1 Extensive genome-wide association analyses identify 2 genotype-by-environment interactions of growth traits 3 in Simmental cattle

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20 **Abstract**

21 **Background:** Understanding the genetic basis of genotype-by-environment interactions
 22 (GxE) is crucial to understand environmental adaptation in mammals and improve the
 23 sustainability of agricultural production. In addition, GxE information could also be
 24 useful to predict the vulnerability of populations to climate change.

25 **Results:** Here, we present an extensive study investigating the interaction of genome-
 26 wide SNP markers with a vast assortment of environmental variables and searching for
 27 SNPs controlling phenotypic variance (vQTL) using a large beef cattle dataset. We
 28 showed that GxE contribute 10%, 4%, and 3% of the phenotypic variance of birth weight,
 29 weaning weight, and yearling weight, respectively. GxE genome-wide association
 30 analysis (GWAA) detected a large number of GxE loci affecting growth traits, which the
 31 traditional GWAA did not detect, showing that functional loci may have non-additive
 32 genetic effects between genotype classes regardless of differences in genotypic means.
 33 We also showed that variance-heterogeneity GWAA can detect loci enriched with GxE
 34 effects without requiring prior knowledge of the interacting environmental factors.
 35 Functional annotation and pathway analysis of GxE genes revealed biological
 36 mechanisms by which cattle respond to changes in their environment, such as neural
 37 signaling, metabolic, hypoxia-induced, and immune system pathways. Knowledge of
 38 these pathways will be important as climate change becomes a burden on animal health
 39 and productivity. In addition, ecoregion-specific GxE SNPs detected in this study may
 40 play a crucial role in identifying resilient and adapted beef cattle across divergent
 41 environments.

42 **Conclusions:** We detected novel trait associations with large GxE effects for birth
 43 weight, weaning weight, and yearling weight. Functional annotation and pathway
 44 analysis uncovered genomic regions involved in response to environmental stimuli. We
 45 unraveled the relevance and complexity of the genetic basis of GxE underlying growth
 46 traits, providing new insights into how different environmental conditions interact with
 47 specific genes influencing adaptation and productivity in beef cattle and potentially
 48 across mammals

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50 **Keywords:** adaptation, beef cattle, environment, ecoregion, variance heterogeneity

51

52 **Background**

53 Genotype-by-environment interactions (GxE) refer to a variable response of genotypes
 54 across environments, resulting in different trait values [1,2]. GxE is a central issue in
 55 genetics, evolution, and ecology [3], and cattle represent a unique opportunity to address
 56 these questions due to a large number of genotyped animals [4] reared over a wide range
 57 of climatic and topographic regions. GxE may contribute to poor adaptation, which
 58 negatively affects the profitability and sustainability of the beef cattle production systems
 59 [1,5]. Studies using the relationship between breeding values and the environment have
 60 reported the existence of GxE for body weight in beef cattle [6,7]; however, limited
 61 research has focused on understanding the genetic loci of GxE underlying such traits.
 62 This knowledge could be used to better drive selection and mating decisions [8] as well
 63 as in statistical models incorporating biological information to accomplish more accurate

64 genomic predictions [9]. In addition, GxE information could also be useful to predict the
65 vulnerability of populations to climate change [3] and to understand environmental
66 adaptation in other mammalian species.

67 Here, we present a comprehensive study of GxE for birth weight (BW), weaning
68 weight (WW), and yearling weight (YW) using SNP marker information and over 13,000
69 Simmental cattle. First, we estimated the contribution of GxE variance on the total
70 phenotypic variances using a restricted maximum likelihood (REML) approach. Then, we
71 searched for SNPs enriched for GxE using four linear mixed model approaches: i) direct
72 GxE genome-wide association analyses (GxE GWAA) using continuous environmental
73 variables; ii) direct GxE GWAA using discrete environmental variables (United States
74 ecoregions); iii) variance-heterogeneity genome-wide association analyses (vGWAA),
75 which enable identification of interactions, using phenotypes adjusted for additive
76 relationship matrix or additive, dominance and epistatic relationship matrices; and iv)
77 meta-analysis of ecoregion-specific GWAA. Lastly, we performed enrichment analyses
78 using GxE candidate genes to investigate the biological mechanisms by which
79 environmental factors modulate such phenotypes. To our knowledge, this is the most
80 comprehensive study investigating the interactions of genome-wide SNP markers with a
81 vast assortment of environmental variables and searching for SNPs controlling
82 phenotypic variance using a large beef cattle dataset. Our results revealed novel trait
83 associations and alternative biological mechanisms involved in shaping the total
84 phenotypic variance of growth traits providing new insights into how the environment
85 influences growth traits and adaptation in beef cattle and potentially across mammals.

86

87 **Results**

88 **Environmental variance component estimates**

89 Phenotypes were pre-adjusted for fixed effect of sex and random effect of contemporary
 90 groups. The amount of BW, WW, and YW variation explained by contemporary group
 91 effects was 19%, 53%, and 61%, respectively, showing that the effect of farm
 92 management on the animal body weight increases with age. After adjusting the
 93 phenotypes, we estimated the contribution of GxE variance on the total phenotypic
 94 variances using additive relationship matrix calculated based on SNP marker information,
 95 and United States ecoregions as environmental factor. Ecoregions were determined based
 96 on the combination of 30-year normal values of mean temperature, precipitation, and
 97 elevation [10]. Each 1 km square of the United States was then assigned to one of 9
 98 resulting ecoregions. We found that GxE variances contribute in 10.1%, 3.8%, and 2.8%
 99 to the BW, WW, and YW total phenotypic variances, respectively, suggesting that there
 100 is a decrease in the effect of GxE on the animal body weight throughout life. These
 101 results indicate an inverse relationship between farm management and environmental
 102 stress and that management practices (e.g. season of birth, forage quality and quantity,
 103 feed supplementation, health programs) are more relevant for later weights than climate
 104 environmental factors. One possible explanation for these findings is that birth weight
 105 reflects intrauterine growth which is highly affected by environmental conditions [11];
 106 however, as the animal ages physiological, morphological, endocrine, cellular and
 107 molecular mechanisms are altered in order to cope up with environmental challenges

108 [12]. These results also suggest that as management inputs are decreased, animals
109 become more sensitive to environmental stress, which has ramifications for animal
110 performance as we seek to decrease inputs to make beef production more sustainable.
111

112 **Genotype-by-environment GWAA**

113 The GxE GWAA for BW, WW, and YW were performed by fitting univariate and
114 multivariate linear mixed models considering continuous or discrete environmental
115 variables. The continuous environmental variables used were minimum, mean,
116 maximum, and mean dew point temperatures; elevation; precipitation; and minimum and
117 maximum vapor pressure deficit, separately (Additional file 1: Figures S1–S8). Discrete
118 environmental variables included United States ecoregions (Desert & Arid Prairie,
119 Southeast, High Plains, Forested Mountains, Fescue Belt, and Upper Midwest &
120 Northeast – Fig. 1F), which are combinations of mean temperature, precipitation, and
121 elevation 30-year normal values (Additional file 1: Figures S9–S14). Descriptive
122 information for growth traits and environmental variables are included in Additional file
123 2: Table S1. Univariate and multivariate models using both continuous and ecoregions
124 environmental variables detected a total of 2,319 GxE SNPs ($P < 1e-5$, Table 1,
125 Additional files 3 and 4). Only 25% of the GxE SNPs detected by the ecoregion GxE
126 GWAA were also identified by the continuous GxE GWAA. These results indicate that
127 the use of ecoregion information as environmental variable allowed us to capture GxE
128 SNPs interacting with region-specific stressors that were not directly measured (e.g.
129 forage differences, water availability, pathogens) and the combination of multiple
130 stressors.

