GENOMIC CONTRIBUTIONS TO BEEF COW STAYABILITY

A Dissertation

by

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ABSTRACT

Beef cow reproductive longevity represents the cumulation of a lifetime of fertility and production traits and is an important contributor to a producer's profitability. Stayability is a component of longevity, and is often considered to be a good representation of a cow's potential for length of productive life. This complex phenotype is known to be highly influenced by environmental factors, and is expected to be the result of a large number of different genomic influences. Therefore, the primary objective of this research was to understand the genomic contributions to beef cow stayability in *Bos indicus-Bos taurus* crossbred cows, for the purpose of improving selection methodologies for this trait. In this study, a structured research herd of Nellore-Angus crossbred cows was predominately used. Long-term production records combined with SNP genotypes were available for this unique mapping population, allowing for the identification of significant genomic variants associated with measures of stayability. These results suggested that the most significant genetic drivers of this phenotype are likely related to heifer age at puberty, which is not surprising considering the well documented differences in maturation rates between *Bos indicus* and *Bos taurus* females. Genomic differences unique to each subspecies were evaluated and appear to have an influence on the expression of heifer productivity phenotypes. Given the observed importance of heifer productivity on stayability, other early life component traits were evaluated. Results suggest that heifers that calve earlier in their first calving season are significantly more likely to experience a long, productive herd life. This makes selection for puberty or other early life characteristics an attractive option in *Bos indicus-Bos taurus* crossbred cattle, and methods for developing selection tools for heifer maturity were explored. Using a population of *Bos indicus*-influenced cows in northern Australia, this work demonstrated that an industry derived phenotyping mechanism for age at puberty, called reproductive maturity score, was an effective way to predict heifer puberty in extensively managed cow herds. Beef cow stayability in *Bos indicus*-crossbred cows is genetically complex and difficult to characterize, but may be selected for by using component traits, such as heifer age at puberty.

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1. INTRODUCTION

1.1. Stayability definitions

Beef cow stayability is defined as a cow's probability of surviving to a specific age, given the opportunity to first reach that age (Hudson and Van Vleck, 1981). In the United States, stayability typically refers to a cow's ability to remain productive in the herd and produce 5 calves by 6 yr of age, provided that she first calved at 2 yr old (Snelling et al., 1995; BIF, 2018). Stayability to 6 yr is commonly cited as a producer's financial breakeven point, where a cow's initial costs of development are offset by her cumulative net income from yearly calf receipts (Rogers, 1972; Snelling et al., 1995). This definition has not only been endorsed by the Beef Improvement Federation (BIF, 2018), but has also been adopted by multiple breed associations, such as the Red Angus Association of America, the American Simmental Association, and the American Gelbvieh Association, in the form of a stayability expected progeny difference (EPD).

Historically, the accepted stayability definition was developed in *Bos taurus* beef breeds. Similar to the BIF definition of stayability (BIF, 2018), some Brazilian studies with Nellore cows used a threshold system, defining stayability as the point where a cow qualifies as meeting the stayability benchmark only if she successfully produces a calf every year until a given age, generally 6 yr (Silva et al., 2003; Van Melis et al., 2007; Santana et al., 2013; Eler et al., 2014). However, the intrinsic reproductive differences between *Bos taurus* and *Bos indicus* females often necessitates modification of the stayability definition to reflect these distinctions. In multiple other studies of reproductive performance of Nellore cattle, stayability has been defined as a cow's ability to produce 3 calves by 76 mo of age (Guarini et al., 2015; Rizzo et al., 2015). This assumed that 3 calves were the economic breakeven point for Brazilian producers and was referenced as being the earliest age at which a cow was expected to produce 3 calves, given the majority of Brazilian beef cows first calve at 32 mo (Rizzo et al., 2015). Similar definitions have been used in the analysis of Brahman cattle, where stayability represented a cow's ability to produce 3 calves by 6 yr of age (Cavani et al., 2015). The definition of stayability utilized in both production and genetic analyses should accurately reflect the considerations of the production environment.

1.2. Importance of stayability

Stayability is an important econometric standard for producers, as longevity in the cow herd is related to lifetime productivity and profitability of the productive asset (Rogers, 1972). As a component of longevity, stayability is often considered to be an indicator for a cow's potential for length of productive life in the herd (Snelling et al., 1995). Increased herd longevity reduces the need and cost of developing or buying replacements, distributes cow maintenance costs over a larger number of calves, and increases the number of mature, previously proven females in the breeding herd. Older, productive cows demonstrate an increase in percentage of calf crop born and weaned, and in total kg of calf weaned (Cundiff et al., 1992). Renquist et al. (2006) observed a 10% increase in calf weaning weight between British breed type cows of 3 versus 5 yr, a 2% increase between 5 and 7 yr of age, and a 12% increase in cows at 3 versus 7 yr old. As a result, increasing the age structure of the herd through retention of older, productive cows will have a positive net influence on overall herd prosperity. In a *Bos indicus*-influenced herd, Garcia et al. (2014) determined that each additional year of peak cow productivity could add $118 to $244 in value per cow per year. Enns et al. (2005) found that a 1 unit increase in overall herd stayability resulted in an increase in profit of $2,700 for herds with 35% of cows remaining in the herd to 6 yr of age (Núñez-Dominguez et al., 1992).

1.3. *Bos indicus* versus *Bos taurus* stayability

There are a limited number of studies directly comparing cow stayability or herd longevity between *Bos indicus* and *Bos taurus* beef breeds. Riley et al. (2001) found that stayability between Angus-Hereford crosses exhibited a decreased survival rate to age 14 in production than *Bos indicus-Bos taurus* crosses, such as Nellore-Hereford, and Gir-Hereford. Plasse et al. (1968) described that straightbred Brahman heifers were older at the onset of puberty than *Bos taurus* and *Bos indicus-Bos taurus* crossbred heifers. In studies of Brazilian Nellore cows, 72.3% of cows met a stayability benchmark of 3 calves by 76 months (Schmidt et al., 2018), and 28.9% to 31.2% for remaining in the herd until 6 yr of age (Silva et al., 2003; Van Melis et al., 2007). Among *Bos taurus* breeds, 35% to 38.5% of cows met or exceeded the stayability threshold of 5 calves by 6 yr (Snelling et al., 1995; Martinez et al., 2005).

Stayability is a comprehensive reproductive trait reflecting longitudinal reproductive performance. There are well established reproductive differences between *Bos indicus and Bos taurus* females that probably contribute to their likelihood for retention in the herd. *Bos indicus* and *Bos indicus-Bos taurus* crossbred females are known to be slower maturing and older at the onset of puberty than straight *Bos taurus* heifers (Gregory et al., 1979; Chenoweth, 1994; Hearnshaw et al., 1994; Thallman et al., 1999). *Bos indicus* heifers are less likely to first calve at 2 yr of age, and if successful, less likely to rebreed during the ensuing breeding period (Chenoweth, 1994).

Bos indicus-influenced, crossbred beef cows show increased potential for productive longevity in the herd, likely as a result of the combined advantage of heterosis and adaptation to the climates in which they are raised (Riley et al., 2001; Thrift and Thrift, 2003). *Bos indicus-Bos taurus* crossbred cows are more likely to avoid culling due to decreased rates of dystocia and decreased tooth loss at advanced ages (Riley et al., 2001; Thrift and Thrift, 2003). Furthermore, Riley et al. (2001) observed that *Bos indicus-Bos taurus* cows were significantly less likely to leave the herd due to reproductive failure than their *Bos taurus* crossbred counterparts. However, *Bos indicus-Bos taurus* crossbred cows are also known to have difficulties with fertility early in life that are largely independent from trends observed in straight *Bos taurus* cows (Plasse et al., 1968; Chenoweth, 1994; Thallman et al., 1999). The slower maturation rate expected in *Bos indicus*-influenced females has a negative impact on the proportion of heifers that calve at 2 yr of age and the subsequent percentage returning to estrus during the following breeding season (Chenoweth, 1994); this decreases their likelihood of meeting the traditional stayability benchmark.

1.4. Heritability

Beef cow stayability is a complex, comprehensive reproductive trait with prior estimates of low to moderate heritabilities in both *Bos indicus* and *Bos taurus* cattle. In the original genetic assessment of beef cow stayability by Snelling et al. (1995) in a herd of Angus cows, the heritability was found to be 0.12, 0.18, 0.18, and 0.19 for stayability to 3, 6, 9, and 12 yr of age, respectively. Similarly, in a mixed herd of Gelbvieh, Red Angus, and Simmental cows, (Brigham et al., 2007) calculated heritabilities were 0.16, 0.17, 0.18, and 0.18 for stayability to 3, 4, 5, and 6 yr of age, respectively. Heritability for stayability to 6 yr of age (5 calves) was estimated at 0.15 in a herd of Canadian Simmentals (Jamrozik et al., 2013), 0.11 and 0.19 in Nellore cows (Santana et al., 2011; Eler et al., 2014), and 0.11 in a mixed breed Czech population (Brzakova et al., 2019). In Nellore and other *Bos indicus* cattle, the definition of stayability often strays from what is deemed traditional. Comparable to previously described studies, in a herd of Nellore females, Silva et al. (2003) estimated heritabilities of 0.12, 0.12, and 0.17 at 5, 6, and 7 yr of age, respectively. Alternatively, when stayability was characterized by successfully having 3 calves by 76 mo of age in Nellore cows, heritabilities of 0.22, 0.20 to 0.25 (Rizzo et al., 2015), and 0.14 (Guarini et al., 2015; Rizzo et al., 2015; Schmidt et al., 2018) were estimated. When defined this way in Brahman cattle, Cavani et al. (2015) reported a heritability of 0.10. Unsurprisingly, stayability traits are largely influenced by broad sense environment making selection difficult (Jamrozik et al., 2013). Consequently, it is expected that the distinguishable genetic influences on stayability will be small and difficult to detect.

The traditional definition of stayability (Snelling et al., 1995) does not give any clarification as to the culling criteria that should be used to designate a stayability threshold and, consequently, there are several culling criteria applied within the prior literature. Martinez et al. (2005) found that the heritabilities for stayability for calving and weaning ($h^2 = 0.35$ and 0.21, respectively) were higher than for stayability to a defined age of 6 yr ($h^2 = 0.17$), indicating that selection for the prior definitions of stayability would be more effective than the latter. In this study (Martinez et al., 2005), “stayability to a specific age” was defined as whether the cow survived to a specific age, “stayability to calving” was based on the number of calves born to each cow, and “stayability to weaning” was based on the number of calves weaned by each cow. Stayability to a specific age may be thought of as general soundness of a cow, whereas stayability to calving represents a cow’s ability to recover and rebreed after each parturition. On the other hand, stayability to weaning measures a cow’s ability to recover to rebreed while raising a calf to weaning (Jamrozik et al., 2013). These distinctions clarify the definitions of stayability, likely reducing the environmental variance for each definition.

1.5. Associated genomic markers and candidate genes

As with most complex, quantitative traits, reproduction and, thus, stayability are assumed to be the result of a large number of genetic influences working in concurrence. In an attempt to identify the most important drivers of stayability, multiple groups have undertaken genome-wide association analyses (GWAS) using SNP genotypes. A GWAS is an analysis that exploits linkage disequilibrium among DNA markers to detect statistical associations between these markers and phenotypes observed in individuals in a population (Visscher et al., 2017). Speidel et al. (2018) used deregressed expected breeding values (EBV) to conduct a GWAS for stayability from cow records provided by the Red Angus Association of America. The Red Angus Association of America defines stayability as successfully having 5 calves by 6 yr of age. Markers tagging quantitative trait loci for stayability were found on bovine chromosomes (BTA) 6, 8, 9, 12, 15, 18, 22, and 23, with the strongest association on BTA 9 at 17 Mb on UMD3.1.1 (Speidel et al., 2018). In a secondary study conducted in Simmental cows, Saatchi and Garrick (2016) found 2 QTL associated with stayability on BTA 6 at 40 and 71 Mb, which is a different genomic location than the previous study. In a population of Nellore cows, Teixeira et al. (2017) conducted a single-step genomic best linear unbiased prediction (ssGBLUP) GWAS for stayability defined as successfully remaining productive in the herd until 65 mo of age. They found 10 windows of 200 SNP each on BTA 1, 2, 5, 6, 9, 20, and X. In a herd of Nellore-Angus crossbred cows, Engle et al. (2018) defined stayability as successfully birthing 5 calves by 6 yr of age, or as successfully weaning 5 calves by 6 yr of age. In a GWAS conducted for the first definition, they found associated SNP QTL on BTA 1, 2, 5, 9, 18, and 21, and in the latter definition QTL associations were identified on BTA 1, 5, 11, 15, and 24, with a critical region on BTA 5 from 43-50 Mb (UMD3.1) in both analyses. There is likely some discordance between the studies in Simmental or Red Angus cows in comparison to a crossbred *Bos indicus-Bos taurus* herd due to differences between the subspecies, as is also observed between the analyses of Nellore and Nellore crossbred cows.

Due to the difficulties of assessing a strict stayability phenotype in a commercially managed cow herd, many alternatives to stayability that are still related to longitudinal cow productivity are often utilized. McDaneld et al. (2014) conducted an association analysis on a trait they called reproductive efficiency in both *Bos taurus* breeds and *Bos indicus* x *Bos taurus* composites. Among the *Bos taurus* herds assessed, a significant SNP was identified on BTA 29, with suggestive positions on BTA 1, 5, 21, and 25. In the composite herds, associations were found on BTA 5 and 25. Hamidi Hay and Roberts (2017) identified genomic positions on BTA 1, 3, 9, 19, and 25 in a population of *Bos taurus* composite cows for cow longevity, as defined as number of months from first calving to disposal.

One of the first large scale association studies for reproductive traits in *Bos indicus* beef cattle was conducted by Hawken et al. (2012) for age at puberty and heifer productivity. In this study, they sought to find genome-wide SNP associations between age at first corpus luteum (AGECL) and postpartum anestrus interval (PPAI) in Brahman or Tropical Composite heifers. They found that there were very few SNP at the same location between breeds, potentially reflecting differences in SNP variability between *Bos indicus* and *Bos indicus* crossbred cattle. For PPAI, most of the significant associations in Brahman heifers were on BTA 3 or BTA 14, near the *PLAG1* gene, and in Tropical Composite heifers the associations were concentrated on BTA 5 and BTA 16. In Brahman cattle, over 40% of the significant markers associated with AGECL mapped to the region on BTA 14 concordant with the *PLAG1* gene. Within the Tropical Composite heifers, 16% of the significantly associated SNP were located on BTA 5, with a critical region from 44 to 50 Mb. The critical region from 44 to 50 Mb on CHR 5 (UMD3) has been associated with many traits in *Bos indicus-Bos taurus* crossbred cattle such as the growth traits, live weight and hump score (Bolormaa et al., 2013), as well as percent intramuscular fat, back fat, and mature hip height (Bolormaa et al., 2014). Additionally, associations to other reproductive traits in similar types of cattle also align to this region, including udder characteristics (Tolleson et al., 2017) and reproductive efficiency (McDaneld et al., 2014).

Assessment of the critical regions in these analyses reveal many candidate genes related to growth, immune response, and hormone signaling (Fortes et al., 2011; Beltman et al., 2013). Within Brahman cattle, one of the most influential genes related to heifer maturity and, thus, subsequent stayability is the highly pleiotropic gene, *PLAG1* (Fortes et al., 2012; Hawken et al., 2012; Melo et al., 2018). In mouse studies, plag1 knockout mice suffer from slow growth rates and dwarfism, and in beef cattle *PLAG1* has widely been attributed to growth rate and body weight (Karim et al., 2011; Bolormaa et al., 2014; Fink et al., 2017; Utsunomiya et al., 2017). This gene has been shown to be introgressed in the Brahman genome from its taurine ancestors (Fortes et al., 2013; Utsunomiya et al., 2017; Koufariotis et al., 2018). The major allele is a functional mutation of taurine origin near *PLAG1* that has been implicated in significantly increased hip height, weight, net food intake, age at puberty in males and females, and decreased IGF-I concentration in blood and fat depth (Fortes et al., 2013). This allele not only appears to be under strong selection in Brahmans, but has been selected almost to fixation in *Bos taurus* cattle (Fortes et al., 2013).

There are multiple studies identifying and assessing candidate genes for stayability in dairy cows (Khatkar et al., 2014). However, there are tremendous biological differences between beef and dairy breeds, especially compared to *Bos* *indicus* cattle, and the expected genetic differences due to differential selection pressures (Bovine HapMap Consortium, 2009) make direct comparisons between the two difficult. Furthermore, milk production is often weighted highly in dairy herd culling criteria, and this is expected to introduce bias into genetic assessment of stayability (Szyda et al., 2011).

1.6. Correlated traits

Stayability, as a reflection of cumulative yearly productivity, may not be measured until a cow reaches an advanced age. This introduces costs into the production system, increases generation interval for the trait, and reduces opportunity for genetic improvement. As a result, creative alternatives to stayability have been developed. Average annual cow productivity (PRODAM) is an index developed in Nellore cattle by Eler et al. (2008) that takes into account cumulative calf weaning weights and adjusts for the preferred age at first calving of 3 yr, and is directly influenced by a cow's ability to begin reproduction earlier in life and to remain productive in the herd. This measure is thought to be advantageous over stayability as it can be used to evaluate the genetic merit of cows with only a few calves (Santana et al., 2013). Average annual cow productivity appears to be a good substitution for stayability, as evidenced by the high genetic correlations observed between the 2 traits. Santana et al. (2013) estimated a genetic correlation between PRODAM and stayability of 0.85 in *Bos taurus-Bos indicus* composite cows, and in Nellore cows, correlations of 0.99, 0.94, and 0.86 (Santana et al., 2013; Eler et al., 2014; Schmidt et al., 2018) have been measured.

Hudson and Van Vleck (1981) argued against direct selection for longevity due to low heritability, increased generation interval, and automatic selection via older cows that contribute more offspring to subsequent generations than short-lived cows. Due to these arguments, it would be advantageous to identify component traits correlated to longevity and stayability that can be measured and utilized as culling criteria earlier in life. Stayability measures at different ages are highly correlated to one another, as evidenced by Jamrozik et al. (2013) in Simmental cattle. The correlation between stayability at 2 yr versus 6 yr was found to be 0.77, at 3 yr versus 6 yr was 0.87, and between 4 yr and 6 yr was 0.94 (Jamrozik et al., 2013). The correlation between stayability and weight traits varies from study to study. Estimated genetic correlations of -0.11, 0.20, and 0.23 between stayability and post weaning gain were observed in Nellore cows (Santana et al., 2011; Santana et al., 2013; Eler et al., 2014), and 0.14 in *Bos indicus-Bos taurus* composite cows. In a separate population of *Bos indicus-Bos taurus* composite cattle, a correlation of 0.09 was found between stayability and body weight at 420 d (Buzanskas et al., 2010). In contrast, a correlation of 0.66 was estimated between stayability and mature cow weight in a Nellore population (Schmidt et al., 2018).

