Grazing personality genetics of beef cattle in New Zealand rangelands

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Cristian Aníbal Moreno García

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Abstract

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The uneven distribution of grazing cattle on pastures and rangelands has been of concern to livestock managers since the very early days of grassland science, not least because of the effects of grazing patterns on ecosystem functions and the sustainability of farming systems. Mountainous terrain imposes additional limitations for free-range grazing animals; which may avoid using vegetation at higher altitudes, on steeper slopes, or at greater distance from drinking water. Consequently, areas that are more easily accessible might be overgrazed, potentially leading to diminished ecological functions and reduced productivity. In recent years, animal personality theory has suggested that individual animals do not behave in the same way, and instead display consistent and distinctive sets of behaviours or 'personality'. Animal personality could explain the distinct grazing patterns reported for free-range cattle, where individual animals have preference for certain habitats over others as a result of their behaviour. Preliminary studies have also reported associations between cattle gene regions (i.e., quantitative trait loci) and indexes that describe terrain use, suggesting the potential for genes that might explain variation in the grazing personality of beef cattle.

This thesis contains the following chapters:

A literature review about grazing behaviour and personality (Chapter 1). While grazing lands can offer a diverse range of forages, individuals within herds appear to prefer to graze some habitats and not others. They can have consistent differences in grazing patterns and occupy specific spatial domains, whilst developing tactics and strategies for foraging that are individual specific. Accordingly, in this chapter, a new understanding of grazing personality was developed. This entailed the development of a 'grazing personality model' (GP-model) that accounts for the personality of individual animals and for the collective behaviour of herds. The GP-model postulates that the grazing personalities of ruminants and other large herbivores are determined genetically and tempered epigenetically in interaction with the social and biophysical environments of the cattle. They may also reflect the emotional state of animals.

While the selection of one grazing personality may be adequate for homogeneous pastoral systems, the design of herds with a range of grazing personalities that are matched to the habitat diversity may be a better approach to improving the distribution of grazing animals, thus potentially enhancing ecosystem services and maximizing productivity.

An investigation of whether the movement of cattle and potential measures of their grazing personalities might be determined genetically, was undertaken in chapter 2. Genetic variation within the glutamate metabotropic receptor 5 gene (*GRM5*), a 'grazing gene' candidate was investigated. Associations between variation in that gene and variation in grazing personality behaviours (GP-behaviours) were tested with mature cows (n = 303) under free-range management during winter grazing in the steep and rugged rangelands of New Zealand. Grazing behaviours were calculated using data from global positioning system (GPS) tracking collars and, satellite-derived data. Eight GP-behaviours were fitted into mixed models to ascertain their associations with variant sequences and genotypes of *GRM5*. Three new *GRM5* variants (A, B and C) were discovered and six possible genotypes were identified in the cattle studied. The mixed models revealed that A was associated ($P < 0.05$) with elevation range, home range and movement tortuosity. Similarly, *GRM5* genotypes were significantly associated ($P < 0.05$) with home range and movement tortuosity, while trends towards association ($P < 0.1$) were revealed for elevation range and horizontal distance travelled. Most of the GP-behaviour models were improved when corrected with the 'cow age-class' factor and the results suggested that grazing personality might be stable when cows reached 4 years of age. Home range and movement tortuosity were not only associated with *GRM5* variation, but also negatively correlated with each other (r = -0.27, $P < 0.001$). Thus, there seems to be a genetically determined trade-off between home range and movement tortuosity that may be useful in beef cattle breeding programmes that aim to improve the grazing distribution and utilisation of steep and rugged rangelands.

The results of Chapter 2 suggested that differences in grazing patterns are associated with nucleotide sequence variation in *GRM5*. Association analyses require large datasets to detect genotype-phenotype associations, hence, most large-scale studies aiming to identify behavioural linkages with grazing genes typically apply random sampling from existing setups without *a priori* control over the genotypic composition of the sample. This can lead to unbalanced experiments with over or under representation of any given group analysed. An alternative approach (Chapter 3) was used to perform a discriminant analysis of a balanced dataset that was generated by under-sampling the larger dataset (n = 303) described in Chapter 2. In this analysis, a training dataset of mature cows (n = 80) that equally represented five of the six *GRM5* genotypes and four farms were selected. The GP-behaviours were derived from 5-min GPS relocations measured over 15 d and the analysis aimed to select a combination of GP-behaviours that assist the identification of specific *GRM5* genotypes, and to investigate behavioural differences between cows of various genotypes of this 'grazing gene'. Two sets of grazing behaviours were selected to build quadratic discriminant models (QDMs) that achieved 87% of accuracy in ascertaining *GRM5* genotype with a training balanced dataset. An ‘exploration discriminant model’ built with the GP-behaviours related to elevation, slope and exploration correctly predicted the genotypes of 85% of the individuals of a testing dataset that were not included in the model’s training. MANOVA and ANOVA analyses highlighted the relative importance of GP-behaviours to discriminate between *GRM5* genotypes and showed behavioural differences between cows of various *GRM5* genotypes. The results extend the list of key behaviours linked to *GRM5* in agreement with genotype-phenotype associations between *GRM5* and GP-behaviours reported in chapter 2 and in the literature. In conclusion, sets of key GP-behaviours might be useful for predicting the variation in putative ‘grazing genes’ and QDMs applied to small-scale experiments with balanced designs seems to be a promising approach for behavioural genetics.

Overall, this research proposed a model for individual and collective grazing personalities for cattle. The analysis suggested consistent differences between individuals associated with *GRM5* variation. Furthermore, linkages between bovine *GRM5* and key grazing behaviours may characterise specific genotypes and assist with their identification. The research provides a conceptual model of grazing personality and experimental evidence suggesting possible applications of behavioural genetics to potentially optimise the distribution of beef cattle in steep and rugged terrain. More research is however needed to validate these findings.

Keywords: Animal personality, Beef cattle, Breeding programmes, Discriminant analysis, Genotype-phenotype associations, Global positioning system tracking (GPS-tracking), Glutamate metabotropic receptor 5 gene (*GRM5*), Grazing behaviour, Linear mixed models, Steep and rugged terrain

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List of Abbreviations

AIC Akaike information criterion

ANOVA Analysis of variance

BIC Bayesian information criterion

CV Coefficients of variation

DEM Digital elevation model

DNA Deoxyribonucleic acid

GLMM Generalised linear mixed model

GP-behaviours Grazing personality behaviour

GP-model Model of grazing personalities

GPS Global positioning system

GRMs Glutamate metabotropic receptor

GRM1 Glutamate metabotropic receptor 1 (G-protein)

GRM5 Glutamate metabotropic receptor 5 (G-protein)

GRM5 Glutamate metabotropic receptor 5 gene

ICC Inter-class correlation coefficient

ICC2 Inter-class correlation coefficient in two-way random effects model

LDA Linear discriminant analysis

LMM Linear mixed model

MAML3 Mastermind-like 3 gene

MANOVA Multivariate analysis of variance

PCR Polymerase chain reaction

QDA Quadratic discriminant analysis

QDM Quadratic discriminant model

QTL Quantitative trait loci

SNP Single nucleotide polymorphism

SSCP Single strand conformation polymorphism

USA United states of America

VIF Variance inflation factor

bp base pairs

d day

ha hectares

ha/d hectares per day

m metres

m/d metres per day

m/ha metres per hectare

s second

h hour

min minute

V volts

° Angular degrees

°C Celsius degrees

adj_dist_ho Adjusted horizontal distance travelled

adj_ele_mean Adjusted elevation

dist_ho Daily horizontal distance travelled

dist_ve Daily vertical distance travelled

dist_3D Daily three-dimensional distance travelled

ele_gain Daily elevation gain

ele_range Daily elevation range

ele_mean_farm Mean elevation centred per farm

ele15_farm 15th quantile of elevation centred per farm

ele85_farm 85th quantile of elevation centred per farm

ele85_farm_cv Coefficient of variation of the 85th quantile of elevation centred per farm

hr_mcp Daily home range

rel_ele_mean Relative elevation mean

rel_ele_range_cv Coefficient of variation of the relative elevation range

rel_ele_mean_cv coefficient of variation of relative mean elevation

rel_ele_range Relative elevation range

rel_slope_range Relative slope range

rel_ele85 Relative 85th quantile of elevation

slope_max Daily maximum slope

slope_mean Daily mean slope

slope_mean_cv Coefficient of variation of the mean slope

slope15 15th Quantile of daily slope

slope85 85th Quantile of daily slope

sp_tortuosity Daily movement tortuosity

3D_dist Daily three-dimensional distance travelled

Chapter 1

On the search for grazing personalities: from individual to collective behaviours

1.1 Introduction

We picture foraging animals distributed throughout grazing lands. Individually or in various sized groups of one or more species, herbivores explore and graze a diverse range of habitats including riparian areas, open flat plains, gentle or steep hills and mountainous lands. Even when considering herds of one single species, individuals show divergent dietary tactics and foraging site preferences resulting in consistently and regularly repeated grazing patterns, like for example in cattle or sheep [8, 9, 10, 11].

Grazing has been described as a process composed of short-term ingestive tactics, and mid- and long-term digestive strategies [12], and its pattern is defined as a cluster of decisions that lead to ingestive actions and digestive strategies that are motivated by the interaction of both internal and external stimuli [13]. Differences in grazing patterns are far from being trivial or random, with individual animals behaving consistently and adopting specific grazing strategies across situations and over time; such that animals are said to display recognizable grazing personalities [14, 15, 16, 4]. The diversity of grazing personalities within herds modulates the intensity and frequency of forage defoliation achieved with recommended stocking rates, the fitness of animals and other production traits such as reproduction success, survival and live-weight changes [17, 7]. As a consequence, differences in grazing patterns and personalities affect ecosystem functions such as speeding up nutrient cycling [18], increasing productivity of grasslands [19] and preventing loss of plant diversity [20].

The concept of animal personality, also referred to as behavioural syndrome [4], copying style [21], and temperament [22] among other closely related terms [23, 5], was developed by integrating correlated traits of behaviour with other traits. For example, Carere and Maestripieri [24] defined animal personality as correlated behavioural and physiological traits that differ among individuals of the same species, and that are temporally stable across different contexts or situations. Gosling and John [25] suggested this concept should not be restricted to differences observed within-species, but rather these are behaviours and patterns that are consistently displayed by individuals regardless of the species identity. Some authors also argue that personality should include traits that account for consistent patterns of feelings and thoughts that affect behaviour [26]. In this way, the concept of animal personality includes emotional and cognitive traits, which can influence animal decision-making and wellbeing. In line with Maderspacher's [27] arguments and Biro and Post's [28] speculations, we have chosen to include morphological traits in our definition of grazing personality, as evidence showed correspondence between behavioural polymorphisms and morphological polymorphisms. Accordingly, we define grazing personality for grazing ruminants and other large herbivores as 'suites of traits of different nature (e.g., behavioural, cognitive, physiological and morphological), which are correlated and often concatenated, to result in specific grazing patterns displayed consistently across contexts and over time'.

Regardless of the species identity, differences in grazing personalities are observed at the individual [29, 30, 15, 25] and collective level; that is in groups, herds and populations of animals [31, 32]. Consequently, we argue that grazing personalities are the result of evolutionary processes that filtered alleles and established allele frequencies of key genes related to behavioural patterns, tactics, strategies and decision-making in the grazing process, hereafter referred to as 'grazing genes'. In addition, interactions with social and biophysical environments, the emotional state of animals and their experiences early in life, might modify the epigenome of grazing genes, thereby modulating their expression.

We support the contention that grazing personalities are observable at individual and collective levels, and suggest that divergent grazing personalities result in distinct grazing patterns and attributes; such as the ability to explore, define a home-range, display a habitat preference and fragment into groups. These all affect the ecological functioning of grazing systems.

We also propose a "grazing personality model" (GP-model). The purpose and context of the GP-model is to represent the genetic elements, the regulatory systems and the phenotypic elements that encompass individual and collective personalities in a context of herds of grazing ruminants and other large herbivores. The objective of the GP-model is to further develop our understanding of distribution of grazing animals following the initial "Ecological-Hierarchical grazing model" [33] and the additional concepts of the "Distribution Patterns and Mechanisms model" [34]. The GP-model represents grazing personalities, which are genetically determined (genotypic personality) and epigenetically modulated through the systems that regulate the expression of grazing genes (personality plasticity) via interactions with the social herd environment and the biophysical features of the grazing environment. The emotional state of animals influences the regulatory systems that modulate gene expression and affects grazing decision-making. In this review, we first deal with grazing personalities at the level of the individual animal, then we deal with collective personalities and finally we illustrate GP-model implications based on movement ecology, genetics and animal personality.

1.2 Individual grazing personalities

> '...from the population optimum perspective [. . .] natural and sexual selection may favor the evolution of multiple responses to environmental challenges, thus resulting in within-population variation in the same behavioural trait, and in whole suites of behavioural traits.' [35].

This section describes the GP-model at the individual level (Fig. 1.1, left side): from individual genotypes of grazing personalities at the top, through regulatory systems that modulate the gene expression and confers the personality plasticity in the middle, to individual phenotypic grazing personalities at the bottom. Thus, in section 1.2.1 The genetics of behaviour and grazing related genes, we present evidence about grazing genes and its heritability. We then investigate regulatory systems that modulate the expression of grazing genes in variable responses to stimuli conferring the personality plasticity (see section 1.2.2 The effect of personality plasticity and regulatory systems on grazing patterns). Finally, in section 1.2.3 Grazing traits of individuals, we present examples of phenotypic grazing personalities and traits at the individual level (Table 1.1).