There was no overlap between the GxE SNPs identified for all traits using univariate models, however, multivariate models detected several GxE SNPs associated with BW, WW, or YW ($P < 1e-5$, Table 1). Multivariate analyses were able to detect GxE SNPs that were not identifiable by standard univariate analyses, indicating advantages of incorporating a multivariate statistical approach to GxE genome-wide association studies of correlated traits. Consistent with our GxE variance component results, a larger number of GxE SNPs was detected for BW than for WW or YW. BW was highly affected by all continuous environmental variables and ecoregions analyzed (Table 1). However, mean dew point temperature was the environmental variable that most interacted with SNPs for BW ($P < 1e-5$, Fig. 1B), and consequently, the ecoregion that identified more GxE SNPs for BW was the Desert & Arid Prairie ($P < 1e-5$, Fig. 1G). The Desert & Arid Prairie ecoregion has both high temperatures and many locations with low absolute humidity measured by dew point temperature (Fig. 1A, Additional file 2: Table S1), which in concert both affect BW. The effect of higher temperature on calf birth weight may be due to the reduced feed intake or the decreased uterine blood flow and placental function often found in heat-stressed cows, factors that contribute to impaired cow-to-fetal nutrient exchange, affecting fetal growth [13–15]. For WW and YW, elevation (Fig. 1C) was found to be the continuous environmental variable that most interacted with SNPs ($P < 1e-5$, Fig. 1 D and E respectively). In agreement, Forested Mountains, the ecoregion with the highest elevation (Additional file 2: Table S1), showed more SNP-by-environment interaction for WW and YW ($P < 1e-5$, Fig. 1 H and I respectively), compared with other ecoregions. Higher altitude regions are associated with reduced atmospheric oxygen (hypoxia), which causes pulmonary hypertension and vasoconstriction leading to right-

side heart failure in cattle [16]. This condition is known as brisket disease and accounts for significant losses in growth and reproductive performance, being one of the top causes of mortality in cattle residing at high altitudes [17].

Only one GxE SNP (*rs133638007*, chromosome 18, 61,721,281 bp) was detected interacting with two different ecoregions. The *rs133638007* T allele increase BW in 1.06 kg in the Desert & Arid Prairie ecoregion, on the other hand, results from the multivariate analysis showed that the *rs133638007* T allele decrease BW by 0.62 kg in Forested Mountains ecoregion. Interestingly, this SNP is located close to the gene *VSTM1*, a member of the leukocyte receptor complex, which is involved in immune responses [18]. In addition, the fact that only one GxE SNP was identified for two different ecoregions indicates that the loci interacting with abiotic and biotic stressors are different across divergent environments, and consequently, the ecoregions have specific genetic architecture involved in GxE.

We explored 10 kb and 100 kb sequence windows that flanked all significant GxE SNPs ($P < 1e-5$) to scan for genes in their vicinity and to identify possible regulatory elements (Additional file 5). Using these GxE gene sets, we performed functional enrichment analysis based on Gene Ontology (GO) and KEGG pathways to get insights into biological mechanisms by which beef cattle respond to changes in their environment. Response to stimulus was the most statistically significant GO term, and its direct descendants GO terms, cellular response to stimulus and response to stress, were also enriched (Additional file 2: Table S2). Additionally, many biological processes involved in metabolism, development, localization, gene expression, transport, signaling, and cell adhesion were enriched. Enriched pathways were related to metabolism; cell proliferation

177 and differentiation; immune and inflammatory responses; hypoxia-induced processes;
178 and mechanisms of neurotransmission in central nervous system (Additional file 2: Table
179 S3). In addition, circadian entrainment, a pathway involved in environmental adaptation
180 to natural periodic changes, including light/dark, temperature, humidity, social activity,
181 and several other cycles [19], was enriched. All GO terms and pathways enriched and
182 their respective GxE genes are available in Additional files 6 and 7, respectively.

183 As the genetic architecture of GxE appeared to be region-specific, we investigated
184 whether they affect the same or different biological functions across ecoregions. To do
185 so, we calculated the percentage of candidate GxE genes for each ecoregion that
186 participate in each biological processes (GO) or pathways enriched (Fig. 2 and 3,
187 Additional file 1: Figures S15 and S16). Even though the ecoregions exhibit mostly
188 unique GxE QTL, the candidate genes associated with these QTLs point toward the same
189 biological processes and pathways, however in different magnitudes. The GxE genes
190 involved in response to stimulus were identified in all ecoregions. In the Southeast,
191 55.8% of the GxE genes detected play roles in the response to stimulus process,
192 suggesting that the combined effects of high temperature and humidity pose a major
193 challenge by affecting how the animal adjusts to external changes and maintains
194 homeostasis. This will become a greater burden on animal health and productivity as the
195 climate continues to warm. Indeed, studies have shown that heat and humidity stress
196 disrupt homeostasis and result in compromised health, growth, fertility, and milk
197 production in several species, including cattle, pig, sheep, mice and humans [12,20–23].