Indicators of puberty are genetically correlated to stayability and lifetime productivity in a wide variety of beef cattle breeds (Morris and Cullen, 1994; Buzanskas et al., 2010; Van Melis et al., 2010; Jamrozik et al., 2013; Eler et al., 2014; Cavani et al., 2015; Guarini et al., 2015; Schmidt et al., 2018). In a mixed herd of *Bos taurus* cattle, Morris and Cullen (1994) estimated the genetic correlation between standardized age at first estrus and lifetime pregnancy rate to be -0.76. When considering the correlation between age at first calving and stayability to 6 yr of age in Nellore herds, Eler et al. (2014) and Schmidt et al. (2018) estimated a genetic correlation of -0.60 and -0.15, respectively. In Brahman cows, Cavani et al. (2015) found correlations of -0.57, and in *Bos indicus-Bos taurus* composite cows Buzanskas et al. (2010) estimated a correlation of -0.63. The negative correlations indicate that decreased lifetime productivity is correlated with older age at first estrus and, thus, older age at first calving. In a population of Simmental cattle, a genetic correlation of 0.77 was estimated between heifer pregnancy and stayability to 6 yr of age (Jamrozik et al., 2013), which is similar to estimations between the same traits in Nellore cows of 0.64, 0.59, and 0.73 (Van Melis et al., 2010; Santana et al., 2011; Eler et al., 2014). The genetic correlation between stayability and heifer rebreeding in Brahman cows is 0.32 (Cavani et al., 2015) and 0.97 in Nellore cows (Guarini et al., 2015). These correlations indicate that heifer performance may be indicative of a cow's potential for stayability. Heifer productivity is a critical period in a cow's productive lifetime and is often reflective of her long-term potential for profitability (Núñez-Dominguez et al., 1991; d'Orey Branco et al., 2016).

When considering the influences of puberty on stayability, it is especially pertinent to consider the innate physiological differences between *Bos indicus* and *Bos taurus* cattle for this phenotype. Age at puberty is defined as the first day that serum progesterone exceeds 1 ng/mL (Schillo et al., 1983). This parameter is difficult to measure, and is often approximated using associated traits such as age at first observed standing estrus (Gregory et al., 1979), estimated age at first conception (Thallman et al., 1999), age at first corpus luteum using manual palpation (Plasse et al., 1968), or age at first corpus luteum using ultrasound examination (Pierson and Ginther, 1984; Johnston et al., 2009), with the latter of these being the most precise estimation. Using age at first observed estrus as a physiological indicator, average age at puberty in Brahman-cross heifers has been reported at 510 d and 398 d by Plasse et al. (1968) and Gregory et al. (1979), respectively, and when considering age at first conception as the physiological indicator of puberty, Riley et al. (2010) reported an average age of 461 d. In a population of Nellore-Angus F_1 heifers, average age at puberty was 405 d using first observed estrus as the determinant (Thallman et al., 1999), and in a separate population of *Bos indicus*-*Bos taurus* crossbred heifers, average age at first corpus luteum as a measure of puberty was 656 d (Hawken et al., 2012).

1.7. Selection for stayability

There is no doubt that stayability and longevity in the cow herd are genetically complex traits with a high degree of environmental influence, making selection for them difficult. However, Paneto et al. (2002) reported that although degree of genetic progress would be small when selecting for stayability, it is possible and warranted. Stayability EPD, predominately determined by a bull's daughter's ability to raise 5 calves by 6 yr of age, have already been adopted by the Red Angus Association of America, the American Simmental Association, and the American Gelbvieh Association. Additionally, selection strategies that utilize component or highly correlated indicator traits may be used to effectively select for stayability earlier in a cow's lifetime. Indices and weighted EPDs are the traditional way in which multiple component traits may be utilized to select for a potentially unmeasured criterion (Hazel, 1943). Furthermore, selection using indices is considered to be one of the most efficient methods for simultaneously improving multiple traits (Costa et al., 2017), which would be advantageous for a complex, multifactorial trait such as stayability. For example, general maternal productivity can be characterized as a summation of successive cow efficiency measures with added components such as reproductive ability and longevity (Crews, 2005). It is often measured in terms of a cow's economic outputs relative to production costs within the beef production system, making it a comprehensive measure for producers to utilize within their own programs, and may be a model by which to approach selection for stayability.

With the widespread adoption of genomic selection, new opportunities are available to select for highly polymorphic traits, such as stayability and cow productivity. Hayes et al. (2019) found that when selecting for female fertility in tropical beef cattle (*Bos indicus* and *Bos indicus*-crossbred cows) that a multibreed reference population is advantageous and high-density SNP markers should be utilized for estimating EPD. In the same population of cattle, Engle et al. (2019) was able to show the advantages of using multi-trait models to improve accuracy of genomic breeding values for beef cow fertility, and this is was further supported within less genetically complex traits such as growth and color, as reported by Porto-Neto et al. (2015). Utilizing a multi-trait model may be one way to harness the phenotypic and genotypic complexities exhibited by stayability, and create effective genomic selection tools.

1.8. Summary

Stayability concatenates a multitude of genetic and environmental influences on reproduction over a period of years into a single, producer friendly representation of cow productivity. This trait has a tremendous impact on cow profitability, representing the cumulation of yearly managerial decisions applied to a cow. Stayability is a low to moderately heritable characteristic, making both selection of the trait and understanding the genetic influences difficult. This matter is further complicated by the acute genetic and reproductive differences that exist between *Bos indicus, Bos taurus,* and *Bos indicus-Bos taurus* crossbred beef cows. Differences between age at maturity between the subspecies has a large influence on a cow's likelihood of meeting a stayability threshold, and it is likely that the genetic architecture driving these traits differs as well. Stayability is genetically associated with a number of genes influencing processes such as growth and hormone signaling. Despite the challenges associated with improving overall herd stayability, selection tools are being developed to accommodate these hurdles.

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2. GENOME-WIDE ASSOCIATION STUDY FOR STAYABILITY MEASURES IN NELLORE-ANGUS CROSSBRED COWS[*]

2.1. Introduction

Beef cow stayability is a complex trait often used as an indicator of a cow's potential lifetime productivity. Stayability was first defined as a cow's probability of surviving to a specific age, given the opportunity to first reach that age (Hudson and Van Vleck, 1981). Now, stayability usually refers to a cow's ability to maintain a perfect weaning record and produce 5 calves by 6 yr of age, typically with respect to *Bos taurus* cattle (Snelling et al., 1995). Heritability estimates for stayability range from 0.1 to 0.22 (Snelling et al., 1995; Van Melis et al., 2007; Cavani et al., 2015). Stayability is an important metric for producers, as longevity in the cow herd is related to lifetime productivity and economic value or profitability of the productive asset (Rogers, 1972).

Bos indicus-Bos taurus crossbred cows are recognized as having high potential for long reproductive lifespans, likely due to the combined advantages of heterosis and adaptation to the climates in which they are raised (Riley et al., 2001; Thrift and Thrift, 2003). However, *Bos indicus*-influenced females reach puberty later than *Bos taurus* heifers, are significantly less likely to first calve at 2 yr of age, and if successful, experience difficulty rebreeding during the subsequent breeding period (Chenoweth,

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1994), decreasing their likelihood of meeting the stayability benchmark. As a popular choice amongst producers in tropical and subtropical climates, it would be economically advantageous to understand the underlying genetic contributors of stayability in *Bos indicus-Bos taurus* crossbred cows. Therefore, the objective of this study was to identify genetic variants associated with measures of beef cow stayability.

2.2. Materials and methods

2.2.1. Population

Cows used in this study were part of the McGregor Genomics Cycle 1 Population, an experimental population housed at the Texas A&M AgriLife Research Center at McGregor, TX. When this population was developed, the primary objective was to understand cow lifetime productivity traits. These females (n = 305) were born in 2003 through 2007 and they were from either 13 full-sibling F_2 families produced through embryo transfer (ET) or 4 paternal half-sibling families produced through natural service (NS) matings. These cows were all *Bos indicus-Bos taurus* crosses, specifically Nellore-Angus F_2 crosses, Nellore-Angus x Brahman-Angus crosses, or Nellore-Angus x Brahman-Hereford crosses. All procedures involving animals were approved by the Texas A&M Institutional Animal Care and Use Committee.

Cows from this population were born either during spring or fall calving seasons. They were vaccinated against clostridial diseases at 2 to 3 mo of age, and then again at weaning. On average, these cows were weaned as calves at 214.8 ± 0.93 d of age. After weaning, but before the first breeding season, they were also vaccinated against bovine viral diarrhea virus, bovine respiratory syncytial virus, infectious bovine rhinotracheatitis, parainfluenza type 3, leptospirosis, vibriosis, and treated for internal parasites. As heifers, they were developed on native, warm season perennial pastures, and were nutritionally managed for a target body condition score of 5 to 6 by the first breeding and calving seasons, and were given a protein supplement as necessary.

On average, heifers were first exposed to Angus bulls at 433.3 ± 0.99 d of age for the opportunity to first calve at approximately 2 yr of age. Spring-born heifers (ET and NS) were all managed to first calve at 2 yr of age. Fall-born heifers (ET only) were exposed to Angus bulls from the first week in December to the second week in February and given the opportunity to first calve at 2 yr of age in the following fall. Those that initially failed to conceive were transitioned to a spring calving schedule, and were bred to first calve at 2.5 yr of age, without a failure to calve counted against them. Any fall-born heifers that first calved during the fall were held through the winter without mating opportunity and rebred in the following spring breeding season to be on a spring calving schedule, with their second calf born at 3.5 yr of age. Subsequently, all cows were managed for spring calving only and they were typically exposed to bulls from the third week in May to the third week in July. Across the study, the average length of the breeding season was 68 d.

Females were allowed to remain in the herd until their second failure to wean a calf. Calves were kept with their dams until weaning at an average of 209.8 ± 0.57 d of age. If a cow was ever deemed unfit to care for her calf, and the calf was removed from her, that was counted as a failure to wean. Records used for this analysis span from the date of this population’s first possible calving season in 2005 through 2014.

2.2.2. Phenotypes

Phenotypes for various measures of stayability to 6 yr of age were produced by artificially imposing culling criteria on data from the population. Any cow that left the herd prior to 6 yr for a reason other than the criterion being considered was omitted from that analysis, so the number of cows included in each analysis differed and ranged from 169 to 300 cows. Cows were scored either as a 1 to indicate a perfect record under each criterion through 6 yr, or as a 0, to indicate failure at or before 6 yr under each criterion (Table 2.1). The first culling criterion corresponded to the actual management of the herd, in which cows were removed from the herd upon their second incidence of failure to wean a calf, regardless of reason (Criterion 1).

The second constructed culling criterion, cows were scored as a 0 (failure) upon their first failure to wean a calf, regardless of reason (Criterion 2*)*. This criterion corresponds to the Beef Improvement Federation (BIF, 2016) definition of stayability. For the third culling criterion, a cow was scored as a 0 upon her first failure to give birth to a calf (Criterion 3*)*. Criterion 3 was used as an indication of pregnancy, so a full term, stillborn calf was not considered a failure.

Under the fourth criterion, a cow was scored as a 0 when she gave birth to a calf and then for any reason failed to wean that calf (Criterion 4). Note that under this criterion a prior failure to calve was ignored. For the fifth criterion, a cow was scored as a 0 upon her first instance of failing to wean a calf, provided that she had no prior instances of calving failure (Criterion 5). For each of these criteria, lifetime productivity records were analyzed and scores were manually assigned.

Table 2.1 Description of culling criteria

Criterion	Description
1	Cows were removed from the herd upon their second incidence of failure to wean a calf, regardless of reason, through 6 yr
2	Cows were scored as culled upon their first failure to wean a calf, regardless of reason, through 6 yr
3	Cows were scored as culled upon their first failure to give birth to a calf, through 6 yr
4	Cows were scored as culled upon their first incidence of failure to wean a calf, but not considering calving failure as a reason, through 6 yr
5	Cows were scored as culled upon their first incidence of failure to wean a calf, provided that they had never previously experienced calving failure, through 6 yr

2.2.3. Genome-wide association study

Filtered genotypes for these cows were the same as those described by Hulsman Hanna et al. (2014). Briefly, DNA was extracted from white blood cells and genotyped using the Illumina BovineSNP50v1 chip (Illumina Inc., San Diego, CA). Chromosomal assignments and positions of SNP were based on the UMD3.1 *Bos taurus* sequence assembly. Genotypes from the whole population (males and females) were filtered in PLINK (Purcell et al., 2007) to remove SNP with completion rates < 90%, minor allele frequencies < 0.05, and those deviating from Hardy-Weinberg equilibrium proportions at $P < 0.0001$. After filtering, average SNP spacing was 75.9 kb with a median of 50.5 kb.

Each of the 5 stayability phenotypes (0 = left herd, 1 = remaining in the herd through 6 yr of age) were pre-adjusted for the fixed effect of contemporary group (birth year and season of birth) using linear model procedures in R, and the residuals from these models were used in GWAS. Genome-wide association studies for beef cow reproductive longevity were performed using the univariate procedures of GEMMA (Zhou and Stephens, 2012) that fitted a single, standardized, genomic relationship matrix to account for genetic covariances among animals. The default SNP filters in GEMMA (missingness 0.05, minor allele frequency 0.01, r-squared threshold 0.999) were used, and because a different number of cows were part of each analysis, some SNP that passed the PLINK filters were subsequently excluded by GEMMA. The Benjamini and Hochberg false discovery rate (Benjamini and Hochberg, 1995) was initially constrained to 0.05 to correct for multiple testing. Given the complex nature of stayability, we anticipated that the SNP heritability would be explained by many small SNP effects (Manolio et al., 2009; Boyle et al., 2017). To minimize failure to detect true associations and reduce the type II error in our study, a false discovery threshold of 0.15 was ultimately used.

2.3. Results and discussion

Stayability typically refers to a cow's ability to remain productive in the herd and produce 5 calves by 6 yr of age, provided that she first calved at 2 yr (Snelling et al., 1995). Although alternatives to this definition at earlier ages have been proposed, a cow's potential to maintain a perfect weaning record through 6 yr is the definition adopted by BIF (2016) and numerous breed associations. Using this definition, EPD for stayability have been developed by the Red Angus Association of America, the American Simmental Association, and the American Gelbvieh Association (Snelling et al., 1995). For these reasons, the analyses were conducted using the most commonly accepted definition of stayability, where a cow must remain in the herd through 6 yr of age.

Under actual management (Criterion 1), just 19% of the cows were removed from the herd before 7 yr of age (Table 2.2). By giving cows 2 opportunities for failure before removing them from production, a similar number of cows (3 to 6%) were removed from the herd each year. Criterion 1 resulted in limited detectable associations (Table 2.3). Although some SNP were found to be significant, no SNP survived correction for multiple testing, and no distinct structure was visible in the corresponding Manhattan plot (Fig. 2.1a). This criterion also gave us no insight into the herd's reproductive performance at 2 yr.

Table 2.2 Proportion of cows remaining in the herd that were culled under each criterion by each age

	Culled					
Criterion[1]	**2 yr**	**3 yr**	**4 yr**	**5 yr**	**6 yr**	**Not Culled**
1	N/A	0.06	0.06	0.05	0.03	0.81
2	0.23	0.20	0.07	0.05	0.06	0.39
3	0.17	0.18	0.06	0.03	0.04	0.52
4	0.07	0.07	0.04	0.05	0.04	0.73
5	0.11	0.07	0.04	0.05	0.04	0.69

[1] 1 = cows were removed from the herd upon their second incidence of failure to wean a calf, regardless of reason, through 6 yr; 2 = cows were scored as culled upon their first failure to wean a calf, regardless of reason, through 6 yr; 3 = cows were scored as culled upon their first failure to give birth to a calf, through 6 yr; 4 = cows were scored as culled upon their first incidence of failure to wean a calf, but not considering calving failure as a reason, through 6 yr; 5 = cows were scored as culled upon their first instance of failing to wean a calf, provided that they had no prior instances of calving failure, through 6 yr.

Table 2.3 Number of significant markers for each culling criterion

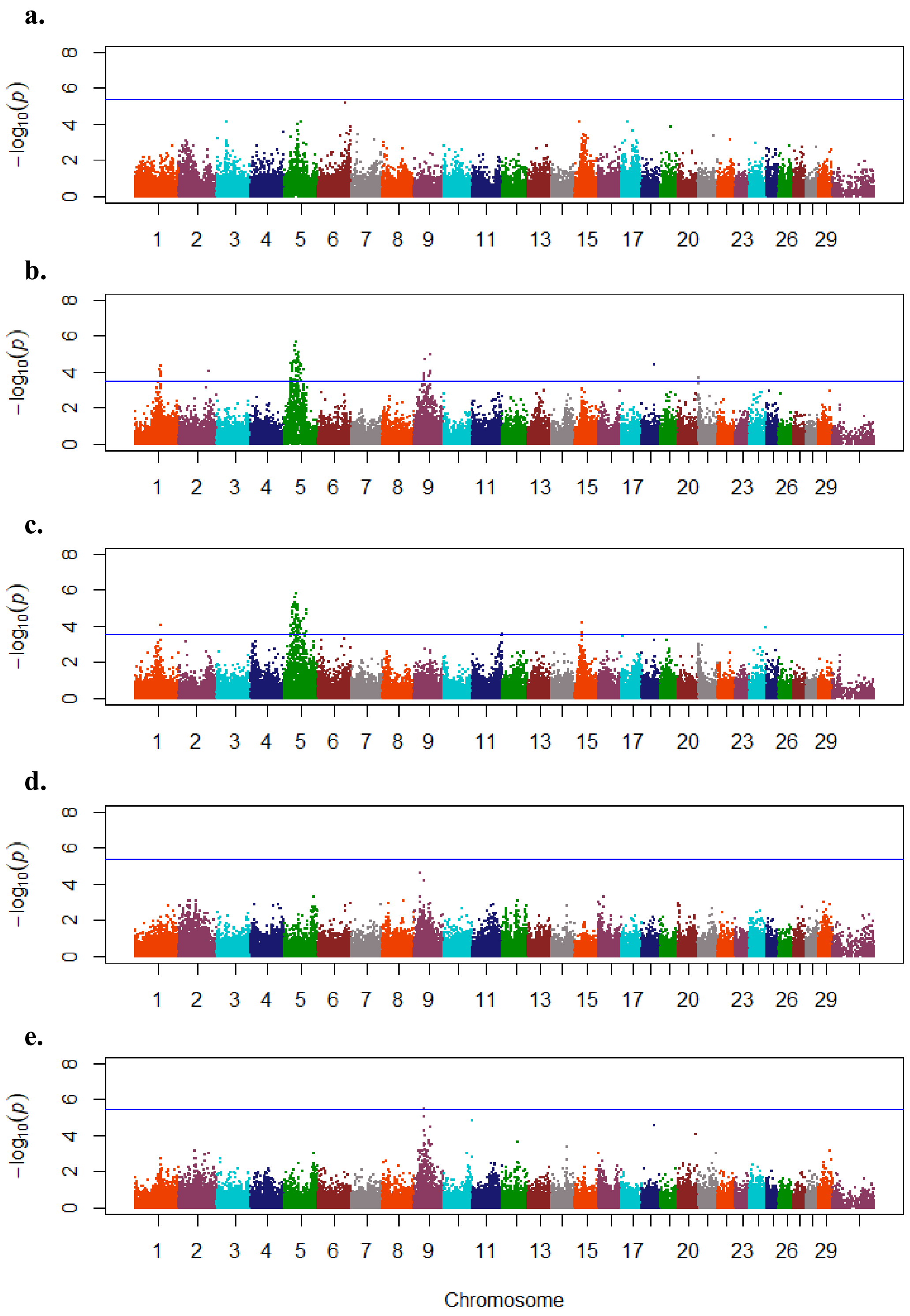
Criterion[1]	n	Counts		Total No. SNP	No. significant SNP		After FDR[2]	PVE[3]
		0	1		$P < 0.01$	$P < 0.0001$		
1	291	60	231	34,640	371	6	0	0
2	300	182	118	34,675	517	46	69	0.218
3	294	141	153	34,632	492	44	61	0.181
4	266	70	196	34,655	306	2	0	0
5	169	51	118	34,596	238	7	1	0

[1] 1 = cows were removed from the herd upon their second incidence of failure to wean a calf, regardless of reason, through 6 yr; 2 = cows were scored as culled upon their first failure to wean a calf, regardless of reason, through 6 yr; 3 = cows were scored as culled upon their first failure to give birth to a calf, through 6 yr; 4 = cows were scored as culled upon their first incidence of failure to wean a calf, but not considering calving failure as a reason, through 6 yr; 5 = cows were scored as culled upon their first instance of failing to wean a calf, provided that they had no prior instances of calving failure, through 6 yr.

