive 1 (AR1) covariance pattern within an animal and a covariance of zero across animals. AR1 is a covariance pattern that estimates one covariance parameter, rho (ρ), which decreases exponentially as hourly body temperature observations get further away from one another. The residual (co)variance matrix was of size 225 (i.e. animals) X 120 (i.e. temperature measurements) and 220 (i.e. animals) X 120 (i.e. temperature measurements) for summer and winter stress events, respectively. A condensed example of the covariance structure used is below, with 3 observations per animal for 2 animals.

$$\text{Residual} = \sigma_{Animal}^2 + \sigma_{Residual}^2 \begin{bmatrix} 1 & \rho & \rho^2 & 0 & 0 & 0 \\ \rho & 1 & \rho & 0 & 0 & 0 \\ \rho^2 & \rho & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & \rho & \rho^2 \\ 0 & 0 & 0 & \rho & 1 & \rho \\ 0 & 0 & 0 & \rho^2 & \rho & 1 \end{bmatrix}$$

Upon inclusion of an AR1 covariance pattern and interaction of trigonometric functions with MG, the following model was generated across all groups within a season:

$$BT_{ijlm} = \mu + M_i + G_j + M_i * G_j + \text{Cos}24H * G_j + \text{Sin}24H * G_j + \text{Cos}12H * G_j + \text{Sin}12H * G_j + \text{Cos}24H * M_i * G_j + \text{Sin}24H * M_i * G_j + \text{Cos}12H * M_i * G_j + \text{Sin}12H * M_i * G_j +$$

$Animal_l + \epsilon_{ijlm}$ (Model 1), where BT was hourly body temperature, μ was average hourly body

temperature, M was MG, G was group and M*G was interaction of MG and group. The interaction of G with Cos24H, Sin24H, Cos12H and Sin12H was interaction of group and trigonometric function periodicities of 24H and 12H. The interaction of M and G with Cos24H, Sin24H, Cos12H, and Sin12H was interaction of MG, group, and trigonometric function periodicities of 24H and 12H. Random effects included animal and a residual (ϵ) with an AR1 covariance structure. The three-way interaction of 12H trigonometric function periodicity, MG, and group was not significant ($P > 0.05$) for summer stress events using Model 1 and was therefore not included in the final analysis.

Covariates of rump fat, body weight, and average DMI nested within group were centered to their respective groups and included in Model 1 for winter and summer stress events. The rump fat and body weight measurements were the ones recorded closest to the temperature related stress period. The average DMI was estimated by averaging over the number of days comprised in the period (s) that the temperature related stress was measured in. The interaction of covariate (i.e. rump fat, body weight, and average DMI) nested within group and the three-way interaction of covariate, group and MG were not significant ($P > 0.05$) for the summer and winter stress events. The effect of pen ($n=2$) was also included in Model 1 and the percent of variation explained by pen for summer and winter stress events were 1.2 and 1 percent, respectively and was therefore not included in the final model. Coat color was investigated and was found not to be significant. Previous studies (Davis et al., 2003; Finch et al., 1984; Brown-Brandl et al., 2006) have found that coat color does have an effect on body temperature, but in our study the number of animals other than black (i.e. white, yellow and red) was small. Furthermore, if an animal did have a coat color other than black it most likely had 1 or 2 copies of the inactive myostatin allele and thus coat color was confounded with MG. Sex was confounded with group and was therefore not investigated.

Body temperature was also analyzed with all groups and stress events combined using the following model:

$$\begin{aligned}
 BT_{ijklm} = & \mu + M_i + G_j + S_k + M_i * G_j * S_k + \text{Cos}24H * G_j * S_k + \text{Sin}24H * G_j * S_k \\
 & + \text{Cos}12H * G_j * S_k + \text{Sin}12H * G_j * S_k + \text{Cos}24H * M_i * G_j * S_k + \text{Sin}24H * M_i * G_j * S_k \\
 & + \text{Cos}12H * M_i * G_j * S_k + \text{Sin}12H * M_i * G_j * S_k + \text{Animal}_{l(k)} + \epsilon_{ijklm} \quad (\text{Model 2}),
 \end{aligned}$$

where BT was hourly body temperature, μ was average hourly body temperature, M was MG, G was group, S was season and M*G*S was interaction of MG, group, and season. The interaction of G and S with Cos24H, Sin24H, Cos12H, and Sin12H was interaction of group, season and trigonometric function periodicities of 24H and 12H. The interaction of M, G, and S with Cos24H, Sin24H, Cos12H and Sin12H was interaction of MG, group, season, and trigonometric function periodicities of 24H and 12H. Random effects include animal nested within season and a residual (ϵ) with an AR1 covariance structure.

The animal variance divided by the total variance (residual plus animal) was used to estimate the repeatability of hourly body temperature recordings within a season. Least-squares means were estimated for each MG and orthogonal contrasts were used to estimate additive $((0\text{-copy} - 2\text{-copy})/2)$ and dominance $[1\text{-copy} - ((0\text{-copy} + 2\text{-copy})/2)]$ effects.

Results and Discussion

Least-squares means by MG along with additive and dominance effects for Model 2 are presented in Table 5. Model 1 least-squares means and additive and dominance effects are not shown due to their high degree of similarity with Model 2. During heat stress conditions, 0-copy animals had significantly ($P < 0.001$) higher body temperatures and were further away from the normal/non-stressed body temperature (38.6 °C) than either 1- or 2-copy animals. During cold stress conditions, 2-copy animals had significantly ($P < 0.01$) lower body temperatures and were further away from the normal/non-stressed body temperature than either 0- or 1-copy animals.

The additive estimate of $0.10\text{ }^{\circ}\text{C}$ ($P = 0.003$) and dominance estimate of $-0.12\text{ }^{\circ}\text{C}$ ($P < 0.001$) were significant during summer stress events. The additive estimate of $0.10\text{ }^{\circ}\text{C}$ ($P < 0.001$) was significant and dominance estimate of $0.054\text{ }^{\circ}\text{C}$ ($P = 0.182$) was not significant during winter stress events. Thus, the fitness of MG differed across environments and the heterozygote appeared to be more robust across environments, while the homozygotes appeared to be more sensitive to environmental extremes. Environmental sensitivity for a genotype can be represented by the slope of a genotypes reaction norm, which graphically displays the effect of different environments on the average phenotypic value for a genotype (Falconer and Mackay, 1998; de Jong and Bijma, 2002). A reaction norm is illustrated in Figure 1 as the deviation of the average phenotypic value for a genotype from normal/non-stressed body temperature ($38.6\text{ }^{\circ}\text{C}$).

The main effect of group ($P < 0.001$) and interaction of group and 24H (cosine $P < 0.001$; sine $P < 0.001$) and 12H (cosine $P < 0.001$; sine $P < 0.001$) trigonometric function periodicities were significant for both winter and summer stress events in Model 1. The main effect of group ($P < 0.001$), season ($P < 0.001$) and interaction of group and season with 24H (cosine $P < 0.001$; sine $P < 0.001$) and 12H (cosine $P < 0.001$; sine $P < 0.001$) trigonometric function periodicities were significant for Model 2. This illustrates that the mean body temperature and shape of the diurnal cycle was different across groups and seasons. The difference across groups may be partially explained by the differences in the severity of the stress event that each group witnessed. The interactions of group with weight, rump fat, and DMI were fitted initially to determine if the phenotypic differences in weight, rump fat, and DMI within a group going into the stress event had an effect on body temperature. They were not significant ($P > 0.05$), which illustrates that phenotypic differences across groups for these traits did not have a significant effect on body temperature. The differences across season may partially be due to differences in sunrise and sunset, which impacts the timing at which an animal begins to warm up or cool

down due to the sun's radiation effects, which had been observed by Lefcourt and Adams (1996, 1998).

The main effect of MG ($P = 0.001$) and interaction of MG and group ($P = 0.005$) were significant for both winter and summer stress events for Model 1. The main effect of MG ($P < 0.001$) and interaction of MG, group, and season ($P < 0.001$) were significant for Model 2. The interaction of the 24H trigonometric function periodicity, group, and MG were significant (cosine $P < 0.001$; sine $P < 0.001$) for summer stress events in Model 1. At least one interaction of 24H (cosine $P = 0.019$; sine $P = 0.006$) and 12H (cosine $P = 0.779$; sine $P = 0.032$) trigonometric function periodicities, group, and MG were significant for winter stress events in Model 1. Furthermore, at least one interaction of 24H (cosine $P < 0.001$; sine $P < 0.001$) and 12H (cosine $P = 0.752$; sine $P = 0.015$) trigonometric function periodicities, group, season and MG were significant for Model 2. This illustrates that the mean body temperature and shape of the diurnal cycle is dependent on MG and the degree of impact that MG has on body temperature varied across groups. The varying impact of MG may be partially explained by the varying intensity of heat or cold stress across groups, where under less severe conditions, the variance across animals is lower, leading to smaller differences in body temperature across MG.