198 Cardiomyopathy related pathways were enriched predominantly in GxE genes
199 interacting with ecoregions in the Western United States, as High Plains, Forested

200 Mountains and Desert & Arid Prairie, ecoregions with higher elevation. Such pathways
 201 were identified likely due to the hypoxia condition exposure, which cause pulmonary
 202 arterial hyperplasia resulting in right ventricular hypertrophy and congestive/dilatory
 203 cardiac failure in cattle living at high altitude [17]. Pathways involved in neural
 204 development and neurotransmission processes were also enriched in GxE genes.
 205 Although these pathways were identified as being affected by GxE genes interacting with
 206 all ecoregions, they seem to be more affected in animals raised in the Fescue Belt and
 207 Southeast ecoregions. This may be due to the consumption of tall fescue, a grass
 208 commonly used in grazing cattle systems in the Southeastern United States [24], which
 209 usually contains an endophytic fungus that produces ergot alkaloids [25]. Ergot alkaloids
 210 have vasoconstrictive effects and interact with GABAergic, glutamatergic and
 211 serotonergic receptors affecting multiple aspects of animal's physiology regulated by
 212 these neurotransmitters [26–28]. Animals consuming the endophyte-infected tall fescue
 213 forage often develop fescue toxicosis, which is characterized by reduced feed intake,
 214 weight gain, and blood flow, as well as elevated body temperature associated with heat
 215 stress [28–31]. Interestingly, the majority of GxE genes for the Fescue Belt and Southeast
 216 ecoregions were also found interacting with temperature. Numerous GxE genes involved
 217 in neural signaling were also detected in the Forested Mountains ecoregion. Hypoxic
 218 environments may affect cells of the nervous system as well. [32] reported that
 219 neuroactive ligand-receptor interaction participates in the regulation of neural stem cells
 220 behavior in mice under hypoxia. Pathways involved in hormone-releasing (oxytocin and
 221 GnRH signaling) related to acute stress in cattle [33] were also identified for high
 222 elevation ecoregions.

GxE genes of all ecoregions drove enrichments in biological pathways related to immune and inflammatory responses (Cytokine-cytokine receptor interaction, Fc epsilon RI signaling, Epstein-Barr virus infection, Tuberculosis, and Hepatitis), except Fescue Belt. This result is unexpected since studies have observed that fescue toxicosis causes hypoprolactemia leading to decreases in immune responses [34,35]. However, our results indicate that this effect may be due to the environmental condition of Fescue Belt ecoregion affecting the activity of GABA and serotonin. Such neurotransmitters are involved in the regulation of prolactin secretion [36,37] and, consequently, may affect the immune system. Many of these immune GxE genes have been associated with diseases caused by pathogens leading to reduced productivity in cattle [38–48]. This result is in agreement that heterogeneity in the immune response to infectious diseases across populations living in different environmental conditions is under genetic control [49]. In addition, pathogen resistance increases host fitness and has driven the evolution of plant and animals through time [50].

GxE genes also participate in pathways involved in the regulation of cell functions including proliferation, growth, gene expression, differentiation, cell survival, and apoptosis, which are crucial processes to proper animal growth and development [51]. Such pathways (PI3K-Akt signaling, Ras signaling, MAPK signaling, Hippo signaling, Focal adhesion, and ErbB signaling) were identified by GxE genes from all ecoregions. However, we identified more GxE genes involved in such pathways in the Southeast, Desert & Arid Prairie, Forested Mountains and High Plains ecoregions.

The majority of GxE SNP associations were identified for BW in this study. Studies have shown that loci identified in GWAA of BW could either represent direct effects of

the fetal genotype, indirect effects of maternal genotype (acting via the intrauterine environment), or some combination of the two [52,53]. According to [54] the uterine environment may even have a greater influence on fetal growth and birth weight than the parental genome. Thus, we investigated whether GxE SNPs affecting BW could be reflecting maternal uterine environmental conditions. To test this, we adjusted BW by including random maternal effects in the model (MAM) and compared the results with those from the GxE GWAA without maternal effects (MA). Results showed that 876 SNPs (out of 1923) were not associated with GxE effects when accounting for the effect of dam, suggesting that those SNPs are interacting with the uterine environment. Candidate maternally-influenced GxE genes (Additional file 8) participate, in particular, in metabolic, insulin signaling, starch and sucrose metabolism, PI3K-Akt signaling and cGMP-PKG signaling pathways; and response to stimulus, blastocyst development, and regulation of signaling processes. These findings support previous investigations suggesting that detrimental environmental factors experienced by the cow during gestation may influence cow-to-fetal nutrient exchange limiting fetal growth and development [13–15,54–56]. Several maternally-influenced GxE genes identified in this study have been previously reported as maternal genes influencing embryonic development and/or response to environmental stress in cows and also in other mammals [57–66]. These results demonstrate that the bovine is an important model to understand the interaction between the physical environment, uterus, and fetus, and could be used in future research.

Variance-heterogeneity GWAA

269 The vGWAA identify differences in variance, rather than differences in mean, between
 270 genotype classes, called vQTLs, which can be explained by GxE or GxG (epistatic)
 271 interactions [67]. Therefore, we performed a REML analysis to estimate how much of the
 272 BW, WW, and YW variance is explained by additive, dominance and epistasis effects.
 273 We fitted models (M) using only additive relationship matrix (MA) or using additive,
 274 dominance, and epistasis relationship matrices (MADE), see Additional file 2: Table S4.
 275 Overall, the heritabilities were greater than the ratio of the variance explained by the non-
 276 additive genetic effects to the phenotypic variances. Proportions of dominance variances
 277 in WW and YW were low (2% and 5%, respectively), whereas no dominance variance
 278 was estimated for BW. Epistatic relationship matrix accounted for 25%, 12% and 11% of
 279 the phenotypic variances for BW, WW, and YW, respectively, suggesting that epistasis
 280 has a substantial contribution to phenotypic variation for body weight traits in Simmental
 281 cattle. Thus, to search for vQTLs we used squared normalized residuals (z^2), which is a
 282 measure of phenotypic variation [68], as dependent variables fitting univariate and
 283 multivariate models. Using z^2 from the MA, we expected to detect loci with both GxE
 284 and GxG effects, since this phenotype was adjusted only for the additive relationship
 285 matrix. However, using z^2 from the MADE, we should detect exclusively GxE loci as the
 286 phenotype was adjusted for additive, dominance and epistatic relationship matrices. In
 287 total, 44 SNPs with effect on z^2 ($P < 1e-5$) were identified on chromosomes 3, 5, 8, 10,
 288 11, 13, 14, 16, 17, 18, 21, 27, and 29 (Additional file 1: Figures S17 and S18, Additional
 289 file 2: Table S5). These 44 SNPs tag 24 putative vQTLs (grouped by LD patterns), with
 290 seven, four, and eleven vQTLs for univariate BW, WW, and YW, respectively. The

291 multivariate analysis identified five vQTLs, of which two vQTLs (chromosome 8 at 4
292 Mb; and chromosome 29 at 39 Mb) were not identified by the univariate analyses.

293 Nine vQTLs were only significant using z^2 from MA (three vQTLs for univariate
294 BW, three vQTLs for univariate WW, two vQTLs for univariate YW, and one vQTL
295 using multivariate analysis). Among these vQTLs likely involved in GxG, one vQTL for
296 BW is located on chromosome 14 at 23 Mb and resides near or within the genes *LYN*,
297 *RPS20*, *MOS*, *PLAG1*, and *CHCHD7*. This region has been reported to have epistatic
298 effects on several chromosomes affecting post-weaning weight in beef cattle [69]. These
299 effects could be due to the gene *PLAG1* which encodes a transcription factor that
300 regulates the expression of several genes involved in a variety of cellular processes [70],
301 and has been previously associated with stature and growth traits in many beef cattle
302 breeds [71–73]. In addition, we detected a GxG vQTL on chromosome 3 at 106 Mb
303 within *TRIT1*, a gene that was also reported to harbor a vQTL involved in epistatic
304 interactions in humans [74].