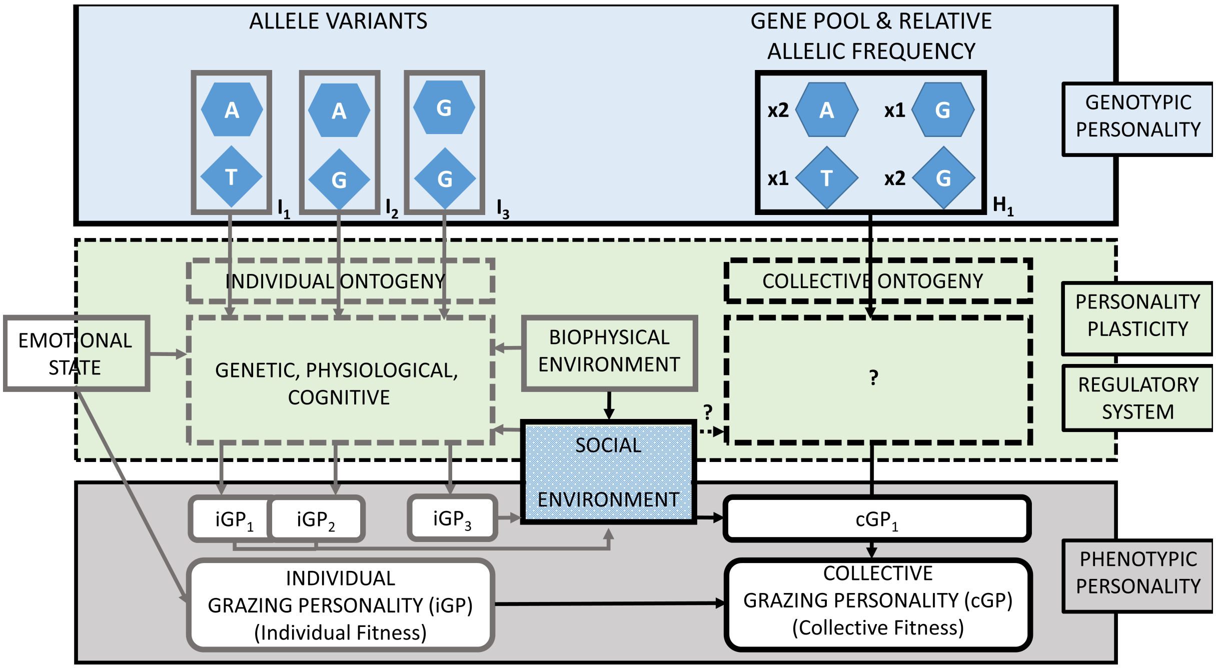

Figure 1.1. The Grazing Personality Model (GP-model) for ruminants and other large herbivores described by three main aspects: the genotypic personality (top), regulatory system conferring personality plasticity (middle), and the phenotypic personality. In the example, three hypothetical combinations of allele variants (I_1, I_2, and I_3) applied to two grazing genes represented at the individual level (left side). The genotype of individuals constitutes the gene pool and the relative allelic frequency (H_1) of grazing genes at the collective level (right side). Individual and collective grazing personalities (iGP and cGP) are phenotypically represented with corresponding fitness. The example shows two grazing genes in beef cattle (32): the glutamate metabotropic receptor 5 gene in chromosome 29 (hexagons) and the mastermind-like 3 in chromosome 17 (diamonds). Allele variants specified by nucleobases adenine [A], guanine [G], and thymine [T]. The interactions between genes and environment regulates the expression of grazing genes and confers personality plasticity. Phenotypic grazing personalities of individuals (iGPs) may overlap (e.g., iGP_1 and iGP_2) or diverge (e.g., iGP_3). A group of individuals co-existing and displaying distinct grazing personalities constitutes a grazing herd with its own collective grazing personality (cGP). Adapted from Bengston and Jandt [1]; with concepts from Koolhaas and van Reenen [2], Robinson [3], and Sih [4].

Individual animals exhibit repeatable differences in their grazing behaviour within populations, within species and across species. These personality differences arise for many reasons, such as differences in permanent environmental effects (e.g., familial, parental, and epigenetic contributions) and the effect of genetic variation. In ruminants, personality differences can influence eating tactics and ingestive behaviors [16, 36]. For example, Gregorini et al. [16] studied a group of 16 dairy cows that were selected as calves (6-8 months old) based on divergence in residual feed intake (i.e., having high and low residual feed intakes) and measured their individual grazing behaviours, eating patterns and ingestion tactics as milking cows. From a grazing behaviour viewpoint, low residual feed intake individuals prioritised grazing and ruminating over idling. They typically took fewer steps when walking during grazing and had a higher ratio of grazing to non-grazing steps when compared with cows with high residual feed intake. From an ingestive viewpoint, low residual feed intake individuals masticated less, but ruminated more intensively, and they had feces with 30% less quantity of large particles size than their counterparts with high residual feed intake. Wesley et al. [37] also pre-classified 18 beef cattle heifers from within 80 animals in two consecutive years ($n = 36$) based on the rate of consumption of supplementary feed (another trait related to the eating tactics). The authors reported divergent grazing behaviour and ingestion tactics; for example, cows with faster rates of consumption of supplement tended to spend less time at water, cover larger areas and exhibit less concentrated grazing search patterns than cows with slower consumption rates. These two studies speculated a link between the divergent phenotypic behaviours (i.e., eating tactics) displayed by the selected animals and their genotype. In the following section, we present the genetics of behaviour and genes related to grazing patterns.

Table 1.1. List of examples used in this review with dichotomous and multiple classifications of animal behavioural types in grazing ruminants (see [5, 6] for extensive list of studies with genetically-associated behaviours and [7] for examples of single personality dimension related to fitness). Behavioural types, behavioural categorizing criteria, type of variable and if behaviour is explained genetically.

Species	Behavioural types	Behavioural categorizing criteria	Continuous and categorical variables	Genetically explained	Reference
Beef cattle	1. Riparian areas users 2. Uplands users	1. Home-range fidelity	Categorical (dichotomous) and continuous	Probably	[9, 38]
Beef cattle	Breeds better suited for mountainous terrain	1. Slope % 2. Horizontal distance 3. Vertical distance	Continuous	Probably	[39]
Beef cattle	1. Bottom dweller 2. Hill climber	1. Terrain-use indexes	Continuous	Probably	[8]
Beef cattle	1. Dominant 2. Subordinate	1. Dominance	Continuous	No	[40]
Beef cattle	1. Fast-eater 2. Slow-eater	1. Supplement intake rate	Continuous	Probably	[37]
Beef cattle	1. Bottom dweller 2. Hill climber	1. Terrain-use indexes	Continuous	Yes	[41]
Beef cattle	1. Bottom dweller 2. Hill climber	1. Terrain-use indexes	Continuous	Yes	[42, 43]
Beef cattle	1. Favourable distribution 2. Unfavourable distribution	1. Terrain-use indexes	Categorical (dichotomous) and continuous	Yes	[44]
Beef cattle	1. Highly exploratory/ bold 2. Slow-exploratory/shy	1. Response to novel object	Categorical (dichotomous) and continuous	No discussed	[45]

Continuation of Table 1.1. List of examples used in this review...

Species	Behavioural types	Behavioural categorizing criteria	Continuous and categorical variables	Genetically explained	Reference
Beef cattle	1. Low residual 2. High-residual	1. Residual feed intake	Continuous	Yes	[16]
Highland beef cattle	1. Initiator 2. Follower	1. Leadership 2. Dominance			[46]
Multiple species (mice, rats)	1. High-aggressive 2. Low (medium)-aggressive	1. Aggressiveness	Categorical (dichotomous) and continuous	Yes	[47]
Multiple species (simulated individual foragers)	1a. Leader 2a. Trailer 1b. Speeder 2b. Laggards	1. Walking speed 2. Accelerations to conspecifics 3. Length of decision zones 4. Sense of orientation	Categorical (dichotomous) and continuous	No discussed	[48]
Sheep	No specified	1. Sagebush consumption/dietary selection	Continuous	Yes	[10]
Sheep	1. Bold 2. Shy	1. Shyness-boldness	Categorical (dichotomous) and continuous	No discussed	[49]
Sheep	1. Bold 2. Shy	1. Shyness-boldness	Categorical (dichotomous) and continuous	No discussed	[50]
Beef cattle	No specified	Consumption of several species of grasses and forbs	Continuous	Yes	[11]
Beef cattle (Nellore)	No specified	1. Crush score 2. Flight speed 3. Movement score 4. Temperament score	Categorical (nominal) and continuous	Yes	[17]

Continuation of Table 1.1. List of examples used in this review...

Species	Behavioural types	Behavioural categorizing criteria	Continuous and categorical variables	Genetically explained	Reference
Deer	Several combinations of multiple dimensions	1. Boldness 2. Dominance 3. Flexibility	Categorical (nominal) and continuous	No discussed	[51]
Multiple species (foragers) with whole spectrum of personality types	1. Superficial explorer/bold/aggressive 2. Thorough explorer/shy/non-aggressive	1. Exploration strategy 2. Boldness 3. Aggressiveness	Categorical (dichotomous) and continuous	Yes	[52]
Multiple species (cattle, horses, pigs)	1. Proactive/bold 2. Reactive/docile 3. Fifteen combinations of three-dimensions personalities	1. Coping style 2. Emotionality 3. Sociality	Categorical (dichotomous and nominal) and continuous	Yes	[2]
Multiple species of foragers with whole spectrum of personality types)	1. Fast-explorer 2. Slow-explorer 3. Home range size and structure 4. Aggressiveness	1. Area-restricted search (fractal movement) 2. Sense of direction	Categorical (dichotomous) and continuous	No discussed	[53]
Multiple species (African elephant, Galapagos tortoises, mule deer)	1. Central place foraging 2. Migration 3. Nomadism	1. Node-level (local) metrics 2. Graph-level (system) metrics	Continuous	No discussed	[31]

1.2.1 The genetics of behaviour and grazing-related genes

Van Oers and Sinn [6] undertook a meta-analysis to quantify the heritability of animal personality in wild, captive and domesticated populations. The statistical meta-analysis included 209 estimates of heritability on 14 taxonomic groups such as Ruminantia, Equidae, Canidae and Hymenoptera, just to mention a few. The authors reported an average heritability of 0.26 for animal personality traits with a cumulative size effect ($E = 0.18$) significantly different from zero. The average heritability in wild populations was 0.36 and in domesticated populations was 0.24, and unweighted heritability estimates for exploration behaviour were 0.58 and 0.21, respectively. These authors concluded that selection of animals based on their personality could be expected in wild populations.

Another meta-analysis of behavioural studies on non-human animals reported estimates of heritability and repeatability of animal personalities [54]. After screening 306 relevant articles, they selected 10 research studies and 71 pairs of estimates for analysis. Their analyses suggested that the repeatability of behavioural responses has a substantive genetic component, with the study revealing that 52% of the phenotypic variation in general behaviours such as aggression, antipredator, foraging, parental effort and mating, was attributable to additive genetic variation (i.e., genotypic personality in the GP-model). The authors also reported a greater and large mean heritability for animal personality (0.52) than for behavioural variation (0.14). Animal personality heritability being inclusive of additive genetic variation, dominance genetic variation and permanent environmental effects, while behavioural variation includes in addition the temporal environmental effects. If genetic dominance (i.e., non-additive genetics) plays a minor role in determining animal personality [54], then one can potentially attribute about half of personality variation to the effects of the social and biophysical environment (e.g., parental care and vegetation characteristics, respectively) and to epigenetics. In the GP-model, these effects are referred to as the regulatory systems and the personality plasticity. However, it remains unclear how much of the non-additive genetics (i.e., allelic interactions at the same locus [dominance] or at different loci [epistasis]) can explain the phenotypic behavioural variation [55]. A summary of the above studies reported that non-additive effects could be as significant as the additive effects in explaining several dimensions of human personalities [56].

Results of Dochtermann's et al. [54] meta-analysis are promising but provisional and need to be taken with caution. They also reported that foraging behaviours had a much weaker genetic component (< 0.2) than aggression and antipredator behaviours (up to 0.6).

Recent studies supported the premise of grazing personality being under genetic control. Howery and Bailey [44] described both genome regions and gene markers associated with grazing distribution patterns in beef cattle. As an example, using collared cows ($n = 87$) that carried global positioning systems (GPS), Bailey et al. [41] investigated the association of several quantitative trait locus (QTL) and genetic markers with the phenotypic variation of grazing patterns of cattle displayed along gradients of steep-sloping terrain, elevation and distance to water sources. These cows were grazed in mountainous and extensive grasslands at five ranches in New Mexico, Arizona and Montana in the United States of America (USA). A high-density single nucleotide polymorphism (SNP) array was used to genotype deoxyribonucleic acid (DNA) samples from these cows. The study then ascertained whether associations existed between variation in the SNP markers and variation in grazing distribution based on indexes of terrain use. Two QTLs overlaying the glutamate metabotropic receptor 5 gene (*GRM5*) accounted for up to 24% of the phenotypic variation in the use of vegetation patches on steep slopes and at high elevations, while another QTL overlaying the mastermind-like 3 gene (*MAML3*) accounted for 23% of the phenotypic variation (Fig. 1.1). These genes have been reported to be involved in locomotion, motivation, and spatial memory as well as in the regulation of neurogenesis, myogenesis, vasculogenesis and other aspects of organogenesis.

Studies conducted by Pierce et al. [42, 43] validated the previously reported genotype-phenotype associations between specific SNPs overlaying grazing genes and indexes of terrain use [41]. While these results are promising and point towards the possible integration of grazing personality into selection programs, Howery and Bailey [44] suggested these studies need to be replicated and/or extended to larger number of animals of different origin and which are grazed in diverse environments, if robust and conclusive conclusions are to be reached. For example, the extended study of Pierce et al. [57] including 330 beef cows from 14 ranches in the western USA reported limited genotype-phenotype associations and pointed towards different candidate genes.

There are two outstanding explanations for the correlation of behavioural traits defining grazing personalities. The first one is pleiotropy, in which one gene could act on two or more traits, which further determine the displayed grazing patterns. If pleiotropy occurs, one single gene would effectively control several traits simultaneously. For example, phenotypic studies corroborated the correlation of distinctive grazing patterns (e.g., fast-explorer cows), growth rates and boldness within relatively small groups of cattle (i.e., 16 and 33 individuals in Gregorini et al. [16] and Wesley et al. [37], respectively).

Kern et al. [58] suggested that pleiotropic effects could explain the correlations between personality, morphological and performance traits on zebrafish (*Danio rerio*), but also did not confirm this possibility. A study with bighorn sheep from Ram Mountain, Alberta, Canada could not find pleiotropic effects at major locus because of the lack of genome-wide QTL overlap on genes related to docility and boldness [59]. Instead, the authors concluded that small pleiotropic effects could have been missed and therefore, results did not confirm pleiotropy. Future studies might give insights of pleiotropy controlling grazing personality traits.

The second explanation for the correlation of traits as observed in grazing personalities is because of a non-random association of alleles at different loci that produce a combination of traits that confers advantage under a specific set of biophysical and social conditions, as is the case of linkage disequilibrium. For example, individuals with certain association of alleles tend to achieve higher reproduction rates than individuals with a different combination of traits. Such allele associations become common and more frequent in a population than other combinations, although traits are controlled by alleles at different loci [6].

Studies suggested that genetic variation might explain different eating tactics linked to distinctive grazing behaviours exhibited in groups of beef heifers [37] and dairy cows [16]. For the latter example, Davis et al. [60] previously confirmed the different genetic basis found on nearly 200 dairy cows that, within a large herd of 3,359 milking cows, displayed extreme residual feed intakes. These genetically tested cows were mother dams of the sixteen calves later on used on Gregorini's et al. [16] research. Future research on grazing personalities and its genetic variation might help to elucidate whether grazing traits are correlated because of genetic pleiotropy, or because of a linkage disequilibrium between grazing traits, or because of both mechanisms acting simultaneously. Both, pleiotropy mechanisms as well as linkage disequilibrium were represented in the hierarchical conceptual model for "Organization of Behavioural Traits" [5] and have implications for the regulation and expression of grazing personalities.

