GENETIC EVALUATION OF COW TEMPERAMENT AT THE TIME OF PARTURITION

A Thesis

by

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ABSTRACT

Temperament is the response of animals to handling. Research has shown that cattle temperament is associated with productive traits. Locating and understanding the genes associated with temperament could help for selection and genetic improvement. The objectives of this research were to estimate the heritability of cow temperament at parturition, conduct a genome-wide association analysis of cow temperament at the time of parturition, estimate the correspondence of cow temperament at the time of parturition with various measures of cow productive performance, and estimate the correspondence of cow temperament at parturition with temperament measured on the same females when they were weaned. Estimates of heritability for highest temperament scores and of the proportions of temperament scores greater than 1 were 0.23 ± 0.07 and 0.12 ± 0.067, respectively. Within generations, 2-yr-old cows had lower temperament score means than cows in most other age categories. Positive estimates of correlation coefficients of unadjusted temperament with weaning weight per cow exposed ($r > 0.249$), weaning weight per 454 kg (1,000 lb) cow weight ($r > 0.166$), birth weight ($r > 0.143$), weaning weight ($r > 0.101$), cow weight ($r > 0.175$) and cow body condition score ($r > 0.026$) were found. Cows with higher temperament scores also showed higher fertility measures (calving rate ($r > 0.173$) and weaning rate ($r > 0.229$)). Although not large in magnitude, they provide evidence that counters other descriptions of relationships of favorable temperament with increased productivity. There were insufficient numbers of cows to estimate genetic correlations between temperament traits and productivity traits. Genome wide association test results associated SNP on BTA 4 and 11 for highest temperament scores; and BTA 14 for proportions of temperament scores greater than 1.

ii

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Contributors

This work was supervised by a thesis committee consisting of Dr. David G. Riley (advisor), and Dr. Andy D. Herring of the Department of Animal Science and Dr. Brian W. Davis of the Department of Department of Veterinary Integrative Biosciences.

The genome wide association analyses described in the Materials and Methods were conducted with the guidance of Dr. Clare Gill of the Department of Animal Science.

All other work conducted for the thesis was completed by the student independently.

Funding Sources

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Temperament is a behavioral characteristic in animals and humans. It is always important in cattle operations because cattle with poor temperament can cause property damages and an unsafe environment. Cattle temperament can be measured a variety of ways, including subjective scores, where the lowest score commonly means docile and higher scores indicate progressively worse temperament meaning that the animal is easily excitable. There is extensive research on cattle temperament and its association with performance in different traits such as reproductive efficiency, growth, weight, meat quality, milk yield, and other traits that could influence the efficiency of a herd. However, there have apparently been no genetic evaluation research efforts to assess cow temperament at the time of parturition. The objectives of this researchwereto: 1) estimate the heritability of cow temperament at parturition, 2) conduct a genome-wide association analysis of cow temperament at the time of parturition, 3) estimate the correspondence of cow temperament at the time of parturition with various measures of cow productive performance, and 4) estimate the correspondence of cow temperament at parturition with temperament measured on the same females when they were weaned.

2. LITERATURE REVIEW

2.1 Temperament in Cattle

Temperament can be defined as the behavioral characteristics resulting from the individual animal's physical, hormonal, and nervous systems (Kilgour, 1975). Temperament in cattle may be defined by response to handling (Burrow and Corbet, 2000). An understanding of the behavior of livestock will facilitate handling, reduce stress, and improve both handler safety and animal welfare (Grandin, 1989). Easily excitable animals compromise their own safety and the safety of stockpersons in charge of raising them. Animals that are calm and docile during handling are said to have good temperaments, while those that are nervous and flighty are said to have poor temperaments (Petherick et al., 2002). Cattle maintain a safe distance from perceived threats such as people and dogs. This distance is known as a flight zone and a major determinant of it is the animal's experience with people and handling (Grandin, 1984). Flight speed is a measure of the time it takes an animal to traverse a certain distance after being restrained in a chute (Burrow et al., 1988; Rolfe et al., 2011). Producers have recognized the importance of temperament in successful management (Busby, 2010). Inherited temperament traits are predictable and observable in early life, but may become gradually overridden as calves age, maybe due to accumulating exposure to environmental stimuli (Littlejohn et al., 2018). According to Piovezan et al. (2013) bovine temperament is distinct by subspecies *Bos taurus taurus*, that is, those cattle originating from Eastern Europe/Western Asia (modern day Turkey) or *Bos taurus indicus*, that is, those cattle with ancestors from the Indian subcontinent. Temperament scores are reasonably stable across time but affected by the individual's experience with humans or other environmental

factors over longer periods (Haskell et al., 2012). Studies in wild animals have shown that temperament is consistent across years (Reale et al., 2000; Gabriel and Black, 2010).

2.2 Correspondence of Temperament with Other Traits

There is much interest in the relationship of temperament with productive performance. According to Reinhardt et al. (2009) animal temperament has a great influence on feedlot performance and carcass value, and because genetic makeup has an effect on this temperament, then breeds should be considered when purchasing feeder cattle for value-added meat programs. Nellore cattle with excitable temperament under grazing conditions had impaired growth performance and showed differences in feeding behavior (Francisco et al., 2020). Grazing may mitigate poor temperament, as was demonstrated in Japanese Black cows (Nakajima et al., 2021). In ewes, temperament influenced entrance order into the milking parlor; animals with worse temperament entered later than ewes with good temperament (Libis-Marta et al., 2021). Those researchers also reported that ewes with lower (better) temperament score had higher milk yields. Cziszter et al. (2016) concluded that selection against animals that are highly reactive to improve welfare and ease of handling would not have detrimental impacts on productivity and reproductive outputs (shorter calving interval and improved milking letdown) of Simmental/Fleckvieh cows.

Aggressiveness was evaluated in Limousin heifers, that is, if the animal threatened the handler (Phocas et al., 2006). Traits investigated included running time as a percentage of 1 minute, the number of escapes in the presence of a motionless handler, and a docility score that was a combination of all the previous traits. Phocas et al. (2006) concluded that non-aggressive heifers were more productive than aggressive heifers, mainly because they were sexually more precocious and fertile and calved more easily, had higher growth and milk yield, and were more attentive to

the new-born calf. Cooke et al. (2017) reported that easily excitable *B. indicus* beef cows may experience reduced pregnancy rates to first timed artificial insemination, increased pregnancy loss, decreased calving and weaning rates, and younger and lighter offspring at weaning compared to cows with adequate cohorts. Favorable genetic relationships between flight speed and scrotal size indicated that selection for improved temperament may also improve indicator traits of male fertility (Burrow, 2001). The calves from cows that allowed the handler to closely approach during tagging (that is, more docile calves) showed their first attempt to stand more rapidly after birth (Turner et al., 2013).

2.3 Cattle Breeds

Nellore cattle originated from India and is one of the breeds that have influenced Zebu cattle breeding in the United States. Many cattle from this breed were imported from Brazil in the early 1900s (Sanders, 1980). The Angus (originally Aberdeen-Angus) breed originates from the counties of Aberdeen and Angus in Scotland. Angus cattle are characterized not only by large amounts of intramuscular fat in the longissimus muscle (marbling) and high energy growth, but also by longevity and good endurance (Smakuyev et al., 2021). It has been well documented that meat from *B. taurus* breeds of cattle was more tender (lower shear force and higher sensory tenderness rating) than meat from *B. indicus* breeds of cattle (Wheeler et al., 1994). Compared to breeds of European origin, Zebu cattle experience less severe reductions in feed intake, growth rate, milk yield and reproductive function in response to heat stress (Hansen, 2004). *Bos indicus* cattle have been shown to have a more excitable temperament compared to *B. taurus* breeds (Brandão and Cooke, 2021).

2.4 Maternal Behavior

Important components of maternal behavior are those that permit proper bonding to take place between mother and offspring, nursing behavior, responsiveness, and attentiveness towards the offspring, and protectiveness towards intruders (Grandinson, 2005) Once the forces of natural selection were eliminated by husbandry, the genetic basis for maternal behavior became quite variable (Sandelin et al., 2005).

2.5 Genetic Control of Temperament in Cattle

Genetic control may be of at least two different forms. According to Rolfe et al. (2011) heterosis had no significant effect on temperament; however, temperament measured in both sexes and at various times in life is responsive to heterosis, and is influenced by the sire's breed, the dam's breed, and their interaction (Riley et al., 2007; 2010; Chase et al., 2017). Temperament is highly heritable and animal selection for temperament may improve production traits because it has been widely reported to have a significant association with growth traits (direct weaning, yearling and mature weight), feed efficiency (residual average daily gain), precocity (scrotal score), and carcass traits (carcass weight, ribeye by area) it also has a low correlation with hair shedding and maternal weaning weight (Alvarenga et al., 2022, 2023; Shen et al., 2022). It is a polygenic trait with responsible genes and QTL broadly spread across the entire genome (Araujo et al., 2021). Estimates of heritability for various temperament traits in cattle ranged from low to moderate (Appendix Table 1). Bovine genomic regions associated with various temperament traits are shown in Appendix Table 2.