[2] False discovery rate = 0.15

[3] Proportion of variance explained

Figure 2.1 Manhattan plots of genome-wide associations for each culling criterion. Horizontal line represents the false discovery rate threshold of 0.15 for each criterion. a) Criterion 1, where cows were removed from the herd upon their second incidence of failure to wean a calf, regardless of reason, through 6 yr; b) Criterion 2, where cows were scored as culled upon their first failure to wean a calf, regardless of reason, through 6 yr; c) Criterion 3, where cows were scored as culled upon their first failure to give birth to a calf, through 6 yr; d) Criterion 4, where cows were scored as culled upon their first incidence of failure to wean a calf, but not considering calving failure as a reason, through 6 yr; e) Criterion 5, where cows were scored as culled upon their first instance of failing to wean a calf, provided that they had no prior instances of calving failure, through 6 yr.


a.
b.
c.
d.
e.
−log10(p)
0
2
4
6
8
1
2
3
4
5
6
7
8
9
11
13
15
17
20
23
26
29
Chromosome

The BIF (2016) definition of stayability was a benchmark of interest for this study, and represents the most severe culling strategy. Criterion 2 corresponds to a common culling policy used by US beef producers, where a cow is removed from production upon her first instance of failing to raise a calf. Under Criterion 2, significant attrition was observed, such that only 39% of the females would have remained in the herd after 6 yr (Table 2.2). Most of the failures were observed in the first 2 yr. Although this is unsurprising in a population produced by the *inter se* mating of *Bos indicus-Bos taurus* cattle, if this culling criterion were to actually have been applied, only 57% of the herd would have remained past 3 yr, diminishing the long-term research potential of these cows. Therefore, implementation of such strict culling policy on a *Bos indicus*-influenced research herd may be impractical.

In the GWAS for Criterion 2 the null model explained 21.8% of the variance observed, and after correction for multiple testing, resulted in 69 significantly associated SNP (Table 2.3). These SNP fell on BTA 1, 2, 5, 9, 18, and 21, with defined peaks due to multiple linked SNP associations on BTA 1, 5, and 9 (Fig. 2.1b). The most highly significant SNP from this analysis fall between 40 and 50 Mb on BTA 5. Independent association between chromosomal peaks was verified by extracting the lead SNP from BTA 5 and modeling it as a covariate in a replication of the Criterion 2 GWAS (Appendix A). Although significant associations were found, Criterion 2 was not specific enough to determine why a cow to left the herd. For example, it did not distinguish between different factors such as pre-weaning calf death loss, failure to maintain pregnancy, or failure to conceive. The purpose of the subsequent analyses was to increase the power of detection by more specifically defining the reasons for reproductive failure and reducing the sources of phenotypic variation.

Criterion 3 was used to identify associations corresponding to failure to give birth to a calf, versus failure to raise and wean a calf. Criterion 3 allowed trends in pregnancy rates to be observed by focusing on yearly calving records. Only 83% of the cows in the herd calved during their first calving season at 2 yr (Table 2.2). After removing the records of cows that failed to calve at 2 yr, 18% of remaining cows failed to successfully rebreed after the birth of their first calf, and experienced calving failure during their second calving season at 3 yr. The strongest associations were observed on BTA 5, and other SNP survived multiple testing correction on BTA 1, 11, 15, and 24 (Fig. 2.1c). These associations were the most significant from 43 to 50 Mb on BTA 5, and this region corresponds to the critical region reported by Hawken et al. (2012) that was associated with measures of age at puberty. These findings are also similar to reports of SNP from 20 to 55 Mb on BTA 5 associated with reproductive efficiency in American *Bos indicus-Bos taurus* composite cattle (McDaneld et al., 2014).

In contrast, Criterion 4 and Criterion 5, which both focus on weaning as the reason for failure, had greater proportions of cows remain in the herd through 6 yr than did the previous 2 criteria, indicating that failure to calve is the primary reason for cows to leave the herd. The purpose of Criterion 4 was to identify associations with culling due to weaning failure that were not associated with calving failure. It was anticipated that genetic associations coinciding with, for example, such traits as mothering ability, maternally inherited health traits, or milking ability would be observed. Once the false discovery rate threshold was applied, no SNP were found to be significant (Table 2.3), and no clear patterns were observed in the corresponding Manhattan plot (Fig. 2.1d). The variance explained by the null model for this analysis was zero, indicating that the model did not adequately capture any genetic variation using this trait.

Criterion 5 focused exclusively on cows that either never failed to raise a calf or those that only ever failed to raise a calf from birth to weaning, but never experienced calving failure. This strict criterion restricted the tested population to only 169 cows. This resulted in an underpowered model, and unsurprisingly, only 1 SNP on BTA 9 survived correction for multiple testing (Table 2.3). Despite the decreased power of detection, a clear peak of linked SNP on BTA 9 was observed, with no peak on BTA 5 (Fig. 2.1e). Comparing this output with results of the GWAS for Criterion 2 and Criterion 3, suggests associations on BTA 5 may be driven by physiological influencers of pregnancy, whereas BTA 9 may be associated with traits related to calf survivability from birth to weaning.

Bos indicus and *Bos indicus* crossbred females are known to be slower maturing and older at the onset of puberty than *Bos taurus* heifers (Gregory et al., 1979; Chenoweth, 1994; Hearnshaw et al., 1994; Thallman et al., 1999). Slower maturation rate in *Bos indicus* influenced cattle, especially straight and high percentage *Bos indicus* heifers, often has a negative impact on the proportion of heifers that calve at 2 yr and the subsequent proportion returning to estrus during the following breeding season (Chenoweth, 1994). Age at puberty in Brahman crossbred heifers has been found to be highly variable depending on proportion of Brahman in the cross, season, and regional weather conditions (Chenoweth, 1994). Using age at first observed estrus as a physiological indicator, average age at puberty in Brahman-cross heifers has been reported at 510 d and 398 d by Plasse et al. (1968) and (Gregory et al., 1979), respectively, and when considering age at first conception as the physiological indicator of puberty, Riley et al. (2010) reported an average age of 461 d. In a population of Nellore-Angus F_1 heifers, average age at puberty was 405 d using first observed estrus as the determinant (Thallman et al., 1999), and in a separate population of *Bos indicus-Bos taurus* crossbred heifers, average age at first corpus luteum as a measure of puberty was 656 d (Hawken et al., 2012).

These last two examples resemble the breed composition of the Cycle 1 herd, and support the theory that substantial variation in the rate of maturity, with late onset of puberty in part of this population, may be the difference between those females that remain productive through 6 yr and those females that skip early in life. Detection of associated SNP is indicative that some of the biological factors influencing stayability are beyond just managerial influences and there is genetic variation within the Cycle 1 cow herd.

Hawken et al. (2012) found that BTA 5: 44 to 50 Mb was significantly associated with age at puberty in *Bos indicus-Bos taurus* composites, as defined by age at first corpus luteum and supported by postpartum anestrous interval and detection of preweaning estrus. This critical interval corresponds to the region on BTA 5 identified for Criteria 2 and 3. Due to the strong parallels between these findings and those associated with puberty, it is hypothesized that the later maturing cows, as indicated by inability to successfully calve at 2 yr or to rebreed after the first calving season (Chenoweth, 1994), drove the strong associations to BTA 5.

In a study characterizing reproductive efficiency, as defined by 2 consecutive years of reproductive success, BTA 5 was the most significantly associated chromosome in *Bos indicus-Bos taurus* composites (McDaneld et al., 2014). Looking more critically at the most highly significant SNP between 26.3 and 48.1 Mb on BTA 5, reported by McDaneld et al. (2014), Psaros et al. (2015) found a large *Bos indicus* derived haplotype in this region in Brahman influenced cattle. They determined that the influence on reproduction was most likely due to additive gene action by SNP within this region on BTA 5, and concluded that greater *Bos indicus* influence on this region was negatively correlated with reproductive efficiency. Future work will be needed to verify the absence or presence of this haplotype in the Cycle 1 population of Brahman- and Nellore-influenced, crossbred cows.

The critical region from 43 to 50 Mb on BTA 5 has been associated with many traits in *Bos indicus-Bos taurus* crossbred cattle, including age at puberty (Hawken et al., 2012), reproductive efficiency (McDaneld et al., 2014), udder characteristics (Tolleson et al., 2017), and growth traits such as live weight and hump score (Bolormaa et al., 2013) and percent intramuscular fat, back fat, and mature hip height (Bolormaa et al., 2014). This gene rich region contains several candidate genes previously implicated in physiological processes associated with reproduction (Fortes et al., 2011; Beltman et al., 2013). There are genes involved in immune response (*IFNG*, *IL22*, *LYZ2*), apoptotic processes (*TMBIM4*), protein dephosphorylation (*PPM1H*, *PTPRR*), signal transduction (*SRGAP1*, *RAB3IP*, *KCNMB4*), DNA replication and RNA processing (*HELB*, *XPOT*), and processes potentially directly influencing reproduction such as cellular response to hormone stimulus (*GRIP1*, *MDM2*), and regulation of intracellular estrogen receptor signaling pathways (*CNOT2*).

Although there are no previously reported SNP directly associated with beef cow stayability in *Bos indicus* influenced cattle, Saatchi and Garrick (2016) recently identified 2 QTL on BTA 6 associated with stayability in Simmental cattle, but did not observe QTL on BTA 5 or 9 as in the current analyses. These QTL were located on BTA 6 at 40 and 71 Mb, and did not concur with SNP associations observed in this study. Hamidi Hay and Roberts (2017) investigated longevity as a continuous trait, measuring the number of months from first calving until disposal. Cows were culled if they failed to become pregnant or failed to wean a calf (Roberts et al., 2016). It should be noted that these *Bos taurus* composite cows were part of a long-term study of supplemental feeding during post-weaning development and winter grazing. There was a trend ($P < 0.07$) for the interaction of dam treatment and heifer treatment to affect pregnancy rate and the proportion of cows retained in the herd at 2.2 and 5.2 yr of age. After correcting the longevity trait for contemporary group and the fixed effects of the 2 treatments, Hamidi Hay and Roberts (2017) reported 5 SNP associated with cow survivability in *Bos taurus* composite cows on BTA 1, 3, 9, 19, and 25. Although the average age for cow disposal in their study was less than 4 yr, compared to 4.3 yr, 3.2 yr, 3.2 yr, 3.7 yr, and 3.5 yr for Criteria 1 to 5, respectively, in the current study, there appears to be limited correspondence in the GWAS results. There were no similarities in the location of significant SNP. It is possible that this is because their population is strictly of *Bos taurus* origin or it may be that the environmental effects of the 2 supplementation treatments masked expression of the natural genetic variation in the phenotype.

Lack of significant associations were likely due to the combination of small sample size and high degree of environmental influence on the phenotypes. Heritability for stayability to 6 yr of age has been estimated to be low to moderate in both *Bos taurus* and *Bos indicus* cattle. Heritability was estimated to be 0.18, 0.18, and 0.15 by Snelling et al. (1995), Brigham et al. (2007), and Jamrozik et al. (2013), respectively, in *Bos taurus* cattle using threshold models. Success rates for cows in these studies ranged from 38% to 62% dependent on breed. Heritability for stayability in *Bos indicus* cattle was estimated at 0.12, 0.22, 0.19, 0.10, and 0.19 using threshold models (Silva et al., 2003; Van Melis et al., 2007; Eler et al., 2014; Cavani et al., 2015; Guarini et al., 2015b). Success rates for cows in these studies ranged from 29 to 31%. In the current study, success rates for Criteria 2 and 3 were comparable to those found in the *Bos taurus* studies, whereas success rates for Criteria 1, 4, and 5 were much higher (Table 2.2) and probably too high to detect genetic variation for the traits in such a small population.

Stayability traits are expected to be largely influenced by environmental factors, so low to moderate heritability estimates are expected (Jamrozik et al., 2013). Martinez et al. (2005) found that the heritability for stayability for calving or weaning ($h^2 = 0.35$ and 0.21, respectively) was greater than for stayability to a defined age of 6 yr ($h^2 = 0.17$), indicating that selection for these definitions of stayability would be more effective. Even with low to moderate heritability estimates, selection for stayability is possible and warranted (Hudson and Van Vleck, 1981; Martinez et al., 2005; Van Melis et al., 2007; Jamrozik et al., 2013; Rizzo et al., 2015).

Single nucleotide polymorphisms included in the genomic relationship matrix were not removed when evaluated for associations because SNP on the Illumina BovineSNP50v1 chip are relatively sparse and are common variants, so few of the SNP are likely to be causative (Wiggans et al., 2016). However, due to proximal contamination the GWAS may be underpowered (Listgarten et al., 2012). Furthermore, Boyle et al. (2017) have recently proposed an "omnigenic" model for complex traits in which all genes expressed in relevant cells have very small effects on phenotypic variation, because gene regulatory networks are interconnected. Thus, regardless of population size, SNP with non-zero effects on stayability may never reach genome-wide significance.

A tendency for *Bos indicus* influenced cattle to experience greater rates of reproductive failure early in life versus straight *Bos taurus* cattle (Chenoweth, 1994) is reflected in the definitions of stayability applied in studies using *Bos indicus* influenced cattle. In multiple studies of reproductive performance of Brazilian Nellore cattle, stayability has been defined as a cow's ability to produce 3 calves by 76 mo (Guarini et al., 2015a; Rizzo et al., 2015). This was referenced as being the earliest age at which a cow was expected to produce 3 calves, given that most cows first calve at 32 mo, and assuming that 3 calves was the breakeven point for Brazilian producers (Rizzo et al., 2015). Similar definitions have been used in the analysis of Brahman cattle, where stayability represented a cow's ability to produce 3 calves by 6 yr (Cavani et al., 2015). Other Brazilian studies with Nellore cows used a system of defining stayability where a cow qualifies as meeting the stayability threshold only if she successfully and successively produces a calf every year until a given age, generally 6 yr (Silva et al., 2003; Van Melis et al., 2007; Santana et al., 2013; Eler et al., 2014). This final definition closely follows the definition most commonly used in the United States and more accurately reflects the goals of a typical American producer. Although not the purpose of the current study, future economic analyses are warranted to determine the optimal ages for stayability benchmarks in *Bos indicus* influenced cattle.

As the average age of a herd increases, herd productivity is expected to peak as well. Older, productive cows demonstrate an increase in percentage calf crop born and weaned, and in total kg of calf weaned (Cundiff et al., 1992). Maintaining productive cows for longer increases economic returns and increases cow value (Garcia et al., 2014). *Bos indicus-Bos taurus* crossbred cows have been reported to have increased reproductive longevity (Riley et al., 2001; Thrift and Thrift, 2003). *Bos indicus-Bos taurus* cows are more likely to survive culling due to decreased rates of dystocia and decreased tooth loss at advanced ages (Riley et al., 2001; Thrift and Thrift, 2003). Furthermore, Nellore-sired cows have been shown to maintain udder integrity, and to have increased survivability and overall lifetime productivity than other *Bos indicus*- or *Bos taurus*-sired females (Riley et al., 2001). In a Brazilian study estimating the influence of popular Nellore founders on the current top 1% of Nellore sires for stayability EPD, the bull Karvadi was the most influential bull and contributed an estimated 8.2% of the genetics in the population subset, mainly through his son Chummak (Marcondes et al., 2007). Karvadi is the great great grand-sire of 2 of the F_1 donor cows through his son Chummak, and an F_1 bull that contributed to 4 ET families and an NS family through his son Chakkar. However, there was no evidence that cows from those families performed differently for stayability ($P > 0.05$).

Beef cow stayability is an important yet complicated measure of cow reproduction and productivity. The large number of sources of variation associated with the trait makes it difficult for producers to select for and for geneticists to understand. It has been shown herein that there is potential to identify genomic regions associated with a complex trait such as stayability in *Bos indicus-Bos taurus* crossbred cows. It has been the long-term goal that the McGregor Genomics herd be used to understand genetic factors influencing cow lifetime productivity traits and to identify important variants that may be applied in the development of genomic selection tools for tropically adapted beef breeds. As the median age of the Cycle 1 herd increases, future analyses will focus on reproductive performance to later ages and lifetime productivity.

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3. FINE MAPPING OF A QTL ASSOCIATED WITH HEIFER PRODUCTIVITY IN NELLORE-ANGUS CROSSBRED COWS

3.1. Introduction

Divergence between *Bos taurus indicus* and *Bos taurus taurus* cattle, commonly known as *Bos indicus* and *Bos taurus* cattle, has been estimated to have occurred between 117,000 to 275,000 or 610,000 to 850,000 years ago based on mitochondrial DNA and microsatellite data, respectively (Bradley et al., 1996; MacHugh et al., 1997). These subspecies have adapted to contrasting environments and, since domestication, have been independently exposed to different selection pressures. Given the estimated time since divergence, it is likely that quantitative trait loci (QTL) mutations have independently arisen in each subspecies, will independently segregate, or have become fixed in either *Bos indicus* or *Bos taurus* (Bolormaa et al., 2013; Koufariotis et al., 2018). This has particular implications in composite or crossbred populations made using *Bos indicus* and *Bos taurus* matings.

Bos indicus crossbred cows are a popular and logical choice for beef cattle producers operating in the Gulf Coast region of Texas and the southeastern United States. These crossbred cows are also economically important in other subtropical and tropical regions of the world; cattle production in subtropical and tropical environments represents an estimated 70% of the world's beef production, and most of these cattle are likely of *Bos indicus*-influence (Robinson et al., 2014). These types of cattle are preferential for production in these regions and are valued for their adaptive tolerance to the physically demanding environmental conditions characteristic of the tropics and subtropics (Bortolussi et al., 2005). However, there is some indication that environmental adaption has come at a cost to productive efficiency in today's production system. For example, Prayaga et al. (2009) found that adaptive traits, such as increased heat tolerance, are negatively correlated to fat thickness, which is an important economic phenotype.

There are obvious phenotypic differences between *Bos indicus* and *Bos taurus* cattle, especially in regards to physical appearance and important production traits. Most of these differences are thought to be due to a differential adaptive response to the environmental conditions in which these subspecies were developed. Maturation rates are known to be different between *Bos indicus* and *Bos taurus* cattle, where heifers with a straight or high percentage of indicine ancestry will be older at the onset of puberty than their more taurine counterparts (Gregory et al., 1979; Chenoweth, 1994; Hearnshaw et al., 1994; Thallman et al., 1999). Delayed onset of puberty in *Bos indicus*-influenced heifers has a negative impact on their early lifetime productivity and potential for long term retention in a managed herd (Chenoweth, 1994). This economically important production trait is differentially expressed between the cattle subspecies, likely as a result of genomic differences arising due to divergent domestication events.

Indicators of puberty – heifer pregnancy, age at first calf, and heifer rebreeding – are genetically correlated to stayability and lifetime productivity in a wide variety of beef cattle breeds (Morris and Cullen, 1994; Van Melis et al., 2010; Jamrozik et al., 2013; Eler et al., 2014; Cavani et al., 2015; Rizzo et al., 2015). Previously, Engle et al.

(2018) conducted a series of genome-wide association studies (GWAS) for stayability in a population of *Bos indicus-Bos taurus* crossbred cows. In this study, Engle et al. (2018) found that the most highly significant SNP associations corresponded to stayability defined as a cow's ability to give birth to 5 calves by 6 yr of age. In this herd, approximately 35% of cows either experienced pregnancy or rebreeding failure as heifers, indicative of the delayed maturation rates expected in an inter se population of *Bos taurus-Bos indicus* females (Chenoweth, 1994).