305 Using z^2 from MADE, 15 vQTLs were detected (four vQTLs for univariate BW; one
306 vQTL for univariate WW; nine vQTLs for univariate YW; and four vQTLs using
307 multivariate analyses, of which three vQTLs were also identified by the univariate
308 analyses), which likely identify GxE. Based on GO information, genes near or within
309 these vQTLs are involved in response to stimulus (*NOS1*, *FBLN1*, *YWHAH*, and
310 *DEPDC5*), inflammation and immunity mechanisms (*LYZL1*, *KSR2*, *FBXO21*, *IL18RAP*,
311 *XCL2*, *XCL1*, *TTYH1*, *LENG8*, *LENG9*, *CDC42EP5*, *LAIR1*, *OSCAR*, and *TARM1*),
312 neurogenesis (*ZHX2*, and *NOS1*), regulation of transcription (*ZHX2*, *YWHAH* and
313 *CNOT3*), developmental process (*FBXW8*, and *CNOT3*), transmembrane transport

(*SLC9A2*, *SLC9A4*, *SLC5A1*, *SLC5A4*, and *TTYH1*), neurotransmission regulation (*SHANK2*), and cell proliferation (*DPT*, *RPS9*, and *TACCI*). Some of those genes were previously reported to be involved in environmental adaptation in cattle [*XCL2* [75], *SLC9A4* [76], *ZMAT4* [77]], and heat tolerance in buffalo [*XCLI* [78]] and dairy cattle [*SHANK2* [79]].

Using z^2 as dependent variables in the analyses allowed the estimation of the proportion of residual variance that is explained by the SNPs in GEMMA (PVE). For BW z^2 , WW z^2 and YW z^2 , SNPs explained 4.9% (SD = 0.8), 4.7% (SD = 0.8), and 5.9% (SD = 1.1), respectively, showing that residual variance is heritable and could be changed by selection, supporting previous studies [80–82]. Residual variability may reflect differences between animals in uniformity for a certain trait, so the reduction of variance could improve economic merit [80]. Rönnegård et al. [81] reported advantages in using breeding values explaining differences in residual variance (vEBV) for milk yield uniformity in Holstein cattle. According to [80] the reduction of phenotypic variance by selecting for reduced residual variance could be more beneficial for low heritability traits.

Meta-analysis of ecoregion-specific GWAA

We performed a meta-analysis of ecoregion-specific GWAA (GWAA for each ecoregion separately) to look for significant differences (Cochran's Q statistics) in effect size of SNPs between the ecoregions, which are interpreted as GxE [83]. In addition, we calculated the m-value statistic for the significant SNPs (Cochran's Q statistic's P -value < 1e-5), which is the posterior probability of an effect being present in an ecoregion given the observations from all other ecoregions [83] allowing us to identify which ecoregions

a given variant has an effect. Southeast and Desert & Arid Prairie ecoregions were not included in the meta-analysis due to their small sample sizes. Meta-analysis of ecoregion-specific GWAA detected 12 GxE SNPs for BW located on chromosome 6 (Additional file 1: Figure S19A); two GxE SNPs for WW on chromosome 15 (Additional file 1: Figure S19B); and two GxE SNPs for YW, one located on chromosome 13 and one on chromosome 15 (Additional file 1: Figure S19C, Additional file 9). Analyzing the PM-plot (P -values against m -values) and PB-plot (P -values against β -values) of the GxE SNPs detected by the meta-analysis, we observed that some GxE SNPs on chromosome 6 at 30 Mb have an effect on animal's BW from Forested Mountain and Upper Midwest & Northeast ecoregions, however with different certainty (Additional file 1: Figures S20A and S21A). For WW and YW, despite that the analysis had shown a significant heterogeneity between the ecoregions, they did not show different posterior probabilities of an effect in any ecoregion (m -value), (Additional file 1: Figure S20B and C, respectively). However, large differences in the SNP effect size (β) were noticed between ecoregions (Additional file 1: Figure S21B and C, respectively).

352

353 **Traditional genome-wide association analyses**

We also performed traditional GWAA using univariate and multivariate linear mixed models in order to compare with the GxE GWAA results. We detected major QTLs on chromosomes 6 (26-48 Mb), 7 (88-90 Mb), 14 (15-30 Mb), and 20 (41-78 Mb) for univariate BW, WW and YW (Additional file 1: Figure S22A-C and Additional file 10). In addition, significant SNPs ($P < 1e-5$) were identified on chromosomes 3 (53.6 Mb) and 17 (73 Mb) for univariate BW; and on chromosomes 1 (81.1-81.3 Mb), 2 (116.1 Mb),

and 5 (96.7 Mb) for univariate WW. The GWAA using multivariate model detected QTL also identified in the univariate GWAA on chromosomes 5, 6, 7, 14, 17, and 20 in addition to on chromosomes 4 (24.5 Mb), 9 (57.9 Mb), 13 (63.3 Mb), 19 (50.5 Mb), and 26 (45.4 Mb) (Additional file 1: Figure S22D and Additional file 11). All genes detected by GWAA are included in the Additional file 12.

Out of 948 total significant SNPs using multivariate GWAA analysis for the growth traits, 531 SNPs were located on chromosome 6 (from 26.8 to 48.8 Mb). Likewise, several SNPs interacting with Forested Mountains ecoregion, elevation, precipitation and, mainly, with the temperatures were also detected in the same positions on chromosome 6. Given the importance of chromosome 6 underlying the growth traits, we repeated the multivariate GWAA including significant SNPs (e.g. *rs109849093*, *rs464458177*, *rs110305942*, *rs43459303*, *rs109278547*, and *rs137209027* located at 37,211,057; 37,234,136; 37,418,164; 37,695,352; 38,139,495; and 41,084,765 bp, respectively) as fixed effects in the model. In this way, if those SNPs are causal mutations accounting for QTL effect the loci association signals would be missed. Results showed that even including six significant SNPs (not in LD) simultaneously as fixed effects in the model, a small number of SNPs were still associated (six out of 531 SNPs), indicating that there are possibly multiple causal mutations in this region.