It has been shown that 2-copy animals are substantially leaner than 0-copy animals (Short et al., 2002; Casas et al., 2004) and this same trend was illustrated by Moore and others (2013) using the same animals as the current study. This led to the hypothesis that decreased fat cover in 2-copy animals allowed them to remove heat at a faster rate than 0-copy animals. A three-way interaction of MG and group with either rump fat or weight was included during model selection and was shown not to be significant ($P > 0.05$), but the main effect of MG was significant. The insignificant three-way interaction of MG, group and rump fat or weight is most

likely attributed to the main effect of myostatin capturing most of the variation, due to the large differences across MG in rump fat and weight.

Variance components for Models 1 and 2 are presented in Table 6. The repeatability of hourly body temperature measurements was low to moderate and was within the range of previous internal body temperature repeatability estimates of 0.15 to 0.385 (Burrow, 2001; Seath and Miller, 1947; Turner, 1982, 1984). When averaged, repeated measurements of body temperature on the same animal reduce temporary environmental variance and relies more on expression of total animal variance, much of which is genetic differences between animals (Falconer and Mackay, 1998; Seath and Miller, 1947). Environmental variance arises from temporary or localized circumstances, which may have large effects on body temperature. Body temperature differences arise from a complex interaction between anatomical, physiological, and behavioral factors which are dependent on the life stage, nutrition, previous degree of heat or cold stress, and health of the animal (McDowell, 1972; Hahn, 1999).

Modeling of continuous body temperature measurements using a trigonometric function provides an assessment of how a particular genotype responds to heat or cold stress through differences in the intercept and shape of the diurnal cycle. Predicted 24-hr cycles by genotype averaged across group are shown graphically in Figures 2 and 3 for summer and winter stress events, respectfully. Figure 2 illustrates that as 0-copy animals warm up during periods of heat stress their slope is steeper and intercept larger than 1- or 2-copy animals, which yields a higher body temperature at the peak of their 24-hr body temperature cycle. Alternatively, Figure 3 illustrates that as 2-copy animals cool down during periods of cold stress, their slope is steeper and intercept lower than 0- or 1-copy animals, which yields a lower body temperature at the trough of their 24-hr cycle.

Implications

The current study illustrated that a genotype by environment interaction exists for MG during periods of heat and cold stress. This knowledge can aid in the management of cattle to ensure optimal performance. This methodology can be transferred to other genetic variants more conducive to mainstream beef production in order to alleviate the effects of cold or heat stress on production traits. Further work needs to be done to better understand the genetic architecture of body temperature regulation under environmental stress conditions in order to inform management decisions of beef cattle and the development of Marker-Assisted Management tools.

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Table 1. Average (\pm SD) age, rump, rib fat, weight, dry matter intake and days on feed prior to each heat stress event by group.

	Group ¹			
	H1	H2	S1	S2
DOF ² prior to heat stress, day	23	32	176	163
Age prior to heat stress, day	501.7 \pm 13.8	498.9 \pm 21.8	428.8 \pm 16.3	433.0 \pm 17.3
Rump fat ³ prior to heat stress, mm	5.88 \pm 2.65	2.80 \pm 1.63	7.06 \pm 3.07	6.10 \pm 3.24
Rib fat ³ prior to heat stress, mm	4.19 \pm 1.81	2.75 \pm 0.96	7.40 \pm 3.07	6.95 \pm 3.40
Weight prior to heat stress, kg	399.0 \pm 28.3	327.9 \pm 36.4	484.6 \pm 41.5	441.9 \pm 46.5
DMI ⁴ during heat stress, kg	8.42 \pm 1.06	8.08 \pm 1.35	8.46 \pm 1.31	7.60 \pm 1.38
Animals	51	59	57	58

¹ Group refers to a set of animals that were placed in the Calan gate feeding facility where H1 = Heifer1, H2 = Heifer2, S1 = Steer1, and S2 = Steer2.

² DOF = days on feed.

³ Measured by ultrasonography.

⁴ DMI = average dry matter intake measured by the Calan gate individual animal feeding system.

Table 2. Average (\pm SD) age, rump fat, rib fat, weight, dry matter intake and days on feed prior to each cold stress event by group.

	Group ¹			
	H1	H2	S1	S2
DOF ² prior to cold stress, day	100	124	23	21
Age prior to cold stress, day	578.7 \pm 13.8	591.9 \pm 21.8	276.8 \pm 16.3	291.0 \pm 17.3
Rump fat ³ prior to cold stress, mm	10.08 \pm 4.36	7.17 \pm 3.58	3.38 \pm 1.81	2.91 \pm 1.07
Rib fat ³ prior to cold stress, mm	8.39 \pm 3.81	6.91 \pm 2.85	3.16 \pm 0.95	2.91 \pm 0.72
Weight prior to cold stress, kg	490.5 \pm 38.0	427.7 \pm 40.9	294.1 \pm 29.9	278.5 \pm 30.2
DMI ⁴ during cold stress, kg	8.61 \pm 1.29	8.08 \pm 1.35	6.58 \pm 1.11	6.86 \pm 1.13
Animals	53	58	53	56

¹ Group refers to a set of animals that were placed in the Calan gate feeding facility where H1 = Heifer1, H2 = Heifer2, S1 = Steer1, and S2 = Steer2.

² DOF = days on feed.

³ Measured by ultrasonography.

⁴ DMI = average dry matter intake measured by the Calan gate individual animal feeding system.

Table 3. Average ($\pm SD$) environmental conditions, hourly animal body temperature and dates for each heat stress event by group.

	Group ¹			
	H1	H2	S1	S2
Ambient Temperature ² , °C	24.3 (± 4.7)	23.7 (± 4.1)	22.8 (± 3.7)	26.8 (± 5.1)
Relative Humidity ² , %	82.2 (± 17.1)	81.7 (± 13.1)	81.5 (± 15.3)	52.7 (± 16.3)
Wind Speed ² , km/h	4.0 (± 2.1)	5.4 (± 3.0)	7.4 (± 3.2)	10.1 (± 5.0)
Solar Radiation ² , kcal/m ² /h	213.0 (± 261.9)	189.2 (± 233.6)	195.5 (± 264.9)	242.4 (± 279.8)
CCI ³ , °C	28.2 (± 6.9)	26.4 (± 5.7)	23.6 (± 6.2)	25.9 (± 6.0)
Animal Body Temperature, °C	38.86 (± 0.48)	39.04 (± 0.62)	38.79 (± 0.43)	38.83 (± 0.43)
Animals	51	59	57	58
Date of Heat Stress	8/20/2010 to 8/24/2010	8/20/2011 to 8/24/2011	6/09/2010 to 6/13/2010	6/04/2011 to 6/08/2011

¹ Group refers to a set of animals that were placed in the Calan gate feeding facility where H1 = Heifer1, H2 = Heifer2, S1 = Steer1, and S2 = Steer2.

² Environmental parameters were taken at the Agricultural Research and Development Center using an automated weather station.

³ CCI = comprehensive climate index (Mader et al., 2010).

Table 4. Average ($\pm SD$) environmental conditions, hourly animal body temperature and dates for each cold stress event by group.

	Group ¹			
	H1	H2	S1	S2
Ambient Temperature ² , °C	10.2 (± 7.7)	4.7 (± 7.0)	-13.9 (± 6.3)	-8.2 (± 5.2)
Relative Humidity ² , %	52.6 (± 19.7)	81.5 (± 16.3)	86.0 (± 7.1)	84.6 (± 5.5)
Wind Speed ² , km/h	7.8 (± 4.1)	6.5 (± 16.3)	7.1 (± 3.8)	7.9 (± 4.2)
Solar Radiation ² , kcal/m ² /h	110.8 (± 161.1)	58.2 (± 103.5)	75.6 (± 120.1)	75.4 (± 125.2)
CCI ³ , °C	5.4 (± 8.0)	-0.6 (± 7.0)	-22.8 (± 7.2)	-16.8 (± 5.6)
Animal Body Temperature, °C	38.70 (± 0.40)	38.66 (± 0.32)	38.14 (± 0.85)	38.15 (± 0.85)
Animals	53	58	53	56
Date of Cold Stress	11/05/2010 to 11/09/2010	11/21/2011 to 11/25/2011	1/08/2010 to 1/12/2010	1/13/2011 to 1/17/2011

¹ Group refers to a set of animals that were placed in the Calan gate feeding facility where H1 = Heifer1, H2 = Heifer2, S1 = Steer1, and S2 = Steer2.

² Environmental parameters were taken at the Agricultural Research and Development Center using an automated weather station.

³ CCI = comprehensive climate index (Mader et al., 2010).

Table 5. Least-squares means for body temperature by myostatin genotype and season and additive and dominance effects for Model 2¹.