Engle et al. (2018) identified a critical region on bovine chromosome (BTA) 5: 40-50 Mb that corresponded to the critical region identified by Hawken et al. (2012) associated with the puberty indicators of age at first corpus luteum and postpartum anestrus interval in a *Bos indicus-Bos taurus* crossbred population. However, these same associations were not observed in Brahman heifers, possibly indicating reduced allele variability at this region within a non-crossbred population (Hawken et al., 2012). Bolormaa et al. (2013) also found QTL within this region for growth rate, frame size, fat deposition, and hump height, but only in *Bos indicus-Bos taurus* composite cattle. These results suggest that there is a pleiotropic QTL on BTA 5 that is influencing phenotypes that are differentially expressed in *Bos indicus* versus *Bos taurus* cattle, including heifer age at puberty.

We hypothesize that there are unique genetic differences between *Bos indicus* and *Bos taurus* cattle at this region. These differences may be the result of divergent domestication events and reflective of adaptive differences between subspecies. Therefore, the objective of this study was to assess this region for differences in the genomic architecture between the subspecies that may explain the differential expression of heifer productivity in *Bos indicus-Bos taurus* crossbred cows. The critical region influencing this trait was refined, and the influence of haplotype breed-of-origin at this location on heifer productivity was evaluated. Sequence differences between the subspecies were also assessed.

3.2. Materials and methods

All procedures involving animals were approved by the Texas A&M Institutional Animal Care and Use Committee.

3.2.1. Population and phenotypes

Cows used in this study were part of the McGregor Genomics Cycle 1 Population ($n = 303$), an experimental population housed at the Texas A&M AgriLife Research Center at McGregor, Texas. These cows were previously described by Engle et al. (2018), but briefly, these females were born either during spring or fall calving seasons from 2003 through 2007. Cows were from 13 full-sibling F_2 families produced through embryo transfer (ET) and 4 paternal half-sibling families produced through natural service (NS) matings. These cows were all *Bos indicus–Bos taurus* crosses, specifically, Nellore–Angus F_2 crosses, Nellore–Angus × Brahman–Angus crosses, or Nellore–Angus × Brahman–Hereford crosses.

On average, heifers were first exposed to Angus bulls for the opportunity to first calve at approximately 2 yr of age. Both spring-born and fall-born heifers (ET and NS) were managed to first calve at 2 yr of age. Fall-born heifers (ET only) were exposed to bulls during the winter, giving them the opportunity to first calve at 2 yr of age in the following fall. Those that initially failed to conceive were transitioned to a spring calving schedule and were bred to first calve at 2.5 yr of age, without a failure to calve counted against them. Any fall-born heifers that first calved during the fall were held through the winter without mating opportunity and rebred in the following spring breeding season to be on a spring calving schedule. Subsequently, all cows were managed for spring calving only. Females were allowed to remain in the herd until their second failure to wean a calf.

The phenotype for stayability to 3 yr of age, or heifer productivity, was produced by censoring lifetime production records from the population. Stayability to 3 yr represents successfully giving birth to 2 calves by the third breeding season. This was scored as a binomial trait, where a cow either successfully met the stayability benchmark (scored 1), or experienced failure to calve at either 2 or 3 yr (scored 0). Heifer productivity was pre-adjusted for the fixed effect of contemporary group (birth year and season of birth) using linear model procedures in R, and the residuals from these models were used in subsequent analyses.

3.2.2. Genotypes, genome-wide association studies, and percent variance explained

Filtered, low-density genotypes for these cows were the same as those described by Hulsman Hanna et al. (2014). Briefly, DNA was extracted from white blood cells and genotyped using the Illumina BovineSNP50v1 array (Illumina Inc., San Diego, CA, United States). SNP positions were mapped to the UMD3.1 *Bos taurus* reference sequence assembly. Genotypes were then filtered using PLINK (Purcell et al., 2007) to remove SNP with completion rates < 90%, minor allele frequencies < 0.05, and those deviating from Hardy-Weinberg equilibrium proportions at $P < 0.0001$, resulting in 34,651 SNP still available for analysis.

Genotype information from the pedigree of the Cycle 1 cows was used to impute genotypes to a higher density. The Nellore and Angus founders and the F_1 generation were genotyped with the Illumina BovineHD SNP array (Illumina Inc., San Diego, CA, United States). FImpute was used to impute the low-density SNP genotypes in the Cycle 1 F_2 cows up to HD density (Sargolzaei et al., 2014; Gill, 2016). After filtering, 555,674 genome-wide SNP were available. Additionally, whole-genome sequences from 4 of the Nellore and 4 of the Angus founders of the McGregor Genomics population were available for imputation to sequence scale. Sequences had been previously generated using Illumina paired-end 100 bp reads to a depth of at least 30x coverage (Gill, 2016).

A genome-wide association analysis (GWAS) for heifer productivity was performed using the previously described residual phenotype and the univariate procedures in GEMMA (Zhou and Stephens, 2012) that fitted a standardized genomic relationship matrix to account for genetic covariances between animals. A GWAS was attempted using both high-density and low-density genotypes. However, imputed HD genotypes were only available for the F_2 cows (n = 189), resulting in insufficient power to detect significant associations. Therefore, results of the GWAS using HD genotypes were not used or reported. The false discovery rate proposed by Benjamini and Hochberg (1995) was constrained to a genome-wide level of 0.01 and 0.001 to correct for multiple testing.

Proportion of variance (PVE) explained by each SNP in the GWAS, or the SNP heritability, was calculated as in Shim et al. (2015), adjusting beta values for sample population minor allele frequency. A sliding window of flexible size was used to average PVE across BTA 5 to determine the optimum window size and location that explained the highest proportion of variance possible. Haploview was used to estimate r^2 as a measure of linkage disequilibrium between SNP across the region with the highest PVE (Barrett et al., 2004).

3.2.3. Haplotypes and breed-of-origin effects

Haplotypes in the Cycle 1 F_2 cows and two generations of their pedigree were phased using FastPhase (Scheet and Stephens, 2006) for the critical region identified through previously described analyses. Haplotypes within the Angus and Nellore founders were identified and manually traced through subsequent generations, so that in the Cycle 1 F_2 cows, the haplotype breed-of-origin of the critical region was characterized. Each cow's haplotypes could either be both of Nellore origin or both of Angus origin, or have a haplotype derived from each breed (with the paternally inherited haplotype listed first). Once genotypes were scored, they were used to test the influence of haplotype breed-of-origin on heifer productivity.

To test the influence of haplotype breed-of-origin, genotype scores representing each of the 4 possible combinations of genotypes (i.e. NN, AN, NA, AA) were used as an explanatory variable in a logistic regression with binary stayability to 3 yr as the response. Given the binary nature of the response variable, a generalized linear model with a logit link function was utilized, using the glm() function in the R package lme4

(Bates et al., 2015). Least squares means for heifer productivity in each of the previously described analyses were estimated using the lsmeans package in R (Lenth, 2016) and back transformed from a logit to a response scale. Pairwise comparisons between breed-of-origin genotypes were conducted using a Tukey's pairwise test and deemed significant when $P < 0.05$. Odds ratios were also calculated.

3.2.4. Variant calling

Sequences from 4 of the Nellore and 4 of the Angus founders were used to identify consensus variants unique to the 4 representatives of each breed. Variant calling was done using mpileup in SAMtools (Li, 2011) against the UMD3.1 reference assembly with masked repeat elements. SNP were filtered for consensus among the 4 individuals within a breed, and variants unique to each breed were isolated. Therefore, the SNP considered for analysis were the same amongst the 4 individuals within a breed, and different between breeds. Polymorphisms were then annotated using SnpEff (Cingolani et al., 2012).

3.2.5. Assembly comparison

The previous analyses suggested that there may be distinct sequence differences between the bovine subspecies across the region of interest, and these differences may not be represented in the current, *Bos taurus* reference assemblies. To identify and compare regions of conservation between different draft reference assemblies for *Bos indicus* or *Bos taurus* cattle, the program MUMmer3 was used (Kurtz et al., 2004). The *Bos taurus* assembly, ARS-UCD1.2 (USDA ARS, *unpublished*) is of Hereford origin and utilized the same reference animal as the original, short read assembly, UMD3.1

(Zimin et al., 2009). The *Bos indicus* assembly is of Brahman origin (University of Queensland, *unpublished*). Both new assemblies were constructed from PacBio long read sequences. The MUMmer function NUCmer, or nucleotide MUMmer, was used to match conserved sequences between the assemblies, and visualized using the function mummerplot.

3.3. Results and discussion

Due to the patterns related to puberty observed in this herd, a GWAS for stayability to 3 yr was conducted. Successfully meeting the stayability threshold at 3 yr indicates that a cow first calved at 2 yr and successfully rebred during the following breeding season to calve again at 3 yr, indicative of heifer productivity. Associations from this analysis mapped to the same peak on BTA 5: 40-50 Mb as Engle et al. (2018), with the lead SNP, ARS-BFGL-NGS-76882, at BTA 5: 46526409 in the UMD 3.1 assembly (Fig. 3.1). In this analysis, 38 SNP surpassed a genome-wide FDR of 0.01, 9 surpassed a genome-wide FDR of 0.001, and all SNP fell within the peak on BTA 5.

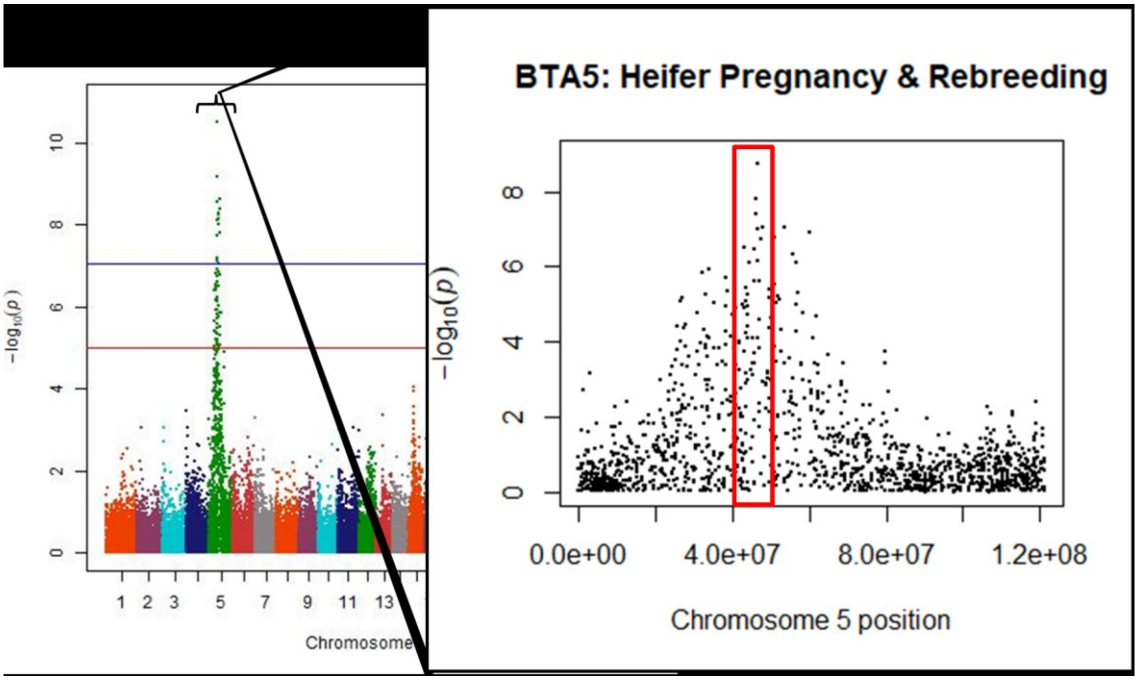

Figure 3.1 Genome-wide association analysis for heifer productivity using 50k density SNP genotypes. A critical region between BTA 5: 40-50 Mb was identified. The blue line on the back Manhattan plot indicates a genome-wide threshold of FDR = 0.001 and the red line represents a threshold of FDR = 0.01.

The PVE of each SNP was used as a metric to identify SNP blocks of importance to heifer productivity. By using a sliding window to average SNP heritabilities across the chromosome, the critical region on BTA 5 was reduced to a 5 SNP block at approximately 46.5 Mb that explained approximately 11.2% of the variance in the model (Fig. 3.2a). This SNP block is approximately 250 kb long and contains the lead SNP from the GWAS. These SNP are all in moderately high linkage disequilibrium (LD) with one another, indicating potential sequence conservation due to a low rate of recombination at this region (Fig. 3.2b). This will be referred to as the critical region hereafter.

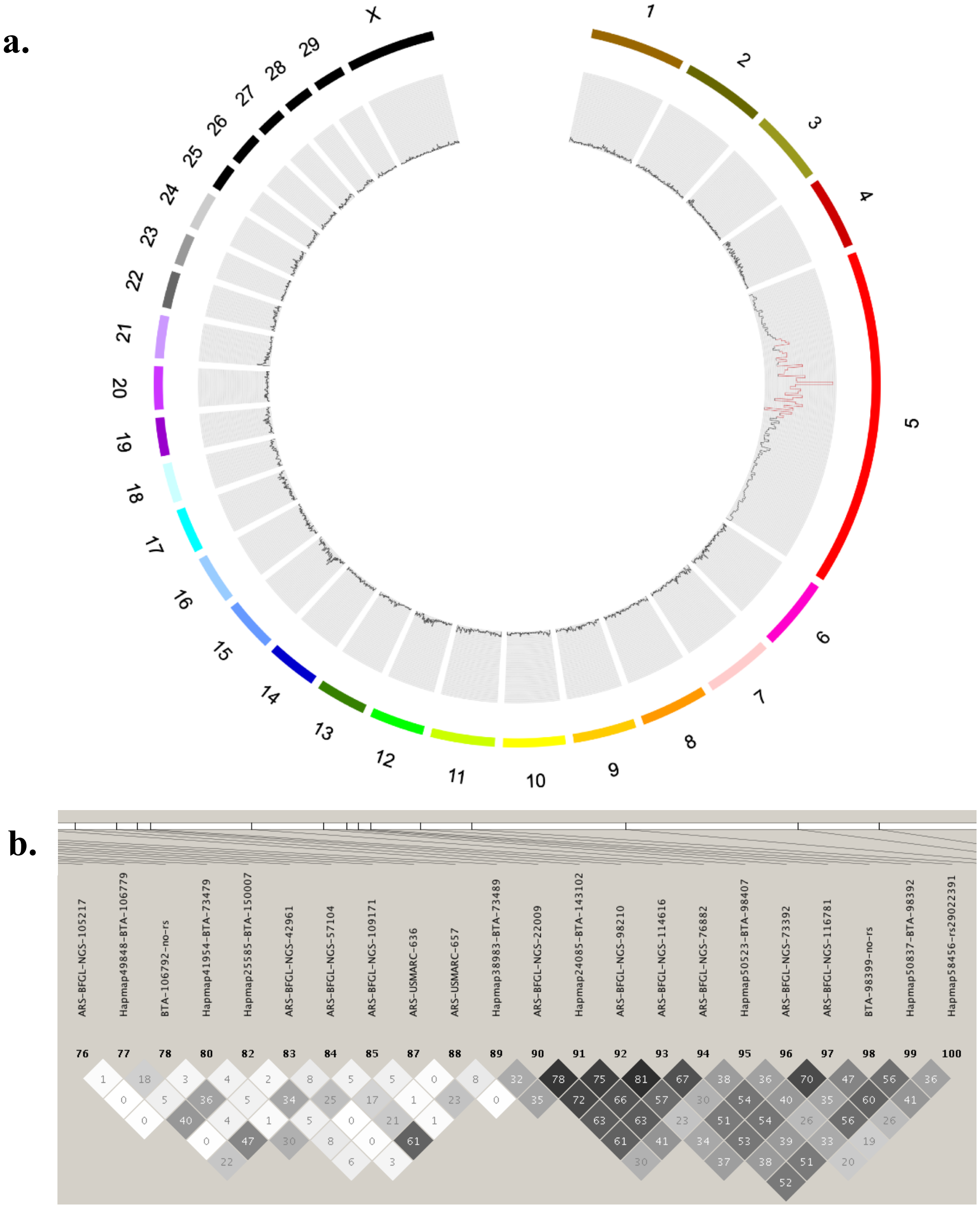

Figure 3.2 Identification of SNP haplotype blocks associated with heifer productivity.

Figure 3.2 Continued. a) Proportion of variance (PVE) explained by each SNP was calculated as in Shim et al. (2015) and then averaged across 5 SNP long sliding windows. The resulting SNP heritabilities are plotted where red indicates ≥ 0.02 PVE. The region with highest PVE is at approximately BTA 5: 46.5 Mb, explaining 11.2% of the variance. BTA 5 is enlarged for clarity. b) Haploview output for the critical region at approximately BTA 5: 46.5, where the lead SNP falls at position 94. Darker grey reflects higher levels of linkage disequilibrium, where r^2 is approaching 1.

With the critical region associated with heifer productivity refined to a 250 kb window, questions relating to the influence of genetic differences between subspecies at this region could be addressed. This region has been implicated in association studies for a wide variety of traits, but exclusively in *Bos indicus-Bos taurus* crossbred cattle (Hawken et al., 2012; Bolormaa et al., 2013; Engle et al., 2018). The noticeable absence of studies mapping QTL to this region for similar traits in either *Bos indicus* or *Bos taurus* breeds is likely indicative of conserved haplotypes across this region within subspecies (Hawken et al., 2012; Porto-Neto et al., 2014; Melo et al., 2018; Speidel et al., 2018). In this study, a structured family-based population was assessed, and as a result, haplotypes could be directly traced through generations, beginning at either the purebred Nellore or Angus ancestors.

The breed-of-origin of haplotypes spanning the critical region at BTA 5: 46.5 were assigned to the F_2 Nellore-Angus cows in the herd and the influence of haplotype breed-of-origin on heifer pregnancy and rebreeding was assessed. There were significant differences in heifer productivity between cows with two Nellore-derived haplotypes at this region, in comparison to cows with either two Angus-derived haplotypes ($P < 0.01$) or those with a haplotype of both Angus and Nellore origin ($P < 0.0001$) (Table 3.1).

Although not significant, these patterns also show improved phenotypic performance in animals with haplotypes derived from each breed. This may potentially be due to advantages of heterosis at points in this region, although at 50k SNP density this is difficult to determine. Comparing odds ratios between genotypes reveal that relative to baseline performance of cows with two Angus derived haplotypes, those with two Nellore derived haplotypes have lower odds of experiencing both heifer pregnancy and rebreeding before 3 yr of age, while those with one haplotype from each breed will be more likely than the others to be productive as heifers (Table 3.1). This result is unsurprising due to known differences in heifer maturation rates between *Bos indicus* and *Bos taurus* cattle (Chenoweth, 1994). These results suggest that the observed phenotypic variation is due to haplotype differences between subspecies.

Table 3.1 Relationship between heifer productivity and haplotype breed of origin in F_2 cows at BTA5: 46.5 Mb

Genotype	n	Probability[1]	Odds ratio
Nellore-Nellore	49	$0.466^{a} \pm 0.09$	0.207
Nellore-Angus	37	$0.889^{b} \pm 0.05$	1.896
Angus-Nellore	43	$0.882^{b} \pm 0.05$	1.772
Angus-Angus	43	$0.809^{b} \pm 0.07$	1.000

[a,b] Means within column with different superscripts differ ($P \leq 0.05$).

[1] Least squared means for heifer productivity are back-transformed from logit to response scale and reported as a probability.