2.6 Evaluation of Temperament at the McGregor Research Center

Bovine temperament has been deliberately investigated at the Texas A&M AgriLife Research Center at McGregor using several generations of a designed population of ½ Nellore ½ Angus cattle; the cows in the present work are part of that population. There were five different aspects of temperament that were evaluated in weaned calves from 2005 to 2013 using subjective numerical scores from 1 to 9, in which higher numbers indicated increasingly worse temperament. Calves were evaluated in pairs in early years, and later singly after being separated from a pen of calves and placed between four evaluators. Each evaluator scored each calf independently; scores were averaged to provide a single number for each attribute for each calf. Aggressiveness was assessment of the calf's willingness to hit or attack the evaluator. Nervousness was indicative of how relaxed the animal was in the presence of humans. Flightiness was also related to the tranquility of the animal, but included jumping and running behavior. Gregariousness was the willingness of the animal to separate from their group and their response to isolation. An overall score of temperament was a distinct evaluation of the ultimate temperament of each calf. High estimates of heritability (aggressiveness = 0.51 ± 0.065; nervousness = 0.40 ± 0.065; flightiness = $0.45 + 0.065$; gregariousness = 0.49 ± 0.066; overall = 0.47 ± 0.066 and 0.361) were observed for these traits of temperament (Riley et al., 2014; 2016; Hanna et al., 2015). These traits were also found to be associated with various genes in different genomic regions, although associations with temperament at the time of weaning and at 1 yr of age were different (Table 1). There was minimal correspondence of these traits with a variety of other beef traits, including carcass traits (slaughter temperament, skeletal maturity, hot carcass weight, ribeye area, fat thickness, USDA yield grade, lean maturity with and without electrical stimulation and their overall maturity, marbling score, and USDA quality grade), palatability traits (Warner-Bratzler shear force, juiciness, muscle fiber

tenderness, connective tissue amount, overall tenderness, and flavor intensity), flavor traits (acid, bitter, bloody/serum, browned, chemical, chemical burn, cooked beef fat, cooked beef lean, grassy, livery, metal, metallic, salt, and sour) and efficiency traits (metabolic mid weight, average daily gain, dry matter intake, residual feed intake, and model predicted residual consumption) (Riley et al., 2019a, b; 2020; Baker et al., 2022).

Table 1. Associated genomic regions with temperament traits in cattle from McGregor Research Center evaluations (Riley et al., 2016)

Trait	BTA	Location (Mb)	Associated gene
Weaning			
Nervousness	1	23.9	roundabout homolog 2-like (*ROBO2*)
	24	13.0	synaptotagmin IV (*SYT4*)
Flightiness	24	14.0	
Aggressiveness	29	3.1	FAT atypical cadherin 3 (*FAT3*)
Yearling			
Aggressiveness	7	1.0	RasGEF domain family 1C (*RASGEF1C*)
	10	100.9	G protein-coupled receptor 65 (*GPR65*)
	12	18.5	cysteinyl leukotriene receptor 2 (*CYSLTR2*)
	20	4.9	stanniocalcin 2 (*STC2*)
	21	5.3	GABAA receptor gamma 3 (*GABRG3*)
Nervousness	5	114.5	tubulin tyrosine ligase-like family 1 (*TTLL1*)
	14	23.0	RB1-inducible coiled-coil 1 (*RB1DC1*)
Overall	3	11.3	olfactory receptor 6P1 (*OR6P1*)
	3	13.0	CD48 antigen-like
	23	48.0	ras responsive element binding protein 1 (*RREB1*)
	26	16.6	PDZ and LIM domain 1 (*PDLIM1*)

3. MATERIALS AND METHODS

3.1 Cattle

The Texas A&M University Institutional Animal Care and Use Committee in multiple Animal Use Protocols were followed through all animal procedures. This project consists of historical data from the McGregor Research Center that includes five generations of ½ Nellore (N) ½ Angus (A) crossbred cattle. Purebred Nellore bulls and Angus cows were bred to produce the F_1 founders (5 bulls and 14 cows; one bull produced only two calves), Nellore-Angus (NA; a pair of letters indicates the sire and dam breeds of crossbred F_1 animals, respectively). Cycle 1 animals were from 14 full sibling families, produced by embryo transfer, and born in the fall or spring from 2003 through 2007. Cycle 1 cows were exposed to bulls as yearlings. There were some Cycle 1 cows that have records from 2005 to 2022. Cycle 2 cows are also F_2 cattle; however, Angus-sired F_1 bulls and cows were also used to produce these. That is, there are cows in Cycle 2 from all possible crosses of F_1s: NA-NA, NA-AN, AN-NA and AN-AN (two pairs of letters used to designate the breed types within this cycle; again, pairs of letters indicate sire and dam breeds, respectively, of the F_1 parents). There are records from Cycle 2 cows from 2010 to 2022. Cycle 1 bulls were bred to Cycle 1 cows to produce F_3 animals in Cycle 3 that were born from 2009 through 2013. Cycle 3 bulls were bred to Cycle 3 females produced F_4 (Cycle 4) calves that were born in 2014 and 2015; those females have records from 2016 to 2022. The last generation were F_5 (Cycle 5) calves produced from mating of F_4 bulls and cows; those were born in 2018 and 2019. Cycle 5 females have records in these data from 2020 to 2022. All females from the different cycles and generations were first exposed to bulls for breeding at approximately 1 yr of age. Fall-born Cycle 1 cows were first exposed to bulls at approximately 18 mo of age in order to align them with a

9

spring calving system. The breeding season in all years was from the first two weeks of May (depending upon the year) until the first two weeks of July. Females were exposed to Angus bulls for their first calves. Subsequently, cows in each generation were exposed to bulls from the same generation for two or more years; inbreeding was avoided as much as possible. After producing calves for the next generation, they were then exposed to the same bulls, usually Angus (in some years both Angus and Hereford bulls were used), in multiple sire pastures (one bull per 20 to 25 cows, depending upon the year). Their calves were spring-born. Cows were removed from the project for health reasons or for two failures to conceive and wean a calf. After 14 yr of age, cows were removed for a single failure to conceive and wean a calf. The majority of the F_3 cows were removed in 2015 from the project after it was determined that sufficient F_4 calves had been produced. Some cows died and some were removed for injury or health reasons. There were a few cows that were so aggressive and dangerous that they were removed from the project.

3.2 Records

Temperament scores were assigned to cows at the time their calves were weighed and tagged (one day after calving) by three evaluators across all years. Scores were assigned based upon the cow's behavior at that time with a range from 1 (very calm) to 5 (easily excitable). Higher scores indicated progressively more active and aggressive behavior. Higher scores were also assigned to cows that displayed more nervous or frightened behavior, evidenced by a variety of actions including running away. Calf weight and cow body condition scores (BCS; values of 1 to 9 per Herd and Sprott, 1986; higher numbers indicate increasing external fat assessed visually) were recorded at the time of calving. Weights and BCS of calves and cows were recorded at

weaning (calves were weaned at an approximate average of 7 mo of age in October). In 2011 and 2022 calves were weaned early (August or September) because of drought conditions.

For each cow a distinct trait was calculated as the proportions of the temperament scores for all calving across her productive lifetime that were greater than 1. Another distinct trait of each cow was her highest temperament score recorded. Because some cows had the same highest score recorded multiple times, the earliest of the those was designated the highest. Means and SD of temperament scores are presented in Table 2.

Table 2. Number of cows and records in each cycle and means (SD) for temperament traits

Cycle	Cows	Records	Temperament score[1]	Highest temperament scores[2]	Proportion of records greater than 1[3]
1	286	2,129	2.64 (1.38)	3.66 (1.34)	0.78 (0.32)
2	228	952	2.34 (1.14)	3.11 (1.22)	0.71 (0.30)
3	273	696	2.38 (1.27)	2.77 (1.44)	0.64 (0.41)
4	103	446	2.17 (1.10)	2.93 (1.18)	0.63 (0.33)
5	56	114	1.37 (0.66)	1.64 (0.79)	0.28 (0.33)
Total	946	4,337	2.45 (1.29)	3.07 (1.40)	0.68 (0.36)

[1]Temperament score; a score from 1 to 5 to describe how calm or easily excitable the cow was at the time of parturition, where 1 = calm and 5 = easily excitable.

[2]Highest temperament scores of each cow at the age of earliest occurrence (some cows had more than one score that was the highest value).

[3]Proportion of records greater than 1; the proportion of all scores greater than 1 (1 represents calm, good temperament) across her lifetime.