Season	Myostatin Genotype						Average Standard Error	Orthogonal Contrasts	
	0-Copies of the inactive myostatin allele		1-Copy of the inactive myostatin allele		2-Copies of the inactive myostatin allele			Dominance Effects ³ ($\pm SE$; °C)	Additive Effects ³ ($\pm SE$; °C)
N	BT ² (°C)	N	BT ² (°C)	N	BT ² (°C)				
Summer	79	39.01 ^a	93	38.79 ^b	53	38.81 ^b	0.033	-0.12 \pm 0.03*	0.1 \pm 0.02**
Winter	77	38.47 ^a	88	38.43 ^a	55	38.27 ^b	0.033	0.05 \pm 0.04	0.1 \pm 0.03**

^{a,b,c} Least-square means within a row with different superscripts differ ($P < 0.05$).

¹ Model 2 refers to the analysis with all groups and seasons combined.

² BT = Body Temperature

³ Orthogonal contrasts of additive and dominance estimates, with * = $P < 0.05$ and ** = $P < 0.001$

Table 6. Variance components for Models 1 and 2.

Group ¹	Model ²	Animal Variance	Residual Variance	Autoregressive	
				Correlation Parameter	Repeatability ³
Summer	1	0.052	0.106	0.79	0.33
Winter	1	0.073	0.231	0.78	0.24
Combined	2	0.063	0.168	0.78	0.27

¹ Group refers to either all groups within a season or all groups and seasons combined.

² Model refers to either Model 1 (i.e. across group within season) or Model 2 (i.e. across group and season).

³ Repeatability was estimated by taking animal variance divided by total variance (i.e. (animal / (animal + residual)).

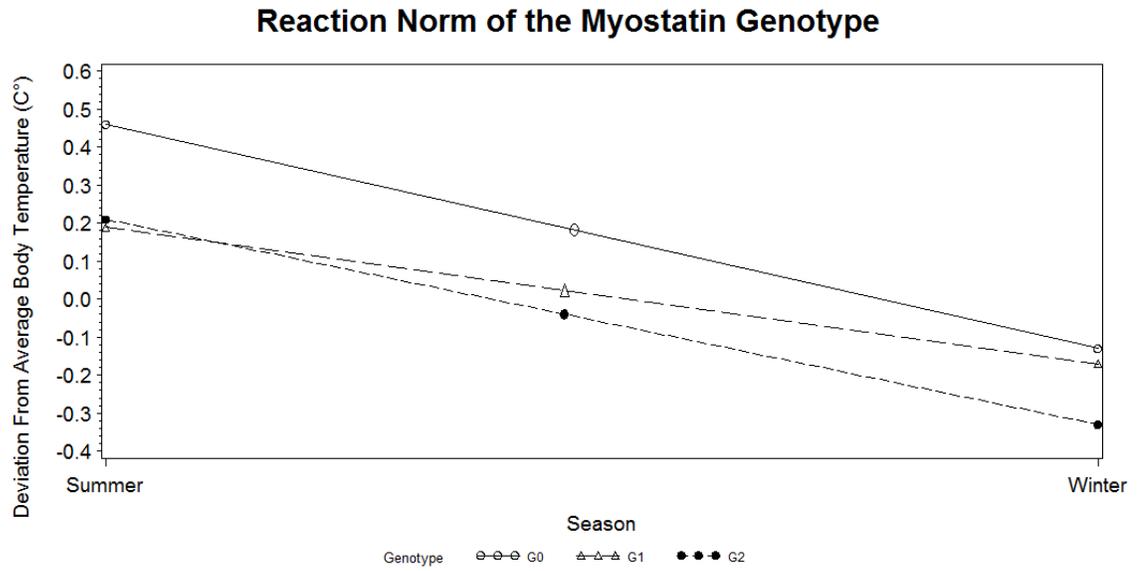


Figure 1. Reaction norm of myostatin genotype¹ during winter and summer conditions.
¹ Genotype refers animal with 0-copies (G0), 1-copy (G1) or 2-copies (G2) of the inactive myostatin allele.

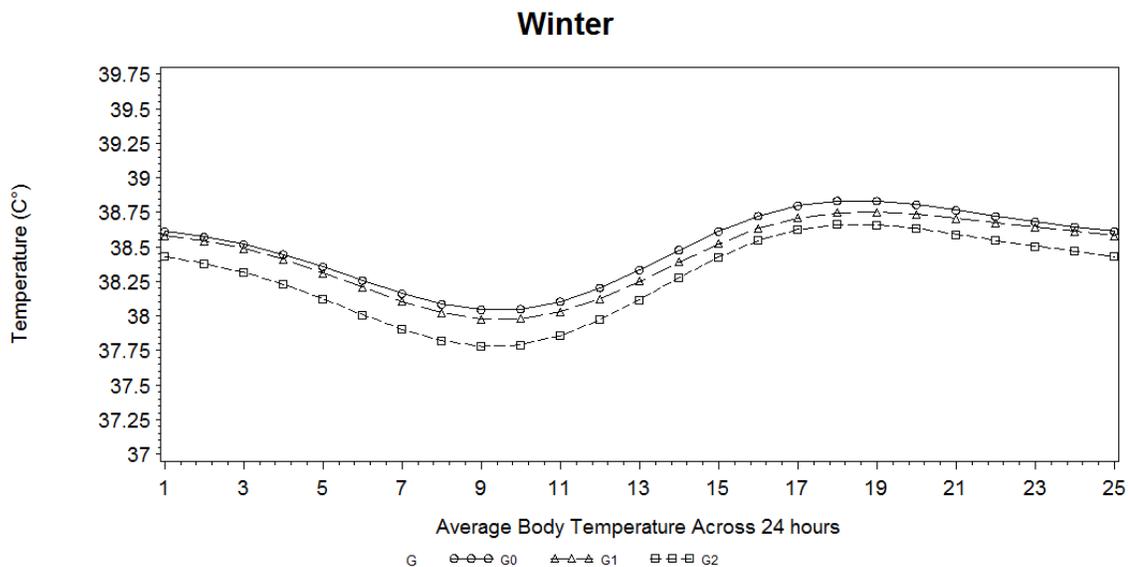


Figure 2. Predicted body temperature averaged across groups by genotype¹ using a trigonometric function (sine + cosine) model during a 24-h period² winter stress event.

¹ Genotype refers animal with zero copies (G0), one copy (G1) or two copies (G2) of the inactive myostatin allele.

² Hour 1, 13, and 25 correspond to midnight, noon and midnight of the next day.

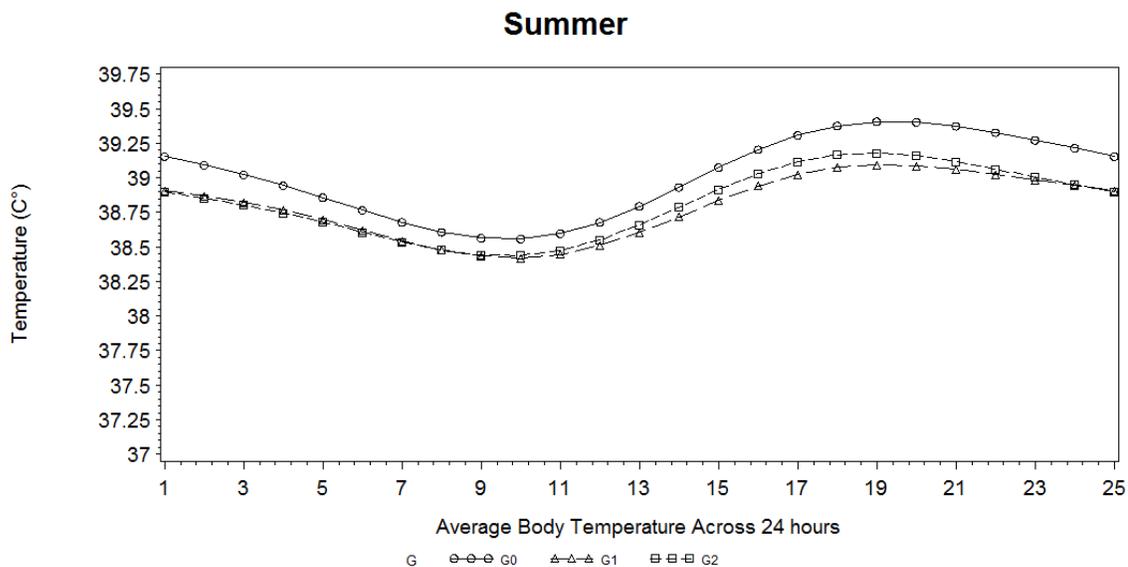


Figure 3. Predicted body temperature averaged across groups by genotype¹ using a trigonometric function (sine + cosine) model during a 24-h period² summer stress event.

¹ Genotype refers animal with 0-copies (G0), 1-copy (G1) or 2-copies (G2) of the inactive myostatin allele.

² Hour 1, 13, and 25 correspond to midnight, noon and midnight of the next day.