The discovery of genetic associations with grazing personalities and thus the identification of specific grazing genes has the potential to assist in breeding programs. However, despite the high heritability of grazing patterns found in cattle, there are other factors controlling them. For example, interactions with the social herd environment (e.g., parental and familial effects), the biophysical environment, and the emotional state as well as the large number of range management practices that influences such interactions. In the next section, we discuss whether non-genetic factors can modulate the expression of grazing genes and if such effects over gene expression are transferable to offspring.

1.2.2 The effect of personality plasticity and regulatory systems on grazing patterns

The section 1.2.1 The genetics of behaviour and grazing related genes, and the section 1.3.1 Gene pools and allele frequencies focused on alleles of grazing genes, their variation and frequency at two levels, individual and collective, respectively. In the GP-model, allele attributes of grazing genes are the ultimate determinants of grazing personalities. These attributes constitute the individual and collective genomes respectively and account for the specific sequence of nucleobases of each gene; that is the genome code. The gene products expressed into RNA and subsequent amino acids and proteins are the ones executing the observed phenotypic traits, such as behavioural traits. In this section, we focus on gene expression and regulatory systems that modulate the expression of behavioural genes related to grazing personalities. Here, we present the ontogeny, the epigenetic inheritance system, and the animal emotional state as the main modulators of behavioural gene expression. These three components of the GP-model create the interface between the genomic determination of grazing personalities and the external and internal stimuli that modulate its gene expression. The expression of grazing genes is variable and responds to changing environmental conditions and emotional states; regulatory systems modulating the gene expression and thus conferring the personality plasticity of the GP-model.

Regulatory systems are an integral part of the pathways between grazing genes and the observed grazing personalities. In the GP-model, grazing personality pathways originate from specific alleles of grazing genes and result in specific phenotypic grazing patterns. Grazing personality pathways involve hierarchical levels of intermediate and concatenated traits with multiple mechanisms that consistently respond to external and internal stimuli modulating the observed grazing patterns. The "organization of behavioural traits" [5] conceptualised genes-neurophysiology-behavioural pathways in a hierarchical model where a few genes are involved in determining a few neurological, physiological and morphological traits. These neurological, physiological and morphological traits further shape the expression of a number of behavioural traits that ultimately result in biological functions, such as herbivore grazing patterns. As the gene expression of intermediate traits is variable in response to stimuli, each adjusted response of intermediate traits is added up and further transferred along pathways of grazing personality [5].

The variable expression of grazing genes modulated by regulatory systems is referred to as grazing personality plasticity. The reaction norm of behavioural traits are examples of behavioural trait plasticity changing along environmental gradients [61]. As the phenotypic response along environmental gradients differs from one individual to another, the grazing personality plasticity might be a trait by itself and even have its own heritability [62]. However, even if environmental conditions stay unchanged, the behaviour of an individual changes as it ages, which is known as ontogeny, and that leads to behavioral development [63].

Ontogeny

Here we discuss two aspects of animal ontogeny related to grazing personalities. Firstly, the ontogeny itself and the changes in behaviour observed in animals over their lifetime. Grazing personalities are consistently observed across situations and over time. However, the behaviour of an animal changes along its behavioural development or maturation. For example, Van Moorter et al. [64] conducted a study at contrasting locations in France to compare the exploration behaviour of yearling (8 to 15 months old) roe deer (*Capreolus capreolus*) prior to the settlement phase of dispersal against the exploration of adult individuals ($>$ 2 year old). Young roe deer had larger exploration behaviour than adult deer. The results proved that yearling roe deer leave their natal home range and display a period of exploration in spring and summer as part of their natural maturation process. Adult individuals settle down later in life and explore smaller areas. The example above shows that individuals display changes in grazing behaviours along their ontogenic development. Furthermore, within behavioural development phases, behavioural differences among animals are maintained from early life and along their lifetime. Finally, behavioural differences among individuals detected early in life can be used as predictors for divergent grazing personalities displayed at mature life phases.

The second aspect regarding animal ontogeny affecting grazing personalities is the importance of environments and emotional states experienced early in life (including experiences of predecessors in preconception) to influence the gene expression of behavioural and personality traits. Maternal effects early in life that induce changes in gene expression and thereby of phenotypic behaviour have been documented in birds. For example, wild females of the altricial canary (*Serinus domesticus*) regulates the use of androgens when laying eggs in a way that late born chicks have higher levels of testosterone [65]. Thus, chicks from late laid eggs showed faster embryonic development, increased muscular development and more begging behaviour than chicks of early laid eggs. All these traits made the younger chicks of the clutch to be more competitive than earlier born chicks. Different hormonal environment experienced early in life can induce changes in the expression of genes controlling physiological and behavioural traits, conferring a social hierarchy, which is maintained later during adulthood [65]. To our knowledge, no study had documented changes of gene expression due to early-in-life experiences in large herbivores (but see study of Candemir et al. [66] with mice).

In the following paragraphs, we explain and exemplify how adaptive responses to early life experiences are determinant in shaping the gene expression of an individual and how such responses can be inherited epigenetically.

Epigenetics

The epigenetic inheritance system of the GP-model is a set of mechanisms that modifies DNA arrangement and that affects the expression of genes related to grazing personalities without causing alterations to the nucleotide sequence. Epigenetic mechanisms stimulate, discourage or inhibit the expression of genes through DNA folding and transcriptional activities. Most known epigenetic mechanisms are DNA methylation and histone alterations [67]. Such mechanisms mediate the interface between the genomic control over grazing behaviours, and responses to stimuli such as the social and biophysical environments and the emotional state of animals. Adaptive and maladaptive responses to stimuli are reflected in the phenotypic grazing personality of individuals that undergo changes to their epigenetic state and thus modulate their gene expression. Thus, alterable epigenomes – i.e., facilitated epigenotypes (probabilistically controlled by the genotype) and pure epigenotypes (not controlled by the genotype outside the affected locus) – depend on stimuli signals and is modified according to each individual's experiences [68]. The transgenerational epigenetic inheritance is the transference to offspring and following generations of adopted epigenetic states in response to stimuli. Steroid hormones mediate a particular case of epigenetic modifications in response to stress [68]. The study conducted by Howery et al. [9] in an extensive grazing allotment in Idaho (USA) reported that the majority of individual beef cows (78%) showed high-fidelity to home range and habitats, returning to these feeding areas in consecutive years. The study was carried on for another four years (1990-1993) to test if offspring and cross-fostered offspring maintained fidelity to the home range and habitats where they were reared and whether grazing behaviour of dams and foster dams influenced their grazing behaviour [38]. These authors reported that home range and habitat fidelity was displayed by dams and foster dams as well as by yearlings and cross-fostered yearlings. They concluded that grazing behaviours experienced early in life conditioned the behaviour in adulthood, and this was observed independently from yearlings being reared by their dams or by foster dams. Habitat fidelity decreased however with a severed drought and in response to the grazing behaviour of other peers. These studies showed that grazing behaviour was consistent over time and it was transferred to the progeny and foster-progeny. While parental effects of dams and foster-dams were corroborated, at that time, genetic heritability of grazing behaviour was not tested and remained unknown. The grazing behaviour of dams and yearlings was affected by a severe drought in 1992, which illustrates the plasticity of grazing behaviours responding to changing biophysical environment. Parental effects and peer effects modulated the grazing patterns of yearlings accordingly to the social herd environment experienced early and in subsequent stages of life. Howery and Bailey [44] attributed these results to a combination of nature (genetic) and nurture (learned), although, the latter could also be attributed to epigenetic inheritance. In the following section, we present examples showing how the emotional state of animals can induce changes on the expression of behavioural genes.

Emotional operating system

Conscious and unconscious internal states of the brain dictate the mental well-being of mammals. While fulfilling their physiological needs, animals can react to external and internal stimuli to attempt to minimize negative emotions and to seek positive emotions [69]. For example, grazing actions and reactions of ruminants and foragers in general are conditioned by their current emotional state, past experiences and expectations [70, 71]; referred to as cognitive mechanisms in the GP-model. Emotions modulate the expression of grazing genes through epigenetic states (inheritable emotional states) and/or affect the observed grazing behaviours directly (i.e., see the two arrows of emotional state in Fig. 1.1). For instance, domestic chickens (*Gallus gallus*), under a social environment of intermittent isolation early in life developed a lowered response to corticosterone, which restrained stress [72]. Using microarrays immediately after the treatment, treated chickens upregulated the function of stress genes. Later in life, chickens treated with social isolation displayed a decreased reactivity of the hypothalamic-pituitary-adrenal axis, increased growth and improved associative learning in comparison with chickens that did not undergo the social isolation. The study provided evidence of transgenerational inheritance triggered by the chickens' emotional state. The emotions and the emotional state of animals affected their immediate behaviours; also experiences early in life might have underpin lifetime "conditioning" that altered the epigenetic environment of specific genes. Such effect was transferred to the progeny. Negative and positive emotions may affect (non-heritable) and modulate (heritable) the behaviour of animals. For example, among these emotions, stress has been studied extensively because of the relevance to animal welfare, health and fitness. As individual animals display different coping styles while facing stressful situations, their emotions, emotional state, and ultimately their welfare, depends upon their individual personalities [47].

1.2.3 Grazing traits of individuals

On the one hand, quantitative and continuous traits are commonly used to describe grazing behaviours along continuum gradients [41, 32]. On the other, grazing personalities as categorical attributes of consistent behaviours may emerge because of the existence of trade-offs among correlated traits. Thus, animals may adopt contrasting strategies [53] such as the contrasting proactive and reactive personalities, *sensu* 'life-history theory' [52] or the fast and slow metabolisms, *sensu* 'pace-of-life syndromes' [73].

Behavioural studies on foraging animals are commonly limited to describe two types of grazing animals, which account for the extreme behaviours observed at the opposite ends of a continuum axis. For example, the residual feed intake was estimated for nearly two thousand dairy cows and a continuous gradient of this parameter was obtained. Then, individuals displaying the lowest and highest residual feed intake within this gradient were selected for further research (i.e., 183 and 16 selected individuals in Macdonald et al. [74] and Gregorini et al. [16], respectively). Similarly, animals of several species have been classified into two contrasting types (Table 1.1). For example, ruminants have been categorised as either riparian or uplands users [9, 38], bold or shy explorers [50, 49], bottom-dwellers or hill-climbers [8, 41, 42, 43].

Alternatively, a diverse range of discrete personalities can be depicted by integrating multiple behavioral 'dimensions' (e.g., grazing traits) to describe and classify animals that show distinctive behaviors [22, 75]. A multi-dimensional approach applied to grazing behaviours allows the conceptualization (and description) of consistent movement patterns both within species and across species. For example, studies have investigated a large diversity of foraging species and thus clustered individuals into four major types of so-called movement syndromes [29], movement strategies [30], or functional movement classes [31]. These studies included, thirteen species of several vertebrate taxa of herbivores and carnivores [29]; large herbivores such as the African elephant (*Loxodonta africana*), giant Galapagos tortoise (*Chelonoidis spp.*) and mule deer (*Odocoileus hemionus*) [30]); and 92 species of marine life with feeding habits of carnivorous, zooplankton and algae feeders [31]. The four movement types of these three studies were described and similarly named as: centered home-range, territorialists, nomads and migrants under movement syndromes [29]; as resident, multi-patch, nomadic and migration under movement strategies [30]); and as resident, occasional, irruptor and roamer under functional movement classes[31]). The studies found four common movement patterns across several taxa that have different modes of movement (e.g., terrestrial locomotion, swimming, flying) and different feeding habits. For example, there were herbivores (e.g., African elephant, plains zebra [*Equus quagga*], springbok [*Antidorcas marsupialis*], mule deer and several algae feeding marine species) and carnivores (e.g., African wild dog [*Lycaon pictus*] and several fish feeding marine species). Furthermore, the authors observed these common movement patterns consistently across situations and over time, a condition for behavioural personalities. We anticipate that grazing ruminants and other large herbivores consistently display such common grazing patterns within herds, populations and species and even across species (i.e., regardless of species identity).

Finally, another alternative would be if grazing patterns and behaviours of grazing ruminants and other large herbivores are displayed as normally distributed variables and genetically independent traits that show no phenotypic correlations [39, 76]. In such a case, conceptualizing categorical grazing personalities might be challenging or even inappropriate.

1.3 Collective grazing personalities

> '[The social environment] interactions have a lifelong influence on what an animal eats and where it goes [. . .]. In herbivores, social organization leads to culture, which is the collective knowledge and habits acquired and passed from generation to generation about how to survive in a particular environment' [77].

In this section, we focused on the collective grazing personalities of the GP-model (Fig. 1.1, right side): from collective genotypes (at the top), through regulatory systems modulating plastic responses (middle), to phenotypic grazing personalities as observed in herds of grazing ruminants and other large herbivores (at the bottom). In section 1.3.1 Gene pools and allele frequencies, we hypothesize that the allelic variation and frequency of grazing genes determine the emergence of grazing personalities at collective level. Section 1.3.2 The social environment of the herd describes the social environment of herds as the main regulatory system that shapes grazing personalities at collective level. In section 1.3.3 Grazing traits of herds, we present examples and discuss the emergence of collective grazing patterns as consistently observed across contexts and over time.

1.3.1 Gene pools and allele frequencies

The existence of divergent grazing personalities among individuals and the coexistence of divergent personalities within populations (so-called behavioural polymorphic populations) are both products of evolutionary processes. Selection acts over phenotype through differential fitness (e.g., individuals achieving different rates of survival and reproduction), which is then reflected in the gene pool of the group [7, 52]. Animals that achieve longer lives, and/or greater reproduction rates under certain social and biophysical conditions, will produce more offspring. In this way, outperforming phenotypes with greater fitness get larger representation within the herd, making their alleles more common in the gene pool. Inversely, phenotypes with lower fitness are less represented in the population and in turn, their alleles become less common. Changes in social or biophysical environments may affect the fitness of distinct grazing personalities and lead, over generations, to changes in the allele frequencies of genes. Despite their lower performance, low fitness phenotypes still reproduce and therefore, their genes are maintained [78]. Mating success of behaviourally distinct individuals would influences the allele frequencies of the population. Populations may have different behavioural morphs that exist at specific ratios. Here are two examples.

Lampert et al. [79] reported genetic associations with divergent behavioural strategies of mating in panuco swordtail fish (*Xiphophorus nigrensis*). Divergent mating-strategy and morphs of panuco swordtail fish are genetically associated with specific alleles and therefore, these populations seems to be genetically and phenotypically polymorphic. The small male morphs have relatively smaller swords, have a female appearance and are less ornate than large males, which are gifted with larger swordtails and are much more decorated. Females prefer mating with large males, which are territorial and court them. The apparent reproductive disadvantage of small swordtail fish morphs does not stop them mating, and instead of undertaking courtship, small males chase and force females to copulate. By adopting a different behavioural mating strategy, small fish morphs successfully passes their genes ensuring the persistence of this morpho-behavioural phenotype. In the second example, Pruitt and Goodnight [80] reported that natural populations of communal spider (*Anelosimus studiosus*) have behavioural polymorphic individuals labeled as aggressive and docile. Populations of spiders growing under contrasting environmental conditions such as high and low availability of resources have different ratios of the aggressive to docile phenotypes. The phenotype ratio largely explained the reproductive success of the colony and determined the behavioural attributes of the colony. The authors concluded that aggressive:docile behavioural ratio would ensure long-term survival at the collective level. The phenotype ratio was site-specific and was the result of a collective-driven selection. On artificially made populations, switches of the phenotype ratio towards the ratio of spiders' origin (and regardless of the environmental conditions i.e., maladaptive responses) can be attributed to collectively controlled inheritance.