3.3 Genotypic Data

Genotypes for 52,785 SNP from Version 1 of the BovineSNP50 array (Illumina Inc., San Diego, CA) were available for 14 Nellore bulls and 9 Angus cows (i.e., grandparents and other ancestors of Cycle 1), 4 Nellore-Angus F_1 bulls and 14 Nellore-Angus F_1 cows (i.e., parents of Cycle 1). Genotypes from the BovineSNP50 array were obtained for 201 F_2 females from Cycle 1 and 118 females produced by natural service using the same 4 sires as the F_2 females. Genotypes for 49,629 SNP from the IDB V.3 array (Weatherbys Scientific, Newhall, Naas, Co. Kildare, Ireland) were available for 246 reciprocal F_2 females from Cycle 2, 306 F_3 females from Cycle 3, and 130 F_4 females from Cycle 4. Genotypes for 47,843 SNP from the GGP 50K array (Neogen Genomics, Lincoln, NE) were obtained for 59 F_5 females. All genotypes were in Illumina top orientation. The SNP manifest for Version 1 of the BovineSNP50 array was based on coordinates from the Btau4.0 assembly of the bovine genome. The SNP manifests for the BovHD array, IDB V.3 array, and GGP 50K array were based on the UMD3.1 assembly. All SNP coordinates were lifted over to the ARS-UCD1.2 assembly based on coordinates from Schnabel (2018). Prior to merging genotypes from different arrays, genotypes were removed for markers and animals with call rates less than 90%, and for markers with a minor allele frequency less than 5%. After merging, duplicates of variants that shared the same coordinate and allele code were removed. Parentage was determined from identity-by-descent computations in PLINK v1.9 (Chang et al., 2015). Across the arrays, there were ~99,000 SNP with unique positions and genotypes for these SNP were extracted from the BovHD data for the founders and F_1s (n = 38), as a reference panel for imputation. Genotypes for each autosome were converted to variant call format (vcf) and phased with eagle v2.4.1 (Loh et al., 2016) using the reference panel by invoking 4 threads and if 1 Mb was equivalent to 1 cM. Phased genotypes were then imputed to 99K density using

Minimac3 (Das et al., 2016). Prior to GWAS, SNP that deviated ($P < 0.0001$) from Hardy-Weinberg proportions were removed (Wigginton et al., 2005).

3.4 Statistical Analyses

Estimates of heritability were generated using animal models in ASReml 4.1 software (Gilmour et al., 2015). The fixed effects investigated include various parameterizations of cow age, year, generation (that is, cycle), and calf sex. Fixed effects were determined with a criterion of $P < 0.15$ of the F statistic while modeling only an additive genetic variance. After the fixed effects were confirmed, various random effect structures were investigated including the additive genetic, permanent environmental, maternal additive genetic, and maternal permanent environmental variances, as well as the additive genetic-maternal additive genetic covariance. Final random structures were determined by likelihood ratio tests of nested models and the fixed effects reconfirmed. All genetic variances and genetic relationships were omitted from final models to generate residuals for each trait for genome wide association analyses.

Residuals for traits were used in genome wide association analyses with univariate procedures of GEMMA (Zhou and Stephens, 2012). Covariances among animals were modeled using a genomic relationship matrix constructed with the SNP markers. The false discovery rate (Benjamini and Hochberg, 1995) was relaxed to control at 0.2 because of the novelty of the trait and preliminary nature of investigations. Candidate genes were identified for each marker with detected associations using the R statistical program package Map2NCBI (Hulsman Hanna and Riley, 2014).

Estimates of correlation coefficients were obtained as Pearson correlation coefficients using R Statistical Software. Estimated correlation coefficients included the relationship of each

cow temperament trait with 1) that animal's temperament scores at weaning (aggressiveness, flightiness, gregariousness, nervousness, and overall temperament, 2) the animal's BCS at the time of parturition, 3) birth weight, weaning weight, and weaning BCS of the calf of the cow, 3) the animal's lifetime calving and weaning rates (each cow was given values of 1 or 0 for success or failure to give birth or wean a calf in a given year), and 4) calf weaning weight per cow exposed to bulls for breeding (that is, those that did not wean a calf were given a weaning weight value of 0), and calf weaning weight per 454 kg cow weight (that is, 1,000 lb) as a measure of efficiency. Correlations coefficients were estimated with unadjusted trait values and with residuals. Residuals were generated for each record from mixed models that included additive genetic and permanent environmental (or maternal permanent environmental effects for calf traits) and parsimonious, appropriate parameterizations of fixed effects, including year, cow age, and generation (cycle). Residuals for the 5 assessments of calf temperament at weaning were obtained from Riley et al. (2014). Residuals or actual measurements were evaluated directly (cow temperament score and other traits at each observed time) or as averaged values for each cow.

4. RESULTS

4.1 Analyses of Single Record Traits

Each cow had a single record of her highest temperament score across all years and of the proportions of all her temperament scores greater than 1 for each generation (Table 3). Inspection of data distribution by age of cow indicated that the 5 categories of cow age that correspond to Beef Improvement Federation guidelines (https://guidelines.beefimprovement.org/index.php/Guidelines_for_Uniform_Beef_Improvement_Programs; accessed February 13, 2023) were an efficient parameterization of this effect. Those categories were 2, 3, 4, 5 to 10, and over 10 years of age. Because cows in later cycles did not have the opportunity to have records in the older age categories, the effect of cow age was nested in levels of generation ($P < 0.001$) in analyses of highest temperament score. It was not included in analyses of the proportion of temperament scores greater than 1 because those values were calculated across all the records a cow had across years and ages. Means for highest temperament score by combinations of generation and cow age category are presented in Table 4. Cows in the age 2 category across generations had lower ($P < 0.00024$ [Bonferroni correction applied]) values of this trait than most other age categories (within generation), indicating more docile temperament. Within the 2- and 3-yr-old age categories, cows in the first three generations had higher means than those in generations 4 and 5 (Table 4). Means for generation (proportion of temperament scores greater than 1) are presented in Table 5. Cows in generations 1 and 2 had higher means for this trait than cows in the other generations, and cows in generation 5 also had a lower ($P < 0.05$) proportion than those in generations 3 and 4. Additive genetic effects were the only random effects included. Estimates of heritability for highest

temperament score and for the proportion of temperament scores greater than 1 were 0.23 ± 0.07 and 0.12 ± 0.067, respectively.

Correlation coefficients of these cow traits with aggressiveness, nervousness, flightiness, gregariousness, and overall total scores at 8 mo of age using unadjusted records (Table 6) and residuals (Table 7) were positive and of similar magnitude (0.2 to 0.3 for correlation coefficients of unadjusted values and slightly lower for correlation coefficients of unadjusted value). Cow productivity traits had moderate positive correlation coefficients of unadjusted values with the cow temperament traits (Table 8). Weaning rate had a correlation coefficient estimate of 0.309 with highest temperament score (Table 8). However, estimates of correlation coefficients for residuals of these traits were much lower in magnitude (Table 9).

Table 3. Summary statistics of the highest temperament score[1] and the proportion[2] of temperament scores greater than 1of each cow by generation

Generation	N	Highest temperament score			Proportion of temperament scores > 1		
		Mean	SD	Maximum	Mean	SD	Range
1	286	3.66	1.34	5	0.78	0.32	0 to 1
2	228	3.11	1.22	5	0.71	0.30	0 to 1
3	273	2.77	1.44	5	0.64	0.41	0 to 1
4	103	2.93	1.19	5	0.63	0.33	0 to 1
5	56	1.64	0.80	4	0.28	0.34	0 to 1
Total	946	3.07	1.40	5	0.68	0.36	0 to 1

[1]Highest temperament scores of each cow at the age of earliest occurrence (some cows had more than one score that was the highest value).

[2]Proportion of records greater than 1; the proportion of all scores greater than 1 (1 represents calm, good temperament) across her lifetime.

Table 4. Adjusted means for highest temperament score[1] by cow age category and generation

Age, yr	Generation				
	1	2	3	4	5
2	$2.82 \pm 0.22^{a\,y}$	$1.86 \pm 0.18^{a\,y}$	$1.78 \pm 0.15^{a\,y}$	$1.14 \pm 0.33^{a\,x}$	$0.83 \pm 0.25^{a\,x}$
3	$3.14 \pm 0.20^{b\,y}$	$3.19 \pm 0.22^{b\,y}$	$2.43 \pm 0.18^{b\,y}$	$1.92 \pm 0.27^{ab\,x}$	$1.31 \pm 0.32^{ab\,x}$
4	3.37 ± 0.22^{b}	3.10 ± 0.16^{b}	3.02 ± 0.19^{c}	2.62 ± 0.27^{bc}	2.33 ± 0.36^{b}
5 to 10	3.72 ± 0.17^{b}	3.74 ± 0.14^{b}	3.48 ± 0.23^{d}	3.20 ± 0.21^{cd}	
> 10	3.67 ± 0.26^{b}				

[a, b, c, d]Means in the same column that do not share a common superscript differ ($P < 0.05$) after correction for multiple comparisons.

[x, y]When superscripts are present, means in a row that do not share a common superscript differ ($P < 0.05$) after correction for multiple comparisons.

[1]Highest temperament scores of each cow at the age of earliest occurrence (some cows had more than one score that was the highest value).

[2]https://guidelines.beefimprovement.org/index.php/Guidelines_for_Uniform_Beef_Improvement _Programs; accessed February 13, 2023.

Table 5. Adjusted means for proportion of temperament scores greater than 1 by generation [1]

Generation	N	Proportion > 1
1	286	0.767 ± 0.0339^a
2	228	0.712 ± 0.0333^a
3	273	0.605 ± 0.0395^b
4	103	0.581 ± 0.0575^b
5	56	0.245 ± 0.054^c

[a,b,c] Means that do not share superscripts differ ($P < 0.05$).

[1]Proportion of records greater than 1; the proportion of all scores greater than 1 (1 represents calm, good temperament) across her lifetime.