378

379 **Comparison of the GWA approaches**

Comparing traditional GWAA with all GxE GWAA results, we verified that 312 SNPs with additive effects (QTL) also have GxE effects (mainly on chromosome 6, but also on chromosomes 7 and 20). Some SNPs showed GxE effects even greater than their additive

effects on the traits. For example, the SNP *rs136523580* (chromosome 6 at 30,358,905) has an additive effect of -0.43 kg for BW in the total dataset, but its GxE effects is even more impactful (-0.67 kg) on the BW of animals from the Forested Mountains ecoregion. The SNP *rs109868461* (chromosome 6, at 36,902,704 bp) has additive effects of -0.69 kg for BW in the total dataset, however it has a GxE effect of 1.11 kg on the BW of animals born in the Southeast ecoregion. These findings show the importance of GxE effects on quantitative traits and the need for precision, ecoregion-specific genomic predictions to optimize animal performance.

Comparing vGWAA with the traditional GWAA results, only one vQTL, on chromosome 14 at 23.1-23.3 Mb harboring the genes *LYN*, *MOS*, *RPS20*, *PLAG1*, and *CHCHD7*, was also found as a direct effect QTL for BW, meaning that this locus affects both mean and variance of BW in this population. Five vQTLs were also identified by the GxE GWAA, which were found to interact with multiple continuous environmental variables as well as interacting with ecoregions. These results agree with [84] in suggesting that vQTL effects are much more enriched with GxE effects than additive effects. However, GxE GWAA detected a greater number of GxE loci when compared with the vGWAA results.

Only few GxE SNPs were detected using the meta-analysis of ecoregion-specific GWAA (Cochran's Q statistic's P -value $< 1e-5$), which were also identified by the GxE GWAA. In addition, using the meta-analysis approach the Southeast and Desert & Arid Prairie ecoregions could not be included in the analysis due to their small sample sizes, suggesting that this was not an optimal approach to detect GxE associations for our dataset. However, creating PM-plots (contrast between environment-specific P -value and

posterior probability of an effect, m-value)[85], and PB-plots (environment-specific P -value and SNP effect size) helped interpret results of our GxE GWAA and vGWAA. For significant GxE SNPs detected by the GxE GWAA, there were clear differences in the SNP effect sizes between the significant ecoregion and the remaining ecoregions (Additional file 1: Figures S23 and S24). For SNPs with significant variance-heterogeneity effects (detected by vGWAA), patterns were not as consistent. While some vQTLs had differences in mean effects, others did not (Additional file 1: Figures S25 and S26).

Discussion

This GxE study provides a more comprehensive understanding of how GxE modulate growth traits in beef cattle and possibly in other mammalian species. We observed that GxE explain substantial proportions of growth traits phenotypic variances, which is likely due to a great number of GxE SNPs with large effects affecting such traits. Detection of large GxE SNP effects indicates that there is considerable opportunity to improve environmental resistance and performance in cattle. Our study also revealed the first evidence of vQTLs controlling phenotypic variation in body weights due to GxG or GxE effects in beef cattle. In addition, we uncovered several biological pathways affected by GxE that likely drive environmental adaptation.

One of our major findings is that the ecoregions showed specific GxE with large effects on the traits. In addition, ecoregion GxE GWAA detected GxE loci that were not identified by the continuous GxE GWAA. One possible explanation for these results is

428 that when analyzing ecoregions, we take into account not only climate and topography,
 429 but also unmeasured variables like forage quality and local pathogens, as well as the
 430 combination of them. This information allowed us to identify environmentally sensitive
 431 genotypes. It indicates that the same trait can be influenced by different genes in the
 432 various ecoregions which may affect particular physiological and behavioral functions
 433 leading to local adaptation. This assumption is supported by the GO and pathways
 434 enrichment analysis of the GxE genes identified for each ecoregion showing that the
 435 diverse ecoregion environment affects different biological mechanisms and in different
 436 magnitude. We also found that major additive QTLs also carry GxE effects on growth
 437 traits and that some of their GxE effects are greater or in opposite directions when
 438 compared with their additive effects. Additionally, novel GxE associations were detected
 439 in multiple genomic regions. These findings support the importance of creating
 440 ecoregion-specific genomic predictions to identify local resilient or adapted animals,
 441 using the GxE SNPs detected in this study to assist selection decisions which would lead
 442 to an increase in animal performance within an ecoregion.

443 Some vQTLs were also identified by the GxE GWAA, indicating that vGWAA can
 444 detect loci enriched with GxE effects without requiring prior knowledge of the interacting
 445 environmental factors. Such GxE loci showed interaction with several continuous and
 446 discrete (ecoregions) environmental variables in the GxE GWAA, suggesting that
 447 vGWAA may capture GxE loci that interact with multiple environmental variables,
 448 possibly even those not included in the GxE GWAA model (e.g. water availability,
 449 pasture condition, pathogens). According to [86] when multiple interacting factors induce
 450 variance heterogeneity, the power of identification of any single one of them or all

451 together may be lower than the power of the variance heterogeneity test, which may
 452 explain why vGWAA identified GxE loci that were not detected by direct GxE GWAA.
 453 We also observed that some vQTLs have differences in means across ecoregions
 454 (Additional file 1: Figure S27), however, not all do. This suggests that some vQTLs
 455 could be the difference between robust genotypes (little variation, flat reaction norms)
 456 across environments while other genotypes at the same locus are more variable across
 457 environments, indicating that selection for low variance vQTL genotypes may provide
 458 sustainability advantages. Many genes harboring the vQTL identified here were
 459 previously reported to be involved in environmental adaptation in cattle [75–79]
 460 supporting that phenotypic variability may be an adaptive evolutionary solution to
 461 environmental changes [87]. However, our results support [88] which reported that when
 462 an environmental factor of interest has been measured on all the genotyped individuals,
 463 the direct GxE GWAA will be able to detect a larger number of GxE loci.

464 We found that environmental conditions modulate growth traits through genes that
 465 participate in a variety of biological mechanisms that are activated to help the body cope
 466 with external stimuli and return to or maintain homeostasis [89,90]. Among them, several
 467 pathways involved in neural signaling and development were detected, which play crucial
 468 roles in the regulation of response to stress [91,92], body temperature, vasoconstriction
 469 [93,94], and circadian rhythms [95]. Specifically, for animals living in higher altitude
 470 ecoregions, we identified pathways related to hypoxia response and secretion of
 471 hormones [33]. Other environmental pathways included immune functions reflecting the
 472 involvement of innate and adaptive immune systems in the activation of host defense
 473 reactions to deal with pathogens [94]. We also identified many metabolic processes that

may be involved in acute stress responses and thermoregulation [12,33,94]. These biological mechanisms are affected differently by the diverse ecoregion environments and have been reported to be involved in resilience [12,92,94], and adaptation processes [10,16,96–98], revealing the role that GxE plays in adaptation and evolution at the level of the genome in cattle and potentially across mammals. It is important to understand such biological mechanisms since the inability of an animal to cope with its environment results in failure to produce optimally leading to lost potential profitability of livestock operations [12,17,33,99]. Further, these biological insights may help us understand how adaptation occurs in wild populations and the potential effect of climate change on animal physiological functions.