To our knowledge, there have not been any studies looking at genotypic diversity, composition and relative frequency of grazing genes in ruminant herds. Since the very beginning of animal domestication, herders are selecting individual animals by their behaviour (e.g., docility). It was only in the last 30 years that scientists started to recommend culling individual animals that display undesired grazing patterns [81, 9]. Certainly, the behavioural selection conducted in the past over domesticated herbivores has shaped the gene pools of present-day herds. However, it is unknown how this selection has affected their grazing patterns. Similarly, environmental changes, such as fragmentation of natural ecosystems, limited animal migration or selective hunting, has affected the gene pools and relative frequency of grazing genes of herds of wild animals and in that way, may have modified their collective grazing personalities. This has been exemplified by the selective capturing of fish with nets over wild fish populations [82]. As seen with the artificially-made colonies of communal spiders [80], we speculate that the ratio of genotypic grazing personalities within a herd of ruminants might be regulated collectively to ensure long-term survival of the group. As the ratio of genotypes within a herd might be site specific, it is possible to speculate that such collective traits are inherited epigenetically.

The recent discovery of nucleotide variation in grazing genes and their association with the grazing patterns of individual animals opens the opportunity to search for an ideal grazer; one that displays the "best" grazing personality [41, 42, 43, 57]. However, large herbivores do not graze alone but in herds of interacting animals, where individuals display a range of distinct grazing personalities that shapes the grazing personality of the herd. In this way, herds have unique attributes of grazing behaviour (see section 1.3.3 Grazing traits of herds). At collective level, genetically similar herds may display different personalities because of the plastic expressions of grazing patterns. This is discussed in the following section.

1.3.2 The social environment of the herd

The interactions among conspecifics constitutes the social environment of herds. Such interactions establish the social status occupied and the behaviours adopted by each individual. For example, the roles of leader and follower [46], dominant and submissive [40], and producer and scrounger [83], are extensively documented in ruminants, birds and other foraging species. Socially responsive individuals adjust their behaviours according to the social context and within the limits of their personality plasticity [84]. Thus, the social herd environment is a major factor of behavioural variation that affects the phenotypic expression of grazing personality and its plasticity at the individual and collective level [61]. In section 1.2.2 The effect of personality plasticity and regulatory systems on grazing patterns, we provided examples of how the social environment (e.g., social isolation and parental care) affects the behaviour of individuals. Similarly, the emergence of socially central individuals (e.g., leader and dominant animals) conditions collective grazing behaviours. For example, in Highland cattle (*Bos taurus*), Sueur et al. [46] reported that castrated mature males provided leadership and promoted group cohesiveness to juvenile cattle. These authors suggested using trained matured castrated males to increase grazing intensity of targeted areas. In another experiment with groups of fallow deer (*Dama dama*), Stutz et al. [85] showed that high aggregation and cohesiveness working towards increasing safety against predators have reduced the individual and collective exploitation of preferred and more nutritious diets. Thus, the collective perceived risk of predators influences collective exploration and utilization of feed sources. Another way to study the effects of collective behaviours is by replacing (or removing) socially central individuals. Vital and Martins [86] removed the key individuals from a group of zebrafish (*Danio rerio*) and reported reduced learning of foraging skills. In bottlenose dolphins (*Tursiops truncatus*) the presence of certain individuals was crucial to maintain interactions between subgroups [87]. However, in beef cattle the effects of socially central individuals might be only relevant in small size herds, for example < 40 individuals, where the fidelity of individuals to the group they belong to is relatively high; on the contrary, social bonds in larger herds are expected to be weaker [88, 89]. In the collective context of colony living organisms, the social environment is crucial for the survival and fitness of the group as well as for the relative success of each individual [1]; to a certain extent, this is also the case for collective grazers such as grazing ruminants and other large herbivores.

The GP-model establishes that stimuli from the social and biophysical environments and the emotional state of animal affects the displayed grazing personalities of individuals, which in turn are transferred to the grazing patterns displayed collectively (see section 1.2.2 The effect of personality plasticity and regulatory systems on grazing patterns). Similarly to the case of individuals, the social environment of the herd might influence the gene expression of collective grazing traits and therefore modulate the phenotypic grazing personalities as observed collectively. However, until now, it is unknown whether there are genes controlling collective behavioural traits in ruminants and, if so, whether the social environment controls its expression. A combination of social learning and a segregation of leader and followers could also explain collective behaviours [86]. We posed these unresolved aspects using question marks in the GP-model (Fig. 1.1). In the next section, however, there are examples of grazing traits measured at collective level.

1.3.3 Grazing traits of herds

Based on behavioural genetics, Gross [90] described three main pathways to explain phenotypic polymorphism of behaviour displayed by individuals within animal populations. Firstly, the so-called "alternative pathway" which considers a frequency-dependent selection of animals that maintains genetically polymorphic populations with individuals displaying behavioural polymorphism and achieving similar fitness. Secondly, the "mixed pathway" occurs in genetically monomorphic populations with individuals displaying mixed behavioural tactics. Finally, the so-called "conditional pathway" occurring in genetically monomorphic populations where individuals display a set of behavioural tactics according to state-dependent conditioning. For the GP-model and for any study of grazing herds in general, it is crucial to bear in mind that herds of ruminants are phenotypically behaviourally polymorphic. Within a herd of ruminants, individuals coexist displaying a range of distinctive grazing personalities. While the alternative pathway attributes the phenotypic behavioural polymorphism to genotype variation (i.e., personality genotype in the GP-model), mixed pathways and conditional pathways apply to populations comprised by genetically monomorphic individuals. As previously presented in section 1.2.2 The effect of personality plasticity and regulatory systems on grazing patterns, the personality plasticity at collective level accounts for the variable gene expression and therefore, different phenotypic outcomes from genetically identical individuals may take place. We hypothesize that the mixed pathway may correspond to variations attributable to the epigenetic system (heritable), and that the conditional pathways may correspond to direct effects over the emotional state. For the previous, adopted behaviours might be transferred to offspring and therefore show transgenerational epigenetic inheritance; for the latter, behavioural polymorphism may be observable only in the animals that adopted such behaviour as a direct response to their emotional states.

We set the GP-model using an individual-based approach of grazing personalities to explain distributional grazing patterns as observed in real herds of ruminants. Gueron et al. [48] presented a model that simulated distributional patterns of grazing herds based on a set of behavioural traits that were applied to individual agents. The authors applied a hierarchical decision-making algorithm, with rules-of-thumb establishing individual sensitiveness to crowding and attraction to conspecifics that applied respectively according to a repulsion zone (animals getting too close), an attraction zone (animals getting too far) and an intermediate buffer zone called neutral zone without response. Simulations were ran for a thousand time-steps of individuals that displayed different behavioural traits, such as walking speed and sense of orientation towards a targeted direction. Gueron's model showed differences in herds distribution and fragmentation as it happens in real herds. The model showed that integrating behavioural, physiological and individual decision-making traits could reproduce attributes of interacting 'grazing' animals. From individual differences in grazing traits emerged collective behaviours of herd fragmentation and distributional patterns.

Gueron's [48] mechanistic simulations were later tested and validated in a similar model using groups of sheep of variable number (two, four, six or eight sheep) of either exclusively bold individuals or exclusively shy individuals [91]. In support of individual-based approaches, the findings of these authors showed that the grazing patterns observed in interacting animals derive from individual behavioural traits and interaction rules; however, behavioural traits at the group level, such as the strength of social attraction, seems to control emergent decision-making mechanisms at collective level. A further step to better simulate grazing herds was achieved by Spiegel et al. [53]. These authors simulated grazing agents with divergent movement traits in variable contexts of vegetation patchiness. With some similarity to the simulations done by Gueron et al. [48], Spiegel and colleagues allocated divergent behavioural traits to grouped individuals 'grazing' along increasing levels of vegetation patchiness, from uniformly distributed, through randomly distributed resources, to discrete patches of nine different vegetation resources. Comparing divergent personalities such as slow and fast explorers, these authors concluded that under low patchiness, fast explorers would achieve higher foraging efficiency than slow explorers. This would be reversed however in grazing lands with discrete vegetation patches. Such results are consistent with real experiments in dairy cows [16]. Spiegel's et al. [53] scenarios showed that seasonal dynamics of vegetation would alternatively benefit one or another grazing personality at different times of the year, highlighting the temporal variation of animal performance in support of the existence of herds with behavioural polymorphism. Finally, these authors pointed out the emergence of a complex group-level structure displaying collective grazing patterns with its own attributes (e.g., clustering of similar phenotypes, home range size and structure), which changed along environmental gradients (e.g., vegetation patchiness). Interestingly, individual-based simulation models set behavioural rules and traits to be repeated over time (i.e., 1,000 and 2,000 time steps in Gueron et al. [48] and Spiegel et al. [53], respectively) and even across different contexts such as a gradient of vegetation patchiness [53]. By allocating different values of behavioural traits to individuals that coexist and interact with each other, simulation models recreated real ruminant herds as mixed behaviours displayed consistently over time and across situations; therefore complying with conditions of grazing personalities used in the GP-model.

Individuals displaying divergent personalities comprise herds of ruminants, which are recognised and described as extended families that maintain cohesiveness and display unique identities [77]. So, how can we characterize and compare the unique identities of ruminant herds (i.e., collective grazing personalities)? One way to value behaviours at collective level is by using grazing traits measured in individuals while performing within the herd and by integrating these individual values into an averaged and/or weighted value. Additionally, the statistical dispersion of behavioural traits (e.g., coefficient of variation) within herds can be used for comparisons among herds. To our knowledge, there are not many studies with such examples. Partially, this might be because of the challenge of measuring grazing behaviours in all members of the herd while grazing as a herd. However, this might be also because of the lack of conceptualizing collective measurements of grazing behaviours, although, this has been proposed for other social living animals such as foraging insects [1]. Sueur et al. [46] studied leadership within four Highland cattle groups (groups ranging from 8 to 21 individuals), but did not compare collective behaviours among groups. Rudin et al. [92] compared behavioural traits on two groups of over 500 Australian field crickets (*Telogryllus oceanicus*) growing under contrasting social environments of 'silent' or 'signing' individuals. Based on statistical differences in the mean value and standard error on distance travelled and speed measured in individuals, these authors concluded that the social environment significantly affected 'the repeatable aspect of behaviour (i.e., personality)', and that behavioral changes were heritable. However, Rudin et al. [92] measured traits in individuals pulled apart from the group rather than on individuals performing within a group. Several studies in the past compared distinct behaviours displayed in ruminants [41, 16, 37] and authors commonly conclude that "individuals" pertaining to a certain group behave differently to "individuals" pertaining to another group rather than assessing collective behaviours. We advocate for comparisons of different groups that display collective grazing personalities with their unique attributes.

Another way applicable to certain scenarios and for certain traits is by representing collective grazing behaviours with monitoring the behaviour of one or a few animals of the herd. For example, Liao et al. [32] studied the grazing behaviour of twenty herds of beef cattle in five different study sites of Southern Ethiopia. These authors derived collective behavioural traits such as herd allocation of time to travel, grazing and resting along the day by averaging the behaviour measured in three cows of each herd with global position system collars. Pastoral people herded their animals to daily foraging areas and brought them back to their camps for overnight. The herd was moved as a relatively compact group, thus, monitoring of any three cows of each herd would be sufficient to provide comparative information among herds. These authors reported different daily patterns of grazing behaviour of monitored herds and provided insights on the different foraging habitats used by different herds with details on greenness, elevation and terrain slope.

Here, we mention attributes of ruminant herds and grazing traits relevant to collective grazing personalities. For example, home range was defined as the spatial expression of behaviours [that individual] animals perform to survive and reproduce [93] in a defined timescale [94]. Thus, a certain number of individuals that comprise a herd occupies, needs or is allocated to an area with features of size, shape and biophysical conditions. Similarly, one could compute the area utilised by a herd, for example, on a daily basis. Fragmentation [48], cohesiveness [95] and assortativity [96, 53] are examples of group-level traits that in a future can be used to study collective and individual grazing attributes as well as the impact of grazing herds to ecosystem functions of grazing lands or to animal welfare. For example, Foister et al. [97] used phenotypic attributes of social interactions measured at group-level (i.e., social network properties) to predict consistent aggressive events (i.e., a personality dimension) among pigs reared as a group in pens. In beef cattle, the centrality of individuals as a specific collective measurement rather than the number of individuals determined the group composition and affected the social stability and stress of the herd [98].

1.4 Illustrations and implications

Figure 1.1 presents a hypothetical example with individuals (left side) differing in the allelic variations of two grazing genes, which comprise a herd of ruminants (right side). Grazing personality pathways between an individual's genotype ($\mathrm{I_x}$) and its displayed grazing personality ($\mathrm{iGP_x}$) involve several intermediate and concatenated traits, which have a regulatory system of the gene expression. Following the GP-model, we described this example starting on the individual genotype (top left), going through stimuli that influence the expression of grazing genes (middle left) to yield in the phenotypic grazing personality of individual grazers (bottom left). As ruminants graze in herds, individual genotypes were aggregated into the collective gene pool (top right), then, we discuss the modulation of the gene expression at group level (middle right) and finally describe the collective personality of the herd.