Within the 250 kb region at BTA 5: 46.5 Mb there are very few candidate genes. This region does contain the genes dual specificity tyrosine phosphorylation regulated kinase 2 (DYRK2) and cullin-associated and neddylation-dissociated 1 (CAND1). Cullin-associated and neddylation-dissociated 1 is a protein coding gene that has been found to be an important effector molecule in adipogenesis (Dubiel et al., 2013). This gene has also been found to be highly expressed in the early development of bovine oocytes (Nemcova et al., 2016). Dual specificity tyrosine phosphorylation regulated kinase 2 falls within a protein kinase family involved with cellular growth, but this gene has not been directly implicated in any relevant or bovine studies. Considering that the effects of this region appear to be pleiotropic in nature, neither of these genes seem to be putative causative candidates, suggesting that structural differences at this region may be driving the variation observed among phenotypes.

Unique SNP variants between Nellore and Angus were then mapped across the critical region and a differential pattern of sequence variation versus the assembly sequence (UMD3.1) was observed (Fig. 3.3). This is not dissimilar to patterns of variation observed between breeds on BTA 5 reported by Porto-Neto et al. (2014). The Nellore founders had 573 unique consensus variants, whereas the Angus founders only had 139 variants between BTA 5: 45.8-46.6 Mb. This difference would be expected given that the reference assembly is of *Bos taurus* origin, so subspecies specific sequence differences would also likely appear as SNP. The SNP variants were binned into 1 kb windows and plotted (Fig. 3.3a). According to the Bovine HapMap Consortium (2009), there should be an average of 0.88 SNP variants per 1 kb in taurine cattle and an average of 1.8 SNP variants per 1 kb in indicine cows. Here, within each breed, a single 1 kb window demonstrated a dramatically increased number of variants, with 11 variants mapping to 1 kb in Nellore and 8 variants mapping within 1 kb in Angus. Interestingly, the patterns of variability appear to be distinctly different between breeds across the region, with most of the variants mapping around the lead SNP at BTA 5: 46,526,409 (Fig. 3.3b). The area immediately surrounding the lead SNP is almost devoid of Nellore specific variants, potentially indicating variant conservation between Nellore founders or a deletion around this SNP.

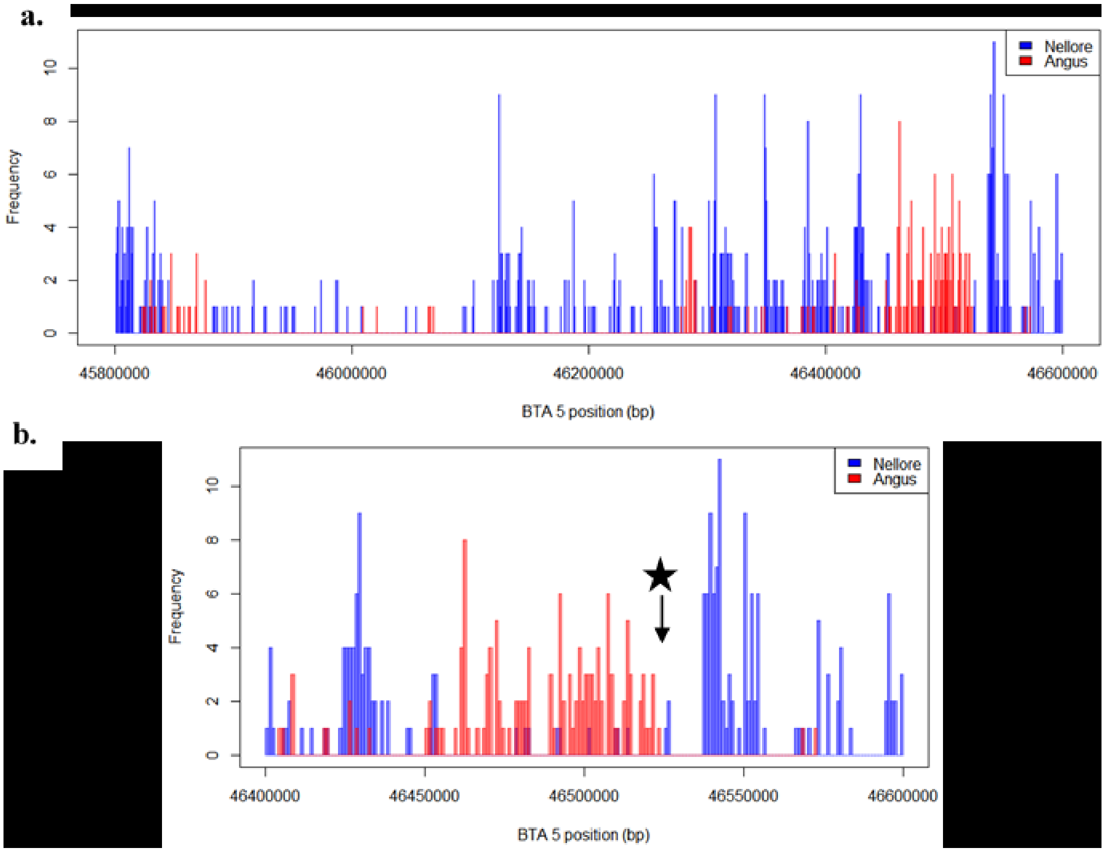

Figure 3.3 Number of de novo SNP mapped within 1 kb windows on UMD 3.1. Each bar represents number of SNP per 1 kb. Red bars indicate uniquely Angus SNP and blue bars indicate uniquely Nellore SNP. a) Number of unique consensus SNP in 1 kb windows for Nellore or Angus cattle between BTA 5: 45.8–46.6 Mb. b) Number of unique consensus SNP in 1 kb windows for Nellore or Angus cattle between BTA 5: 46.4–46.6 Mb. The star denotes the approximate location of the lead SNP associated with heifer productivity.

Within the set of 50k density SNP genotypes used for GWAS, the SNP immediately downstream from the lead SNP was approximately 2 Mb away. Due to the sparsity of SNP following the lead SNP, the sequence immediately following the critical region was explored. Within the 2 Mb space between SNP is an area with a high level of repeats, with the lead SNP falling immediately before an 8 kb long interspersed element (LINE). This region is also characterized by areas of high GC content, simple tandem repeats, and gaps in the assembly. The lead SNP is upstream from a copy number variant (CNV) detected in this population at approximately BTA 5: 46.7 Mb (Xing, 2018), and a CNV associated with traits of tropical adaption, naval length, reported by Aguiar et al. (2018) at BTA 5: 48 Mb. Further, previous studies have identified pleiotropic SNP downstream from the lead SNP at BTA 5: 47,727,773 (UMD3.1) (Bolormaa et al., 2014). Bolormaa et al. (2014) found that this SNP at BTA 5: 47.7 Mb clusters tightly with 4 other lead SNP on BTA 6: 40.1 Mb, BTA 14: 25.0 Mb, and BTA 20: 4.9 Mb with a high level of correlation between them. These 4 SNP have been shown to be associated with increases in height and weight, as well as decreases in fatness, RFI, and blood concentration of IGF1. (i.e.: all changing mature size) (Bolormaa et al., 2014). Additionally, the critical region and its flanking sequences falls within a 20 Mb region on BTA 5 that appears to be under genetic selection and associated with parasite resistance, yearling weight, body condition score, coat color and penile sheath score in both *Bos indicus* and *Bos taurus* cattle (Porto-Neto et al., 2014).

Linkage disequilibrium in cattle is expected to decay at a distance of approximately 50 kb, meaning that variation across this region has not been accurately accounted for by using low density SNP genotypes (Bovine HapMap Consortium, 2009). To address this, imputed HD genotypes were extracted from this region, including the CNV, and were then individually modeled as covariates in GWAS for heifer productivity. There was no change in the outputs of these association analyses, likely indicating that this region is not the primary driver of the phenotypic variation observed in this population. These results suggest that the 2 Mb area of repeat elements following the critical region on BTA 5 does not have a significant impact on heifer productivity in this population of crossbred cows.

Within the downstream region adjacent to the lead SNP there was a series of assembly gaps observed in the UMD3.1 reference assembly. To assess potential differences between subspecies at this region, and to validate the existence of these assembly gaps, the new long-read *Bos indicus* and *Bos taurus* reference assemblies were assessed. These two assemblies were compared at the region of interest for areas of conserved sequence between the two. Comparisons between the long-read assemblies and UMD3.1 confirmed the assembly gaps observed in UMD3.1, and showed that these gaps were resolved in the long-read assemblies (results not shown). When the long-read Brahman and Hereford assemblies were compared, the results indicate that there is an inverted, translocated sequence conserved between the two subspecies and mapping to chromosome 5 at approximately 46.33 and 46.37 Mb on the ARS-UCD1.2 taurine assembly, and at approximately 46.53 and 46.59 Mb on the UMD3.1 taurine assembly

(Fig. 3.4). This conserved, inverted, and translocated sequence maps to the same location as the lead SNP associated with heifer productivity at BTA 5: 46,526,409 bp (UMD3.1).

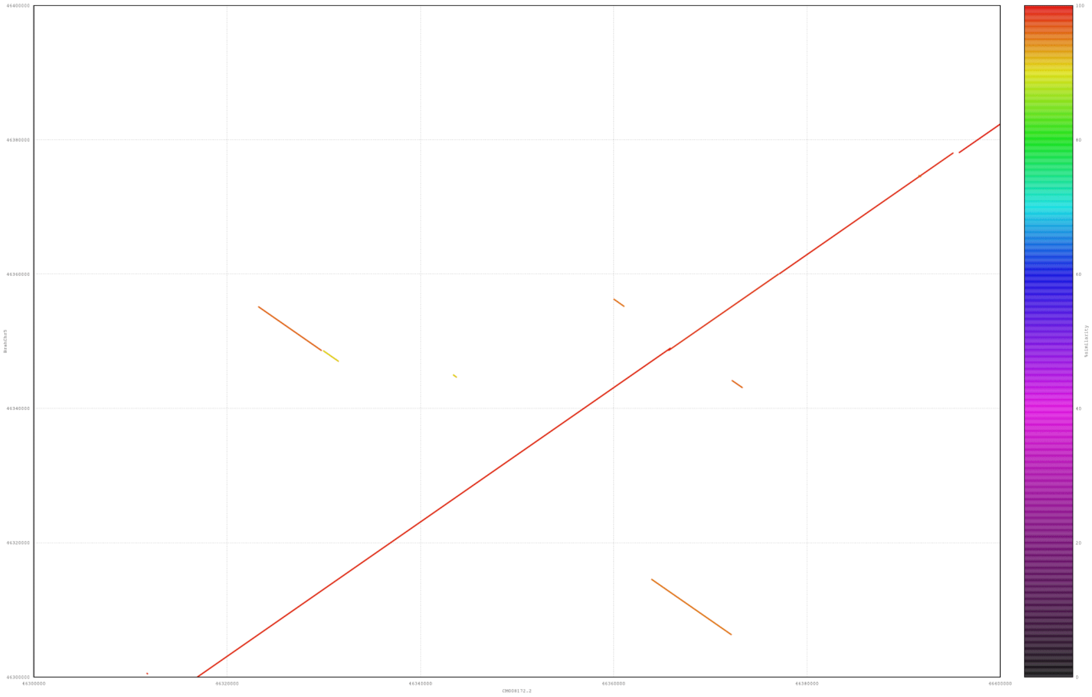

Figure 3.4 MUMmer3 plot visualizing the consensus sequence between the bovine ARS-UCD1.2 long-read reference assembly and the University of Queensland Brahman (bovine) long read reference assembly, between 46.3-46.4 Mb on chromosome 5 of each assembly.

3.4. Conclusion

The objective of this study was to refine and assess a critical region on BTA 5 associated with heifer productivity in a population of *Bos indicus-Bos taurus* crossbred cows. The central hypothesis was that there are unique genetic differences between *Bos indicus* and *Bos taurus* cattle at this region that may be reflective of adaptive differences between the subspecies, and have an impact on economically important traits such as heifer productivity or heifer age at puberty. These results suggest that there are genetic differences between subspecies both at the critical region, and likely downstream from it as well, and that these differences do have an effect of heifer pregnancy and rebreeding before 3 yr of age. Differences in patterns of variation unique to Nellore and Angus surrounding the lead SNP may correspond to the observed translocated inversions observed between the Brahman (University of Queensland, *unpublished*) and Hereford (USDA ARS, *unpublished*) assemblies. There are a large number of repeat elements immediately downstream from the lead SNP, some of which have been previously associated with pleiotropic (Bolormaa et al., 2014) or adaptive traits (Aguiar et al., 2018). Given the apparent pleiotropic nature of the region surrounding the critical interval assessed in this study, it is not unlikely that variants within this interval may also be associated with a number of other traits. Future work will be needed to verify these findings and validate the genetic mechanisms underlying these observations.

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4. INFLUENCE OF FIRST CALVING DATE ON STAYABILITY AND COW PRODUCTIVITY IN *BOS INDICUS* CROSSBRED COWS

4.1. Introduction

Beef cow reproductive longevity is related to a cow's lifetime productivity and cumulative economic value, making it one of the single most important factors influencing herd profitability (Rogers, 1972). However, Hudson and Van Vleck (1981) argued against direct selection for longevity due to low heritability, increased generation interval, and automatic selection via older cows that contribute more offspring to subsequent generations than do short-lived cows. Due to these arguments, it would be advantageous to identify component traits correlated to longevity that can be measured and applied as culling criteria earlier in a cow's life.

It is anticipated that when heifers conceive earlier in their first breeding season, they will calve earlier in the subsequent calving season. This lengthens the postpartum recovery period, increasing the likelihood that the cow will return to estrus in time to rebreed during the following breeding season. Cows are then more likely to calve early in the following calving seasons, thus, repeating the cycle. Prior research has shown that *Bos taurus* heifers that calved in the first 21 d of their first calving season experienced increased longevity compared to heifers that calved later in their first calving season (Cushman et al., 2013; Damiran et al., 2018). This is expected to be significant in *Bos indicus*-influenced herds, as these heifers are known to be older at the onset of puberty than their *Bos taurus* counterparts. Delayed puberty has a negative effect on a female's ability to first calve at 2 yr of age, rebreed, and calve again at 3 yr, depressing their potential for long term productivity and life in the herd (Chenoweth, 1994).

The timing of when a heifer gives birth during her first calving season is predictive of the performance of her future progeny (Lesmeister et al., 1973; Funston et al., 2011; Mousel et al., 2012). Heifer calves born in the first 21 d of the calving season are more likely to be cycling at the beginning of their first breeding season, and their first calf progeny have increased weights at weaning compared to heifer's born later in the season (Funston et al., 2011). Regardless of when the heifers were born, the date of first parturition has also been shown to influence the weaning weights of their first, and subsequent calves (both steer and heifer calves) (Lesmeister et al., 1973; Arthur et al., 1993; Funston et al., 2011; Mousel et al., 2012). Calves born earlier in the season will be older at weaning and are therefore more likely to be heavier at weaning (Lesmeister et al., 1973; Arthur et al., 1993). These studies suggest that the relative calving date in the calving season has an influence on cow profitability in kg calf weaned in *Bos taurus* females. However, these results have not yet been confirmed in *Bos indicus* or *Bos indicus*-influenced herds.

We hypothesize that date of first calving will be negatively correlated with reproductive longevity, where an earlier calving date and increased longevity is desirable. Therefore, the objective of this study was to characterize the relationship between first calving season period in *Bos indicus-Bos taurus* crossbred heifers with herd longevity and subsequent cow productivity. Secondly, the influence of the calving period in which a cow was born on her potential for stayability and productivity was assessed.

4.2. Materials and methods

All procedures involving animals were approved by the Texas A&M University Institutional Animal Care and Use Committee.

4.2.1. Population

Cows assessed in this study were part of the McGregor Genomics Cycle 1 Population (n = 241), an experimental herd housed in McGregor, Texas at the Texas A&M AgriLife Research Center. This population has previously been described by Engle et al. (2018). Briefly, these cows were all *Bos indicus–Bos taurus* crosses, specifically, Nellore–Angus × Brahman–Hereford crosses, Nellore–Angus × Brahman–Angus crosses, and Nellore–Angus F_2 crosses; all were 50% *Bos indicus* and 50% British (*Bos taurus*). Cows are from either 4 paternal half-sibling families produced through natural service or 13 full-sibling, embryo transfer F_2 families. From 2003 through 2007, cows were born either during spring or fall calving seasons. Records used for this analysis span from the date of this population's first possible calving season in 2005 through 2015, which is when the project ended for a portion of the population, and when the youngest cows in the herd were at least 8 yr.

All heifers were exposed to Angus bulls for the opportunity to first calve at approximately 2 yr of age (Table 4.1). Fall-born heifers were exposed to bulls from the first week in December to the second week in February and given the opportunity to first calve at 2 yr of age in the following fall. Those that initially failed to conceive were transitioned to a spring calving schedule and were bred to first calve at 2.5 yr of age, without a failure to calve counted against them. Any fall-born heifers that first calved during the fall were held through the subsequent winter without mating opportunity, and then rebred in the following spring breeding season to be on a spring calving schedule, with their second calf born at 3.5 yr of age. Spring-born heifers were all managed to first calve at 2 yr of age. Subsequently, all cows were managed together for spring calving only. Therefore, any cows that first calved in the fall were removed from consideration. Across the study, the average length of the breeding season was 68 d. Once a cow experienced two incidences of failure to wean a calf, under actual management criteria the cow was removed from production.

Table 4.1 Distribution of first calving season period by heifer age at first calving.

	Calving period[1]				
Age at first calving, yr	1	2	3	4	Subtotal (n)
2	0.36	0.37	0.15	0.12	185
2.5	0.45	0.38	0.14	0.04	56
Subtotal (n)	92	89	35	25	241

[1] First calving season periods of 21-d intervals where Period 1: ≤ 21 d, Period 2: 22 to 43 d, Period 3: 44 to 63 d, Period 4: ≥ 64 d

4.2.2. Phenotypes and covariates

Calving period was assigned by splitting each of the calving seasons into 21-d periods. The average length of the calving season for the 5 seasons evaluated was 80 d, so each season was split into 4 sections and treated as a categorical variable. Period 1 represents the first 21 d of the season, Period 2 spans d 22-43, Period 3 equates to d 44 through d 63, and Period 4 included everything after d 64. The calving season that the cow was born in was categorized using the same 21-d scheme, and each cow was assigned a score of 1-4 for the time period of her birth.

Stayability is defined as a cow's probability of surviving to a specific age, given the opportunity to first reach that age (Hudson and Van Vleck, 1981), and was used as a proxy for longevity. Stayability was evaluated to ages 3, 4, 5, 6, and 7 yr, provided that a cow calved each year starting at 2 yr of age (Snelling et al., 1995). Stayability was evaluated as a binary threshold trait where records were censored and cows were scored as either a 1, representing successfully reaching a given age, or a 0, representing calving failure at or before the given age. Due to the way that stayability is scored in these analyses, any individual that did not calve during their first possible calving season at approximately 2 yr of age would not have met any of the stayability benchmarks, and were therefore removed from consideration.

First calving interval was measured from the birth date of the first calf to the birth date of the second calf. Average calving interval over the cow's lifetime was estimated by calculating in days the mean difference between subsequent calf birth dates. The body weights of the cow's first calf at both birth and weaning were evaluated. Average kg of calf weaned over the course of each cow's productive life was evaluated up to 8 yr of age under two culling criteria, the actual criteria of 2 failures to wean a calf and a second, where records were censored upon a cow's first failure to wean a calf. This was the maximum age that each cow was maintained to, prior to the project ending for a portion of the herd. Consequently, a portion of the cow's records were censored for this analysis. Each measure was tested for normality using a Shapiro-Wilk test, and outliers were removed if they exceeded 1.5 times the interquartile range.