Conclusions

In summary, GxE GWAA and vGWAA detected several loci affecting growth traits that the traditional GWAA did not, showing that functional loci may have non-additive genetic effects between genotype classes regardless of differences in genotypic means. We revealed biological mechanisms by which beef cattle respond to changes in their environment. Neural signaling, metabolic, hypoxia-induced, and immune system pathways were highlighted, indicating that climate change may become a burden on animal health and productivity. In addition, ecoregion-specific GxE SNPs detected in this study may play a crucial role in resilience and adaptation of beef cattle to divergent environments. Although conventional genomic selection has shown great promise in improving genetic gain, it only considers additive effects and, according to our findings,

ecoregion-specific genomic predictions should be created to identify animals best suited for given environments. This could help producers in making optimal decisions given their geographical location. Our results revealed novel trait associations and alternative genetic mechanisms involved in shaping the total phenotypic variance of growth traits providing new insights into how the environment influences such traits and adaptation in beef cattle and other mammalian species.

Methods

Phenotype, genotype and environmental data

Data from the American Simmental Association males and females born between 1975 and 2016 and genotyped for genomic-enhanced EPDs were transferred to the University of Missouri. The traits studied were birth weight (BW), 205-d adjusted weaning weight (WW) and 365-d adjusted yearling weight (YW), with the traits also adjusted for age of dam as per breed association practice. The animals were assigned to contemporary groups (CG), defined by the combination of farm, season (spring vs. fall) and year of birth.

Phenotypes were pre-adjusted for fixed effect of sex estimated using ‘*--reml-est-fix*’ option, and random effect of contemporary groups (CG) was predicted by the BLUP method using ‘*--reml-pred-rand*’ flag while controlling for population stratification using single-trait animal model implemented in GCTA software [100]. CG were included in the univariate model as random effect since 19% of individuals analyzed belong to CG with

517 only a single animal [101]. The random maternal permanent environmental effect was
518 also included in the WW adjustment.

519 The DNA samples were genotyped with various low-density assays and were imputed
520 to the combination of Illumina BovineHD (Illumina, San Diego, CA) and the GeneSeek
521 Genomic Profiler F250 (GeneSeek, Lincoln, NE) according to [102]. Genotype filtering
522 was performed to remove non-autosomal variants, as well as both variants and
523 individuals with call rates less than 0.90, using PLINK 1.9 [103]. SNP positions were
524 based on the ARS-UCD1.2 Bovine reference genome assembly [104]. The genotypes
525 were then phased using Eagle 2.4 [105] and imputed using Minimac3 [106]. The final
526 imputed genotype data contained a total of 835,947 bi-allelic variants with imputation
527 accuracy of 99.6% [102]. Variants with minor allele frequency less than 0.01 were
528 removed using PLINK 1.9 for further analysis, leaving 710,202, 709,054, and 706,771
529 SNP markers for 13,427, 11,847, and 8,546 animals for BW, WW and YW, respectively.

530 Eight continuous environmental variables were used for the GxE GWA analyses,
531 including 30-year normal of minimum, mean, maximum and mean dew point
532 temperatures; elevation; precipitation; and minimum and maximum vapor pressure
533 deficit, which were drawn from the PRISM climate dataset [107]. The United States was
534 partitioned into nine regions (Fig. 1F *Top*) based on similar topographic and
535 environmental conditions (ecoregions), including mean temperature, elevation and
536 precipitation information, using k-means clustering implemented in ‘*RStoolbox*’ R
537 package [108,109]. The optimal ecoregions were identified using ‘*pamk*’ function from
538 the R package ‘*fpc*’ [110]. The ecoregions were named Desert (D), High Plains (HP),
539 Arid Prairie (AP), Forested Mountains (FM), Upper Midwest & Northeast (UN),

Southeast (SE), Rainforest (R), Foothills (FH), and Fescue Belt (FB) [10]. Animals were assigned to the ecoregions based on the zip code of their breeder (Fig. 1F *Bottom*), and only ecoregions with more than 200 records were analyzed. Thus, animals located in the Rainforest ecoregion were removed from the dataset, and those that were reared in the Desert and Arid Prairie ecoregions were analyzed together (called DA) due to their similar environmental conditions. No animal record was assigned to the Foothills ecoregion. A complete description of the dataset, including weight records and environmental variable measurements is shown in Additional file 2: Table S2.

548

549 **Estimation of variance components**

Variance components for pre-adjusted BW, WW, and YW were estimated using multi-component restricted maximum likelihood (REML) approach, implemented in GCTA software, in univariate linear mixed models that included only additive effects (MA), or additive, dominance and epistasis effects (MADE) as follows:

$$554 \quad y^* = 1\mu + Z_a a + e \text{ (MA)}$$

$$555 \quad y^* = 1\mu + Z_a a + Z_d d + Z_{ep} ep + e \text{ (MADE)}$$

where y^* is the vector of pre-adjusted phenotypes; μ is the overall mean; a is the vector of random additive genetic effects; d is the vector of random dominance effects; ep is the vector of random epistatic effects; and e is the vector of random residuals. Z matrices are incidence matrices relating observations to animals. Assuming that $a \sim N(0, G\sigma_a^2)$, $d \sim N(0, D\sigma_d^2)$, $ep \sim N(0, E\sigma_{ep}^2)$, $e \sim N(0, I\sigma_e^2)$, where σ_a^2 , σ_d^2 , σ_{ep}^2 , and σ_e^2 are the additive, dominance, epistatic, and residual variances, respectively. I is the identity matrix; and G ,

D , and E are the additive, dominance and epistatic genetic relationship matrices, respectively. The genomic (G) and the dominance (D) relationship matrices were calculated using SNP marker information according to [111] as: $G = W_G W_G' / N$, and $D = W_D W_D' / N$, where $w_{G(i)} = (x_{G(i)} - 2p_i) / \sqrt{2p_i(1 - p_i)}$ and , $w_{D(i)} = (x_{D(i)}^2 - 2p_i^2) / [2p_i(1 - p_i)]$ with x_G being 0, 1, or 2 and x_D being 0, 2p, or (4p - 2) for genotypes AA, AB or BB, respectively; p_i is the allele frequency at SNP i ; and N is the number of SNPs. According to Henderson [112] the epistatic relationship matrix (E) can be derived from the additive genomic relationship matrix as: $E = G \# G$, where $\#$ denotes the Hadamard product operation.

The variance of GxE interaction was also estimated considering the ecoregions as an environmental factor and treated as random effect in the model in order to estimate the amount of phenotypes that are explained by the GxE interaction among the ecoregions, as implemented in GCTA.