1.4.1 Grazing personality genotype

Individuals with allelic variations I_1 and I_2 display shy grazing personalities named iGP_1 and iGP_2, respectively. Individuals with shy personalities occupy relatively small home ranges, stay at relatively short distances from one another and prefer grazing flat terrain in low altitude habitats. As personalities are phenotypically plastic, under certain conditions, iGP_1 and iGP_2 cannot be differentiated because of phenotypic overlap. Individuals with allelic variation I_3 are associated with animals displaying a bold grazing personality named iGP_3. Such herbivores show relatively large home ranges, they graze alone or at relatively large distance from one another and show grazing preference for steep slope terrain in high altitude habitats. Regardless of conditions, IGP_3 always display discernible grazing patterns from the previously described personalities. For example, iGP_1 and iGP_2 could be similar to bottom dweller cattle and, iGP_3 to hill climber cattle, which display divergent indexes of landscape use and exhibit divergent grazing patterns [8]. These cattle have genetic associations to gene markers overlaying the glutamate receptor 5 gene (*GRM5*) and the mastermind-like 3 gene (*MAML3*) [41]. In the example, these genes are represented with hexagon and triangle shapes in Fig. 1.1. For simplicity, only two of the five genes reported by Bailey et al. [41] are represented in the GP-model. Applying individual-based models, grazing patterns of herbivores can be simulated by using traits such as walking speed and sense of direction towards a preferred habitat and by applying variable responses to stimuli such as to vegetation patchiness, like variable walking acceleration or proximity to conspecifics [48, 91, 53]. In our example, iGP_1 and iGP_2 have equal allelic variation as I_1 *GRM5*[A] = I_2 *GRM5*[A]. This genotype determines animals to have low concentrations of blood cortisol that makes them to display low walking speed and travel relatively short distances [99, 100, 37]. For this example, we establish that *GRM5*[A] animals prefer grazing in flat terrains. Walking acceleration and attraction zone to conspecifics are also similar ($iGP_1 \approx iGP_2$) making them quickly accelerate towards conspecifics that get away and to do so at relatively short distances. These personalities differ in their allelic variation I_1 *MAML3*[T] $\neq$ I_2 *MAML3*[G], responsible of sense of orientation towards preferred areas. For example, *MAML3*[T] animals display a high sense of orientation and *MAML3*[G] express a low sense of orientation ($iGP_1 > iGP_2$). I_3 animals differ from both previous genotypes by having *GRM5*[G], which is phenotypically expressed with a high blood cortisol concentration. *GRM5*[G] animals display fast walking speed, and therefore I_3 animals travel relatively long distances. For this example, we establish that *GRM5*[G] animals prefer grazing in steep slope terrain in high altitudes. iGP_3 walking acceleration is low and attraction zone to conspecifics is long, therefore, iGP_3 individuals accelerate slowly towards conspecifics that get away and do so when conspecifics are relatively far away. iGP_3 has equal allelic variation to iGP_2 animals for the sense of orientation trait (I_2 *MAML3*[G] = I_3 *MAML3*[G]), therefore show low sense of orientation towards its preferred mountainous terrain.

In a herd of ruminants, allelic diversity is defined as the number of different alleles of a grazing gene

present when accounting for all individuals. Allelic composition refers to which alleles in particular are represented. Finally, relative allelic frequency refers to the proportion of each allelic variant of grazing genes. While these two previous attributes do not necessarily depend of the number of members but on their genotype, the latter, depends on combining the genotype of members and their proportional representation. Finally, the total size of the herd, at equal proportion of individual grazing personalities, affects the collective personality (not considered in this example). In our example in Fig. 1.1, two grazing genes, *GRM5* and *MAML3*, are shown in three grazing personalities I_1, I_2 and I_3 that comprise herd one (H_1). Each gene has two variants. Therefore, the allelic diversity for either of these genes in H_1 is two. The allelic composition of *GRM5* is Adenine and Guanine, while for *MAML3* is Thymine and Guanine. Note that the total existing allelic variation for these genes is much larger than in our example; Bailey et al. [41] reported four possible nucleobases (adenine, cytosine, thymine and guanine) at six different positions in *GRM5*, and the nucleobases thymine and guanine for *MAML3*. In Fig. 1.1, we did not specify the number of individuals of each genotype nor total number of individuals comprising the herd. However, we represented the relative allelic frequency of grazing genes *GRM5* and *MAML3* establishing equal number of individuals ($n = 10$) of each genotype. For example: If I_1 $n = 10$; I_2 $n = 10$; I_3 $n = 10$, then the relative allelic frequency in H_1 would be:
GRM5 x2[A]: x1[G]; *MAML3* x1[T]: x2[G].

1.4.2 Personality plasticity

Despite the differences in cortisol concentrations in blood in individual animals, which might be genetically controlled [101], it has also been revealed that it may be affected by other stimuli, such as during experiments involving social isolation (see Goerlich et al. [72] in section 1.2.2 The effect of personality plasticity and regulatory systems on grazing patterns). For example, regardless of an animal's genotype, the use of low-stress herding techniques might reduce cortisol concentration in the blood of ruminants and foster the use of targeted areas because of emotional state of lower predation risk, as compared to animals managed under 'traditional' herding techniques [102]. However, cortisol levels are believed to be affected by an animal's environment and its emotional state [103], which may result in variable behavioural responses observable phenotypically and despite genetic control. In our example, low concentrations of cortisol in blood is established to reduce walking speed and also daily travelled distance. We represented personality plasticity on the phenotype of the hypothetical individuals. In Fig. 1.1, iGP_1 and iGP_2 overlap each other and under certain conditions it will not be possible to distinguish them by simple phenotypic observation of behaviours. On the other end, iGP_3 is separated towards the right of the GP-model and representing therefore that differences in grazing personalities are phenotypically observable.

1.4.3 Grazing personality phenotype

The GP-model as shown in Fig. 1.1 represents genetically polymorphic individuals (i.e., individuals with different alleles) that comprise the collective gene pool and relative allelic frequency of grazing genes of a herd. Phenotypically, in such a herd coexist individuals that display distinct grazing personalities. iGP_1 and iGP_2 individuals display slow walking speed and travel short distances. As soon as conspecifics move away a relatively short distance, these individuals will accelerate and reduce distance to conspecifics. These grazing personalities prefer flat and low altitude habitat, where they graze more intensively and spend more time than on steep slopes located in high altitude habitats. iGP_1 individuals will return quicker and more often to vegetation patches of their preferred habitat than iGP_2, because of the lower sense of orientation of the latter. Therefore, iGP_1 tends to utilize its preferred habitat for a longer time. Herds comprised purely of either iGP_1 or iGP_2 individuals are less fragmented, move slowly and have smaller home ranges (slow-explorer *sensu* Spiegel et al. [53]). In grazing lands where patches of vegetation are small and homogeneously distributed, these two personalities may display similar grazing patterns because the sense of orientation would not make a difference in distribution where non-conspicuous patches of vegetation exist. In grazing lands where significantly big patches of vegetation are heterogeneously distributed, iGP_1 will utilize more intensively its preferred habitat, taking advantage of its better sense of orientation in comparison with iGP_2 individuals. Herds comprised purely of iGP_3 individuals are highly fragmented, move faster, and individuals graze at greater distances from one another. iGP_3 individuals graze alone or in relatively small groups that occupy larger home ranges than iGP_1 or iGP_2 individuals. iGP_3 individuals prefer steep slope areas in high altitude habitats and have low sense of orientation. As per their low sense of orientation, these animals will show similar grazing patterns in homogeneous and heterogeneous grazing lands.

1.4.4 Implications

The GP-model proposes a novel understanding of social foragers: grazing is a social activity performed by herds of interacting ruminants that display collective grazing personalities with their own distinctive attributes. Individuals that display distinct grazing personalities comprise behavioural polymorphic herds of ruminants. Grazing personalities of ruminants are controlled by their genetic composition and are modulated by their epigenetic states in response to the social herd environment, biophysical environment and the emotional state. Adaptive and inheritable epigenetic states confers plasticity to grazing personalities at individual and collective levels.

Selecting for grazing personality

Farmers, ranch managers and breeders may adopt the concept of grazing personalities and select for animals according to the desired and needed distinctive behaviours. By so doing, we forecast a genetic gain on herds to address major challenges faced by the pastoral livestock production industry. The identification of grazing personality genotypes and the development of the corresponding genetic markers can be used to determine the grazing personality composition of herds and to further assist in applying goal-oriented selection of animals using a relatively simple and inexpensive genetic test such as single-strand conformation polymorphism (SSCP) [104, 105].

Enhancing the expression of grazing personalities

The GP-model establishes that grazing personalities of ruminants and other large herbivores are plastically displayed in response to stimuli (e.g., social herd environment, biophysical environment and animals' emotional states). Such responses might be adopted and shown for the entire lifespan of animals and, can be farther transferred to their progeny through transgenerational epigenetic inheritance. This is particularly relevant for experiences occurred early in life. Exposing grazing ruminants and other large herbivores to the biophysical environmental conditions where they are targeted to perform may trigger epigenetic mechanisms and regulatory systems that foster the expression of grazing genes towards desired behaviours of individual grazers and herds. As per the GP-model, the social context in which an animal and its predecessor grow (i.e., the social herd environment) modulates the expression of grazing genes and therefore the displayed grazing personalities. For example, social environments of isolation, crowdedness, threats and fearfulness, as well as the aggressiveness of herds, affect the emotional states and modulates the individual and collective grazing behaviours and associated decision-making. Similarly, the biophysical environment might shape the expression of grazing genes.

Influencing grazing personalities through emotions

Grazing management practices such as fasting, supplementation or herding techniques alter animal internal states (e.g., hunger, emotions), influence animal decision-making and ultimately, modify their grazing patterns.

Designing behaviour-customised herds

The composition and relative frequency of grazing personalities of domesticated ruminant herds has been manipulated and shaped for millennia to produce docile and manageable individuals and herds suitable for living alongside and under management of humans. The GP-model proposes to apply behavioral-based selection for the design of ruminant herds matching the spatial diversity and the temporal variety of forages, foodscapes and landscapes. Pastoral livestock production systems are heterogeneous in space and time. Despite efforts to create “simple and homogeneous” systems, individualities and collective attributes of grazing patterns emerge. Herds are comprised of a mix of individuals displaying distinctive grazing personalities. Therefore, grazing patterns of ruminant herds can be manipulated through designing and deciding the relative frequency of individual grazing personalities along with the adoption of grazing management practices that foster the desired behaviours.

1.5 Conclusions

The GP-model proposes that genetic effects (alleles, allelic variation and the frequency of alleles) and epigenetic modulation (via regulatory systems that modulate the gene expression) conditions grazing behaviours of ruminants and other large herbivores, so that, animals display grazing personalities at individual and collective levels. The interactions with the social herd environment and the biophysical environment shape the phenotypic grazing personalities of individuals. Collective grazing personalities emerge from the social interaction of individuals and their grazing personalities. The social herd environment mediates between the individual and the collective grazing personalities. This is because interacting individuals constitute the herd and creates its environment. And in turn, the social herd environment influences both, the grazing personality of individuals and the grazing personality of the herd.

The allelic composition and the relative allelic frequency of grazing genes characterize the collective genotypic of grazing personalities and, therefore, there is the opportunity to develop breeding programs aiming to influence grazing patterns of ruminant herds applying behavioural selection. Because of the genetic basis of grazing behaviour, animal selection maybe a useful tool to improve grazing distribution of habitat-heterogeneous livestock systems. The displayed grazing personality of herds of ruminants and other large herbivores results from their genome and their personality plasticity. Grazing management, herding techniques, feeding strategies and rearing practices that affect animal welfare and the gene expression of grazing traits have the potential to foster desirable grazing personalities. Managers that account for the variety of individual grazing personalities naturally displayed in ruminants, and that manipulate its proportion, can enhance ecosystem services and improve animal welfare while maintaining the productivity of livestock production systems.

The need for further investigation into key areas of behavioural genetics emerged from this review. From an animal personality perspective, there is a need to recognise sets of correlated grazing behaviours measured over time and across contexts with the ultimate objective of identifying distinctive grazing personalities in large herbivores. The neuro-physio-morphological pathways of grazing behaviours remain largely unknown. Studies with relatively coarse genetic markers (e.g., QTL) suggested possible candidate genes which may be associated with grazing patterns. However, research with finer resolution and with larger datasets could discover new genes and variant sequences thereof and provide more certainty about the genetic control of grazing behaviours. This may result in the identification of specific gene markers for animal selection programmes. It is also unknown if grazing behaviours are controlled by pleiotropic gene(s), through combined effects, or by both.

The novelty and uniqueness of the GP-model lies in the role that social environmental factors play in modulating the genetic expression of grazing personalities and in highlighting the collective nature of large herbivores and foragers in general. However, there is still very little knowledge about the genetic control of grazing behaviours and personalities at the collective level, and hence the GP-model might remain less robust until research into that is undertaken. We still need to identify and develop robust metrics to measure collective grazing behaviours and also design studies that aim to detect behavioural differences at the collective level in large herbivores.

The grazing personality model presented here further develops our understanding of the distribution of ruminants and large herbivores by integrating discoveries from the past few decades into models of grazing distribution and behaviour [33, 34]. The GP-model was inspired from and supported with scientific works conducted with a diverse range of taxa from the animal kingdom, namely bees, birds, marine species, large herbivores, ruminants and other ungulates. Future research on grazing personalities at the individual and collective levels may confirm the hypotheses posed in our 'grazing personality model' and thus contribute to a better understanding of livestock production systems, grassland science and animal behaviour.

Chapter 2

The glutamate metabotropic receptor 5 gene (*GRM5*) is associated with beef cattle home range and movement tortuosity

2.1 Introduction

The selection and breeding of animals with desirable morphological and physiological characteristics has led to greater fitness and productivity in livestock farming systems. During the domestication of these animals and the establishment of livestock systems, behavioural characteristics have been equally or even more important than other production characteristics because of the close interaction with humans [78], yet measuring behaviour and objectively quantifying differences among individuals remains a challenge.

While the selection for animals with sets of behaviours suitable for safe handling and production has been practiced along the animal domestication [78]; the realisation, conceptualisation and acceptance of animal personality is rather new [24]. This stated, similar (and probably) more accepted concepts such as temperament and behavioural syndromes [5, 4] have been around for longer. Regardless, we have limited knowledge of how individual personalities affect livestock production systems, the welfare of non-human animals, and the ecological functions and services associated with livestock production, such as the carbon cycle, nutrient redistribution and the quality of water.

The evidence shows that animal personality varies among individuals and that it affects livestock production and animal fitness [17, 7]. It can also be stated that to some extent, heritable factors determine behavioural characteristics and even personalities [26, 55, 106]. Thus, animal personality is becoming an important criterion in livestock breeding programmes [107, 108].

The concept of animal personality in foragers [109, 37] is rather novel. It is therefore not surprising that there are few reports describing genetic effects in the grazing personality of cattle [110], as well as a lack of candidate genes that might control such behaviours (but see [41, 57]). However, in one suggested model of grazing personalities (the GP-model; [109]) it is postulated that distinctive grazing personalities might be determined by variations in 'grazing-related genes'. What-is-more, these genes could be modulated by epigenetic mechanisms that control their activities through interactions with the social and biophysical environments, with this ultimately affecting the animal's behaviour.

Genetic models are useful tools for the identification and study of candidate genes related to the physiology, behaviour and cognitive abilities. For example, Bakker and Oostra [111] studied the Drosophila model for fragile-X syndrome and found individuals with arrhythmic and erratic patterns of locomotor activities and abnormal circadian behaviour, which were regulated by the glutamate metabotropic receptor 5 protein (GRM5). Subsequently, Jew et al. [112] suggested that GRM5 controlled neural synaptic plasticity, and that in turn this modulated the locomotor reactivity of mice to novel environments. Jew's et al. results showed direct association between GRM5 and locomotor reactivity to a novel environment, increased and decreased exploratory behaviour and, activity levels of mice. Subsequently, Wu et al. [113] reported that GRM5 in the forebrain GABAergic neurons of mice modulated locomotor activity, and in this way it affected their horizontal and vertical distances travelled and the time spent moving. These authors reported that mice with genotypic variation in the glutamate metabotropic receptor 5 gene (*GRM5*) displayed different levels of activity in familiar and unfamiliar environments.