Chapter 2

A genome-wide association study for body temperature regulation during periods of heat and cold stress in beef cattle.

Abstract

Cattle are reared in environments that differ greatly in multiple environmental parameters making the ability to regulate body temperature across multiple environments essential. Collecting phenotypic body temperature measurements is difficult and expensive, thus a genomics approach is highly applicable. The population utilized to locate genomic regions responsible for body temperature regulation included cross-bred Piedmontese influenced steers and heifers (n=239) with varying copies of the inactive myostatin allele. Four groups across two years were placed in a feedlot, and during predicted heat and cold stress events hourly tympanic and vaginal body temperature devices were placed in steers and heifers, respectively. A GWAS was conducted for area under the curve (AUC) using hourly body temperature observations for five days (i.e. AUC 5-d summer (AUC5DS) and AUC 5-d winter (AUC5DW)) and during the maximal stress cycle (i.e. AUC 1-d summer (AUC1DS) and AUC 1-d winter (AUC1DW)) to where body temperature equals zero. Animals were genotyped with the BovineSNP50 assay and data analyzed using Bayesian models. Posterior heritability estimates were 0.68, 0.55, 0.21, and 0.20 for AUCS5D, AUCS1D, AUCW5D, and AUCW1D respectively. Phenotypic correlations were lowly negative between AUCS5D and AUCW5D (-0.16) and AUCS1D and AUCW1D (-0.22). Moderately negative Genomic-EBV correlations were found between AUCS5D and AUCW5D (-0.40) and AUCS1D and AUCW1D (-0.50), although a small percentage of the top 5% 1-Mb windows were in common between winter and summer stress events. Genomic heritability estimates were moderate to high and genetic antagonisms were shown to exist between heat and cold stress.

Key Words: beef cattle, body temperature, genome-wide association study

Introduction

Cattle are reared in environments that differ greatly in temperature, humidity, and wind speed, which has forced cattle to be regionally adapted, thus creating sensitivity to environments that differ greatly from the adapted environment. This potentially decreases their production efficiency in un-adapted environments and usefulness across multiple regions or in international breeding programs (Hahn, 1999; Young, 1983). Consequently developing breeds of cattle that can tolerate extremes in both directions while maintaining a high level of productivity and possessing superior carcass attributes is advantageous (Scharf et al., 2010).

One possible way to increase environmental tolerance is to characterize animals within a population based on their inherent differences for body temperature regulation using continuous internal body temperature measurements. A simulated selection scheme by Nardone and Valentini (2000), compared selection for heat tolerance within a high milking breed and milk production within a highly adapted breed. The authors found that selection for heat tolerance within the high milking breed was more efficient due to the adapted breed needing several generations (30 plus) to reach comparable levels of milk production. Animal variation has been shown to exist for body temperature regulation during periods of external temperature related stress in beef cattle with heritability estimates ranging from 0.11 to 0.44 (Burrow, 2001; Da Silva et al., 1973; Turner, 1982, 1984).

Internal body temperature measurements are difficult and expensive to measure in a production setting. Thus identifying and using genetic variants that impact body temperature regulation for selection and management purposes is highly applicable. A few genetic variants that impact an animal's ability to cope with heat stress have been identified, including the slick hair gene found in Senepol and Criolle cattle (Olsen et al., 2003) and a variant in the *ATP1A1* gene found in Holstein cattle (Liu et al., 2011). Selection for decreased heat and cold

susceptibility would broaden the temperature threshold for a population, which in turn would reduce the occurrence of the deleterious effects during heat or cold stress conditions and increase international germplasm exchange. Also, results can be used to inform management decisions of beef cattle dependent on upper and lower critical threshold temperature. Our objective was to conduct a genome-wide association study (GWAS) to discover the genetic basis of body temperature regulation during periods of heat and cold stress and to better understand the genetic relationship between heat and cold stress.

Materials and Methods

Experimental Design

This project was approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. Prior to arrival crossbred Piedmontese influenced animals were genotyped to confirm myostatin genotype (MG) as either homozygous normal (0-copy, n=84), heterozygous (1-copy, n=96), or homozygous for inactive myostatin (2-copy, n=59). Cattle were fed in four groups over a 2-yr period where groups 1 (S1) and 3 (S2) consisted of calf-fed steers and groups 2 (H1) and 4(H2) consisted of yearling heifers and were fed as described by Howard et al. (2012). Ultrasonic rump fat, rib fat, ribeye area, intramuscular fat percentage and live BW were recorded monthly. Cattle were harvested as a group based on average body weight and external fat.

Individual animal body temperatures were recorded per Howard et al. (2012). In brief, during anticipated times of heat and cold stress, body temperature recording devices were placed for a minimum of 5 d inside the ear canal (tympanic) for steers or intra-vaginally using a modified blank (i.e. did not contain hormones) controlled internal drug release (CIDR) for heifers. Body temperature was recorded via data loggers every hour with a resolution of 0.0625 °C. A subset of heifers (n=8) had both tympanic and vaginal body temperature recorded and a

correlation of 0.98 was estimated between the two. Tympanic temperature averaged 0.163°C greater than vaginal temperature and to account for this steer body temperature was subtracted by 0.163 °C. In a study by Bergen and Kennedy (2000), the authors found a high phenotypic correlation (0.77; $P < 0.05$) between vaginal and tympanic temperature.

Ambient temperature (T_a , °C), relative humidity (RH, %), wind speed (WS, km/h), and solar radiation (SR, kcal/-m²) were taken hourly at a nearby automated weather station as described by Howard et al. (2012). These parameters were used to compute a Comprehensive Climate Index (CCI) that was effective for winter and summer conditions (Mader et al., 2010). The animals were housed in a partially enclosed cement floor barn with a flush system. The open side of the barn faced the south and led to a small dirt floored pen. Due to the type of housing, the effects of the environmental parameters may not be as severe as animals on pasture without access to shade or wind protection.

Tissue was extracted from an ear notch (EN) taken from the tip of the ear with an appropriate sized ear notcher. Once the EN was collected it was placed in a 2.0-ml plastic tube and stored at -20 C°. DNA was extracted from 10 to 25 mg of tissue from each animal using the DNeasy or puregene blood and tissue kit (Qiagen). The quantity and quality of the DNA sample was assessed by NanoDrop Spectrophotometer (Thermo Scientific) and agarose gel electrophoresis. 1 μ g of total DNA from samples that were deemed acceptable were sent to GeneSeek Inc. (Lincoln, NE) and genotyped using the Illumina BovineSNP50 Bead-Chip (Illumina Inc., San Diego, CA).

Phenotypic Traits

Hourly body temperature observations (n=120 per animal) were used to approximate area under the curve (AUC) across 5-d to where body temperature equals zero during winter (AUCW5D) and summer (AUCS5D) conditions. Additionally, hourly body temperature

observations ($n=24$ per animal) for the 24-hr cycle at which heat or cold stress was maximal were used to approximate the AUC to where body temperature equals zero. AUC for both 5-d and 1-d was approximated using the Trapezoid rule which is the average of the left and right hand sums. The maximal heat or cold stress cycle was chosen based on the highest and lowest maximum CCI index temperature for summer (AUCS1D) and winter (AUCW1D) stress events, respectively. The hour at which the 24-hr cycle began and ended within a group was determined using a trigonometric function (sine + cosine) to smooth out the observed hourly body temperature cycle, as modeled by Howard et al. (2012). A high AUC value for a heat stress event or a low AUC value for a cold stress event indicates poor body temperature regulation. The average (\pm SD) CCI and AUC for AUCW5D, AUCS5D, AUCW1D, and AUCS1D are provided in Table 1.

Animals were removed from the analysis for summer ($n=14$) and winter ($n=13$) stress events, due to missing hourly body temperature observations. Additional steer observations ($n=5$) were removed from the analysis for winter stress events due to body temperature observations not following a cyclical pattern similar to other animals in the group, likely due to data logger malfunctions.

Statistical Analysis

A GWAS using AUC for winter and summer stress events was undertaken to estimate the proportion of phenotypic variation in AUC for both stress periods that was due to additive genomic variation. Estimates of marker effects and variances were obtained by fitting all markers simultaneously using Bayesian methods via GenSel (Version 0.9.2.045; Fernando and Garrick, 2011). Illumina data analysis software was used to assign quality scores (GenCall) for each genotype. If genotypes were missing or a GenCall score was below 0.20, they were replaced with the mean allele frequency across all animals. All SNP were utilized for analysis,

and none were culled based on MAF. The mixed model to determine marker effects and variances can be represented as: $y = X\beta + \sum_{i=1}^I z_i \alpha_i \delta_i + e$, where y is the vector of AUC phenotypic observations, X is a incidence matrix of the fixed effects in β including Group (1 to 4), I is the number of markers, z_i is a vector of genotype scores (-10, 0, 10) at marker i , α_i is the random additive effect of marker “ i ”, δ_i is an indicator for whether marker “ i ” was included ($\delta=1$) or excluded ($\delta=0$) in the model for a specific iteration of the Markov Chain Monte Carlo (MCMC) algorithm, and e is the random residual. The model to estimate marker effects was implemented using Bayes C as outlined in Habier et al. (2011). The proportion of markers having a null effect (π) was set to 0.995. A chain length of 150,000 iterations was run with the first 50,000 discarded as burn-in. Group was included as a fixed effect in the GWAS because group had a significant effect on AUC ($P < 0.05$). Of the total AUC phenotypic variance, group accounted for 29.0, 11.6, 48.5 and 42.3 percent of the total variance for AUCS1D, AUCS5D, AUCW1D, and AUCW5D, respectively. The genomic estimated breeding value (GEBV) of the i th animal was calculated as: $GEBV_i = \sum_{k=1}^I z_{ik} \hat{a}_k$, where z_{ik} is the genotype score (-10, 0, 10) for the i th animal at the k th marker and \hat{a}_k is the posterior mean effect at the k th locus.