Genome-wide association analyses (GWAA)

Univariate linear mixed model analyses were performed for the pre-adjusted BW, WW or YW using GEMMA software [113] as follows:

$$y^* = 1\mu + X\beta + u + e$$

where y^* is an n -vector of pre-adjusted phenotypes; μ is the overall mean; X is the incidence matrix of genotypes; β is the genotype effects; u is an n -vector of random additive genetic effects; and e is an n -vector of random residual. Assuming that $u \sim MVN_n(0, GV_g)$ and $e \sim MVN_n(0, IV_e)$, G is the $n \times n$ genomic relationship matrix

(GRM); V_g is the residual additive genetic variance; V_e is the residual variance component; $\mathbf{I}$ is an $n \times n$ identity matrix. MVN_n denotes the n -dimensional multivariate normal distribution. GRM were estimated using the standardized genotypes of 710,202, 709,054, and 706,771 SNP markers for 13,427, 11,847, and 8,546 animals for BW, WW and YW, respectively.

We also fitted a multivariate linear mixed model for the pre-adjusted BW, WW and YW using GEMMA [114] in the following form:

$$Y^* = 1\mu + X\beta + U + E$$

where Y^* is an $n \times d$ matrix of d pre-adjusted phenotypes for n individuals; μ is the overall mean; X is the incidence matrix of genotypes; β is a d vector of the genotype effects for the d phenotypes; U is an $n \times d$ matrix of random additive genetic effects; and E is an $n \times d$ matrix of random residuals. $U \sim MN_{n \times d}(0, G, V_g)$ and $E \sim MN_{n \times d}(0, I_{n \times n}, V_e)$, G is the $n \times n$ genomic relationship matrix (GRM); V_g is the $d \times d$ matrix of additive genetic variance component; V_e is a $d \times d$ matrix of the residual variance component; $\mathbf{I}$ is an $n \times n$ identity matrix. $MN_{n \times d}$ denotes the $n \times d$ matrix normal distribution with mean 0, row covariance matrix G or $\mathbf{I}$ ($n \times n$), and column variance matrix V_g or V_e ($d \times d$). For all GWA analyses performed, single-marker P -values were used to generate Manhattan plots using ‘manhattan’ function implemented in ‘qqman’ R package [115].

601

602 **Genotype-by-environment GWA analyses**

603 The GxE GWAA were performed using GEMMA through two approaches: using eight
 604 continuous environmental variables (i.e. minimum, mean, maximum and mean dew point
 605 temperatures; elevation; precipitation; and minimum and maximum vapor pressure
 606 deficit) separately; and using the ecoregions as discrete environmental variable, where
 607 each ecoregion was compared against the total dataset using 0 and 1 dummy coding. In
 608 the GxE GWAA, for each SNP in turn, GEMMA fits a linear mixed model that controls
 609 both the SNP main effect and environmental main effect, while tested for the interaction
 610 effect and controlling for population stratification, evaluating the alternative hypothesis
 611 ($H_1: \neq 0$) against the null hypothesis ($H_0: x_j \beta_{ij} = 0$) for each interaction, therefore the
 612 resulting P -values correspond to the significance of the GxE interaction [71]. This model
 613 can be described as:

$$y^* = 1\mu + x_i\beta_i + x_j\beta_j + x_i * x_j\beta_{ij} + u + e$$

614 where y^* is an n -vector of pre-adjusted phenotype; x_i is an n -vector of the genotypes of
 615 the SNP i ; β_i is the effect size of the SNP i ; x_j is an n -vector of environmental variable j ;
 616 β_j is the fixed effect of the environmental variable j ; β_{ij} is the interaction effects between
 617 the genotypes of the SNP i and the environmental variable j ; u is an n -vector of random
 618 additive genetic effects; and e is an n -vector of random residual. GxE GWA analyses
 619 using multivariate linear mixed model were also performed.

620

621 **Variance-heterogeneity GWAA**

622 We performed vGWAA to detect locus affecting the difference in variance between
 623 genotypes (vQTL). Residual effects (e) from the MA and MADE models were

standardized to z score by the rank-based inverse-normal transformation and squared (z^2), which is a measure of phenotypic variance [84,116]. The vGWAA were performed for BW z^2 , WW z^2 , and YW z^2 using univariate and multivariate linear mixed models implemented in GEMMA software as denoted in “Genome-wide association analyses” section, where y^* is an n -vector of z^2 ; and β_i is the effect size of the SNP i on z^2 . Linkage disequilibrium (LD) analyses were performed using PLINK 1.9. All vQTL were defined by SNP markers with r^2 greater than 0.6 [117].

631

632 **Meta-analysis of ecoregion-specific GWAA**

In order to evaluate how significantly the effect size of SNPs (Cochran’s Q test) are between the ecoregions (indicative of GxE) [83] we combined the outputs from univariate within-ecoregion GWAA into a single meta-analysis for each phenotype using METASOFT software [118]. Only ecoregions with more than 1000 animals were analyzed. In addition, the statistic m-value for the significant SNPs (Cochran’s Q statistic’s P -value $< 1e-5$) were calculated, which is an estimative of the posterior probability of a locus having an effect in a particular ecoregion [119]. The results were visualized through PM-plots and PB-plots, in which P -values are simultaneously visualized with the m-values and beta-values at each tested locus, respectively.

642

643 **False discovery rate (FDR) estimation**

FDR of the nominal significant threshold ($P < 1e-5$) was estimated for all analyses performed (Additional file 2: Table S6), based on [120], as:

$$FDR = P \left(1 - \frac{A}{T} \right) / \left(\frac{A}{T} \right) (1 - P),$$

where P is the nominal significant threshold (e.g., $1e-5$); A is the number of SNP that were significant at the nominal significant threshold; and T is the total number of SNPs tested. Although the true FDR cannot exceed 1, the estimated FDR can exceed 1 if the number of significant SNPs is smaller than expected by chance, meaning that all SNPs that met the nominal significant threshold are false positives [69].

Functional enrichment analyses

We explored 10 kb and 100 kb sequence windows that flanked the significant SNPs ($P < 1e-5$) to scan for GxE genes located in their vicinity and to identify possible regulatory elements, respectively, based on the ARS-UCD1.2 bovine reference genomic positions. Enrichment analysis using the GxE genes based on Gene Ontology (GO) and KEGG pathways were performed using ShinyGO v0.60 [121], in which the analyses were adjusted for FDR of 5%. GO annotations were also retrieved from the AmiGO browser [122].