The glutamate metabotropic receptors (GRMs) are G-protein coupled receptors that have been categorised into three groups according to their sequence similarity and intracellular signalling mechanisms. The GRMs 1 (GRM1) and 5 (GRM5) are members of receptor group 1, which couple with phospholipase C and have similar functions or effects. Bossi et al. [114] reported GRM5 interactions with GRM1 that affected the motor coordination of mutant mice, and in a recent study, Gray et al. [115] concluded that the stimulation of GRMs Group 1 increased the activity of Cav2.3 R-type voltage gated Ca^{2+}-channels in hippocampal neurons. This led to hyperactivity at the neural synapses and aberrant calcium spiking in both male and female, and it caused deficient short-term memory, increased activity, and increased exploratory behaviour.

Earlier work with *GRM5*-knockout mice also suggested effects related to spatial cognitive ability. For example, Lu et al. [116] observed impairment in the acquisition and use of spatial information and persistent strengthening of neural synapses (i.e., long-term potentiation). Such results were consistent with Bliss and Collingridge's findings [117], which linked neural potentiation with memory and spatial learning. Taken together, the literature would suggest that GRM5 may either directly or indirectly control animal activity and cognitive behaviours related to the exploration and use of space.

In 2015, Bailey et al. [41] conducted a study seeking genetic associations with the grazing behaviour of beef cows recorded using GPS-tracking collars on five farms in the United States of America (USA). The cattle were screened to identify quantitative trait loci (QTL) related to terrain-use indices. Two of the QTLs overlapped *GRM5* on bovine chromosome 29, and these explained 18% and 24% of the total variation in the study's so-called 'rough' grazing index. The reported associations between a QTL overlapping *GRM5* and the rough index made *GRM5* a candidate gene to explain the phenotypic variation in GP-behaviours of beef cattle.

Accordingly, for this study, we hypothesize that nucleotide sequence variation in bovine *GRM5* may be associated with behaviours that underpin the grazing personalities displayed by beef cattle, and hence research was undertaken to ascertain whether genetic variation exists in *GRM5*, and if it existed, to explore its association with grazing personality behaviours in beef cattle.

2.2 Materials and methods

2.2.1 Cattle investigated and phenotypic data collection

The Lincoln University Animal Ethics Committee approved all procedures before sampling and handling animals for this research (AEC 2018-16, AEC 2018-16 extension and AEC 2020-02).

The cows studied ($n = 306$) ranged in age from three to ten years, and they were categorised into three age groups: 'class 1' (under four-years of age), 'class 2' (four to five-years of age) and 'class 3' (six or more years of age). They were either Hereford cattle ($n = 224$) or Angus x Hereford cross cattle ($n = 82$), with the crosses only present on two farms. For the Hereford cattle, most of the cows were from registered studs, and pedigree information (i.e., sire and dam identities) could be gathered from publicly available sources [118], or directly from the farmer. For cows with unknown pedigree ($n = 82$), three 'notional sire' identities were allocated in three of the fourteen mobs (i.e., cattle groups between and within farms and sampling years) to avoid redundancy among herds (i.e., cattle groups within a given farm), farms ($n = 4$) or years ($n = 2$).

For the 2019 sampling, fifteen cows were selected within the existing breeding herds at four farms and GPS-tracked. This was undertaken with modified tracking collars that contained i-gotU GT-600 GPS data loggers (Mobile Action Technology Incorporated, Taipei, Taiwan) and additional rechargeable batteries to prolong running time in the field. Subsequently, in 2020, ninety cows were selected within a single herd from each farm for GPS-tracking and the tracking collar deployments were carried out, one farm after the other, during the grazing season. Due to failure of some GPS devices, several deployments did not yield usable data (see details below).

Grazing behaviour was recorded in steep and rugged rangelands of Canterbury, New Zealand, over the autumn and winter period (approximately between April and August). As is commonly practiced on New Zealand commercial farms, mated cows were moved to graze higher rangelands immediately after weaning (in April). They remained in these uplands until commencement of the calving season in spring (August-September), and were grazed in a 'free-range' system, on the relatively large (average size 34.5 ha) and uncultivable paddocks of the so-called 'New Zealand hill country' (see Tozer et al. [119] for terrain description). The data set for statistical analysis comprised 303 cows (except for results in Table 2.3) from four farms, sampled over two years that sum up to fourteen mobs (i.e., different herds within and between farms and years) from 73 sires and five *GRM5* genotypes (genotype *AA* excluded).

For each collar deployment, individual cow trajectories for the duration of the grazing period were created using the R package ‘adehabitatLT’, which contains functions capable of dealing with the analysis of animal movement [120]. In these analyses, a combination of turning angle and speed between geolocations was used to identify GPS outliers [121], and then the trajectories excluding the outliers were recalculated. The Shuttle Radar Topography Mission digital elevation model (DEM) raster of New Zealand (16 metres (m) resolution) was downloaded from Land Information New Zealand [122] and additional rasters were created for slope and aspect using the 3D Analyst toolbox of ArcMap™ [123]. The annotation with data of elevation, slope and aspect for each GPS data point was obtained by extracting values using the R package ‘raster’ [124].

With assistance from the R package ‘dplyr’ [125], a number of behaviours describing grazing personalities (GP-behaviours) were calculated for each cow. First, they were calculated on a daily basis, but days with a recording rate under 75% (i.e., less than 216 data points recorded out of 286, for locations recorded at a 5-min intervals) were not included. Next, the mean of each GP-behaviour was calculated across the days for each cow. The GP-behaviours included: the daily horizontal distance travelled, the daily vertical distance travelled, the daily three-dimensional distance travelled, the daily elevation range, the daily elevation gain, the relative elevation mean, the relative elevation 85th quantile, the relative elevation range, the daily slope 85th quantile, the daily home range (using the minimum convex polygon method) and the daily movement tortuosity (using the spatial search pattern [37, 126]). See 2.1 for a detailed descriptions of GP-behaviours.

Table 2.1. List of grazing personality behaviours with abbreviations, units, data transformations and description of calculations.

Grazing personality behaviours	Abbreviations	Units	Transformation	Description
Daily horizontal distance travelled	dist_ho	m/d	Log	Distance calculated as the sum of distances between consecutive GPS[1] data points per day using two dimensions (i.e., Easting and Northing) of the UTM[2] projection
Daily vertical distance travelled	dist_ve	m/d	Log	Distance calculated as the sum of the absolute difference in elevation between consecutive GPS data points per day using a DEM[3]
Daily three-dimensional distance travelled	dist_3D	m/d	Log	Distance calculated as the sum of distances between GPS data points per day using three dimensions (i.e., Easting and Northing (UTM) and elevation difference (DEM)
Daily elevation range	ele_range	m	Log	Range of elevation computed as calculated as the difference between the daily maximum and minimum elevation
Daily elevation gain	ele_gain	m/d	Log	Sum of positive changes of elevation between consecutive GPS data points as depicted from a DEM
Relative elevation mean	rel_ele_mean	0-1 scale		In any given day, ratio between the cows' mean elevation minus the minimum elevation of the herd and the elevation range of the herd
Relative elevation 85th quantile	rel_ele85	0-1 scale		In any given day, ratio between the cows' 85th quantile of the elevation minus the minimum elevation of the herd and the elevation range of the herd
Relative elevation range	rel_ele_range	0-1 scale		In any given day, ratio between the cows' elevation range and the elevation range of the herd
Daily slope 85th quantile	slope85	Percentage		85th quantile of the slope across GPS data points per day as depicted from a DEM
Daily home range	hr_mcp	ha/d	Log	Explored area estimated by calculating the minimum convex polygon depicted from all GPS data points per day using the R package 'adehabitatHR'
Daily movement tortuosity	sp_tortuosity	m/ha	Log	Movement tortuosity estimated as the ratio between daily horizontal distance and daily home range

[1] GPS: Global Positioning System fixes recorded with i-gotU GT-600, Mobile Action.

[2] UTM: Universal Transverse Mercator.

[3] DEM: Digital Elevation Model with a 16 m x 16 m spatial resolution.

A minimum of 7 days (d) of GPS tracking data were deemed sufficient to represent consistent grazing behaviours, thus any cow with six or fewer days of data collection was excluded from the study. For each cow, the first seven to 28 daily trajectories recorded were analysed from the start of GPS deployment, when herds grazed in rolling or steeper rangeland terrain (i.e., when the median daily slope for the relocations of the herd was greater than eight degrees) based on slope classes for New Zealand [127]. Overall, GP-behaviours for 303 cows were analysed. GPS data deposited under Movebank ID 1321429570 and Movebank ID 1321461925.

2.2.2 Blood sampling and polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis of *GRM5*

Individual blood samples from the nicked ears of the cattle were collected onto TFN paper (Munktell Filter AB, Sweden), and genomic deoxyribonucleic acid (DNA) used for polymerase chain reaction (PCR) amplification was purified from the dried blood spot using a two-step procedure described by Zhou et al. [128].

Human *GRM5* was first described as having nine coding exons, with lengths ranging from 96 to 940 base pairs (bp) [129]. Based on this, a human *GRM5*-202 (ENST00000305447.5) sequence was analysed to ascertain which region of bovine *GRM5* may be suitable for further molecular analysis. Exon V (247 bp) (hereafter referred to as exon 5) was chosen for analysis, as this exon encodes part of the receptor-binding region [129] and has more sequence variation described in Ensembl (ENSBTAG00000048061) than other regions of the gene.

A pair of PCR primers were then designed to amplify the *GRM5* exon 5 region based on the sequence ENSBTAG00000048061. These primer sequences were 5'-AGAATCCATAAAGAGCTACAG-3' and 5'-GATCAGGCTCTGGTGTCTAG-3', and the primers were synthesised by Integrated DNA Technologies (Coralville, IA, USA).

The PCR amplifications with these primers were performed in a 15-µL reaction. These contained the DNA of one punch of TFN paper, 150 µmol of each deoxyribonucleoside triphosphate (dNTP) (Bioline, London, United Kingdom), 0.25 µmol of each primer, 0.5 U of Taq DNA polymerase (Qiagen, Hilden, Germany), 2.5 mmol Mg^{2+}, 1× reaction buffer supplied with the enzyme and distilled water to make up volume. The thermal profile for amplification consisted of 2 min at 94 Celsius degrees (℃), followed by 35 cycles of 30 seconds (s) at 94 ℃, 30 s at 60 ℃, and 30 s at 72 ℃, with a final extension of 5 min at 72 ℃.

The PCR amplicons were screened for sequence variation using single strand conformation polymorphism (SSCP) analysis. Each amplicon (0.7 µL) was mixed with 7 µL of loading dye (98% formamide, 10 mmol EDTA, 0.025% bromophenol blue, and 0.025% xylene cyanol). After denaturation at 95 ℃ for 5 min, the samples were rapidly cooled on wet ice and then, electrophoresed in 16 cm × 18 cm, 14% acrylamide: bisacrylamide (37.5:1) (Bio-Rad) gels in 0.5× TBE buffer at 6 ℃ and 370 Volts (V) for 19 hours (h). The gels were silver-stained according to the method of Byun et al. [130].

2.2.3 Sequencing of variants and sequence analysis

PCR amplicons representing different SSCP banding patterns from cattle that appeared to be homozygous were sequenced in both directions using Sanger sequencing at the Lincoln University DNA Sequencing Facility (Lincoln University, Canterbury, New Zealand). Nucleotide sequence alignments and translation to amino acid sequences were undertaken using DNAMAN (version 5.2.10, Lynnon BioSoft, Vaudreuil, QC, Canada).

2.2.4 Statistical analyses

Statistical analyses were conducted using R [131]. For data aggregated at the daily level, skewness, kurtosis and normality were graphically evaluated by plotting histograms with their corresponding theoretical normal distribution curves and with Q-Q plots. When needed, logarithmic transformation was utilised to better fit the data into a normal distribution. Overall, the dataset comprised 6142 daily-aggregated observations.

For data aggregated at the cow level (i.e., averaged across 7-28 d of records), Pearson correlation coefficients were calculated between the eleven GP-behaviours using 'rcorr()' from the R package 'Hmisc' [132]. The correlations were calculated based on data from the 303 cows (three cows with *GRM5* genotype *AA* excluded from analysis), except for home range and movement tortuosity (n = 299) because of missing values.

Linear mixed models (LMM) and generalised linear mixed models (GLMM) were fitted to the GP-behaviours to assess their associations with *GRM5* variants and genotypes using the R package 'lme4' [133, 134]. The LMMs were fitted to GP-behaviours with a Gaussian distribution (e.g., daily horizontal distance traveled), whereas the GLMMs were used with bounded GP-behaviours (i.e., those scaled between 0 and 1, e.g., relative elevation mean) set with binomial distributions, which apply a logit transformation. The GLMM-binomial distribution was preferred over using beta distribution [135], in order to correct for random factors.

Unbalanced repeated measurements were nested into a cow identity factor. The effects of farm, sampling year, mob, sire and genotype were tested as potential random explanatory factors. Breed effect (i.e., Hereford vs Angus x Hereford cross) was not independently assessed because the number of Angus x Hereford cattle was small (n = 82) and some of the genotypes were rare, and whilst breed might be affecting the various phenotypic measures the cattle studied cannot be claimed to be representative of the breed as a whole. There were 29 half-sister cows that shared the same sire but were part of different mobs.

For each GP-behaviour, the random and fixed explanatory factors were selected in two steps. First twelve models were run with several combinations of random factors only (i.e., cow identity, farm, sampling year, mob, and *GRM5* genotype). The model with the best compromise of statistics (i.e., least degrees of freedom, lowest Akaike information criterion (AIC) [136], lowest Bayesian information criterion (BIC) and lowest factor significance evaluated using the 'anova()' function), was selected for further evaluation. In a second step, the random factor selected models were then fitted with the corresponding fixed factors, i.e., presence/absence of the variants or *GRM5* genotype (the predictor variables under evaluation), to create variant and genotype models respectively, and with cow age-class. Using the same criteria as above to reach the best compromise of AIC, BIC and ANOVA, a final model with or without the cow age-class factor was fitted.

To carry out the comparison of models, the maximum likelihood method was used. Once the random and fixed factors were set, models were fitted using restricted maximum likelihood procedures [137]. The suitability of the selected models was assessed with plots of residual versus fitted data, and with the criteria of accepting models with up to 5% of scaled residuals beyond the ± 3 limits. Associations of fixed factors (variants, genotypes and cow age-classes) were assessed with the Satterthwaite's method using the R package 'emmeans' [138], and *post-hoc* analyses (pairwise comparisons) were undertaken using the Benjamini-Hochberg method [139, 140] in 'emmeans'. Groups that were different at $P < 0.05$ were labelled with different letters using the 'multcompView' package in R [141].

Dominance models were fitted by testing the effect of the presence/absence of variants and genotype models were fitted with the identified genotypes. Cows with low genotype frequency in the cattle studied (i.e., <5%) were excluded from the various statistical analyses.