Table 6. Estimates of correlation coefficients ($P < 0.05$) of unadjusted temperament scores at the time of calving and unadjusted temperament traits when cows were approximately 8 mo of age[1]

	Agg	Ner	Fli	Gre	Ovt
Highest temperament score[2]	0.262	0.235	0.247	0.221	0.259
Proportions of temperament scores > 1[3]	0.281	0.289	0.291	0.265	0.291
Temperament score average[4]	0.288	0.265	0.278	0.252	0.287

[1]Agg = aggressiveness; Ner = nervousness; Fli = flightiness; Gre = gregariousness; Ovt = overall temperament (Riley et al., 2014).

[2]Highest temperament score at parturition of each cow at the age of earliest occurrence (some cows had more than one score that was the highest value).

[3]Proportion of records greater than 1; the proportion of all scores greater than 1 (1 represents calm, good temperament) across her lifetime.

[4]Cow temperament scores at parturition averaged

Table 7. Estimates of correlation coefficients ($P < 0.05$) of residuals of cow temperament score at parturition and residuals of temperament traits when cows were approximately 8 mo of age[1]

	Agg	Ner	Fli	Gre	Ovt
Highest temperament score[2]	0.249	0.223	0.219	0.228	0.234
Proportions of temperament scores > 1[3]	0.238	0.246	0.237	0.224	0.239
Repeated records analyses[4]					
PE and MPE	0.184	0.185	0.186	0.181	0.190
PE	0.184	0.184	0.187	0.180	0.190

[1]Agg = aggressiveness; Ner = nervousness; Fli = flightiness; Gre = gregariousness; Ovt = overall temperament (Riley et al., 2014).

[2]Highest temperament score at parturition at the age of earliest occurrence (some cows had more than one score that was the highest value).

[3]Proportion of a cow's scores greater than 1 (at parturition) across her lifetime

[4]Averaged temperament score residuals were obtained from two separate repeated records analyses. Both included the additive genetic component. The first also included permanent environmental (PE) and maternal permanent environmental (MPE) components. The second included only the PE component.

Table 8. Estimates of correlation coefficients ($P < 0.05$) of unadjusted temperament scores and unadjusted cow productivity traits[1]

	Calving rate	Weaning rate	WWCE	WW1K
Highest temperament score[2]	0.232	0.309	0.365	0.235
[2]Proportion of temperament scores > 1[3]	0.094	0.223	0.245	0.155
Temperament score average	0.173	0.229	0.249	0.166

[1]WWCE = weaning weight per cow exposed; WW1K = weaning weight per 454 kg (1,000 lb) cow weight.

[2]Highest temperament scores of each cow at the age of earliest occurrence (some cows had more than one score that was the highest value).

[3]Proportion of records greater than 1; the proportion of all scores greater than 1 (1 represents calm, good temperament) across her lifetime.

Table 9. Estimates of correlation coefficients of residuals of temperament scores[1] and cow productivity traits

	Calving rate[5]	Weaning rate	WWCE	WW1K
Highest temperament score[2]	0.061	0.093	0.095	0.073
Proportions of temperament scores > 1[3]	0.035	0.130	0.105	0.109
Repeated records analyses[4]				
PE and MPE	−0.005	0.067	0.091	0.087
PE	−0.003	0.068	0.087	0.085

[1]WWCE = weaning weight per cow exposed; WW1K = weaning weight per 454 kg (1000 lb) cow weight

[2]Highest temperament scores of each cow at the age of earliest occurrence (some cows had more than one score that was the highest value).

[3]Proportion of records greater than 1; the proportion of all scores greater than 1 (1 represents calm, good temperament) across her lifetime.

[4]Averaged temperament score residuals were obtained from two separate repeated records analyses. Both included the additive genetic component. The first also included permanent environmental (PE) and maternal permanent environmental (MPE) components. The second included only the PE component.

[5]Correlation coefficients for calving rate with cow temperament traits did not differ from 0 ($P > 0.05$).

4.2 Repeated Records Analyses

Each cow had a record of her temperament score each time she calved. The fixed effect of cycle (generation) was included in all models ($P < 0.0001$). Inspection of data distribution by age of cows resulted in creation of 5 categories of cow age that correspond to Beef Improvement Federation guidelines (https://guidelines.beefimprovement.org/index.php/Guidelines_for_Uniform_Beef_Improvement_Programs; accessed February 13, 2023): 2, 3, 4, 5 to 10, and over 10 years of age to reduce the number of categories compared. Not all cows in all cycles had records in each age category. Again, an efficient parameterization was cow age category nested in levels of the fixed effect generation. This effect was included ($P < 0.001$) in the repeated records analysis and means are presented in Table 10. Within generations, 2-yr-old cows had lower ($P < 0.00024$) temperament scores than those in other age categories. First generation 2-yr-old cows had higher mean than cows in generation 4. The random effects considered were additive genetic, maternal additive genetic, the covariance of the additive genetic and maternal additive genetic effects, permanent environmental, and maternal permanent environmental. Likelihood ratio tests supported the additive genetic component ($P < 0.001$), the permanent environmental ($P < 0.001$), and the maternal permanent environmental component ($P = 0.047$). Because the maternal permanent environmental component P value was close to 0.05, and because the SE of the maternal permanent environmental variance as a proportion of the phenotypic variance suggested that the component was not different from 0, data were analyzed using models with and without this random effect. Residuals were produced from each of these models and a fixed effect model (only used in GWAS) for GWAS. Estimates of heritability from models that did not and did include the maternal permanent environmental term were 0.11 ± 0.028 and 0.09 ± 0.029, respectively. The estimate of permanent environmental variance as a proportion of the phenotypic variance was 0.15

± 0.024 in analyses that excluded or included the maternal permanent environmental effect. The

estimate of maternal permanent environmental variance as a proportion of the phenotypic variance

was 0.02 ± 0.013. The estimate of repeatability from each model was 0.26 ± 0.014. Analyses to

produce residuals from other traits for correlation coefficient estimates were done with models

similar to those above. Calf traits included a maternal permanent environmental effect only

because there were not enough records to separately estimate the maternal additive genetic

component. It is likely that the maternal permanent environmental component includes most or all

of that variation.

     The estimates of correlation coefficients of unadjusted and residual temperament records

are shown in Table 11. All estimates of these correlation coefficients were low in magnitude. Calf

and cow body condition score had correlation coefficients with cow temperament that did not

differ from 0 (P = 0.88 and 0.13, respectively).

Table 10. Adjusted temperament score[1] means by Beef Improvement Federation[2] age categories and generation

	Generation				
Age, yr	1	2	3	4	5
2	$1.96 \pm 0.17^{a\,y}$	$1.38 \pm 0.12^{a\,xy}$	$1.61 \pm 0.13^{a\,xy}$	$1.07 \pm 0.18^{a\,x}$	$1.35 \pm 0.23^{a\,xy}$
3	2.34 ± 0.16^{b}	2.02 ± 0.14^{b}	1.78 ± 0.14^{a}	1.76 ± 0.18^{b}	1.89 ± 0.23^{ab}
4	2.44 ± 0.15^{b}	2.58 ± 0.12^{c}	2.26 ± 0.15^{b}	2.08 ± 0.18^{b}	2.65 ± 0.27^{b}
5 to 10	2.58 ± 0.13^{b}	2.96 ± 0.11^{c}	2.54 ± 0.15^{b}	2.82 ± 0.17^{c}	
>10	2.82 ± 0.15^{b}	2.95 ± 0.26^{bc}			

[1]Temperament Score; a score from 1 to 5 to describe how calm or easily excitable the cow was at the time of parturition, where 1 = calm and 5 = easily excitable.

[2]https://guidelines.beefimprovement.org/index.php/Guidelines_for_Uniform_Beef_Improvement_Programs; accessed February 13, 2023.

Table 11. Estimates of correlation coefficients of unadjusted and residual cow temperament at the time of parturition records[1] with unadjusted records and residuals of calf or cow traits

	Unadjusted records	Residuals[2]	
	Temperament[1]	PE and MPE	PE
Calf birthweight	0.143	0.0258	0.0260
Calf Weaning weight	0.101	0.0410	0.0412
Calf BCS[3, 4]	−0.003	−0.0030	−0.0027
Cow weight	0.175	0.0444	0.0443
Cow BCS[3, 4]	0.026	0.0072	0.0075

[1]Temperament Score; a score from 1 to 5 to describe how calm or easily excitable the cow was at the time of parturition, where 1 = calm and 5 = easily excitable.

[2]Temperament score residuals were obtained from two separate repeated records analyses. Both included the additive genetic component. The first also included permanent environmental (PE) and maternal permanent environmental (MPE) components. The second included only the PE component.

[3]BCS = Body condition score; subjective scores from 1 to 9 in which higher values indicate more visual fat cover.

[4]Correlation coefficients for BCS with cow temperament score did not differ from 0 ($P > 0.05$).