Supplementary information

Additional file 1: Supplementary Figures. Supplementary Figures S1-S26. (DOCX 5.0 MB)

Additional file 2: Supplementary Tables. Supplementary Tables S1-S6. (DOCX 272.9 KB)

667 **Additional file 3:** Significant SNPs ($P < 1e-5$) using univariate GxE GWAA for Birth
668 Weight, Weaning Weight and Yearling Weight. (XLSX 248.7 KB)

669 **Additional file 4:** Significant SNPs ($P < 1e-5$) using multivariate GxE GWAA for Birth
670 Weight, Weaning Weight and Yearling Weight. (XLSX 40.7 KB)

671 **Additional file 5:** Candidate genes identified by GxE GWAA using 10 kb and 100 kb
672 region flanking the significant SNPs. (XLSX 253.8 KB)

673 **Additional file 6:** Enrichment analyses based on Gene Ontology terms using GxE genes
674 for 10 kb and 100 kb region flanking the significant SNPs. (XLSX 1.2 MB)

675 **Additional file 7:** Enrichment analyses based on KEGG pathways using GxE genes for
676 10 kb and 100 kb region flanking the significant SNPs. (XLSX 86.1 KB)

677 **Additional file 8:** Candidate maternally-influenced GxE genes using 10 kb and 100 kb
678 region flanking the significant SNPs. (XLSX 77.3 KB)

679 **Additional file 9:** Significant SNPs (Cochran's Q statistic's P -value $< 1e-5$) using meta-
680 analysis of ecoregion-specific GWAA for Birth Weight, Weaning Weight and Yearling
681 Weight. (XLSX 5.4 KB)

682 **Additional file 10:** Significant SNPs ($P < 1e-5$) using univariate GWAA for Birth
683 Weight, Weaning Weight and Yearling Weight. (XLSX 128.8 KB)

684 **Additional file 11:** Significant SNPs ($P < 1e-5$) using multivariate GWAA for Birth
685 Weight, Weaning Weight and Yearling Weight. (XLSX 93.6 KB)

686 **Additional file 12:** Candidate genes identified by GWAA for Birth Weight, Weaning
687 Weight and Yearling Weight. (XLSX 19.6 KB)

688

689 **Declarations**

690 **Ethics approval and consent to participate**

691 Animal Care and Use Committee approval was not required or obtained for data that
692 were extracted from the existing American Simmental Association database.

693

694 **Consent for publication**

695 Not applicable.

696

697 **Availability of data and materials**

698 The raw data used in this research are available from the American Simmental
699 Association under a Data Use Agreement, but are not publicly available. Derived data
700 (analytical results) are however available as supplementary files associated with this
701 publication.

702

703 **Competing interests**

704 The authors declare that they have no competing interests.

705

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710

711 **Author's contributions**

712 CUB and JED conceived the study. CUB analyzed the data and interpreted the results and
713 wrote the manuscript. TNR and RDS managed data and imputed variants. JED assisted
714 with analyses, preparation of the manuscript and interpretation of results. All authors read
715 and approved the final manuscript.

716

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721

722

723 **References**

724

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1046 **List of figures**

1047

1048 **Fig. 1.** Genotype-by-environment interaction genome-wide association analyses (GxE
1049 GWAA) for growth traits in Simmental cattle. (A) Mean dew point temperature average
1050 annual over the most recent three full decades covering the conterminous United States.
1051 (B) Manhattan plot of GxE GWAA of mean dew point temperature for birth weight. (C)
1052 Elevation of the conterminous United States. (D) Manhattan plot of GxE GWAA of
1053 elevation for weaning weight. (E) Manhattan plot of GxE GWAA of elevation for
1054 yearling weight. (F) Boundaries for ecoregion assignments in the United States; (*Top*)
1055 United States partitioned into nine ecoregions based on similar topographic and
1056 environmental conditions; (*Bottom*) location of beef farms for which data was retrieved.
1057 (G) Manhattan plot of GxE GWAA of Desert & Arid Prairie ecoregion for birth weight.
1058 (H) Manhattan plot of GxE GWAA of Forested Mountains ecoregion for weaning
1059 weight. (I) Manhattan plot of GxE GWAA of Forested Mountains ecoregion for yearling
1060 weight. In Manhattan plots, horizontal red line indicates a significant threshold ($P < 1e$ -
1061 5). Environmental continuous variables were drawn from the PRISM climate dataset
1062 (<http://prism.oregonstate.edu>). The United States was partitioned into nine regions using
1063 k-means clustering.
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1065

1066 **Fig. 2.** Percentage of candidate genes with genotype-by-environment effects for each

1067 ecoregion that contribute to the enriched gene ontology (GO) terms. Genes were located

1068 100 kb from significant SNPs ($P < 1e-5$).

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1071 **Fig. 3.** Percentage of candidate genes with genotype-by-environment effects for each
 1072 ecoregion that contribute to the enriched biological pathways. Genes were located 100 kb
 1073 from significant SNPs ($P < 1e-5$).

Table 1. Number of significant SNPs ($P < 1e-05$) and magnitude of the allele substitution effects by environmental variables/factors for each trait.

Env	Birth Weight		Weaning Weight		Yearling Weight		Mv	Total
	N	ASE	N	ASE	N	ASE	N	N
Elev	142	-0.001–0.001	28	-0.008–0.002	15	-0.005–0.006	50	216
Precip	338	-0.001–0.002	4	-0.004– -0.004	1	-0.02	12	349
MT	555	-0.170–0.201	1	0.49	4	-1.240–1.704	69	611
MinT	756	-0.159–0.212	6	-0.868–0.439	-	-	88	823
MaxT	408	-0.175–0.207	1	0.43	4	-1.267–1.848	23	427
MDPT	941	-0.178–0.168	4	-0.516–0.409	1	-1.11	94	1018
MinVPD	152	-1.399–1.831	2	-3.254– -3.242	1	-6.62	10	165
MaxVPD	224	-0.213–0.262	10	-0.546–0.556	1	1.08	17	236
SE	54	-2.078–1.649	-	-	2	-11.120– -10.500	7	59
HP	40	-1.381–1.132	16	-5.353–3.930	4	-22.900–6.722	23	63
FM	90	-3.135–1.215	31	-6.785–4.737	49	-9.397–17.690	148	221
FB	47	-2.145–0.953	1	-2.94	1	18.53	11	56
UN	24	-0.709–0.872	-	-	1	8.18	10	33
DA	125	-8.243–6.043	8	-11.570–6.456	6	-16.450–23.620	*	125
Total	1923		101		78		353	2319

Results of the genotype-by-environment genome-wide Association Analysis using environmental variables (Env), such as elevation (Elev), precipitation (Precip), mean temperature (MT), minimum temperature (MinT), maximum temperature (MaxT), mean dew point temperature (MDPT), minimum vapor pressure deficit (MinVPD), and maximum vapor pressure deficit (MaxVPD), and using the United States ecoregions (Southeast [SE], High Plains [HP], Forested Mountains [FM], Fescue Belt [FB], Upper Midwest & Northeast [UN], and Desert & Arid Prairie [DA]) as environmental factors (Env. Factor) in univariate (Birth Weight, Weaning Weight, and Yearling Weight) and multivariate (Mv) linear mixed models. The number of significant SNPs (N) at the significant threshold of $P < 1e-05$ and the magnitude of the allele substitution effects (ASE, kg) were also provided.