2.3 Results

Eleven grazing personality behaviours were derived by combining GPS DEM-annotated data from free-ranging cows grazing rangeland. These were calculated on a daily basis, and subsequently averaged across 7-28 d for each individual cow.

2.3.1 Correlation of grazing personality behaviours

The Pearson correlation coefficients (r) between the grazing personality behaviours are summarised in Table 2.2.

Table 2.2. Pearson correlation coefficients for the grazing personality behaviours (GP-behaviours).

GP-behaviours[1]	dist_ho	dist_ve	dist_3D	ele_range	ele_gain	rel_ele_mean	rel_ele85	rel_ele_range	slope85	hr_mcp
dist_ve	**0.70*****[2]									
dist_3D	**1.00*****	**0.72*****								
ele_range	0.07	**0.41*****	0.08							
ele_gain	**0.70*****	**1.00*****	**0.72*****	**0.41*****						
rel_ele_mean	- 0.13*	0.19**	0.00*	**0.68*****	0.19**					
rel_ele85	- 0.14*	0.11*	- 0.13*	**0.71*****	0.11	**0.97*****				
rel_ele_range	**0.41*****	0.12*	**0.40*****	0.28***	0.12*	0.18**	**0.36*****			
slope85	- 0.26***	**0.38*****	- 0.23***	**0.60*****	**0.38*****	**0.43*****	**0.42*****	-0.09		
hr_mcp	**0.32*****	- 0.13*	**0.30*****	-0.09	- 0.12*	- 0.24***	-0.08	**0.54*****	- 0.27***	
sp_tortuosity	**0.48*****	**0.56*****	**0.49*****	-0.07	**0.55*****	-0.03	- 0.15*	- 0.17**	-0.04	- 0.27***

[1] Daily horizontal distance travelled (dist_ho), daily vertical distance travelled (dist_ve), daily three-dimensional distance travelled (dist_3d), daily elevation range (ele_range), daily elevation gain (ele_gain), relative elevation mean, relative elevation 85th quantile (rel_ele85), relative elevation range (rel_ele), 85th quantile of daily slope (slope85), daily home range (hr_mcp) and daily movement tortuosity (sp_tortuosity).

[2] Bolded values indicate moderate (r = 0.3 - 0.5) and strong ($r > 0.5$) correlations. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Daily horizontal distance travelled, daily three-dimensional distance travelled, daily vertical distance travelled and daily elevation gain were highly positively correlated with each other, and they had positive correlations that ranged between $r = 0.48$ and $r = 0.56$ with daily movement tortuosity. Moderate positive correlations were found between the daily horizontal distance travelled and the daily home range ($r = 0.32$) and the daily three-dimensional distance travelled and the daily home range ($r = 0.30$). The daily horizontal distance travelled was moderately positively correlated with relative elevation range ($r = 0.41$) and the daily three-dimensional distance travelled was moderately positively correlated with relative elevation range ($r = 0.40$). The daily vertical distance travelled had a moderate positive correlation ($r = 0.38$) with the 85th quantile of daily slope, and the daily elevation range and daily elevation gain were also positively correlated with the 85th quantile of daily slope ($r = 0.60$ and $r = 0.38$ respectively).

When looking at 'relative' GP-behaviours (i.e., those calculated by comparing the individual behaviour with the mean behaviour displayed by animals of the same herd), strong positive correlations were revealed between daily elevation range and relative elevation mean ($r = 0.68$), as well as with the 85th quantile of relative elevation ($r = 0.71$). Similarly, the 85th quantile of daily slope correlated positively ($r = 0.43$ and $r = 0.42$ respectively) with relative elevation mean and the 85th quantile of elevation. Finally, the daily home range was strongly correlated ($r = 0.54$) with the relative elevation range.

2.3.2 Genetic variation in *GRM5* exon 5

The nucleotide sequence variation in exon 5 of *GRM5* was investigated in 306 adult cows, albeit only 303 of these were subject to further statistical analyses. After PCR-SSCP analyses of the *GRM5* exon 5 region, three distinctive banding patterns corresponding to homozigous variants named *A*, *B* and *C*) were identified. Fig. 2.1 shows the three homozygous and several heterozygous banding patterns.

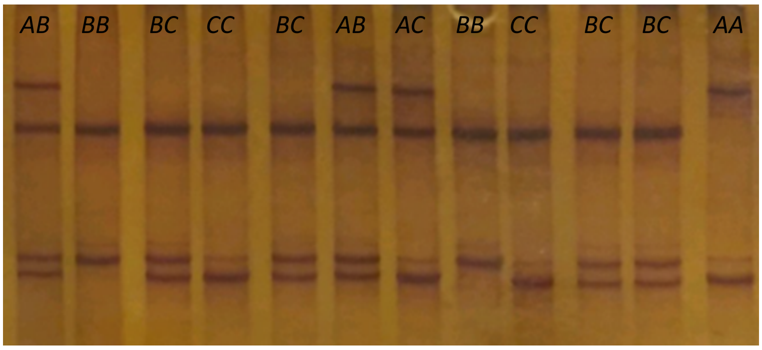

Figure 2.1. Banding patterns of *GRM5* genotypes. Banding patterns of various genotypes of the bovine glutamate metabotropic receptor 5 gene (*GRM5*) exon 5 region obtained from Polymerase Chain Reaction – Single Strand Conformation Polymorphism (PCR-SSCP) analyses.

DNA sequencing of the PCR products of these three variants revealed three new and different nucleotide sequences (GenBank accessions numbers OK078019, OK078020 and OK078021) with two previously reported nucleotide substitutions (rs43744222 and rs210610001). These substitutions, if expressed, would not change the amino acid sequence.

The six possible genotypes of the three variants were all identified (Table 2.3). Genotype *AA* was the least common, and across the four farms was present in only 1% ($n = 3$) of the cows. Genotypes *BC* and *CC* were the most common, with frequencies of 36% and 35% respectively, and together these genotypes accounted for 65-79% of cows on any given farm.

Table 2.3. Number of cows (and percentage frequency) per farm of each *GRM5* genotype of four beef farms in the Canterbury Region of New Zealand.

Genotype	Farm								Total	
	1		2		3		4			
AA	0	(0%)	1	(2%)	1	(1%)	1	(1%)	3	(1%)
AB	4	(5%)	3	(5%)	4	(5%)	7	(9%)	18	(6%)
AC	11	(13%)	6	(10%)	5	(6%)	9	(12%)	31	(10%)
BB	5	(6%)	3	(5%)	20	(24%)	10	(13%)	38	(12%)
BC	36	(43%)	23	(38%)	25	(30%)	25	(32%)	109	(36%)
CC	27	(33%)	25	(41%)	29	(35%)	26	(33%)	107	(35%)
Total	83		61		84		78		306	

2.3.3 Selecting random explanatory factors for the linear mixed models

For each GP-behaviour, twelve different combinations of random factors were assessed, and the best combination of the lowest AIC (Table S1) and BIC (not shown), lowest number of degrees of freedom (Table S1) and those that were statistically significant by an ANOVA comparison of models (results not shown) was selected. The selected combination of random factors for each GP-behaviour are indicated with bolded and underlined AIC values (Table S1). The models for horizontal distance travelled and three-dimensional distance travelled were 'best' corrected using sampling year as a random factor (i.e., 2019 or 2020) and within each year of the farm (farm: sampling year) (RF4 in Table S1). Models for vertical distance travelled, relative elevation, 85th quantile of relative elevation and 85th quantile of slope were 'best' corrected with the factor mob, and for the remaining five GP-behaviours a combination of mob and sire was the selected random factor correction.

Nearly 75% of the cows tracked in this study were Herefords and the rest Angus x Hereford crosses mostly from farm 2 (16%). Thus, farm effects could have been confounded with breed effects if the crossbred cows actually differed from the purebred cows. However, since purebred cows dominated the dataset, differences in breed are considered small in our dataset (in comparison to other factors such as the farm effect) and therefore negligible for the correction of models. The models were therefore corrected for farm effects only and breed was not included as factor. As such, the results chiefly represent variability within Hereford cattle.

Selected genotype models were assessed by plotting residuals versus fitted values. All models had less than 1% of the scaled residuals exceeding the ±3 limits and the residuals were mostly randomly distributed (Figs. S1 and S2).

2.3.4 Cow age-class as a fixed explanatory factor

Tables 2.4 and S2 reveal summaries for correcting the *GRM5* variant and genotype models respectively with cow age-class as a fixed explanatory factor. Out of eleven variant and genotype models and for the same GP-behaviours, seven were improved by correcting for cow age-class: horizontal and 3D distances, relative elevation, 85th quantile of relative elevation, 85th quantile of daily slope, home range and movement tortuosity.

For any given GP-behaviour, the three *GRM5* variant models showed similar results, with an explanatory factor correcting all three models, or none of them. *P*-values ranged between 0.01 and 0.05 for horizontal and 3D-distance travelled, relative mean elevation, relative 85th quantile elevation and 85th quantile slope (Table 2.4). For home range and movement tortuosity, *P*-values were below 0.01 (Table 2.4). The significance level for each GP-behaviour was similar for the variant and the genotype models.

Table 2.4. Associations between bovine variants of the glutamate metabotropic receptor 5 (*GRM5*) and grazing personality behaviours (GP-behaviours).

GP-behaviours[1]	Cow age class (*P*-value)[2]	*GRM5* variant	Marginal mean[3] (standard error)		*P*-value[4]
dist_ho (m/d)	**0.038**	*A*	3837	(±1120)	0.847
	0.039	*B*	3843	(±1120)	0.741
	0.037	*C*	3874	(±1130)	0.213
dist_ve (m/d	0.697	*A*	566	(±76)	0.461
	0.701	*B*	557	(±74)	0.638
	0.703	*C*	555	(±73)	0.916
dist_3D (m/d)	**0.044**	*A*	3900	(±1141)	0.870
	0.044	*B*	3903	(±1139)	0.749
	0.043	*C*	3935	(±1149)	0.223
ele_range (m)	0.413	*A*	71.0	(±9.0)	**0.008**
	0.407	*B*	66.3	(±8.4)	0.833
	0.402	*C*	66.3	(±8.4)	0.729
ele_gain (m/d)	0.697	*A*	283	(±38)	0.452
	0.699	*B*	279	(±37)	0.485
	0.704	*C*	277	(±37)	0.963
rel_ele_mean (0-1)	**0.018**	*A*	0.42	(±0.06)	0.212
	0.017	*B*	0.44	(±0.05)	0.263
	0.018	*C*	0.46	(±0.05)	0.556
rel_ele85 (0-1)	**0.006**	*A*	0.67	(±0.05)	0.934
	0.006	*B*	0.67	(±0.05)	0.274
	0.006	*C*	0.68	(±0.05)	0.749
rel_ele_range (0-1)	0.286	*A*	0.51	(±0.09)	0.354
	0.266	*B*	0.50	(±0.09)	0.610
	0.277	*C*	0.50	(±0.09)	0.971
slope85 (0-1)	**0.023**	*A*	0.47	(±0.08)	0.138
	0.026	*B*	0.44	(±0.08)	0.848
	0.025	*C*	0.43	(±0.08)	0.205
hr_mcp (ha/day)	**0.008**	*A*	7.86	(±0.83)	**0.021**
	0.008	*B*	7.22	(±0.74)	0.285
	0.008	*C*	7.39	(±0.75)	0.273
sp_tortuosity (m/ha)	**0.006**	*A*	556	(±74)	**0.003**
	0.006	*B*	611	(±79)	0.139
	0.007	*C*	598	(±77)	0.476

[1] See GP-behaviours abbreviations and details in Table 2.1.

[2] Significance level of ANOVA test for comparison of models with and without cow age class as fixed factor. Values below 0.05 indicated with bold font.

[3] Marginal mean for variant presence in measured units (back-transformed from the log scale as required).

[4] Significance level for Satterthwaite's method t-test of presence/absence of variant. Values below 0.05 indicated with bold font.

2.3.5 Association of ***GRM5*** variants and genotypes with grazing personality behaviours

Using linear mixed models, associations between the presence/absence of the *GRM5* variants and GP-behaviours were investigated. The presence of variant A was associated with elevation range, home range and movement tortuosity (Table 2.4). Trends to association ($P > 0.2$) were found for variant A and the 85^{th} quantile of slope ($P = 0.138$) and for variant B with movement tortuosity ($P = 0.136$). No associations were detected with variant C.

The *GRM5* genotype models (Table S2) revealed associations with home range and movement tortuosity (see below genotypes *post-hoc* analysis). Trends suggesting association ($P < 0.1$) were found between the *GRM5* genotypes and elevation range, as well as with the horizontal distance travelled and three-dimensional distance travelled. Overall, this suggested a high degree of consistency between the variant presence/absence models and the genotype models.

The effect of the fixed factors was consistent in the variant and genotype models. Thus, if cow age-class had an effect in the variant presence/absence models, this effect was observed in the corresponding genotype model. Similarly, when genetic associations were revealed in the variant models, such genetic effects were reflected in the genotype models too.

2.3.6 ***Post-hoc*** comparisons of ***GRM5*** genotypes and cow age-class

The *GRM5* genotypes associations with home range and movement tortuosity (Table S2) were related to cow age-class. For these two GP-behaviours, an analysis of the main effect of genotype across cow age-class and *post-hoc* analyses were conducted. Results of *post-hoc* analyses revealed significant differences in the combined effects of *GRM5* genotypes and cow age-classes for both GP-behaviours (Table 2.5).

Table 2.5. *Post-hoc* comparisons between groups of *GRM5* genotypes and cow age classes that had significant association with grazing personality behaviours (GP-behaviours)

GRM5 genotype	Cow age class	Marginal mean[1] (standard error)	Degrees of freedom	BH[2] (P <0.05)
		Home range (ha/day)		
AB	1	9.74 (±1.52)	62	a
AC		9.67 (±1.48)	55	a
BB		8.16 (±1.26)	57	bcd
BC		8.96 (±1.32)	50	ab
CC		9.01 (±1.34)	51	ab
AB	2	7.43 (±0.83)	23	abc
AC		7.37 (±0.77)	18	ab
BB		6.22 (±0.65)	18	de
BC		6.83 (±0.67)	14	bcd
CC		6.87 (±0.68)	14	bcd
AB	3	6.77 (±0.80)	29	bcd
AC		6.72 (±0.76)	24	bcd
BB		5.67 (±0.64)	24	e
BC		6.23 (±0.66)	20	cde
CC		6.26 (±0.67)	20	cde
		Movement tortuosity (m/ha)		
AB	1	434 (±73)	35	j
AC		456 (±75)	33	ij
BB		534 (±89)	34	cdefgh
BC		495 (±80)	31	ghi
CC		484 (±79)	31	hij
AB	2	595 (±81)	17	efghi
AC		626 (±83)	15	cdefgh
BB		732 (±97)	15	ab
BC		679 (±88)	14	abcd
CC		663 (±86)	14	abcdef
AB	3	610 (±86)	19	dfghi
AC		641 (±88)	17	bcdefg
BB		751 (±103)	17	a
BC		696 (±93)	16	abce
CC		680 (±91)	16	abcdef

[1] Response marginal mean in measured units (back-transformed from the log scale as needed).