Possible covariates available for analysis included the contemporary groupings of breed, cow sire, and concatenated birth year/birth season, or traits reflective of individual cow maturity such as cow weight at first calf weaning and cow age at first calving. Prior to analysis, all continuous traits, including both response and independent variables, were tested for normality using a Shapiro-Wilk test, and outliers were removed if they exceeded 1.5 times the interquartile range.

4.2.3. Modeling and variable selection

To assess binomial stayability to each age, a generalized linear model with a probit link was utilized, using the glm() function in the lme4 statistical package for R (Bates et al., 2015). The model used for the analysis was: $f(\mu_Y) = X\beta + \varepsilon$, where $f(\mu_Y) = \Phi^{-1}(\mu_Y)$ and is the inverse normal distribution of the binomial response variable Y, X is the explanatory variable, β is the coefficient of change for the explanatory variable, and ε is the model error term. Models for stayability to each age were individually fit for independent variables using a backward stepwise variable selection methodology, and then verified using forward selection. Goodness of fit for each model was confirmed using a combination of AIC comparison and likelihood ratio testing. All stayability models fit the same fixed effects and in addition to either cow or first calf calving period, included cow weight at calf weaning as a covariate.

To assess calving interval and cow productivity in the form of kg of calf, a linear model was utilized using the lm() function in the R package lme4 (Bates et al., 2015). Assessment of these productivity traits fit an individualized, model specific combination of explanatory variables, potentially including cow weight at calf weaning, cow breed, concatenated cow birth year/birth season, or cow sire id, in addition to first calving period. The effect of cow age at first calving was assessed and found not to be significant in any stayability or productivity model and was therefore not included as a covariate.

Least squares means for calving period in each of the previously described analyses were estimated using the lsmeans package in R (Lenth, 2016) and back transformed from a probit to a response scale. Pairwise contrasts between levels of calving period were conducted using a Tukey pairwise comparison, and significance was declared if $P < 0.05$.

4.3. Results and discussion

The first objective of this study was to assess the effect of a heifer's first calving season period on her subsequent stayability in the herd. Heifers that first calved within the first calving period were significantly more likely to rebreed and calve again at 3 yr of age in comparison to heifers that first calved at the end of the breeding season (Table 4.2). Heifers calving in Period 1 of the calving season had a 92% chance of rebreeding, in comparison to either a 65 or 55% chance in calving Periods 3 or 4, respectively. This trend was again observed for stayability to 4 yr, provided the cow gave birth to 3 calves, where cows that first calved within Period 1 were more likely to meet this stayability threshold. The benefit of first calving within the first calving period became more pronounced as stayability was assessed to later ages, to 5, 6, and 7 yr; Those heifers that first calved in Period 1 were significantly more likely to meet these stayability benchmarks than heifers that first calved within any of the other calving periods. For example, heifers calving in Period 1 were 32% more likely to achieve stayability to 7 yr than heifers first calving in Period 2.

Table 4.2 Probability of females meeting stayability benchmark at various ages, based on when they gave birth to their first calf

	Calving period[1]				
	1	2	3	4	% successful[3]
Heifers (n)	92	89	35	25	
Stayability: 3yr[2]	0.92 ^{a} ± 0.03	0.78 ^{ab} ± 0.05	0.65 ^{b} ± 0.09	0.55 ^{b} ± 0.11	0.82
Stayability: 4yr[2]	0.82 ^{a} ± 0.04	0.70 ^{ab} ± 0.05	0.61 ^{ab} ± 0.08	0.50 ^{b} ± 0.10	0.73
Stayability: 5yr[2]	0.83 ^{a} ± 0.04	0.66 ^{b} ± 0.05	0.52 ^{b} ± 0.09	0.38 ^{b} ± 0.10	0.68
Stayability: 6yr[2]	0.78 ^{a} ± 0.04	0.60 ^{b} ± 0.05	0.49 ^{b} ± 0.09	0.34 ^{b} ± 0.10	0.63
Stayability: 7yr[2]	0.73 ^{a} ± 0.04	0.53 ^{b} ± 0.05	0.46 ^{b} ± 0.09	0.33 ^{b} ± 0.10	0.57

[1] First calving season periods of 21-d intervals where Period 1: ≤ 21 d, Period 2: 22 to 43 d, Period 3: 44 to 63 d, Period 4: ≥ 64 d

[2] Indicates a binary trait, where a 1 = success and 0 = failure to meet each threshold, presented as a probability (back transformed least squares means to response scale from a probit link)

[3] Proportion of cows from entire herd meeting each stayability benchmark, where whole herd n = 241

[a,b] Indicate significant difference of at least $P < 0.05$

In this analysis, all heifers had to have first calved at approximately 2 yr of age in order to be considered for any further analyses. Under actual management practices, 17% of the heifers were unable to calve from their first breeding season, and first calved at approximately 3 yr (Engle et al., 2018). In this population, heifer pregnancy rate is much higher than the 16% heifer pregnancy rate reported by Van Melis et al. (2010) in a herd of Nellore heifers first exposed at 14 mo, but more comparable to the 77% heifer pregnancy rate observed in Angus females (Snelling et al., 1995). Of the heifers that did calve at 2 yr, 18% of the heifers were unable to successfully rebreed to again calve at 3 yr of age (Table 4.2). The proportion of heifers in this population that successfully rebred is higher than observed by Cavani et al. (2015) within a Brazilian Brahman herd (68% success) and higher than in herds of Nellore (52-71% success) (Guarini et al., 2015; Valente et al., 2017).

No puberty measures were collected in this population, so the best estimate of age at puberty in these females is heifer pregnancy and rebreeding rates. Only 65% of all the cows in this population met the stayability threshold of 2 calves by 3 yr of age (Engle et al., 2018), which is lower than the calving rates at 3 yr reported by Brigham et al. (2007) in Gelbvieh, Simmental, and Red Angus cows (82%, 71%, and 77%, respectively). However, given the 50% *Bos indicus* influence in this population, an older age at the onset of puberty would have been expected in comparison to a straight *Bos taurus* herd (Chenoweth, 1994), impacting both heifer pregnancy and heifer rebreeding rates.

These results suggest that first calving period has an impact on stayability to a variety of ages, where an earlier calving period may be indicative of increased potential for longevity in the herd. These results are comparable to previous studies that found *Bos taurus* heifers that calved earlier during their first calving season, or the first 21-d period of the season, had an increased productive lifespan and herd retention in comparison to those that first calved in later periods (Mousel et al., 2012; Cushman et al., 2013; Damiran et al., 2018). Bourdon and Brinks (1983) found that on average, a *Bos taurus* cow's subsequent calving date was delayed 0.11 d for each 1-d delay in the previous calving date. If this trend were to manifest in a cow's reproductive timeline, at some point she would be biologically unable to recover after parturition and return to estrus before the end of the breeding season and would therefore fall out of production (Bourdon and Brinks, 1983). Heifers in this study that calved in the first calving period had a longer calving interval between their first and second calf (Table 4.3). This does not appear to have negatively impacted their potential for longevity. Rather, this likely reflects the added time allowed for these early calving heifers to recover before rebreeding, aiding in their future success in achieving stayability at different ages. However, the average calving interval in days over the course of a cow's lifetime tends to increase with later calving periods (Table 4.3), potentially reflecting the patterns observed by Bourdon and Brinks (1983).

Table 4.3 Least squares means for cow productivity traits relative to their first calving season period

	Calving period[1]			
	1	2	3	4
Heifers (n)	92	89	35	25
First calving interval, d	$384.4^{a} \pm 1.9$	$371.6^{b} \pm 2.2$	$356.7^{c} \pm 3.7$	$335.2^{d} \pm 5.2$
Lifetime avg calving interval, d	383.0 ± 3.5	389.3 ± 3.8	397.9 ± 7.7	399.9 ± 5.8
1st calf birth wt, kg	$27.8^{a} \pm 0.4$	$29.7^{b} \pm 0.5$	$30.5^{b} \pm 0.7$	$30.6^{b} \pm 0.9$
1st calf weaning wt, kg	$207.2^{a} \pm 2.4$	$194.4^{b} \pm 2.5$	$181.0^{c} \pm 3.9$	$167.4^{c} \pm 5.0$
1: Avg. lifetime calf wwt[2], kg/cow	$211.7^{a} \pm 2.0$	$203.3^{b} \pm 2.1$	$196.6^{b} \pm 4.3$	$196.4^{b} \pm 3.3$
2: Avg. lifetime calf wwt[3], kg/cow	$217.4^{a} \pm 2.1$	$213.7^{ab} \pm 2.3$	$210.7^{ab} \pm 3.3$	$204.9^{b} \pm 3.9$

[1] First calving season periods of 21-d intervals where Period 1: ≤ 21 d, Period 2: 22 to 43 d, Period 3: 44 to 63 d, Period 4: ≥ 64 d

[2] Average yearly weaning weights up to 8 yr of cow age, where cows were culled after 1 failure to wean a calf

[3] Average yearly weaning weights up to 8 yr of cow age, where cows were culled after 2 failures to wean a calf

[a,b,c,d] Indicate significant difference of at least $P < 0.05$

The second objective of this study was to evaluate the influence of a first calf heifer's first calving period on her productivity in kg of calf raised. A heifer's first calving period had a significant effect on the performance of her first calf (Table 4.3). The birth weight and weaning weight for calves born in the first period were significantly different from calves born later in the season. The birth weights were significantly lighter in early-born calves from Period 1 than calves born in all other calving periods. Additionally, the weaning weights were significantly different between calves born at the beginning, versus the end of the calving season, where weaning weights decreased by advancing periods. These patterns in first calf performance mirror those observed by Funston et al. (2011), who also observed that calves born later in the season were lighter at weaning. Over the course of a cow's lifetime, the average weaning weight of her calves tended to decrease based upon her first calving period, and this was maintained regardless of culling criteria applied to the data. Unsurprisingly, the average, lifetime calf weaning weights for each cow differed between first calving period and last calving period (Table 4.3). The date of first parturition for first calf heifers has also been shown to influence the weaning weights of not only their first calf, but also the weaning weights of their subsequent calves (Lesmeister et al., 1973; Arthur et al., 1993; Funston et al., 2011; Mousel et al., 2012). Given the relationship between first calving period and stayability observed in this herd, it is expected that those heifers that calve earlier will stay in the herd longer, providing more opportunity to wean more, and heavier, calves.

Funston et al. (2011) has reported that the calving period in which a breeding female was born can have an impact on the performance of her offspring. They found that when the birth date of an early-born heifer's first calf was earlier, the birth weight was reduced, and calf weaning weight was greater than progeny of later born, first calf heifers. Funston et al. (2011) also reported additional benefits of an early calving date in the form of improved heifer body weight at both prebreeding and precalving, greater percent prebreeding cycling, and as a result, greater pregnancy rates than those heifers born later in the season. Given these benefits, the influence of a cow's calving date relative to the calving season was explored in this population. The calving period a cow was born in was not shown to have a significant impact on their ability to successfully meet a stayability threshold to ages 3, 4, 5, 6, and 7 yr (results not shown). This is likely due to managerial influences, as fall born females were allowed a second breeding opportunity if unsuccessful during their first, winter breeding season, so some later born cows may have been given more time to mature (Table 4.1).

These results suggest that in a *Bos indicus-Bos taurus* crossbred population, the birth date of a heifer's first progeny has a significant impact on the performance of the calf, and on the long-term productivity and potential of the cow. Similar to previous studies in *Bos taurus* herds, these results show an implied economic advantage to first calving within the first 21 d of the calving season as heifers' first calves are heavier at weaning and demonstrate an increased preweaning weight gain. This is further reflected in the long-term productivity of a breeding female, as cows that calved in the first 21-d period of their first calving season produced significantly higher average calf crop weaned than those cows that first calved in the last 21-d period of their first calving season. These early-calving heifers were more likely to meet stayability benchmarks at 5, 6, and 7 yr than heifers calving at any other time in the season. Due to the advantages to both maternal productivity and calf performance, calving within the first 21 d of a breeding season is expected to increase the herd longevity of these females. Therefore, first calving season period may be considered as an early-in-life evaluation criterion when selecting for longevity or lifetime productivity in *Bos indicus-Bos taurus* crossbred beef cows.

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5. MULTIVARIATE GENOMIC PREDICTIONS FOR AGE AT PUBERTY IN TROPICALLY ADAPTED BEEF HEIFERS[*]

5.1. Introduction

Cow and heifer fertility are of critical concern to beef cattle producers, as reproductive performance is a key driver of farm profitability. Heifer productivity is the critical first stage of a cow's productive lifetime and is often reflective of her future long-term profitability and potential in the herd (Núñez-Dominguez et al., 1991; d'Orey Branco et al., 2016). *Bos indicus*-infused females, such as those most commonly raised in Queensland and northern Australia, and in many other tropical regions, tend to be later maturing and older at the onset of puberty than 100 percent *Bos taurus* breeds (Gregory et al., 1979; Hearnshaw et al., 1994; Thallman et al., 1999), reducing heifer productivity in herds with these genotypes (Chenoweth, 1994). However, cattle with *Bos indicus* content are often preferred for production in tropical and subtropical climates because of their adaptive tolerance to the harsh environmental conditions characteristic of these regions (Bortolussi et al., 2005).

Selection has been shown to be an effective way to reduce age at puberty and improve heifer and lifetime pregnancy rates (Mackinnon et al., 1990; Schatz et al., 2010) as a result of the favorable genetic correlation between these traits and age at puberty.

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However accurately measuring heifer age at puberty requires regular ovarian scanning (Pierson and Ginther, 1984; Johnston et al., 2009). This limits the feasibility of large-scale phenotype collection. A new scoring system to assess variation in heifer age at puberty has been proposed for use within extensively managed, northern Australian commercial beef operations (Burns et al., 2016). In this system, a reproductive maturity score of 0-5 was assigned to represent ovarian activity at approximately 600 d of age by real-time ultrasound scanning for presence of corpus luteum (CL), and accounting for potential pregnancy status (obviously a pregnant heifer has definitely reached puberty). Reproductive maturity score should not be confused with reproductive tract score, where palpation rather than ultrasonography of the reproductive tract is used to assess uterine size, uterine tone, and ovarian structures in heifers, and includes no specific assessment of pregnancy status (Anderson et al., 1991; Perry and Cushman, 2016).

While a single ovarian ultrasound scan will be less informative than the serial ultrasounds used to estimate AGECL, reproductive maturity score (RMS) is more commercially feasible as a trait. By eliminating the necessity of additional musters to collect additional measurements, costs associated with mustering, technician travel, and loss of condition due to handling stress may be reduced. The goal of the reproductive maturity score (RMS) was that it may be used for the development and eventual implementation of a puberty genomic estimated breeding value (GEBV) for northern Australian, *Bos indicus-infused* beef cattle (Burns et al., 2016). Recently, Hayes et al. (2019) demonstrated that moderately accurate genomic predictions for CL score

(CLscore, 0 = no CL, 1 = CL present), which is closely related to RMS, could be generated for multi-breed, tropical beef populations.

There have been several large-scale projects collecting AGECL phenotypes and RMS in *Bos indicus* and *Bos indicus-infused* beef cattle (eg. Johnston et al., 2009; Corbet et al. 2018). However, the two traits have not been measured on the same animals, or even on animals with known pedigree relationships between them. Using single nucleotide polymorphism (SNP) genotypes, genomic relatedness matrices may be utilized to estimate the relationship between individuals with minimal or unknown pedigree linkages between them, allowing for multi-breed and across population estimated of genetic parameters via multi-trait predictive modeling (Karoui et al., 2012; Visscher et al., 2014; Porto-Neto et al., 2015; Wientjes et al., 2015). This methodology represents an opportunity to validate how RMS (Burns et al., 2016) genetically correlates with AGECL (Johnston et al., 2009), and allows evaulation of the utility of an industry derived puberty phenotype in increasing accuracy of genomic predictions for AGECL, a more accurate research derived phenotype. Others have found evidence that including highly correlated traits in multi-variate, across population analyses can increase the accuracy of genomic predictions (Karoui et al., 2012), warranting the exploration of different approaches for future development of an age at puberty GEBV using this scoring system.

We hypothesize that reproductive maturity score is reflective of the same biological processes as age at puberty and is therefore analogous in the underlying genetic mechanisms driving the trait. Hence, the primary objective of this research is to determine if using reproductive maturity score phenotypes, and genotypes for the phenotyped cattle, in a multi-trait genomic analysis can improve the accuracy of genomic predictions (GEBV) for age at puberty in *Bos indicus* and *Bos indicus-infused* beef heifers. Increasing the reference population size can increase the accuracy of genomic predictions (VanRaden et al., 2009; Lund et al., 2011). Therefore, the second objective is to explore if the addition of correlated, research derived phenotypes (AGECL) improves the accuracy of predicting age at puberty in commercial herds scored using the proposed reproductive maturity scoring system.

5.2. Materials and methods

Animal Care and Use Committee approval was not obtained for this study as no new animals were handled in this experiment. Analyses were performed using production records and DNA samples previously collected with approval by the J.M. Rendel Laboratory Animal Experimental Ethics Committee (CSIRO, Queensland) as approvals TBC107 (1999-2009) and RH225-06 (2006-2010), and by the University of Queensland Production and Companion Animal Ethics Committee as Approval QAAFI\050\13\Smart Futures.

5.2.1. Animals, phenotypes, and covariates

Cattle used in this study represent a subset of two larger collaborative research herds: The Northern Breeding Project resource population established by the Cooperative Research Centre for Beef Genetic Technologies (Beef CRC) and the Queensland Smart Futures resource population assembled through the Next Gen Beef Breeding Strategies project (Burns et al., 2016). The purpose of the Next Gen Beef

Breeding Strategies project was to evaluate the value of the reproductive traits first identified in the Beef CRC and now in industry herds under commercial management. Both resource populations are typical of the tropical regions of northern Australia and comprised of breeds that are widely used in this production environment, specifically Brahman, Santa Gertrudis, Droughtmaster, and Tropical Composite cattle.

A total of 1872 records from the Beef CRC herd were considered, consisting of 882 Brahman (CRCBRAH) and 990 Tropical Composite (TCOMP) females. The management of these heifers and the phenotypes collected has been extensively described (Burrow et al., 2003; Barwick et al., 2009; Johnston et al., 2009). Briefly, estimations of age at puberty were taken using age at first CL (AGECL). AGECL was defined as age in days at first observed CL, as determined using real-time ultrasound scanning every 4-6 we (Johnston et al., 2009).

A total of 3682 complete records from the Smart Futures herd were utilized, with 974 Brahman (SFBRAH), 1798 Santa Gertrudis (SG), and 910 Droughtmaster (DM) females considered. Heifers were in 7 different commercial seedstock herds from 8 different property locations distributed across northern, central and southern Queensland. Selection and management of these heifers was previously described by Burns et al. (2016). To summarize, all operations managed their animals in mobs that were relatively stable over time, allowing for straightforward segregation of management cohorts based upon year of birth (2011-2014) and property of origin. There were no sires in common between the different breeds and no two breeds were run on the same property. Mating programs were implemented so that cohorts were given an equal opportunity to conceive, at ages ranging between approximately 1-2 yr of age, adhering to the standard practices of the region. At pregnancy test, approximately 5 we after removing bulls, the fetus was aged in weeks.

A reproductive maturity score (RMS) of 0-5, as developed by Burns et al. (2016) was derived and assigned to each heifer where ovarian function was assessed at approximately 600 d of age by a single real-time ultrasound scan (Table 5.1). RMS is a proxy trait for age at puberty, which is the (sole) focus of this paper. RMS is evaluated from a single ovarian scan on a female animal at approximately 600 d of age. The trait attempts to measure how early the animal cycled, a proxy for age at puberty. If pregnant, the animal has definitely cycled, likely earlier than her contemporaries who are in the same management group (paddock), and therefore were also exposed to a bull. If pregnant, the animal is given a 5 for pregnancy > 10 wk (cycled very early) or 4 for earlier pregnancy < 10 wk (likely cycled later). If the animal is not pregnant, but has a CL, she is given a 3 (definitely cycled, but likely later than her contemporaries who are pregnant). If she has no CL, then she is given a 2 (reasonable size follicles, will likely cycle soon), or a 1 (less likely to cycle soon). Table 5.2 gives the distribution of RMS, and age, in each herd.