4.3 Genome Wide Association

There was a single marker on Chromosome 4 statistically significant at the genome wide level for highest temperament score and temperament score residuals from fixed effect models only (Figures 1 and 2). A single SNP on BTA 3 met the significance threshold for analyses of residuals from a fixed effect model (Figure 2). There were no detected SNP with association to proportion of scores > 1 or residuals from models that included the permanent environmental variance or both the permanent environmental variance and the maternal permanent environmental variances. Those models must be preferred over the fixed effect model (Figure 3) results and indicate that their omission in generation of residuals results in inappropriate detections. Additional markers were associated with highest temperament scores, proportions of the temperament scores greater than 1, and fixed effects model when a less stringent false discovery rate (Table 12) was applied. The closest candidate gene to each associated SNP are shown in Table 13.
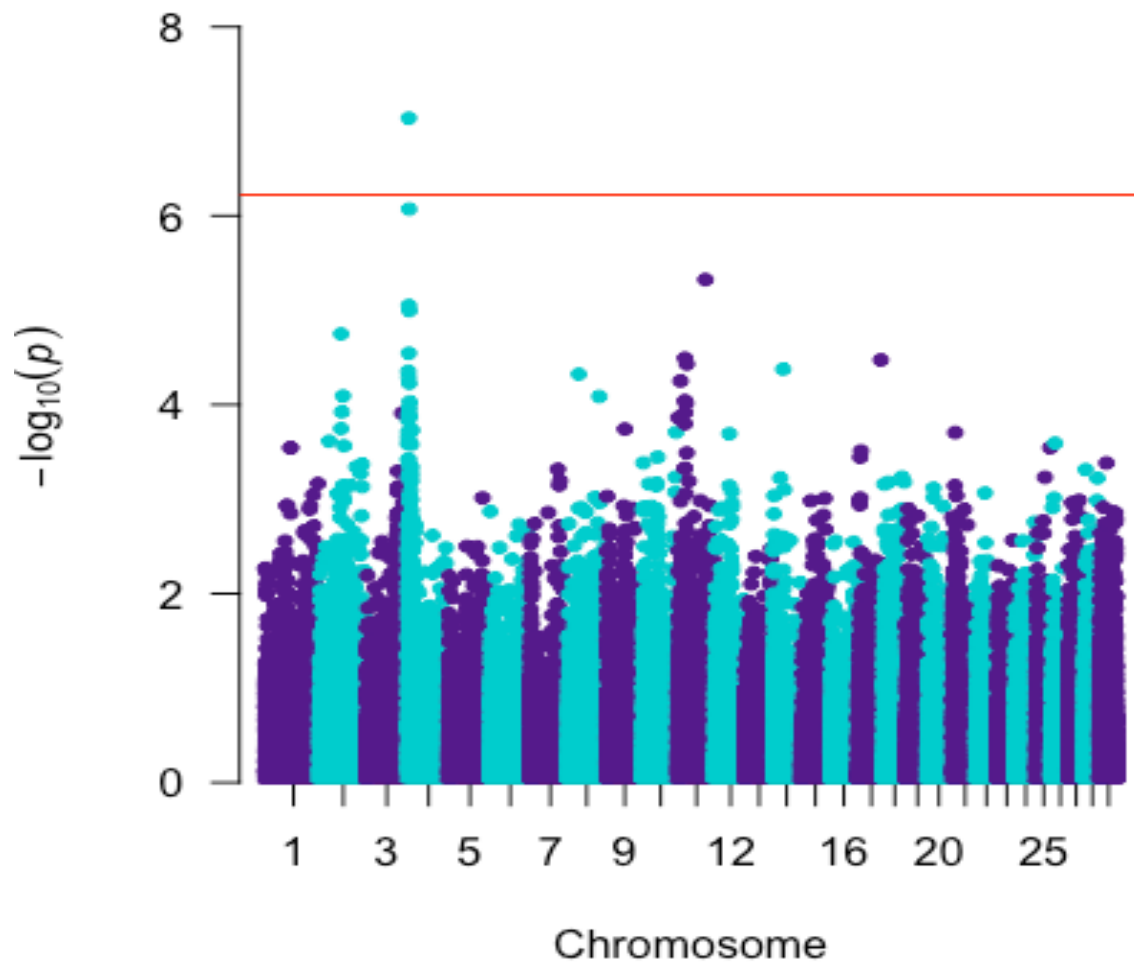
Figure 1. Genome wide association results of highest temperament score. The horizontal line represents Bonferroni corrected significance threshold.
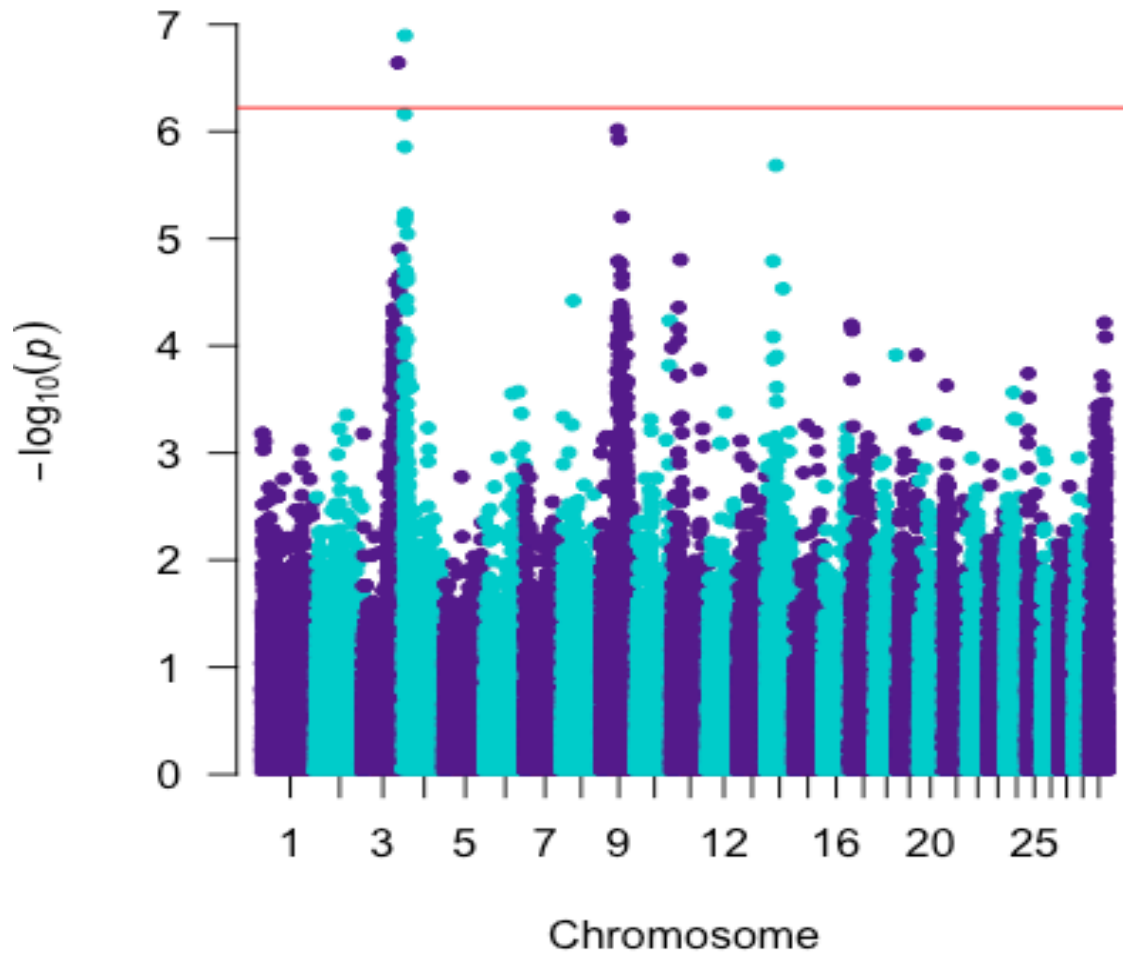
Figure 2. Genome wide association results of residuals from analyses of cow temperament from repeated records models without either the permanent environmental or maternal permanent environmental components included (fixed effects model). The horizontal line represents Bonferroni corrected significance threshold.
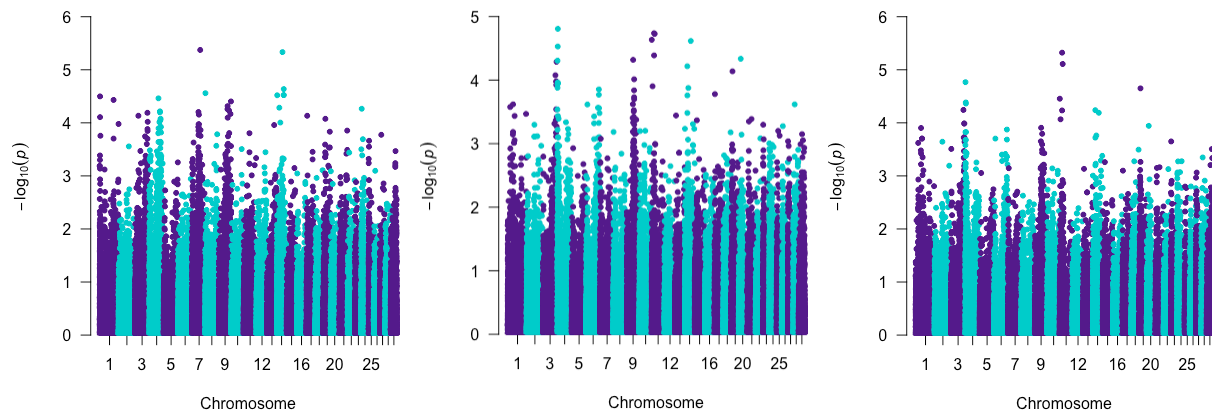
Figure 3. Genome wide association results of residuals of proportions of the temperament scores greater than 1 (single record per cow; left), residuals from analyses of cow temperament from repeated records models including permanent environmental variances (middle), and permanent environmental variances and maternal permanent environmental variances (right).