Convergence was met for all analyses by starting with high and low *a priori* heritability estimates until the posterior heritability estimates were trending down and up, respectively. When the posterior heritability estimates were trending towards each other a value in the middle was chosen as the *a priori* heritability. The *a priori* heritability estimates used for final analyses were 0.2, 0.2, 0.55, and 0.68 for AUCW1D, AUCW5D, AUCS1D, and AUCS5D respectively.

The phenotypic and genetic relationship between winter and summer or 1- and 5-d stress events within a season were investigated with the following correlations: 1.) AUCW1D and AUCW5D; 2.) AUCS1D and AUCS5D; 3.) AUCW1D and AUCS1D; 4.) AUCW5D and AUCS5D.

The phenotypic correlation was estimated using multivariate analysis of variance (MANOVA) procedures with group fitted as a fixed effect. The genetic correlation was estimated using the predicted GEBV. Additionally, SNP were blocked into 1 Megabase (Mb) windows and the marker specific posterior variance across SNP within a window was summed to give an estimate of the total genetic variance for each window (n=2,678). The top 5% windows (n=131) within each trait were then compared in a similar fashion as the phenotypic and genetic correlation, to determine the percentage of windows in common between two particular traits.

Gene Ontology

The top 0.5% 1 Mb windows (n=13) that accounted for a large proportion of the additive genetic variance were extended by 1 Mb in both directions and a positional candidate gene approach was conducted using *Bos taurus* build UMD_3.1 assembly (Zimin et al., 2009). Human orthologs of beef cattle positional candidate genes were obtained using Ensembl Genes 69 database and the BioMart data mining tool

(<http://www.ensembl.org/biomart/martview/dd0c118c99ed15210cc6e97131d873fb>).

Functional annotation of human orthologs, identification of overrepresented gene ontology terms, and pathway analysis was performed using DAVID (<http://david.abcc.ncifcrf.gov>.)

Results and Discussion

The phenotypic and genetic correlations along with percent of 1 Mb windows in common between traits are presented in Table 2. Phenotypic correlations were highly positive between AUCS5D and AUCS1D (0.887; $P < 0.001$) and AUCW5D and AUCW1D (0.895; $P < 0.001$), indicating that an animal responds in a similar fashion under high stress conditions and during successive stress periods. Correlations between GEBV were highly positive for AUCS5D and AUCS1D (0.904; $P < 0.001$) and AUCW5D and AUCW1D (0.935; $P < 0.001$), indicating that similar genes are controlling how an animal responds to high stress conditions and successive stress

periods. This was confirmed with a moderate percentage of the top 5% 1-Mb windows being in common between AUCS5D and AUCS1D (43.5%) and AUCW5D and AUCW1D (58.7%).

Phenotypic correlations were lowly negative between AUCS5D and AUCW5D (-0.16; $P = 0.0161$) and AUCS1D and AUCW1D (-0.22; $P = 0.0014$), indicating an animal that responds well in summer stress conditions is more likely to be more susceptible to winter stress conditions or vice versa. Correlations between GEBV were moderately negative for AUCS5D and AUCW5D (-0.40; $P < 0.001$) and AUCS1D and AUCW1D (-0.50; $P < 0.001$), indicating that selection for heat tolerance may be antagonistic to selection for cold tolerance. The use of marker-assisted selection (MAS) can circumvent these antagonisms by selecting for markers that have an effect on heat tolerance independent of cold tolerance or vice versa. This is possible due to a low percentage of the top 5% 1-Mb windows being in common between AUCS5D and AUCW5D (7.6%) and AUCS1D and AUCW1D (7.6%).

The posterior mean heritability (\pm SE) estimated for AUCS1D (0.55 ± 0.10) and AUCS5D (0.68 ± 0.11) were high in comparison to previous estimates ranging from 0.11 to 0.44 (Burrow, 2001; Da Silva et al, 1973; Turner, 1982, 1984). In a study conducted by Howard et al. (2012), it was found that the MG had an impact on body temperature. The percentage of phenotypic variance in AUC explained by MG was estimated to be 11 and 13 percent for AUCS1D and AUCS5D, respectively. The posterior mean heritability (\pm SE) estimate for AUCW1D and AUCW5D was $0.20 (\pm 0.08)$ and $0.21 (\pm 0.09)$, respectively. The percentage of variance in AUC explained by MG was estimated to be 3 and 4 percent for AUCW1D and AUCW5D, respectively. The inflated posterior heritability estimate may be attributed to associations between markers and AUC phenotypes occurring due to using an admixed population or attributed to the small sample size.

The genetic variance explained by 1-Mb windows, based on the posterior marker specific additive genetic variance estimate, uncovered regions that had a large effect on heat stress. The windows for AUCS5D included BTA1 (90 -91 Mb), BTA8 (43 -44 Mb), BTA10 (91-92 Mb), BTA11 (80-81 Mb), BTA12 (23-24, 25-26, 30-31 Mb), BTA20 (17-18 Mb), BTA22 (10-11 Mb), BTA23 (50-51 Mb), BTA25 (4-5 Mb), BTA26 (49-50 Mb), and BTA27 (12-13 Mb). The windows for AUCS1D included BTA4 (47-48, 82-83 Mb), BTA7 (39-40 Mb), BTA8 (43-44 Mb), BTA10 (32-33 Mb), BTA12 (30-31 Mb), BTA20 (43-44, 50-52 Mb) BTA22 (57-58 Mb), BTA23 (22-23 Mb), BTA27 (12-13 Mb), and BTA29 (1-2 Mb). The windows for AUCW5D included BTA5 (8 -11 Mb), BTA7 (88 -89 Mb), BTA8 (82-83, 86-87), BTA9 (22-23 Mb), BTA18 (11-12 Mb), BTA21 (42-43 Mb), BTA25 (22-23), and BTAX (65-66, 117-118, 142-143 Mb). The windows for AUCW1DW included BTA4 (76-77 Mb), BTA5 (9-11 Mb), BTA7 (70-71, 88-89 Mb), BTA8 (82-83 Mb), BTA9 (22-23 Mb), BTA10 (52-53 Mb), BTA20 (51 – 52 Mb), BTA21 (44-45 Mb), BTA29 (32-22 Mb) and BTAX (65-66, 142-143 Mb). The SNP name, location, and frequency that explained the greatest proportion of additive genetic variance within each of the top 0.5% 1-Mb windows for AUCS1D, AUCS5D, AUCW1D, and AUCW5D are detailed in Table 3.

One window that had had a large impact on body temperature regulation was in the vicinity of previously reported QTL. The region on BTA23 from 22-23 Mb for AUCS1D is 3 Mb away from the heat shock protein 90-kDa beta gene (*HSP90AB1*). A mutation (g.4338T>C) within the *HSP90AB1* gene was found to have an effect on heat susceptibility in two native indigenous Thai breeds (White Lamphun and Mountain cattle) and crossbred Holsteins (Holstein × Thai indigenous breed) (Charoensook et al., 2012). The mutation within the *ATP1A1* gene found in Holstein cattle (Liu et al. 2011) was not found to be associated with heat stress in the current study.