Table 5.1 Description of reproductive maturity score[1], assessed via ovarian scanning

0 = infantile tract or free-martin
1 = small ovarian follicles
2 = ovarian follicle >10 mm diameter
3 = corpus luteum present
4 = pregnancy to 10 wks
5 = pregnancy >10 wks

[1] As defined by Burns, et al. (2016).

Table 5.2 Proportion of each Smart Futures herd assigned each Reproductive Maturity Score (RMS), and mean age and standard deviation of age in each herd.

		RMS				
Herd[1]	Mean, age at scanning and sd, d	1	2	3	4	5
Brah1	585.7 ± 65.6	0.39	0.12	0.23	0.03	0.22
Brah2	664.7 ± 71.2	0.57	0.15	0.28	0	0
Brah3	618.7 ± 20.5	0.20	0.21	0.50	0.05	0.02
SG1	516.0 ± 74.3	0.29	0.14	0.20	0.14	0.22
SG2	525.8 ± 31.5	0.25	0.25	0.39	0.11	0.01
DM1	605.7 ± 47.4	0.68	0.21	0.11	0.01	0
DM2	592.0 ± 25.7	0.55	0.24	0.21	0.01	0

[1] Abbreviations: Brah = Brahman, SG = Santa Gertrudis, DM = Droughtmaster

Previous studies reported that average age at puberty in tropically adapted composite breeds occurred between 580 and 650 d (Burns et al., 1992; Johnston et al., 2009), rationalizing the use of this score as a relative reflection of puberty. Furthermore, this timing was conducive to standard enterprise management, and coincided with the presence of a CL in approximately 40% of individuals across the herds studied. It is noted that a single scan for CL presence has an error rate of about 14% associated with "false negatives" in cycling females ultrasounded at approximately day 21 of the estrus cycle (Bicalho, et al., 2008). This loss of accuracy will likely reduce the heritability estimate of the trait, the accuracy of the resulting GEBVs of the females measured, and correlations with other traits. However, it is not commercially viable to pay contractors to do multiple scans on a large scale, nor is it commercially feasible to muster and process cattle as frequently as is possible in research facilities or intensive operations.

In this analysis, the frequency of heifers assigned a zero RMS was low, thus more likely reflecting an anomaly rather than true biological state, and these individuals were removed from all subsequent analyses.

Significant fixed effects for each population were separately identified in previous analyses (Johnston et al., 2009; Burns et al., 2016) and then confirmed for each population using linear mixed modeling procedures in R. Variables such as herd of origin, birth month, and management cohort were concatenated into a single classifier of contemporary group that was modeled as a factor in the analysis. Other fixed effects were age at scanning (in Smart Futures herds only), age of dam, and *Bos indicus* content, confirmed using the linear mixed modeling procedures of R. Age of dam was available for CRCBRAH and all Smart Future individuals, but not TCOMP heifers, so the average of the combined herds was assigned to TCOMP, and modeled as a covariate in the analysis. Breed was not modeled as a separate factor as it is confounded with herd in all populations and was not estimable in the ensuing analyses. Age at scanning was perhaps the most important fixed effect in the Smart Futures data. Initial attempts to fit the full bivariate model had difficulties with age as a covariate, due to the absence of age as a covariate in the AGECL part of the model. Therefore, we took the approach of precorrecting for age for the RMS data. An analysis of the estimate of effect of the age when analyzing RMS by itself (univariate) showed the estimate of the effect was almost identical whether a pre-correction was used or whether age was fitted in the linear mixed model. Pre-correction for age in days at scanning was done using linear model procedures in R and the resulting residuals were used as the puberty phenotype for all Smart Futures heifers.

In the TCOMP herd there was a high degree of variation in *Bos indicus* content. The *Bos indicus* content of these individuals was estimated by Farah et al. (2016) using a supervised ADMIXTURE analysis and was used as a covariate in this study. The composition of Santa Gertrudis cattle was historically considered to be ⅜ *Bos indicus* ⅝ *Bos taurus* (Santa Gertrudis Breeders International) and Droughtmaster were ½ *Bos indicus* ½ *Bos taurus* (Droughtmaster Stud Breeders Society). These approximations of *Bos indicus* content were modeled as a covariate for each SG and DM, respectively. Although an estimated 10% of the Australian Brahman genome is of taurine origin (Bolormaa et al., 2011), the *Bos indicus* content of both CRCBRAH and SFBRAH was assumed to be 1.

5.2.2. Genotypes

All Beef CRC heifers were genotyped using the BovineSNP50 BeadChip (Illumina, San Diego, CA) (Hawken et al., 2012). Quality control and quality assurance for SNP genotypes is described in Erbe et al. (2012) and Hayes et al. (2019). Briefly, all Smart Futures heifers were genotyped with 24,121 genome wide SNP using the Geneseek GGP-LD array. SNP were evaluated for average GC score (measure of genotyping quality), and SNP with more than 10% of animals with GC score less than 0.6 were excluded from further analysis. Monomorphic SNP were also excluded (where the SNP were monomorphic across the entire population). 20,414 SNP remained. Of the remaining SNP, if individual genotype calls had GC score less than 0.6, they were set to missing and genotypes were recovered with imputation.

The heifer genotypes were imputed up to 728,785 SNP (Bovine HD array), using 3456 Brahman, Droughtmaster, Santa Gertrudis, Tropical Composites and other relevant breeds genotyped for the Bovine HD array. The 728,785 SNP remained from 777K after a similar QC process as described above, with the addition that mis-mapped SNP were also excluded as described in Erbe et al. (2012). The Fimpute software was used for imputation (Sargelozei 2014).

Genomic relationship matrices G were constructed from the SNP genotypes for the combined reference and validation populations using GCTA (Yang et al., 2011) and as described by Yang et al. (2010). Allele frequencies for centering G were calculated from the whole population. G was adjusted by adding 0.05 to the matrix diagonal to improve matrix stability and making it easier to invert. The reference and validation populations were a combination of both Brahman and composite cattle. Principle components from the G matrix were obtained using the eigen() function in R.

5.2.3. REML estimation of genetic parameters and genomic correlations

Each phenotype (AGECL, RMS) was treated as a separate measure of age at puberty and was analyzed using a multivariate linear mixed model that included the fixed effects of contemporary group, age of dam, and percent *Bos indicus* content as covariates (RMS was pre-corrected for age of animal). Random genetic additive (animal) effects were fitted using G, constructed following model 1 by VanRaden (2008). **G** containing both Smart Futures and Beef CRC heifers had a diagonal mean of 1.085 (σ = 0.01) and off-diagonal mean of -0.0002 (σ = 0.02). AGECL and RMS were then analyzed using the following bivariate linear mixed model, including the previously outlined random and fixed effects:

$$\begin{bmatrix} \boldsymbol{y}_{\mathbf{AGECL}} \\ \boldsymbol{y}_{\mathbf{RMS}} \end{bmatrix} = \begin{bmatrix} \boldsymbol{X}_{\mathbf{AGECL}} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{X}_{\mathbf{RMS}} \end{bmatrix} \begin{bmatrix} \boldsymbol{\beta}_{\mathbf{AGECL}} \\ \boldsymbol{\beta}_{\mathbf{RMS}} \end{bmatrix} + \begin{bmatrix} \boldsymbol{M}_{\mathbf{AGECL}} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{M}_{\mathbf{RMS}} \end{bmatrix} \begin{bmatrix} \boldsymbol{u}_{\mathbf{AGECL}} \\ \boldsymbol{u}_{\mathbf{RMS}} \end{bmatrix} + \begin{bmatrix} \boldsymbol{\varepsilon}_{\mathbf{AGECL}} \\ \boldsymbol{\varepsilon}_{\mathbf{RMS}} \end{bmatrix}$$

Where:

y_{AGECL} and y_{RMS} = vectors of the cows with the respective phenotypes

X_{AGECL} and X_{RMS} = incidence matrices relating y_{AGECL} and y_{RMS} with fixed effects in β_{AGECL} and β_{RMS}

M_{AGECL} and M_{RMS} = incidence matrixes relating y_{AGECL} and y_{RMS} with the random additive genetic animal effects in u_{AGECL} and u_{RMS} using a marker-based relationship matrix

$\varepsilon_{\mathrm{AGECL}}$ and $\varepsilon_{\mathrm{RMS}}$ = vectors of random residual effects associated with measurements in y_{AGECL} and y_{RMS},

The random effects u_{AGECL} and u_{RMS} were distributed as $\begin{bmatrix} \boldsymbol{u}_{\mathbf{AGECL}} \\ \boldsymbol{u}_{\mathbf{RMS}} \end{bmatrix} \sim N(0, \boldsymbol{G} \otimes \boldsymbol{T})$, where $\boldsymbol{T} = \begin{bmatrix} \sigma^2_{u_{AGECL}} & \sigma_{u_{AGECL}, u_{RMS}} \\ \sigma_{u_{RMS}, u_{AGECL}} & \sigma^2_{u_{RMS}} \end{bmatrix}$, $\sigma^2_{u_{AGECL}}$ is the genetic variance of AGECL, $\sigma^2_{u_{RMS}}$ is the genetic variance of RMS, and $\sigma_{u_{AGECL}, u_{RMS}}$ is the genetic covariance between the two traits. The random residual effects were distributed as

$\begin{bmatrix}\boldsymbol{\varepsilon}_{\mathbf{AGECL}}\\ \boldsymbol{\varepsilon}_{\mathbf{RMS}}\end{bmatrix} \sim N(0, \boldsymbol{\Sigma}\otimes\boldsymbol{I})$, where $\boldsymbol{\Sigma} = \begin{bmatrix}\sigma^2_{\varepsilon_{AGECL}} & 0\\ 0 & \sigma^2_{\varepsilon_{RMS}}\end{bmatrix}$, $\sigma^2_{\varepsilon_{AGECL}}$ is the residual variance of AGECL, $\sigma^2_{\varepsilon_{RMS}}$ is the residual variance of RMS, and $\boldsymbol{I}$ is an identity matrix. The off-diagonals of $\boldsymbol{\Sigma}$ are zero because no animal had both traits in our analysis.

Although the Beef CRC and Smart Futures herds are unrelated via known pedigrees, it is expected that due to breed similarities between populations they should be related through common ancestors. These relationships are captured in G and allow for estimation of $\sigma_{AGECL,RMS}$ (Karoui et al., 2012; Porto-Neto et al., 2015; Visscher et al., 2014; Wientjes et al., 2015). For example, the standard deviation of the genomic relationships between the Beef CRC and Smart Futures data was 0.15, mean -0.002, while the standard deviation for the off-diagonal elements corresponding to CRCBrah and SFBrah animals was 0.22 with mean 0.23. This demonstrates that there is information (variation) in these coefficients.

Variance components and correlations for RMS and AGECL were estimated using REML methods in MTG2 (Lee and van der Werf, 2016). Heritability for each phenotype and in each of the Smart Futures herds was also individually estimated using separately calculated **G** matrices specific to each herd.

5.2.4. Reference and validation populations

The primary objective of this research was to determine if using phenotypes from this new scoring system, and genotypes for the phenotyped cattle, in a multi-trait analysis can improve accuracy of GEBV for age at puberty in *Bos indicus* and *Bos indicus-infused* beef heifers. To address this objective, ten-fold cross-validation was used by dividing the Beef CRC herd into 10 parts representing 20% of the population, maintaining the proportion of TCOMP and CRCBRAH represented within the whole population. The analysis was performed 10 times using each division of the data in turn as a validation group and including the corresponding division representing 80% of the herd in the reference population. The Beef CRC and Smart Futures herds were allocated into reference and validation groups using the following 2 schemes:

1. Prediction of AGECL in Beef CRC heifers (n = 1872), using the Smart Futures herd only as an across herd reference (n = 3682).
2. Prediction of AGECL in 20% of the Beef CRC heifers (n = 374), either using the Smart Futures herd (n = 3682) as a reference, or 80% of the Beef CRC alone (n = 1498) as a reference, or including 80% of the Beef CRC with the Smart Futures herd as a combined reference (n = 5180).

The second objective was to explore if the addition of correlated, research derived phenotypes improves the accuracy of predicting age at puberty in commercial herds scored using RMS. To address this objective, the Beef CRC and Smart Futures herds were allocated into prediction reference and validation populations following 3 general schemes:

1. Prediction of RMS in Smart Futures heifers (n = 3682), using the Beef CRC as a reference (n = 1872).
2. Prediction of RMS in 2014 born Smart Futures heifers (the youngest cohort, n = 1324), using either the Beef CRC (n = 1872) as a reference, the 2011-2013 born Smart Futures alone as a reference (n = 2358), or including the

2011-2013 born Smart Futures heifers together with the Beef CRC as a combined reference (n = 4230).

3. Prediction of RMS in each Smart Futures herd, using either the remaining Smart Futures herds as a reference or including the Beef CRC as a combined reference (across herd prediction).

5.2.5. Genomic prediction

Multi-trait genomic best linear unbiased prediction (GBLUP) was implemented to calculate genomic predictions for AGECL and RMS using the different reference populations using REML in MTG2 (Lee and van der Werf, 2016). For all GBLUP analyses, the validation animals were included in G but had unknown phenotypes in the calculation of GEBV. When the reference dataset included both the Beef CRC and Smart Futures herds, the previously described bivariate model was fitted to the population. The univariate model (when the phenotype in the reference was either AGECL or RMS) included the fixed effects of contemporary group, age of dam, and percent *Bos indicus* content, and random additive genetic effects based on a marker-based relationship matrix. The model was:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e}$$

Where:

y = vector of phenotypes

X= design matrix allocating phenotypes to fixed effects

β = vector of fixed effects

Z= design matrix of SNP marker genotypes

u = vector of additive SNP effects, distributed $N(0, \mathbf{G}\sigma_u^2)$

e = vector of residual errors, distributed $N(0, \sigma_e^2)$

Separate G matrices were calculated based upon the individuals present in each combination of reference and validation datasets, with the first among both populations and additional matrices based exclusively on one herd or the other. Prediction accuracy was calculated as the correlation between the genomic estimated breeding values and the residual phenotype (phenotype adjusted for fixed effects using linear modeling) and divided by the square root of the estimated heritability. When using ten-fold cross-validation, accuracy was calculated in each of the 10 validation groups and then averaged. Prediction accuracy for the Smart Futures population was calculated separately in each of the 7 Smart Futures herds and averaged by breed.

5.3. Results

The genomic heritability of RMS was estimated at 0.23 (± 0.03) with an additive variance of 0.30 (± 0.04). Through the use of bivariate modeling, the genetic correlation between RMS and AGECL was estimated to be -0.83 (± 0.17). Increased AGECL reflects a slower rate of maturity, whereas reduced RMS is indicative of a later age at puberty, so a negative correlation between the phenotypes was expected. The genetic correlations between RMS in each breed in the Smart Futures population and AGECL in the Beef CRC population maintained this trend (Table 5.3). However, the genetic correlations between RMS in the entire Smart Futures herd and each breed in the Beef CRC herd were lower. Although the TCOMP, SG, and DM have some Brahman ancestry, the composite populations are genetically distinct from CRCBRAH and SFBRAH, with TCOMP and DM sharing some similarities (Fig. 5.1).

Table 5.3 Genetic correlations between entire herds and breed subsets scored using the alternative phenotyping method

RMS herd[1]	*AGECL herd*	*correlation*
All SF	TCOMP	-0.70 ± 0.23
All SF	CRCBRAH	-0.70 ± 0.21
SFBRAH	All Beef CRC	-0.66 ± 0.34
DM	All Beef CRC	-0.41 ± 0.58
SG	All Beef CRC	-0.68 ± 0.22
SFBRAH	CRCBRAH	-0.85 ± 0.28

[1] Abbreviations: SF = Smart Futures, RMS = (residual) reproductive maturity score, AGECL = age at first corpus luteum, TCOMP = Tropical Composite, CRCBRAH = Beef CRC Brahman, SFBRAH = Smart Futures Brahman, DM = Droughtmaster, SG = Santa Gertrudis

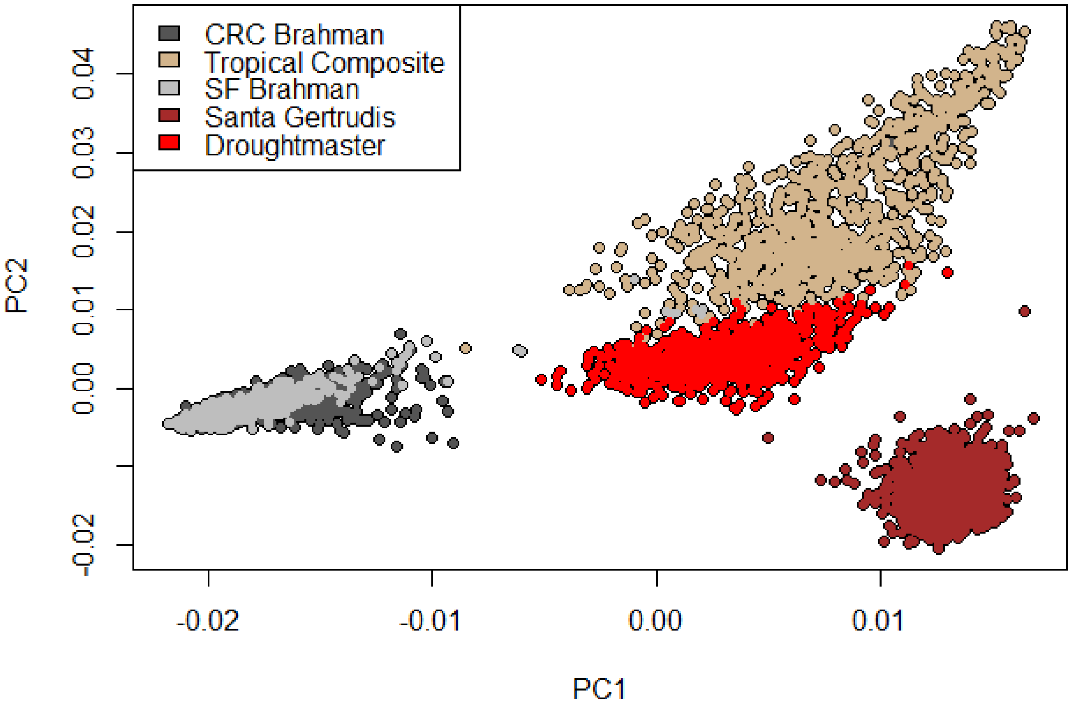

Figure 5.1 Relationships between CRC Beef and Smart Futures heifers. Shown are principal components 1 and 2 for the genomic relationship matrix (Yang et al., 2010; Yang et al., 2011) constructed from all CRC Beef (n = 1872) and all Smart Futures (n = 3682) heifers. Principle components were obtained using the eigen() function in R.

Prediction accuracy of AGECL in Beef CRC heifers when using exclusively Smart Futures heifers (phenotyped for RMS) as a reference was low (Table 5.4). Accuracy was on average 40% of the accuracy for the within-Beef CRC predictions. When the Smart Futures heifers (with RMS) were added to the reference for predicting AGECL, along with the 80% Beef CRC heifers, accuracy of prediction had a 17% increase for TCOMP and 11% increase for CRCBRAH, although this increase was not significant for CRCBRAH.