Table 12. Markers associated with cow temperament traits

BTA	SNP	bp	MAF[1]	$\hat{\beta}$	SE	P	FDR
Highest temperament score							
4	BovineHD0400000882	3,636,604	0.43	0.30	0.06	0.0000001	≤ 0.05
	BovineHD0400001166	4.473,358	0.387	0.29	0.06	0.0000009	≤ 0.05
	BovineHD0400000863	3,569,103	0.22	0.32	0.07	0.0000089	≤ 0.2
	BovineHD0400001472	5,243,454	0.424	−0.25	0.06	0.0000101	≤ 0.2
11	Hapmap51367-BTA-99264	7,9691,780	0.039	−0.69	0.15	0.0000047	< 0.15
Proportions of the temperament scores greater than 1							
14	ARS-BFGL-NGS-30322	47,131,561	0.159	−0.52	0.11	0.0000046	≤ 0.2
Residuals from fixed effect model							
3	ARS-BFGL-NGS-109983	104,642,202	0.253	−0.29	0.05	0.0000002	≤ 0.05
4	BovineHD0400001166	4,473,358	0.387	0.25	0.05	0.0000001	≤ 0.05
	BovineHD0400000863	3,569,103	0.22	0.29	0.06	0.0000007	≤ 0.05
	BovineHD0400000882	3,636,604	0.43	0.22	0.04	0.0000014	≤ 0.05
9	BTB-00391548	48,441,405	0.434	−0.22	0.05	0.0000010	≤ 0.05
	BovineHD0900031483	52,557,567	0.423	0.22	0.04	0.0000012	≤ 0.05
14	BovineHD1400008954	29,325,534	0.071	−0.43	0.09	0.0000021	≤ 0.05

[1]MAF = Minor allele frequency

Table 13. Candidate genes for SNP associated with cow temperament traits at the time of parturition

SNP	Location		Candidate gene	Distance kb[1]
	BTA	Mb		
ARS-BFGL-NGS-109983	3	104.6	HIVEP zinc finger 3 (*HIVEP3*)	inside
ARS-BFGL-NGS-109983	3	104.6	endothelin 2 (*EDN2*)	37.1
BovineHD0400000863				
BovineHD0400000882	4	3.6	*LOC112446320*[2]	179.2
BovineHD0400001166	4	4.5	cordon-bleu WH2 repeat (*COBL*)	109.2
BovineHD0400001472	4	5.2	growth factor receptor bound 10 (*GRB10*)	inside
BTB-00391548	9	48.4	glutamate receptor, ionotropic, kainate 2 (*GRIK2*)	inside
BovineHD0900031483	9	52.6	MMS22 like, DNA repair (*MMS22L*)	inside
Hapmap51367-BTA-99264	11	79.7	*LOC112448881*[2]	273.9
BovineHD1400008954	14	29.3	basic helix-loop-helix family member e22 (*BHLHE22*)	48.2
ARS-BFGL-NGS-30322	14	47.1	solute carrier family 30 member 8 (*SLC30A8*)	inside

[1]Distance to closest boundary of the candidate gene. Inside indicates that the SNP was within the gene boundaries.

[2]Pseudogene

[3]Long non-coding RNA (lncRNA)

5. DISCUSSION

5.1 Analyses of Single Record Traits

According to the results, cows that had their highest temperament score at the age of 2 had lower means across generations compared to other ages, this may be related to cows being first time dams with strong maternal instinct not fully developed. Means of the proportions of temperament scores greater than 1 were lower in more recent generations. Although all animals were ½ Angus and ½ Nellore based on pedigree averages, the actual content may be different, and it may result in favorable temperament. Cows in later generations could have higher Angus influence resulting in docile temperament. Residuals and unadjusted temperament traits of these cows at 8 mo of age (aggressiveness, nervousness, flightiness, gregariousness, and overall total) had positive (unfavorable) estimates of correlations with highest temperament scores, proportions of temperament scores greater than 1, and temperament scores averages. Although inbreeding was avoided, it accumulated in this closed populations; estimates of inbreeding from the genomic relationship matrix ranged from 0.02 for first generation crosses (including males) to 0.03 in the F_5 animals (unpublished results). It is difficult to attribute improved to temperament to inbreeding.

Residual correlation estimates were closer to 0 than the unadjusted estimates of correlation; this may suggest that temperament is mostly related to the fixed effects or random additive genetic component, as those effects were removed to produce the residuals. Opposite to what other studies have reported, there were positive correlations between productivity traits and temperament scores, that is, unfavorable relationships of cow temperament with various attributes of productivity. Essentially all reported research asserts that good animal temperament is associated with higher levels of productivity.

Docile animals are inclined to improve welfare and ease of handling without having detrimental impacts on productivity (Alvarenga et al., 2022; Cziszter et al., 2016). According to Cooke et al. (2017), *B. indicus* beef cows with high temperament at the beginning of breeding season may experience reduced pregnancy rates to first timed artificial insemination, increased pregnancy loss, that could reduce calving and weaning rates and wean earlier offspring with a lower body weight compared to cows with adequate cohorts. The results presented show a moderate to high correlation ($P < 0.05$) and suggest that docile cows are not associated with good performance. Highest temperament scores estimate of heritability (0.23 ± 0.07) was higher than the estimate for the proportions of temperament scores greater than 1 (0.12 ± 0.067).

5.2 Repeated Records Analyses

Repeated records results were consistent with the single records results. There were positive estimates of correlation coefficients of cow temperament score with calf birth weight, calf weaning weight, cow weight, and cow body condition score indicating no strong relationships of temperament with these traits.

5.3 Estimates of Heritability

Estimates of heritability for analyses for single record traits (highest temperament scores and of the proportions of temperament scores greater than 1) were 0.23 ± 0.07 and 0.12 ± 0.067, respectively and estimates of heritability for repeated record analyses (models that did not and did include the maternal permanent environmental term) were 0.11 ± 0.028 and 0.09 ± 0.029, respectively. The estimates of heritability for cow temperament in repeated records analyses were similar for those models yet, modeling the maternal permanent effect variances resulted in a lower

estimate of heritability. This might suggest that some of the maternal permanent environmental variance was improperly included in the heritability estimate. There were no big differences in results due to inclusion of the maternal environmental effect. The estimates of permanent environmental variance and maternal permanent environmental variance indicated that nongenetic influences on cow temperament were relatively more important than the additive genetic component. Especially the maternal permanent environmental influence provides first evidence that there is a learned component to temperament that may persist across a cow's lifetime. Since previous genetic work in bovine temperament had a wide range of heritability estimates (0.07 to 0.70) (Appendix Table 1), it is reasonable to suggest that the results agree with previous heritability estimates.

5.4 Genome Wide Association

Highest temperament score was associated with SNP markers on BTA 4 (FDR ≤ 0.2; Table 14). Kolbehdari et al. (2008) reported an association of temperament on Holstein cows with a region on BTA 4 and suggested neuronal cell adhesion molecule (*NRCAM*) as a candidate gene. Cow temperament residuals from a fixed effect model were associated ($P \leq 0.05$) with a marker on BTA 3. Markers on BTA 3 were reported as associated with Holstein cattle temperament (Chen et al., 2012). A marker on BTA 11 79.7 Mb was associated ($P \leq 0.05$) with highest temperament score. Chen et al. (2021) also identified markers on BTA 11 as associated with Holstein temperament. Candidate genes from other cattle temperament studies were also on BTA 11. Brahman exit velocity was associated with SNP in the VRK serine/threonine kinase 2 (*VRK2*), FA complementation group L (*FANCL*) and transfer RNA cysteine (anticodon ACA) (*TRNAC-ACA*) genes (Paredes-Sánchez et al., 2020). Nellore flight speed (Valente et al., 2016) and Charolais

temperament were associated with gene 5-hydroxytryptamine receptor 2A (*HTR2A*) located on BTA 11 (Garza-Brenner et al., 2017). A marker on BTA 14 was associated with the proportion of temperament scores greater than 1 (*FDR* ≤ 0.2). Regions on BTA 14 have previously been associated with Guzerat temperament with a proposed candidate gene KIAA1429 ortholog (*KIAA1429*) (Dos Santos et al., 2017). Regions on BTA 14 were also reported as associated with Angus temperament; candidate genes included thymocyte selection associated high mobility group box (*TOX*), Zinc finger and AT-hook domain containing (*ZFAT*), Family with sequence similarity 135 member B (*FAM135b*), and Potassium two pore domain channel subfamily K member 9 (*KCNK9*) (Alvarenga et al., 2023). The review of Alvarenga et al. (2021) also identified BTA 14 with associated markers and candidate genes.