Functional annotation, enrichment and pathway analysis of the extended top 0.5% 1-Mb windows resulted in significant enrichments for multiple biological processes and pathways. A significant enrichment for AUCS5D was cellular response to stress ($P = 0.032$; e.g. *HMGB1*, *RIPK1*). The genes listed have key functions in the extrinsic apoptotic pathway (death receptor pathway) regulating apoptosis of a cell (Elmore, 2007). Furthermore, the extended region on BTA12 from 30-31 Mb and BTA25 from 4-5 Mb, contained the heat shock protein 110-kDa (*HSP110*) and heat shock protein 75-kDa (*HSP75*), respectively. Heat shock proteins (HSPs) are highly conserved ubiquitous stress proteins occurring from bacteria to yeast and humans and comprise of several families (Richter et al., 2010). They are present under normal cellular conditions and situations involving both systematic and cellular stress (Kregal, 2002). During cellular stress HSPs function as molecular chaperones, which enhance the protein folding capacity of a cell, thus counteracting the stress and promoting cell survival (Fulda et al., 2009). In a study by Ju Oh et al. (1997), it was shown that *in vivo* overexpression of *HSP110* conferred substantial heat resistance to both Rat-1 and HeLa culture cell lines. The region on BTA10 91-92 Mb contained the type II iodothyronine deiodinase gene (*DIO2*), which has important functions in the thyroid gland to produce T3 and T4. The thyroid hormones have critical roles in thermogenesis and metabolism (Silvestri et al., 2005). The process enriched at the suggestive level for AUCS1D included intracellular signaling ($P = 0.062$; *TRH*). The extended region on BTA12 (30-31 Mb) contained *HSP110* and *HMGB1*. Furthermore, genes related to apoptosis were within the extended region on BTA4 (47 – 48 Mb; *RAD50*, *BCAP29*) and BTA22 (22-23 Mb; *MBP4*).

Significant enrichments for AUCW5D were metal ion transport ($P = 0.032$; e.g. *ATP2C2*, *SCNN1G*, *SCNN1B*) and calcium (Ca^{2+}) ion transport ($P = 0.044$; e.g. *CACNG3*, *PRKCB*). Pathways enriched for AUCW5D include Aldosterone-regulated sodium reabsorption ($P = 0.048$; *PRKCB*,

SCNN1G) and the pentose phosphate pathway ($P = 0.019$; *FBP2*, *PRPS2*). The genes involved in ion transport involved the directed movement of Ca^{2+} , sodium (Na^{+}), and potassium (K^{+}), all of which have important functions in increasing heat production via ion leaks (Himms-Hagen, 1976). The increased ATP requirement from the ion leaks results in increased ATP consumption and thus metabolic pathways need to be adjusted to account for this (Lowell et al., 2000). The extended region on BTA18 from 11-12 Mb contained the heat shock factor-binding protein 1 (*HSBP1*). The extended region on BTA7 (88-89 Mb) contained the *COX7C* and *RASA1* gene, which have important functions related to metabolism and vascularity, respectively. A mutation within the *RASA1* gene in humans brings about the Parkes Weber Syndrome, which is characterized by capillary malformations (Boon et al., 2005). Significant enrichments for AUCW1D included glucose metabolic process ($P = 0.0076$; *FBP2*, *PGM3*) and vasculogenesis ($P = 0.042$; *RASA1*). The Pathways enriched for AUCW1D include Glycolysis / Gluconeogenesis ($P = 0.017$; *GCK*, *GAPDH*) and the pentose phosphate pathway ($P = 0.021$; *FBP2*, *PRPS2*).

Functional annotation, enrichment and pathway analysis for both AUCS5D and AUCS1D uncovered regions that involved genes underlying how cells respond to heat stress, either through protective roles (i.e. HSPs) or involved in cell death (i.e. genes involving apoptosis). Differences in genetic resistance to environmental stress have been seen in comparisons between *Bos indicus* and *Bos taurus* breeds (Hansen, 2004; Kamwanja et al., 1994), but identifying genes or genetic pathways within a population on a genome-wide level is novel. Furthermore annotation, enrichment and pathway analysis for AUCS5D and AUCS1D uncovered regions involved in metabolic processes related to either, ion movement or enzymes involved in metabolic pathways.

Implications

Medium-density genomic information was able to describe a moderate to large proportion of the phenotypic variation in body temperature during periods of heat and cold stress. Multiple genomic regions contributing to body temperature regulation during periods of heat and cold stress have been located in a crossbred population. The regions need to be further scrutinized in order to locate the causal gene/variant due to multiple candidate genes being in the extended 1-Mb regions. Furthermore, the impact that the genetic variant has on maintenance energy requirements need to be taken into account if a variant is to be used for selection. There was a moderate negative genetic correlation between heat and cold stress, with relatively few genomic regions that had an effect on both heat and cold stress. Thus, simultaneous selection for decreased heat and cold tolerance is possible and MAS can be used to increase the accuracy and efficacy of decreased heat and cold tolerance.

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Table 1. Average ($\pm SD$) CCI¹ and AUC² across 5 d and during the maximal cycle for summer and winter stress conditions.

	Trait ³			
	AUCW5D	AUCS5D	AUCW1D	AUCS1D
CCI	-1.80 (± 11.71)	24.40 (± 4.64)	-13.07 (± 12.51)	29.44 (± 6.72)
AUC	4570.9 (± 49.44)	4627.1 (± 34.37)	919.1 (± 14.86)	936.9 (± 9.68)

¹ CCI = comprehensive climate index (Mader et al., 2010).

² AUC = area under the curve and it was approximated using hourly body temperature observations for 5 d and during the maximal 24 hr stress cycle to where body temperature equals zero.

³ Trait refers to a specific AUC season and observation length where AUCW5D = AUC across 5 d during winter conditions, AUCS5D = AUC across 5 d during summer conditions, AUCW1D = AUC maximal stress cycle during winter conditions, and AUCS1D = AUC maximal stress cycle during summer conditions.

Table 2. Phenotypic and genetic correlations and percent of 1-Mb windows in common between winter and summer stress and 1- and 5-d stress events.

Trait ¹	Phenotypic Correlation	Genetic Correlation	Percent of 1Mb Windows in Common
AUCW1D, AUCW5D	0.887	0.904	43.5
AUCS1D, AUCS5D	0.895	0.935	58.7
AUCW1D, AUCS1D	-0.167	-0.406	7.6
AUCW5D, AUCS5D	-0.221	-0.506	7.6

¹Trait refers to a specific AUC season and observation length where AUCW1D = AUC during the maximal cycle during a winter stress event; AUCW5D = AUC across 5 d during a winter stress event; AUCS1D = AUC during the maximal cycle during a summer stress event; AUCS5D = AUC across 5 d during a summer stress event.

Table 3. Summary statistics for large effect SNP within the top 0.5% 1-Mb windows.

llumina BovineSNP50 SNP ID	Trait ¹	Chromosome	Base Pair	Window ²	Allele Frequency ³
BTA-41479-no-rs	AUCS5D	1	90503071	91	0.611
ARS-BFGL-NGS-86183	AUCS5D	8	43497231	948	0.522
BTA-80379-no-rs	AUCS5D	10	91982227	1218	0.391
ARS-BFGL-NGS-41612	AUCS5D	11	80730546	1312	0.304
ARS-BFGL-NGS-95608	AUCS5D	11	80774399	1312	0.307
ARS-BFGL-NGS-59877	AUCS5D	12	23991213	1363	0.673
ARS-BFGL-NGS-10862	AUCS5D	12	25746796	1365	0.562
ARS-BFGL-NGS-58970	AUCS5D	12	25775588	1365	0.407
ARS-BFGL-NGS-57156	AUCS5D	12	30992759	1370	0.5
ARS-USMARC-Parent- DQ888313-no-rs	AUCS5D	20	17837675	1993	0.353
Hapmap34041- BES1_Contig298_838	AUCS5D	20	17837675	1993	0.353
Hapmap38236-BTA- 55228	AUCS5D	22	10502283	2130	0.358
ARS-BFGL-NGS-11502	AUCS5D	23	50443262	2232	0.46
Hapmap25777-BTC- 071814	AUCS5D	25	4779974	2303	0.229
Hapmap34836- BES2_Contig346_959	AUCS5D	26	49620433	2392	0.516
Hapmap38845-BTA- 63388	AUCS5D	27	12739324	2407	0.622
BTA-63401-no-rs	AUCS5D	27	12818675	2407	0.504
ARS-BFGL-NGS-20161	AUCS1D	4	47396270	475	0.284
BTB-00183730	AUCS1D	4	47463674	475	0.716
BTA-70505-no-rs	AUCS1D	4	47501491	475	0.289
ARS-BFGL-NGS-108354	AUCS1D	4	82167575	510	0.396
ARS-BFGL-NGS-115370	AUCS1D	7	39226471	831	0.609
ARS-BFGL-NGS-119880	AUCS1D	7	39371602	831	0.547
ARS-BFGL-NGS-35666	AUCS1D	7	39392834	831	0.438
ARS-BFGL-NGS-19606	AUCS1D	7	39620003	831	0.458
ARS-BFGL-NGS-88753	AUCS1D	7	39679687	831	0.293
UA-IFASA-8863	AUCS1D	7	39944515	831	0.373
ARS-BFGL-NGS-86183	AUCS1D	8	43497231	948	0.522
BTA-62321-no-rs	AUCS1D	10	32677973	1159	0.46
ARS-BFGL-NGS-4838	AUCS1D	12	30099199	1370	0.198
ARS-BFGL-NGS-57156	AUCS1D	12	30992759	1370	0.5
ARS-BFGL-NGS-98332	AUCS1D	20	43757926	2019	0.451
ARS-BFGL-NGS-113078	AUCS1D	20	50828117	2026	0.224
Hapmap39571-BTA- 50761	AUCS1D	20	50935019	2026	0.22