Table 5.4 Accuracy[1] of genomic estimated breeding values for age at first corpus luteum (AGECL)

Validation:	All Beef CRC (n = 1872)		20% Beef CRC (n = 374)			
Reference:	SF[2] (n = 3682)	n_{val}	SF	80% Beef CRC	80% CRC + SF	n_{val}
Tropical Composite	0.14	990	0.09 ± 0.02	0.42 ± 0.02	0.49 ± 0.06	205
Brahman	0.22	882	0.21 ± 0.04	0.47 ± 0.04	0.52 ± 0.07	169

[1] Accuracy was calculated using $\mathrm{acc} = \frac{\mathrm{r(GEBV,AGECL}_{res})}{\sqrt{\mathrm{h}^2}}$, where residuals from linear modeling - taking into account the fixed effects of contemporary group, *Bos indicus* content, and age of dam – were used as the phenotype, and where estimated $h^2 = 0.48$. Accuracies were calculated and averaged across a ten-fold cross validation. Accuracy is reported in absolute terms and is accompanied by the SEM for cross validated accuracies.

[2] Abbreviations: SF = Smart Futures, n_{val} = number in validation population

The ability for AGECL in the Beef CRC to predict RMS was directly reflected by the degree of genetic similarity between breeds within each herd (Fig. 5.1). This was also observed in the ranking of average GEBV accuracies for each Smart Futures breed (Table 5.5). In comparison to predictions based on RMS only, when the validation set was 2014 born Smart Futures heifers, prediction accuracies decreased for all herds, even if only slightly, when using exclusively the Beef CRC as a reference. With the addition of Beef CRC records to the Smart Futures reference herd, the GEBV accuracy for RMS in the 2014 born SFBRAH, SG, and DM either showed only slight decline, did not change, or only slightly improved, respectively. However, given the accuracy in which AGECL records from the Beef CRC was able to predict GEBV in SFBRAH, it was surprising that SFBRAH had the lowest within herd prediction accuracy.

Table 5.5 Accuracy[1] of genomic estimated breeding values for residual reproductive maturity score[2] in Smart Futures heifers

Validation:	All SF[3] (n = 3682)		2014 born SF (n = 1324)			
Reference:	Beef CRC (n = 1872)	n_{val}	Beef CRC	SF[4]	Beef CRC + SF[4]	n_{val}
Brahman	0.30 ± 0.11	979	0.20 ± 0.04	0.21 ± 0.08	0.20 ± 0.09	384
Santa Gertrudis	0.11 ± 0.11	1803	0.17 ± 0.16	0.31 ± 0.10	0.31 ± 0.05	619
Droughtmaster	0.16 ± 0.11	914	0.14 ± 0.16	0.29 ± 0.02	0.31 ± 0.05	321

[1] Accuracy was calculated using $\text{acc} = \frac{\text{r}(\text{GEBV},\text{RMS}_{res})}{\sqrt{\text{h}^2}}$, where residuals from linear modeling - taking into account the fixed effects of contemporary group, *Bos indicus* content, and age of dam – were used as the phenotype, and where estimated $h^2 = 0.23$. Accuracies were calculated by herd and the mean was taken across breed. Accuracy is reported in absolute terms and is accompanied by the SEM.

[2] Tract score (0-5 score) was pre-adjusted for age in days at scanning using linear modeling and the resulting residuals (RMS) were used as the puberty phenotype.

[3] Abbreviations: SF = Smart Futures, n_{val} = number in validation population

[4] Denotes the reference populations containing the 2011-2013 born Smart Futures heifers

To investigate the surprising trends in prediction accuracy for SFBRAH, each herd was individually evaluated (Table 5.6). Heritability of RMS was independently estimated in each herd, ranging from 0.11-0.35. One herd, Brah3, had a notably low heritability. Compared to other Smart Futures herds, the adjusted RMS for Brah3 contained more outliers and the median deviated greater from an expected value of 0 (Fig. 5.2), which may explain the low heritability estimate and prediction accuracy.

Table 5.6 Accuracy of genomic estimated breeding values for residual reproductive maturity score[1] in each Smart Futures herd using two different reference populations

			Smart Futures		CRC + Smart Futures	
Herd[2]	heritability[3]	n_{val}	n_{ref}	acc[4,5]	n_{ref}	acc[4,6]
Brah1	0.35 ± 0.12	397	3285	0.15	5157	0.23
Brah2	0.22 ± 0.12	371	3311	0.26	5183	0.32
Brah3	0.11 ± 0.13	206	3476	0.01	5348	0.11
SG1	0.22 ± 0.06	1022	2660	0.18	4532	0.19
SG2	0.33 ± 0.08	776	2906	0.36	4778	0.27
DM1	0.24 ± 0.16	222	3460	0.23	5332	0.22
DM2	0.32 ± 0.09	688	2994	0.21	4866	0.18

[1] Tract score (0-5 score) was pre-adjusted for age in days at scanning using linear modeling and the resulting residuals (RMS) were used as the puberty phenotype.

[2] Abbreviations: Brah = Brahman, SG = Santa Gertrudis, DM = Droughtmaster, n_{val} = number in validation population, n_{ref} = number in reference population, CRC = Beef CRC

[3] Individual herd h^2 was independently calculated from individuals in validation herd. Includes SEM.

[4] Accuracy was calculated using $\mathrm{acc} = \frac{r(\mathrm{GEBV}, \mathrm{RMS}_{res})}{\sqrt{h^2}}$, where residuals from the linear modeling- taking into account the fixed effects of contemporary group, *Bos indicus* content, and age of dam – were used as the phenotype, and where estimated $h^2 = 0.23$.

[5] Standard error of all correlations is ± 0.16

[6] Standard error of all correlations is ± 0.13

GEBVs were calculated for each Smart Futures herd by sequentially omitting one herd in turn, using the remaining Smart Futures herds as the reference (Table 5.6). When averaged across the herds, results were very similar to those reported in Hayes et al. (2019), using a CLScore. For individual herds, accuracy was low for Brah1 and very low for Brah3. With the inclusion of Beef CRC records into the training population, the accuracy of predictions for the Brah3 herd improved slightly, but was still very low. Although change in accuracy for each herd was highly variable, overall, the SFBRAH exhibited a positive change in accuracy of 64% with the addition of Beef CRC animals to the reference, SG decreased by 16%, and DM decreased by 10%. This trend again corresponds to the degree of similarity between each breed in the Smart Futures population and that of the breeds in the Beef CRC (Fig. 5.2). A combination of the low heritability for RMS in Brah3 and the low within herd prediction accuracies for Brah1 and Brah3 likely drove the low GEBV accuracies for 2014 born SFBRAH (Table 5.6). Furthermore, Brah3 is the smallest herd included in the analysis and only contains records from 2013 and 2014, which may have impacted prediction accuracy. However, Brah3 was kept in the analysis to maintain consistency between evaluations of RMS.

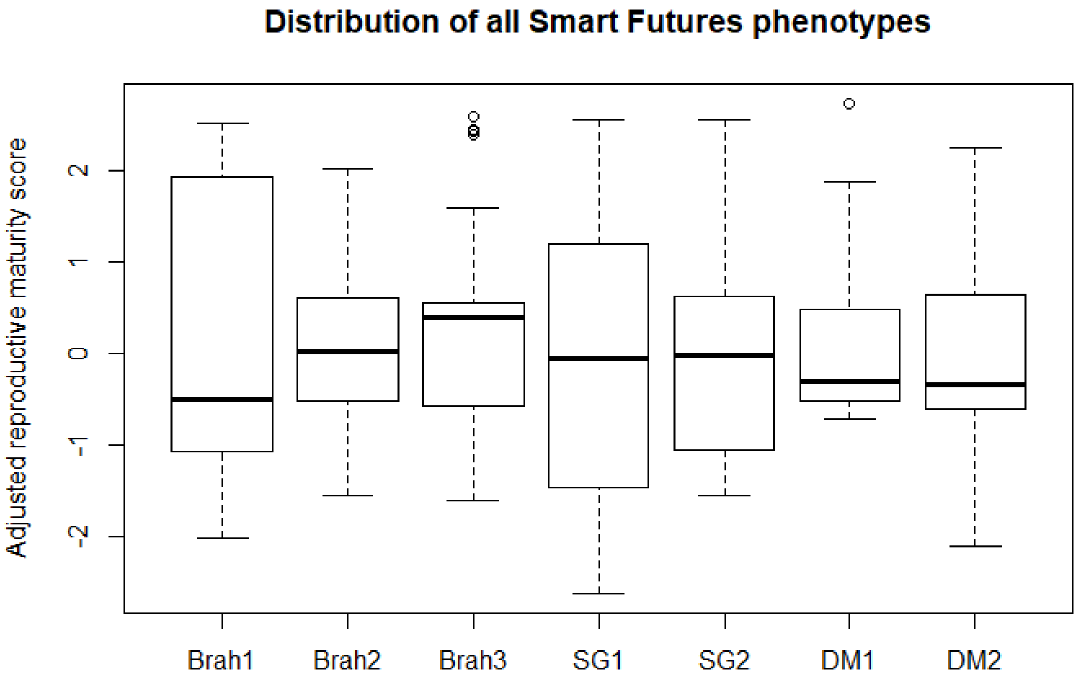

Figure 5.2 Distribution of adjusted puberty phenotypes in the Smart Futures herds. Reproductive maturity score, as defined by Burns et al. (2016), was adjusted for the fixed effects of age in days at scanning, contemporary group, age of dam, and *Bos indicus* content using linear modeling, and the resulting residuals were plotted as the puberty phenotype.

5.4. Discussion

The primary objective of this study was to establish the value of RMS for its ability to improve accuracy of GEBV for age at puberty in beef heifers with high *Bos indicus* content, using a multi-trait analysis. To determine the efficiency of this scoring system for predicting puberty, RMS was evaluated based upon change in GEBV accuracy for AGECL when animals with RMS phenotypes were included in the reference herd. Heritability estimates from this study corroborate the pedigree-based heritability for associated measures reported by Corbet et al. (2018), of 0.18-0.32, but are lower than the estimated heritabilities for AGECL (0.49-0.56) in Beef CRC herds (Zhang et al., 2014). This discrepancy between traits is likely due to the improved precision in which AGECL covariates and phenotypes were measured, where AGECL is a more biologically accurate reflection of true heifer age at puberty.

RMS is feasible for measurement in commercial (or at least stud) herds, and is an adaptation of AGECL developed with the intent of capturing the same biological process as AGECL. The degree of similarity between the traits is reflected in the genomic correlation between them. A high genetic correlation may indicate that LD and QTL architecture is maintained between the breeds in each population, or that QTL and allele substitution effects do not differ between breeds (Karoui et al., 2012; Bolormaa et al., 2013; Wientjes et al., 2016). Karoui et al. (2012) determined that the genetic correlation between populations or traits must be greater than 0.6 to observe an increase in prediction accuracy via use of a multi-trait model. The correlation between RMS and AGECL (-0.83) in our results suggests that there is potential that they may be used together in a multi-trait prediction model to increase the accuracy of prediction for age at puberty, as demonstrated by our increases in accuracy of GEBV for AGECL when the RMS data is included.

When RMS was used to supplement a training population for prediction of AGECL GEBV, accuracy did not significantly improve for CRCBRAH but did for TCOMP. This corresponds to previous reports where adding individuals of a different breed or cross to the reference herd improved accuracy of predicting crossbred performance, but may not have had an influence on purebred prediction accuracies (Bolormaa et al., 2013; Zhang et al., 2014; Esfandyari et al., 2015; Farah et al., 2016). These results were comparable to other studies that found adding crossbred or additional breeds into a reference herd did not improve prediction accuracy in purebred individuals (Erbe et al., 2012; Karoui et al., 2012; Weber et al., 2012).

There is evidence that using crossbred training groups on validation populations of purebred animals comprised of the same breeds used to make the reference population resulted in similar prediction accuracies as when predicted by a purebred reference (Toosi et al., 2010; Weber et al., 2012). This trend was not observed when Santa Gertrudis and Droughtmaster, breeds with Brahman origins, were used to train for CRCBRAH. In this model, the benefits of including additional Brahman and crossbred individuals in the reference appeared to be offset by the increased error variance from using across-population prediction. However, the accuracy for CRCBRAH did not decrease, implying that using a multi-breed, composite reference population would not be detrimental. Together, these results suggest that RMS adequately reflects variation in puberty in *Bos indicus* and *Bos indicus*-infused beef cattle and can be used to increase accuracy of GEBV for predicting age at puberty where AGECL is not available. Future efforts may focus on further validating this use of RMS for commercial application by comparing it to similar scores measured at a series of time points. A potential challenge may be lack of recording of birth date so that age cannot be fitted in the model. Some novel approaches to resolving this, including walk over weighing, ear tags that record calving date, and DNA methylation predictions of age are all in development.

The second objective of this study was to determine if the addition of correlated, research derived phenotypes could improve prediction accuracy for age at puberty in industry herds characterized using RMS. This score is an indicator trait for the phenotype of interest, puberty. While RMS is a significantly less expensive to collect and easily incorporated into commercial herd management, AGECL more accurately represents puberty compared to RMS. It would therefore be advantageous to include AGECL in multi-trait GEBV predictions with RMS as it would more directly tie the predictions to the true biological puberty phenotype. Previous research indicates that increasing reference population size can increase the accuracy of GEBV predictions (VanRaden et al., 2009; Lund et al., 2011). Considering the previously discussed results of this study, it was expected that the addition of AGECL phenotypes would either have a positive or no effect on prediction accuracy in Smart Futures heifers. All herds demonstrated this pattern.

This work demonstrates that there is potential for RMS and AGECL to be used together in a multi-trait prediction model for the of prediction of heifer age at puberty in

Bos indicus-infused beef cattle. When RMS was included in the reference population for predicting AGECL GEBV, accuracy improved for both purebred and crossbred validation populations. This implies that the utilization of a composite/crossbred training population would not be detrimental to prediction accuracy. Additionally, the inclusion of both AGECL with RMS in multi-trait GEBV predictions will more directly tie the predictions to the true biological puberty phenotype, which is practical for application in industry. Collectively, these results suggest that RMS adequately reflects variation in puberty of heifers with high *Bos indicus* content and is an informative trait in the analysis of age at puberty as a component of heifer fertility. The use of RMS to improve heifer fertility in extensively managed beef cattle herds is not only valuable to the region it was developed for, northern Australia, but also other tropical and sub-tropical areas of the world. An estimated 70% of the world's cattle are raised in tropical and subtropical environments (Robinson et al., 2014), elevating the value and practicality of this scoring system and prediction methodology to a global scale.

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6. CONCLUSION

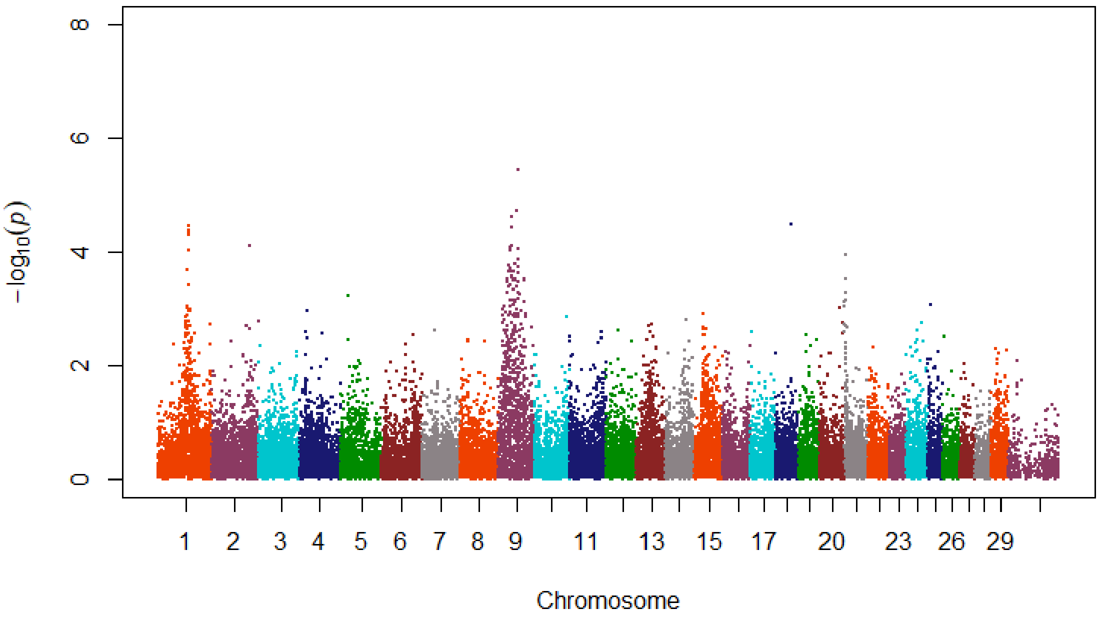
Beef cow stayability is an important yet complicated measure of cow reproduction and productivity. The high degree of environmental variation and genetic complexity associated with the trait makes it a difficult selection target. It has been shown herein that there is potential to identify genomic regions associated with a complex trait such as stayability in *Bos indicus-Bos taurus* crossbred cows. These results suggest that there are structural genetic differences between the subspecies, predominantly on bovine chromosome 5, and that these differences have an effect on heifer pregnancy and rebreeding before 3 yr of age. Differences in patterns of allelic variability unique to Nellore and Angus surrounding the lead SNP associated with heifer productivity may correspond to the translocated inversions observed between the Brahman (University of Queensland, *unpublished*) and Hereford (USDA ARS, *unpublished*) reference assemblies. This region has been previously associated with pleiotropic or adaptive traits in *Bos indicus* x *Bos taurus* crossbred cattle, so it is not unlikely that genetic variation within this interval may also be associated with a number of other traits. However, additional research will be needed to corroborate these findings and validate the genetic mechanisms underlying these observations.

These results suggest that in a *Bos indicus-Bos taurus* crossbred population, relative to the calving season, the timing of parturition for a heifer's first progeny has a significant impact on the performance of the calf. Additionally, this may have an effect on the long-term productivity of the cow and her potential for long-term herd retention.

Similar to previous studies in *Bos taurus* herds, these results show an implied economic advantage of calving within the first 21 d of the calving season as heifers' first calves are older and heavier at weaning. This is further reflected in a cow's potential for long-term productivity, as these early calving females were more likely to meet stayability bench marks at 5, 6, and 7 yr than heifers calving at any other time in the season. Given the advantages with both maternal productivity and calf performance, calving within the first 21 d of a breeding season is expected to increase the productive longevity of these breeding females. Heifer calving date relative to the calving season may therefore be considered as an early-in-life evaluation criterion when selecting for either longevity or lifetime productivity in *Bos indicus*-*Bos taurus* crossbred beef cows.

Lastly, this work demonstrates that there is potential for on-the-farm and research-derived measures of heifer maturity to be used together in a multi-trait prediction model for the prediction of heifer age at puberty in *Bos indicus*-infused beef cattle. When reproductive maturity score was included in the reference population for predicting age at first corpus luteum genomic estimated breeding values, accuracy improved for both purebred and crossbred validation populations. The inclusion of both age at first corpus luteum and reproductive maturity score in multi-trait genomic estimated breeding value predictions is expected to more directly tie the predictions to the true biological puberty phenotype. This increases the potential for impactful industry application of such selection tools. Collectively, these results suggest that reproductive maturity score adequately reflects variation in puberty of heifers with high *Bos indicus* content and is an informative trait in the analysis of age at puberty as a component of heifer fertility.

APPENDIX A



Appendix A. Independent association between chromosomal peaks was verified by extracting the lead SNP from the GWAS for Criterion 2, located on BTA 5, and modeling it as a covariate in a replication of the Criterion 2 GWAS.

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