It should be noted that the fixed effects model (no permanent environmental variances or maternal permanent environmental variances) undoubtedly is the incorrect model to generate residuals for use in GWAS; it is coincidental that some detections were in similar regions on the same BTA as other analyses. There was a single detection on BTA 9 with residuals from the fixed effects model. Regions on this chromosome were reported as associated with Holstein temperament (Chen et al., 2021). Those researchers identified these candidate genes: SFT2 domain containing 1 (*SFT2D1*), TBC1 domain family member 32 (*TBC1D32*), and TBC1 domain family member 32 (*TBC1D32*). This chromosome was the location of regions associated with Nellore flight speed (Valente et al., 2016) and Charolais aggressiveness (Vallée et al., 2016). A marker on BTA 14 was associated with cow temperament using residuals from a fixed effect model. Although these associations were identified, they are only reported here because some are in reasonably similar parts of the genome. The fixed effect model results are not correct.

There were no apparent differences in GWAS results based on inclusion of maternal permanent environmental variance; no detections were made with residuals for cow temperament from either model.

5.5 Candidate Genes

The candidate genes detected in each of the chromosomes associated with this study (Table 15) have not been identified in other studies for temperament for cattle. HIVEP zinc finger 3 (*HIVEP3*) encodes large zinc finger proteins and regulates transcription via the kappaB enhancer motif. It may produce protein isoforms that contain or exclude the carboxyl DNA binding domain and the leucine zipper by alternative RNA splicing and differential polyadenylation and also has a novel gene regulatory mechanism as well as the generation of structural and functional diversity. (Hicar et al., 2001). There may be a novel role of ZAS3 (*HIVEP3*) as a positive regulator of osteoclast differentiation (Liu et al., 2011). Endothelin-2 (*EDN2*) is a macrophage chemoattractant, a hypoxia-induced autocrine survival factor for breast tumor cells and is considered to be protective in cardiovascular disease (Grimshaw et al., 2002a, b; Nagai et al., 2007). The protein produced by the gene Cordon-Bleu (*COBL*) participates in neural tube closure (Carroll et al., 2003; Gasca et al., 1995). Expression of *COBL* in neuronal cells promotes branching of neurites while its loss leads to decreased arborization (Ahuja et al., 2007; Kessels et al., 2011). Growth factor receptor binding protein 10 (*GRB10*) is a cellular partner of several receptor tyrosine kinases and other signaling mediators compatible with a role in a variety of signaling mechanisms, it is also a key negative regulator of β-cell proliferation, function, and survival. β-Cell failure, which is largely due to impairment in cell proliferation and increased cell death, is a key component in the pathogenesis of both type 1 and type 2 diabetes. (Riedel, 2004; Zhang et al., 2012). Glutamate

receptor, ionotropic, kainate 2 (*GRIK2*) mediates the excitatory neurotransmission in the central nervous system. This gene has been associated with schizophrenia (Begni et al., 2002; Ozawa et al., 1998). The gene *MMS22* like, DNA repair (*MMS22L*) is highly expressed in the majority of clinical lung and esophageal cancers. It is involved in an important signaling pathway in cancer cells (Nguyen et al., 2012). Basic helix-loop-helix family member e22 (*BHLHE22*) acts as a transcriptional repressor and has a role in cell differentiation in neuron development and shows compelling genetic evidence of directly impacting the severe depression phenotype (Darmawi et al. 2022; Hupalo et al. 2022). Solute carrier family 30 member 8 (*SLC30A8*) is a transporter localized in insulin secretory granules, and plays a major role in movement of zinc from the cytoplasm to intracellular insulin containing vesicles for insulin maturation, storage and secretion of insulin (Chimienti et al. 2004; 2005) it also regulates hepatic insulin clearance and that genetic dysregulation of this system may play a role in the pathogenesis of type 2 diabetes (Tamaki et al. 2013). The genes *COBL, GRIK2*, and *BHLHE22* are of particular interest because they are related to behavioral diseases and brain development which could explain some reactions towards handling from our cattle.

6. SUMMARY AND CONCLUSIONS

Breeders should understand how temperament influences production to be able to select without having a negative effect on their herd. Estimates of heritability for highest temperament scores and of the proportions of temperament scores greater than 1 were 0.23 ± 0.07 and 0.12 ± 0.067, respectively. Within generations, 2-yr-old cows had lower temperament score means than cows in most other age categories. Positive estimates of correlation coefficients of unadjusted temperament with weaning weight per cow exposed ($r > 0.249$), weaning weight per 454 kg (1,000 lb) cow weight ($r > 0.166$), birth weight ($r > 0.143$), weaning weight ($r > 0.101$), cow weight ($r > 0.175$) and cow body condition score ($r > 0.026$) were found. Cows with higher temperament scores also showed higher fertility measures (calving rate ($r > 0.173$) and weaning rate ($r > 0.229$)). Although not large in magnitude, they provide evidence that counters other descriptions of relationships of favorable temperament with increased productivity. There were insufficient numbers of cows to estimate genetic correlations between temperament traits and productivity traits. Genome wide association test results associated SNP on BTA 4 and 11 for highest temperament scores; and BTA 14 for proportions of temperament scores greater than 1.

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Table 1. Estimates of heritability for temperament traits of cattle

Breed	Age	Heritability	Trait	Reference
Bos taurus	2 to 4 yr	0.08 to 0.35	Many	Silva et al. (2002)
Angus	< 2 yr	0.38 ± 0.01	Temperament	Alvarenga et al. (2022)
Angus	> 2 yr over the years	0.38 ± 0.02 to 0.53 ± 0.03	Temperament	Alvarenga et al. (2023)
Bos indicus	Weaning	0.54 ± 0.16	Flight speed	Burrow et al. (1988)
	18 mo	0.26 ± 0.13		
Zebu	12 mo	0.08 to 0.35	Flight speed	Burrow and Corbet (2000)
Zebu	Weaning	0.39	Flight speed	
	12 mo	0.33		
	18 mo	0.29		
Zebu	Weaning, 12 and 18 mo	0.44	Flight speed	Burrow (2001)

Brahman, Belmont Red, Santa Gertrudis	Post-weaning	0.31 ± 0.04	Flight speed	Johnston et al. (2003)
European and Zebu		0.20 ± 0.03	Flight speed	Prayaga and Henshall (2005)
Brahman, Santa Gertrudis, Belmont Red	19 mo	0.30 ± 0.02	Flight speed	Kadel et al. (2006)
	8 mo	0.34 ± 0.03	Flight speed	
Angus, Charolais and beef hybrid	8 mo	0.49 ± 0.18	Flight speed	Nkrumah et al. (2007)
Bos taurus: Hereford, Angus and others	Finishing phase	0.34 ± 0.11	Flight speed	Rolfe et al. (2011)
Angus Charolais	5 to 11 mo	0.20 ± 0.08 0.25 ± 0.10	Flight speed	Hoppe et al. (2010)

Breed	Age	Value	Measure	Reference
Hereford		0.36 ± 0.06		
Limousin		0.11 ± 0.07		
German Simmental		0.28 ± 0.07		
Limousin	10 mo	0.22	Docility score	Le Neindre et al. (1995)
Limousin	10 to 14 mo	0.18 ± 0.01	Docility test	Phocas et al. (2006)
Bos indicus	Weaning	0.40 ± 0.15	Flight distance	Fordyce et al. (1996)
	12 mo	0.32 ± 0.14		
	18 mo	0.70 ± 0.23		
Limousin	8 mo	0.17 ± 0.08	Flight distance	Benhajali et al. (2010)
Holstein		0.12 ± 0.02	Milking temperament [1]	Lawstuen et al. (1988)
Holstein Friesian		0.22 ± 0.03	Milking temperament	Visscher and Goddard (1995)
Jersey		0.25 ± 0.06		
Holstein Friesian		0.15	Milking temperament	Schrooten et al. (2000)
Holstein Friesian		0.07 ± 0.001	Milking temperament	Pryce et al. (2000)
Holstein		0.07	Milking temperament	Hiendleder et al. (2003)
Holstein		0.13 ± 0.01	Milking temperament	Sewalem et al. (2011)

Nellore		0.21	Flight speed	Valente et al. (2016)
Angus	Yearling	0.38 ± 0.001	Temperament	Alvarenga et al. (2022)
Brahman	Weaning	0.27 ± 0.1	Exit velocity	Schmidt et al. (2014)
		0.49 ± 0.1	Pen score	
		0.43 ± 0.1	Temperament	
Bos taurus		0.34 ± 0.1	Flight speed	Chase et al. (2017)

[1]Milking temperament can be defined in terms of milking behavior, ease of handling, or aggressiveness at feeding (Sewalem et al., 2011)

Table 2. Associated genomic regions with temperament traits in cattle

Breed	Trait[1] [2]	BTA	Mb[3]	Candidate gene[4]
Brahman	TMP	6	36.65	GPRIN family member 3 (GPRIN3)
Angus			36.67	alpha-synuclein (*SNCA*)
				multimerin 1 (*MMRN1*)
Paredes-Sanchez et al. (2023)				
Bos taurus		2	112.6	cullin 3 (*CUL3*)
		21	3.8	gamma-aminobutyric acid type A receptor subunit beta3 (*GABRB3*)
Costilla et al. (2020)				
Brahman	EV	11	41.0	VRK serine/threonine kinase 2 (*VRK2*)
			40.9	FA complementation group L (*FANCL*)
				transfer RNA cysteine (anticodon ACA) (*TRNAC-ACA*)
		13	82.2	docking protein 5 (*DOK5*)
		15	56.1	tsukushi, small leucine rich proteoglycan (*TSKU*)