BTB-00787483	AUCS1D	20	50969848	2026	0.216
ARS-BFGL-NGS-70574	AUCS1D	20	51118749	2027	0.558
BTB-00787333	AUCS1D	20	51143018	2027	0.431
ARS-BFGL-NGS-112879	AUCS1D	22	57035240	2177	0.422
Hapmap45569-BTA-55944	AUCS1D	23	22916565	2204	0.478
Hapmap38845-BTA-63388	AUCS1D	27	12739324	2407	0.622
BTA-63401-no-rs	AUCS1D	27	12818675	2407	0.504
BTB-01316281	AUCS1D	X	1270607	2541	0.562
BTB-01347248	AUCS1D	X	1692506	2541	0.591
UA-IFASA-4572	AUCW5D	5	8562667	557	0.457
BTA-121674-no-rs	AUCW5D	5	9708718	558	0.302
BTB-00221620	AUCW5D	5	10066097	559	0.566
UA-IFASA-4222	AUCW5D	5	10511028	559	0.502
BTB-00324772	AUCW5D	7	88094903	880	0.34
BTB-00324984	AUCW5D	7	88291043	880	0.664
Hapmap35978-SCAFFOLD186218_5128	AUCW5D	7	88922026	880	0.411
ARS-BFGL-NGS-4606	AUCW5D	8	82125433	987	0.58
ARS-BFGL-NGS-67447	AUCW5D	8	82157258	987	0.466
ARS-BFGL-NGS-22890	AUCW5D	8	82249679	987	0.348
ARS-BFGL-NGS-107062	AUCW5D	8	86245016	991	0.386
BTB-00383268	AUCW5D	9	22864414	1043	0.842
ARS-BFGL-NGS-14524	AUCW5D	18	11836226	1856	0.511
Hapmap60703-rs29010376	AUCW5D	21	42089249	2090	0.707
Hapmap50414-BTA-59706	AUCW5D	25	22238007	2321	0.248
ARS-BFGL-NGS-26893	AUCW5D	X	65813619	2605	0.493
Hapmap50310-BTA-30575	AUCW5D	X	117213812	2657	0.686
ARS-BFGL-NGS-117757	AUCW5D	X	142747536	2682	0.62
Hapmap38268-BTA-09661	AUCW5D	X	142828641	2682	0.573
Hapmap53819-rs29009941	AUCW5D	X	142850548	2682	0.568
ARS-BFGL-NGS-117534	AUCW1D	4	76545016	504	0.591
BTA-121674-no-rs	AUCW1D	5	9708718	558	0.302
BTB-00221620	AUCW1D	5	10066097	559	0.566
Hapmap49738-BTA-75486	AUCW1D	5	10335741	559	0.595
UA-IFASA-4222	AUCW1D	5	10511028	559	0.502
Hapmap54764-rs29012507	AUCW1D	7	70669704	862	0.361
BTB-00324772	AUCW1D	7	88094903	880	0.34
BTB-00324984	AUCW1D	7	88291043	880	0.664
BTA-86098-no-rs	AUCW1D	7	88859896	880	0.255

Hapmap35978- SCAFFOLD186218_5128	AUCW1D	7	88922026	880	0.411
ARS-BFGL-NGS-4606	AUCW1D	8	82125433	987	0.58
ARS-BFGL-NGS-67447	AUCW1D	8	82157258	987	0.466
ARS-BFGL-NGS-22890	AUCW1D	8	82249679	987	0.348
BTB-00383268	AUCW1D	9	22864414	1043	0.842
Hapmap39044-BTA- 85348	AUCW1D	9	22961361	1043	0.336
ARS-BFGL-NGS-14607	AUCW1D	10	52836817	1179	0.414
BTB-00425187	AUCW1D	10	52860808	1179	0.618
BTA-69292-no-rs	AUCW1D	10	52955736	1179	0.73
ARS-BFGL-NGS-118883	AUCW1D	10	52998549	1179	0.343
ARS-BFGL-NGS-70574	AUCW1D	20	51118749	2027	0.552
BTB-00787333	AUCW1D	20	51143018	2027	0.439
ARS-BFGL-NGS-118121	AUCW1D	21	44907303	2092	0.436
BTB-01020151	AUCW1D	29	32070762	2520	0.416
UA-IFASA-7281	AUCW1D	29	32594274	2520	0.691
ARS-BFGL-NGS-87575	AUCW1D	29	32621489	2520	0.675
ARS-BFGL-NGS-26893	AUCW1D	X	65813619	2605	0.493
ARS-BFGL-NGS-117757	AUCW1D	X	142747536	2682	0.62
Hapmap38268-BTA- 09661	AUCW1D	X	142828641	2682	0.573
Hapmap53819- rs29009941	AUCW1D	X	142850548	2682	0.568

¹ Trait refers to a specific AUC season and observation length where AUCW5D = AUC across 5 d during winter conditions, AUCS5D = AUC across 5 d during summer conditions, AUCW1D = AUC maximal stress day during winter conditions, and AUCS1D = AUC maximal stress day during summer conditions.

² Window refers to the 1-Mb window (n=2,678) the SNP was in.

³ Allele frequency of the SNP allele.

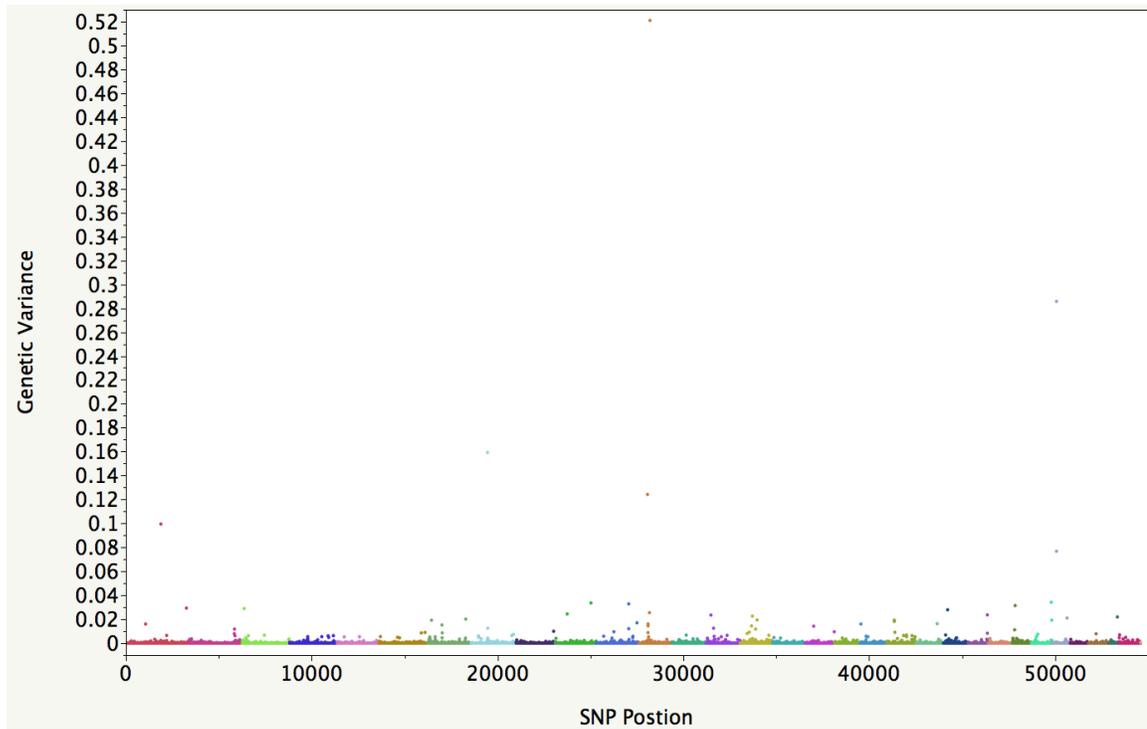


Figure 1. Manhattan plot using 54,609 SNP and area under the curve across 5 d during summer conditions (AUCS5D). Alternate colors represent different autosomes from BTA1 to BTA29, followed by unknown SNP locations and the X chromosome.