			56.1	guanylate cyclase 2E (*GUCY2E*)
			56.0	leucine rich repeat containing 32 (*LRRC32*)
			55.8	EMSY transcriptional repressor, BRCA2 interacting (*EMSY*)
			56.3	alkaline ceramidase 3 (*ACER3*)
		21	15.2	solute carrier organic anion transporter family member 3A1 (*SLCO3A1*)

Paredes-Sanchez et al. (2020)

Holstein	TMP	1	60.5	
		2	41.4	
		3	13.0	
		6	32.8	
		8	89.5	
		9	29.9	TBC1 domain family member 32 (*TBC1D32*)
			101.3	SFT2 domain containing 1 (*SFT2D1*)
		10	14.6	mitogen-activated protein kinase kinase 5 (*MAP2K5*)
			20.2	REC114 meiotic recombination protein (*REC114)*
		11	59.6	

12	80.3	
14	52.0	
16	18.8	
17	58.4	
18	58.3	
21	30.3	ADAM metallopeptidase with thrombospondin type 1 motif 7 (*ADAMTS7*)
22	59.6	kelch repeat and BTB domain containing 12 (*KBTBD12*)
	59.5	eukaryotic elongation factor, selenocysteine-tRNA specific (*EEFSEC*)
23	2.3	
24	19.5	
27	35.4	
	35.4	zinc finger matrin-type 4 *(ZMAT4)*
	39.7	zinc finger protein 385D (*ZNF385D*)
	44.2	zinc finger protein 385D (*ZNF385D*)
29	24.0	

Chen et al. (2021)

Guzerat	TMP	1	35.4	POU class 1 homeobox 1 (*POU1F1*)
			40.7	
			59.8	zinc finger and BTB domain containing 20 (*ZBTB20*)
		5	60.2	
		14	69.8	KIAA1429 ortholog (*KIAA1429*)
		25	14.5	ATP binding cassette subfamily C member 1 (*ABCC1*)
			19.8	von Willebrand factor A domain containing 3A (*VWA3A*)
Dos Santos et al. (2017)				
Limousin	TMP	2	3.5	Myostatin (*MSTN*)
Esmailizadeh et al. (2008)				
Angus	TMP	29	4.9	
Glenske et al. (2011)				
Charolais	SSWR	6	4.0	
Holstein		8	51.9	
		10	52.5	
		16	7.6	

		19	25.1	
	FLFF	20	45.6	
		25	19.6	
		28	6.2	
		29	29.4	
Gutierrez-Gil et al. (2008)				

Holstein	TMP	4	49.3	neuronal cell adhesion molecule (NRCAM)
		13	59.5	cleavage stimulation factor subunit (*CSTF1*)
		2	31.4	calcium channel, voltage-dependent, L type, alpha 1D subunit (*CACNA1D*)
		23	15.7	bystin like (*BYSL*)
		26	37.6	solute carrier family 18 (vesicular monoamine transporter), member 2 (*SLC1*
		29	27.7	
			34.7	neurotrimin (*NTM*)
			43.3	coiled-coil domain containing 88B (*CCDC88B*)
			44.5	dipeptidyl peptidase 3 (*DPP3*)
Kolbehdari et al. (2008)				

German Holstein	TMP	29	8.3	
Hiendleder et al. (2003)				
Nellore	FLSPD	1	72.7	
		2	64.7	
		5	22.4	
		9	97.3	
		11	67.4	
		15	16.3	
		17	0.7	
		26	46.7	
		5	94.7	RAS like estrogen regulated growth inhibitor (RERG)
		7	2.0	ADAM metallopeptidase with thrombospondin type 1 motif 2 (*ADAMTS2*)
			96.0	Calpastatin (*CAST*)
		8	100.2	Muscle associated receptor tyrosine kinase (MUSK)
		10	51.4	ADAM metallopeptidase

domain 10 (*ADAM10*)

11	74.1	Proopiomelanocortin (POMC)
13	0.93	Chromosome segregation 1 like (*CSE1L*)
		Phospholipase C beta 1 (PLCB1)
	7.5	Mono-ADP ribosylhydrolase 2 (MACROD2)
14	0.7	Zinc finger and AT-hook domain containing (*ZFAT*)
	4.3	Family with sequence similarity 135 member B (*FAM135b*)
	3.6	Potassium two pore domain channel subfamily K member 9 (KCNK9)
15	50.3	Olfactory receptor family 51 subfamily E member 2 (OR51E2)
	49.8	Olfactory receptor family 52 subfamily J member 3 (EN SBTAG000000380
	46.9	Olfactory receptor family 56 subfamily A member 1 (OR56A1)
	81.3	Olfactory receptor family 9 subfamily
		Q member 2 (OR9Q2)
17	17.8	Mastermind like transcriptional coactivator 3 (MAML3)
		Nuclear receptor subfamily 3 group C member 2 (NR3C2)

18	9.5	Solute carrier family 6 member 2 (SLC6A2)
19	40.5	Cell division cycle 6 (*CDC6*)
	25.3	PITPNM family member 3 (PITPNM3)
	25.1	Solute carrier family 13 member 5 (SLC13A5)
	26.8	Solute carrier family 16 member11 (SLC16A11)
	24.9	Sphingolipid transporter 3 (putative) (SPNS3)
21	45.3	Family with sequence similarity 177 member A1 (FAM177A1)
23	30.8	Olfactory receptor family 2 subfamily B member 6 (OR2B6)
25	27.5	Solute carrier family 5 member 2 (SLC5A2)
26	37.5	Solute carrier family 18 member A2 (SLC18A2)
27	15.4	Solute carrier family 25 member 4 (SLC25A4)
2	6.5	Glutamate metabotropic receptor 5 (GRM5)

Valente et al. (2016)

5	94.7	RAS like estrogen regulated growth inhibitor (RERG)
7	2.0	ADAM metallopeptidase with thrombospondin type 1 motif 2 (*ADAMTS2*)
	96.0	Calpastatin (*CAST*)

8	100.2	Muscle associated receptor tyrosine kinase (MUSK)
10	51.4	ADAM metallopeptidase domain 10 (*ADAM10*)
11	74.1	Proopiomelanocortin (POMC)
13	0.93	Chromosome segregation 1 like (*CSE1L*)
		Phospholipase C beta 1 (PLCB1)
	7.5	Mono-ADP ribosylhydrolase 2 (MACROD2)
14	0.7	Zinc finger and AT-hook domain containing (*ZFAT*)
	3.6	Potassium two pore domain channel subfamily K member 9 (KCNK9)
	4.3	Family with sequence similarity 135 member B (*FAM135b*)
15	50.3	Olfactory receptor family 51 subfamily various members
17	17.8	Mastermind like transcriptional coactivator 3 (MAML3)
		Nuclear receptor subfamily 3 group C member 2 (NR3C2)
18	9.5	Solute carrier family 6 member 2 (SLC6A2)
19	40.5	Cell division cycle 6 (*CDC6*)
	25.3	PITPNM family member 3 (PITPNM3)
	25.1	Solute carrier family 13 member 5 (SLC13A5)

			26.8	Solute carrier family 16 member11 (SLC16A11)
			24.9	Sphingolipid transporter 3 (putative) (SPNS3)
		21	45.3	Family with sequence similarity 177 member A1 (FAM177A1)
		23	30.8	Olfactory receptor family 2 subfamily B member 6 (OR2B6)
		25	27.5	Solute carrier family 5 member 2 (SLC5A2)
		26	37.5	Solute carrier family 18 member A2 (SLC18A2)
		27	15.4	Solute carrier family 25 member 4 (SLC25A4)
		2	6.5	Glutamate metabotropic receptor 5 (GRM5)
Alvarenga et al. (2021)				
Angus	TMP	10	2.2	Neuronal regeneration related protein (*NREP*)
			45.6	Thyroid hormone receptor interactor 4 (*TRIP4*)
			45.7	Casein kinase 1 gamma 1 (*CSNK1G1*)
			88.7	Serine palmitoyltransferase long chain base subunit 2 (*SPTLC2*)
		18	24.7	Carboxylesterase 5A (*CES5A*)
Alvarenga et al. (2023)				

Charolais	TMP	1	58.8	proopiomelanocortin (*POMC*)
		11	74.1	5-hydroxytryptamine receptor 2A (*HTR2A*)
		12	16.8	dopamine receptor D2 (*DRD2*)
		15	24.0	solute carrier family 18 (vesicular monoamine transporter), member 2 (*SLC18A2*)
		26	37.6	dopamine receptor D3 (*DRD3*)
	FLSPD	1	58.8	
		12	16.8	

Garza-Brenner et al. (2017)

Charolais	AGGR	6	33.8	
		9	28.4	
		17	47.9	
		20	69.2	
		25	12.5	
		26	31.1	

33.8

28.4

Vallée et al. (2016)

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[1]TMP = temperament; SSWR = Social Separation - walking/ running; FLFF = flight from feeders; AGGR = aggressive behavior; FLSPD = flight speed; EV = exit velocity.

[2]Bovine (*Bos taurus*) chromosome

[3]In some cases more detections (without candidate genes) were reported on the same chromosome. Here the most acrocentric of those is reported.

[4]Not all published associations included candidate genes.