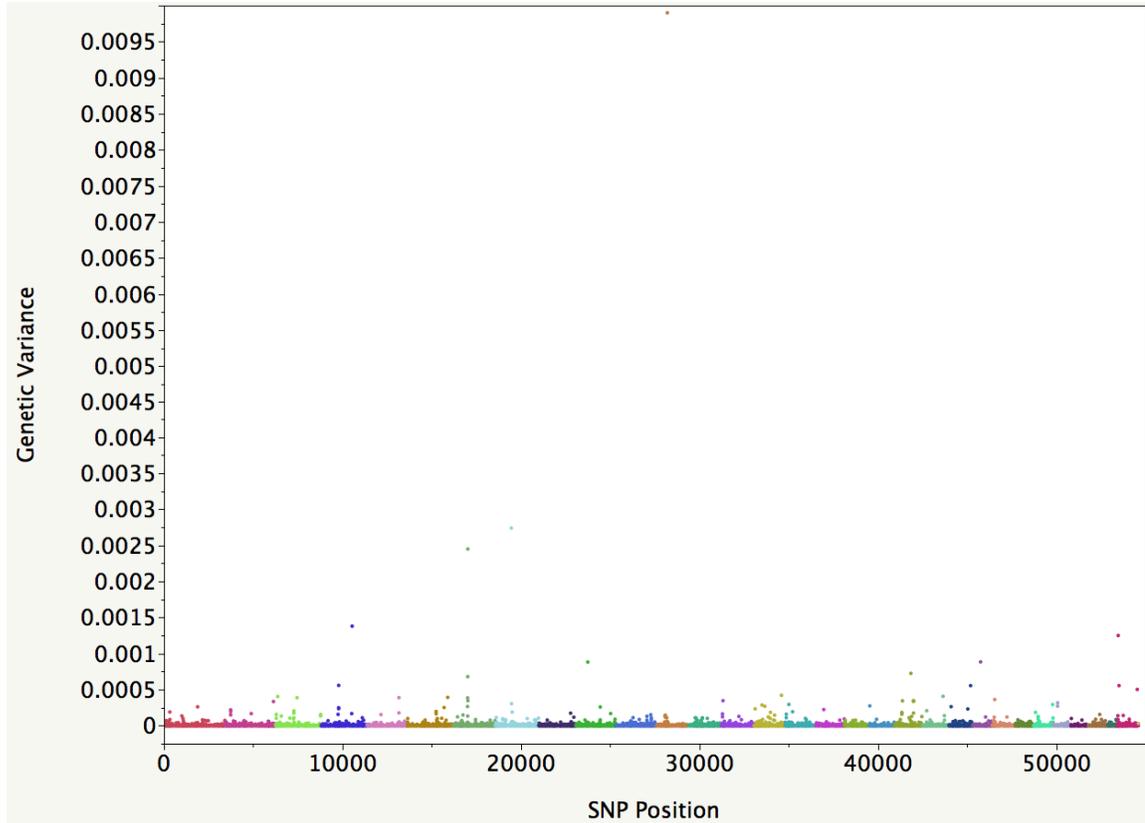


Figure 2. Manhattan plot using 54,609 SNP and area under the curve during maximal summer stress cycle (AUCS1D). Alternate colors represent different autosomes from BTA1 to BTA29, followed by unknown SNP locations and the X chromosome.

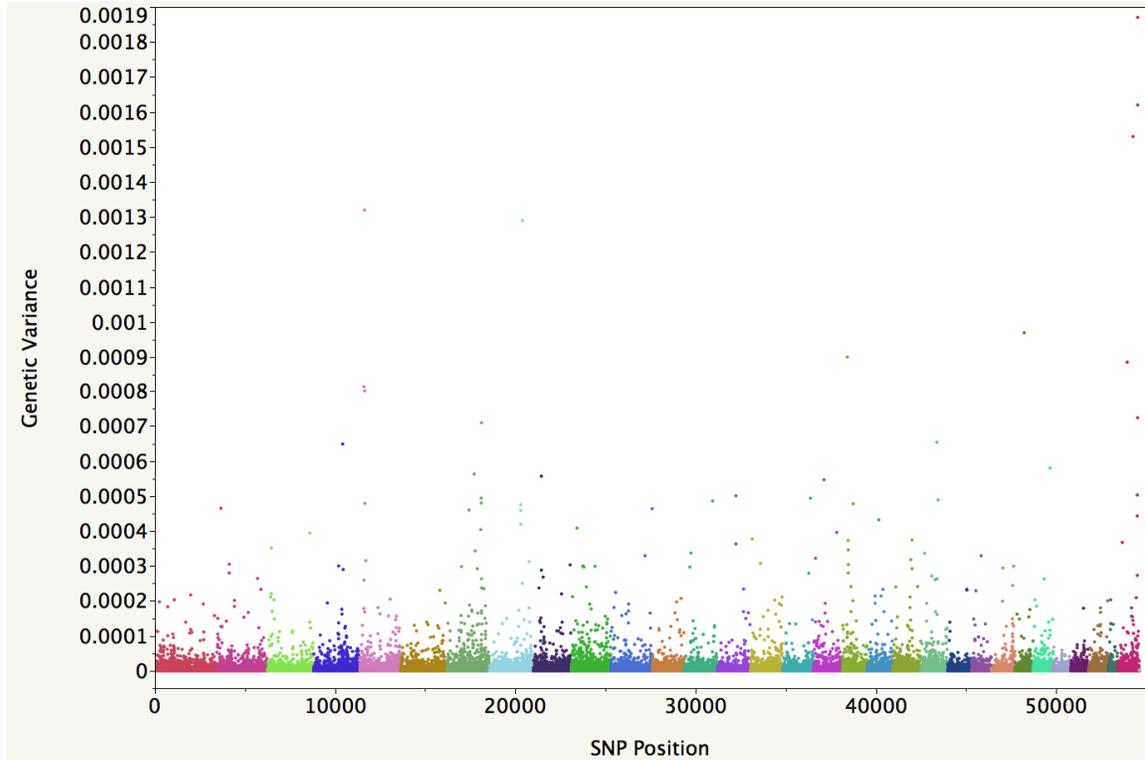


Figure 3. Manhattan plot using 54,609 SNP and area under the curve across 5 d during winter conditions (AUCW5D). Alternate colors represent different autosomes from BTA1 to BTA29, followed by unknown SNP locations and the X chromosome.

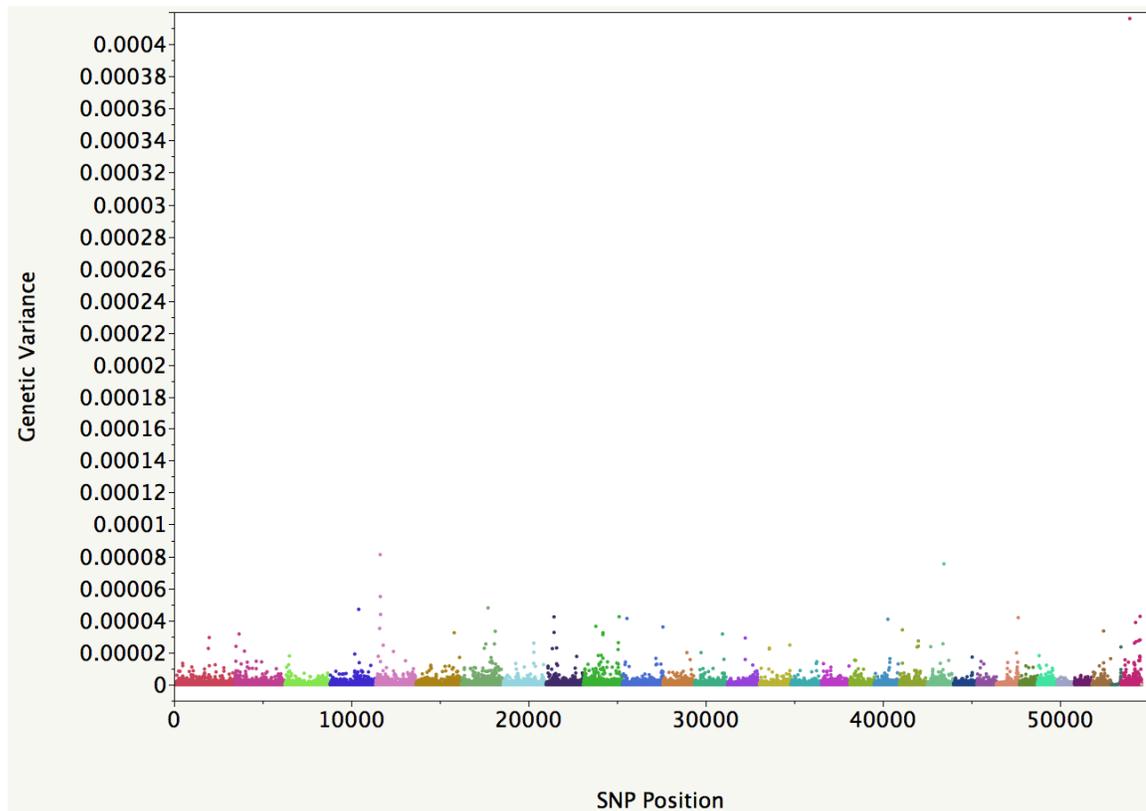


Figure 4. Manhattan plot using 54,609 SNP and area under the curve during maximal winter stress cycle (AUCW1D). Alternate colors represent different autosomes from BTA1 to BTA29, followed by unknown SNP locations and the X chromosome.