[2] Different letters indicate significantly different groups calculated with a pairwise comparison using the Benjamini-Hochberg method ($P < 0.05$).

Home range

Here is presented the selected linear mixed model for home range:

Response variable: log(hr_mcp)

Fixed factors: GRM5_genotype + cow_age_class +

Random factors: (1|cow_id) + (1|mob_id) + (1|sire_id)

Where hr_mcp refers to home range and id denotes the identifier.

The main effects of cow age and genotype were assessed with a mean comparison using the Benjamini-Hochberg method for the adjustment of P-values. When the daily home range by cow age (i.e., home ranges averaged over genotype levels) was analysed, cows younger than four years of age (class 1) explored larger areas (9.09 hectares per day (ha/d)) than cows of 4-5 years of age (class 2; 6.93 ha/d; $P = 0.039$) and older cows of six or more years of age (class 3; 6.32 ha/d; $P = 0.014$). The daily home range explored by the 4-5 years old cows was also larger than that of the older cows ($P = 0.046$). The main effects of genotype in daily home range across cow age-classes (i.e., home ranges averaged over levels of cow age-class) was not significant ($P > 0.05$), but it tended to decrease, with AB and AC being greater than CC and BC, which were greater than BB (7.88 ha/d, 7.82 ha/d, 7.29 ha/d, 7.25 ha/d and 6.60 ha/d, respectively).

The results of the combined effects of genotype and cow age revealed that the daily home range of an individual cow calculated with the minimum convex polygon method ranged between 5.67 ha/d (genotype BB, cow age-class 3) and 9.74 ha/d (genotype AB, cow age-class 1) (Table 2.5). Cows with genotype BB displayed among the lowest daily home ranges in age-classes 2 and 3, but for the cows under four-years of age (class 1), genotype BB cows had similar daily home ranges to the BC and CC genotype cows, but significantly smaller daily home ranges than the AB ($P = 0.047$) and AC cows ($P = 0.032$). Young cows (class 1) with genotypes AB and AC had larger daily home ranges than cows of any other genotype of age-classes 2 and 3; and within cow age-class 1, the daily home ranges of the AB and AC cows were larger than BB cows (see above). For age-classes 1, 2 and 3, cows of genotype AC displayed slightly smaller, but statistically similar daily home ranges to AB cows ($P = 0.933$, $P = 0.933$ and $P = 0.933$, respectively). Cows with genotypes BC and CC had intermediate values for their daily home ranges.

Movement tortuosity

Here is presented the selected linear mixed model for movement tortuosity:

Response variable: log(sp-tortuosity)

Fixed factors: GRM5_genotype + cow_age_class +

Random factors: (1|cow_id) + (1|mob_id)

Where sp_tortuosity refers to movement tortuosity and id denotes the identifier.

Analysis of the main effects of genotype and cow age on daily movement tortuosity, revealed that cows in class 3 (six-years and older) displayed similar tortuous trajectories (674 metres per hectare (m/ha) to those shown by the middle-aged cows (class 2; 657 m/ha; $P = 0.517$). Younger cows of class 1 (less than four-years of age) had less tortuous trajectories (479 m/ha) than cows in both of the older age-classes ($P = 0.005$). The genotype main effects averaged by cow age-class revealed that cows with the genotype *BB* had the most tortuous trajectories (665 m/ha). This was followed by cows with genotypes *BC* and *CC* (616 m/ha; not significant $P = 0.123$, and 602 m/ha; a trend at $P = 0.061$, respectively). Cows with the genotypes *AC* (578 m/ha) and *AB* (540 m/ha) had straighter trajectories than *BB* genotypes ($P = 0.021$ and $P = 0.012$, respectively). Differences in movement tortuosity between cows of *AC* and *AB* genotype were trending towards a difference ($P = 0.061$).

When accounting for the combined effects of genotype and age, daily movement tortuosity estimated with the spatial search pattern ranged from 434 m/ha (genotype *AB*, age-class 1) to 751 m/ha (genotype *BB*, age-class 3) (Table 2.5). Cows of genotype *BB* and age-classes 2 and 3 displayed among the largest daily movement tortuosity, but having the *BB* genotype did not lead to much distinction in the young cows (age-class 1). In turn, young *BB* cows displayed similar movement tortuosity to other genotypes in age-classes 2 and 3, as well as to cows with genotypes *BC* and *CC* in age-class 1.

Cow age-class as main factor

Results presented above showed some of the differences among age-classes when computed as main factor. For example, home range significantly ($P < 0.05$) decreased from cow age-class 1 (9.09 ha/d) to classes 2 (6.93 ha/d) and 3 (6.32 ha/d). Movement tortuosity was significantly ($P < 0.01$) smaller for cows age-class 1 (479 m/ha) than both older classes suggesting that trajectories of the youngest class was straighter. Differences on movement tortuosity were not significant between cows of age-classes 2 (657 m/ha) and 3 (674 m/ha), albeit values tended to increase. This lack of difference might be largely explained by the significantly ($P < 0.05$) shorter horizontal distance travelled by cows in class 3 (3689 m/d) compared to cows in class 2 (3941 m/d) and, in lesser extent, by their difference in home range. On the contrary, the slope 85th quantile significantly ($P < 0.01$) increased from the youngest cow age-class 1 having 37.5% (17 °(angular degrees)) to age-class 2 with 45.8% (21 °) while even steeper 85th quantile slope was recorded in cows of age-class 3, which reached 52.9% (24 °).

2.4 Discussion

2.4.1 Sets of correlated grazing personality behaviours

Senft et al. [142] provided clues about which grazing personality behaviours were relevant to describe grazing patterns and predict the distribution of cattle. Accordingly, eleven behaviours related to the grazing personality of beef cattle were measured or calculated in this study. In some cases, these behaviours were correlated and provide insights into behavioural 'trade-offs' that could be affected by genetics. In other cases, the correlations between behaviours might be explained with other reasons or factors, and might not have their roots in behaviour or animal personality.

In the following paragraphs, we discuss in detail some examples of such correlations. We report correlations that do suggest behavioural trade-offs and even concatenated behaviours that ultimately might resemble differences in grazing personality. For example, cows that travelled longer distances on a daily basis (i.e., both horizontal distance travelled and three-dimensional distance travelled) had increased daily elevation gains and displayed more tortuous trajectories. Longer distances travelled travelled (i.e., horizontal distance travelled and three-dimensional distance travelled) were moderately and positively correlated with daily movement tortuosity ($r = 0.48$ and $r = 0.49$, respectively in Table 2.2), while daily vertical distance travelled had an even stronger correlation ($r = 0.56$) with daily movement tortuosity, meaning more twisted trajectories when elevation gains (and losses) increased. It was also found that cows that travelled longer distances (i.e., horizontal distance travelled and three-dimensional distance travelled), typically had larger daily home ranges ($r = 0.32$ and $r = 0.30$, respectively in Table 2.2). In contrast, cows with greater vertical distance travelled (i.e., the sum of elevation gain and loss), typically explored smaller home ranges (r = -0.13 in Table 2.2). Despite this, the daily horizontal and vertical distances travelled were positively correlated, and increased in both of these behaviours with larger or smaller home ranges, respectively. This suggests a trade-off between the size of the area explored and the extent of elevation change. Similarly, the negative correlation (r = - 0.27 in Table 2.2) between the daily home range and daily movement tortuosity suggests another trade-off, where the smaller the area explored, the more crooked the trajectories.

Browning et al. [126] analysed the grazing behaviour of mature Angus x Hereford cross cows grazing in the northern Chihuahuan Desert (NM, USA). They reported that as pasture regrew, the movement tortuosity estimated during grazing activity periods (75.3 m/ha) tended to increase ($r = 0.62$), while home range decreased (r = -0.38). These results agree with our findings and further support the existence of a trade-off between the extent of the home range and the nature of the movement tortuosity. It does however need to be noted that there was a marked difference between the land being grazed in the two studies. Browning's et al. [126] experiment was set up on flat desert land with a mean distance travelled of 6100 m/d and explored areas of 81.1 ha/d, versus our study, which was conducted in steep and rugged terrain where the average distance travelled was 3700 m/d and cattle explored 12.77 ha/d. Overall, the study undertaken here, revealed an average daily home range of nearly 13 ha/d, with a notably higher movement tortuosity of 629 m/ha, eight-fold larger than the value of 75.3 m/ha reported in Browning et al. [126]. Another difference between these studies is that Browning's et al. [126] measurements accounted exclusively for grazing time, while our study used the total daily movement regardless of activity, (i.e., it was not just time spent grazing). Additionally, given the difference in latitude of these two studies, the effect of day-time and night-time temperatures in the areas being grazed may also affect grazing behaviour, albeit unfortunately this variable was not measured.

Another study conducted in central New Mexico (NM, USA) by Wesley et al. [37] studied the grazing behaviours of free-ranging beef cows. Based on their findings, two contrasting grazing personality types were described: type 1 cows, which used larger areas and displayed less twisted trajectories (21 ±0.3 ha/d, 264 ±8.9 m/d, respectively); and type 2 cows, which covered smaller areas and exhibited more tortuous trajectories (17 ±2 ha/d, 314 ±2.6 m/d, respectively). These results exemplified the home range versus movement tortuosity trade-off. It is noteworthy that the values reported by Wesley et al. [37] were closer to the values we recorded, than to those in the study of Browning et al. [126]. This may be because Wesley et al. [37] included all-of-day movement trajectories (as we did), instead of the grazing-time movement trajectories described by Browning et al. [126].

A similar analysis was also reported by Pauler et al. [143] for Swiss alpine pastures grazed with three different breeds of beef cattle. They observed that what are considered to be the more productive breeds (Braunvieh and Angus x Holstein cross cattle), took many more steps and covered longer distances but in much smaller areas, with this suggesting greater movement tortuosity. In contrast, the less productive Highland cattle appeared to explore larger areas but with fewer steps and shorter distance travelled, suggesting reduced movement tortuosity. While the grazing pattern of productive breeds suggested much greater grazing intensity (*sensu* Pauler et al. [143]) and therefore a high movement tortuosity, it also implied a much smaller portion of the accessible land was utilized, and probably high selectivity to graze a preferred and small area. The grazing pattern associated with the 'less productive breed' reflected individuals that explored a larger proportion of the available land, but with fewer steps. This suggests lower movement tortuosity and, as Pauler et al. [143] described, a much greater 'evenness' (i.e., on average all accessible vegetation patches were visited with similar frequency) denoting lower selectivity within the accessible land. The findings reported by Pauler et al. [143] suggest once again a trade-off between home range and movement tortuosity between breeds that displayed distinctive GP-behaviours.

Given the differences in experimental conditions between Browning et al. [126] and Wesley et al. [37], Pauler et al. [143] and the study here presented, it is notable that there are apparently similar trade-offs between home range and movement tortuosity. Furthermore, the genetic associations revealed here suggest that such trade-offs might be genetically controlled.

We acknowledge that some correlations between GP-behaviours might not have purely behavioural roots, but can instead be explained by other things such as through being mathematically related or being related through some environmental effect. For example, it is not surprising that the daily horizontal distance travelled and the daily three-dimensional distance travelled were highly correlated, because the former accounts for distance between two points in the same plane (a two-dimensional measure) and the latter accounts for changes in elevation as well as horizontal movement (a three-dimensional measure) by using the hypotenuse of the relocations. Equally, the vertical distance travelled is the sum of both elevation gains (ascent) and losses (descent), which while perhaps not equal, were nevertheless very similar in absolute values. Accordingly, the correlation of either with vertical distance travelled should be close to 100%. Additionally, in hill country rangelands steeper slopes occur at higher elevations, which is an environmental preconditioning for an animal grazing at higher elevation and steeper slopes as it is reflected with moderately positive correlations between the 85^{th} quantile of slope and relative elevation as well as between the daily slope 85^{th} quantile and the 85^{th} quantile of relative elevation. Overall, it could be concluded that not all correlations between GP-behaviours are necessarily meaningful from a behavioural viewpoint.

From an animal personality perspective, it is important to validate the correlation between grazing personality behaviours because this is a key premise to comply with the definition of animal personality [144]. Our results included correlation between several GP-behaviours measured on 303 individual cows over time (i.e., 7 to 28 d of recording GPS positions) and across contexts (i.e., different paddocks following the grazing rotation established by each farmer). Correlations among GP-behaviours have been reported in the past in beef cattle [145, 143, 37], dairy cattle [146, 147, 148], sheep [91, 49] and other domesticated livestock [23] and all support our findings. There is also evidence of the temporal consistency of such correlations in livestock [81, 107, 149, 150]. Changes in GP-behaviours have been also reported in dairy cattle [151], where they have been called personality developments and which are likely explained by regulating mechanisms such as animal maturity [150]. It might be concluded then that the behaviours investigated here comply with a definition of animal personality and might therefore be useful permanent descriptors of grazing personality for beef cattle and other foragers (see Moreno

García et al. [109]). However, further investigation of behaviours that describe key traits of grazing personality (GP-traits) also seem to be warranted [22, 144, 152].

2.4.2 Variation in the bovine *GRM5* gene

Investigation of the variation revealed in bovine *GRM5* exon 5 resulted in the discovery of three variant sequences, which were the result of two 'silent' nucleotide substitutions registered previously (rs43744222 and rs210610001). The presence of three synonymous single nucleotide polymorphisms (SNPs) that do not change the amino acid coding in *GRM5* exon 5 does not mean these changes in nucleotide sequences are benign or innocuous, but rather they might lead to deleterious outcomes as exemplified with other genes [153]. Such silent substitutions might affect an animal's functioning by a number of means that have been well described by Hurst [153]. For example, the nucleotide change might affect the rate of transcription and translation, and hence the folding of the peptide produced into a three-dimensional structure, which in turn may affect its function. Additionally the nucleotide changes may be linked to sequence variation elsewhere in the gene that has functional effect, or sequence variation in another closely linked gene that has a functional effect, given the linear arrangement of genes on chromosomes. Alternatively, nucleotide changes can affect the splicing and processing of the primary transcript, and thus modify the mRNA (and thus amino acid sequence produced at translation) or the regulation of translation. In the behavioural context, Fu et al. [154] illustrated that the effects of silent mutations on *Drosophila*'s circadian rhythm and thus, its potential implications on the regulation of animal daily and seasonal behaviours in general, which could apply to free-range cattle in steep and rugged terrain [155]. Considering the previously reported associations of *GRM5* with behaviour, movement and cognitive abilities in several animal species [41, 111, 114, 115, 112, 113], the variants of bovine *GRM5* reported here are notable, because of their potential for use in cattle selection programmes that could target particular grazing patterns and cattle distribution in rangelands.

The proportion of genotypes was asymmetrical (Table 2.3) reflecting the low frequency of *GRM5* variant *A*. The *AA* genotype was only present in three cows from three different farms, and this did not allow any sensible comparison with others genotypes as any analysis is likely biased by the small sample-size. Moreover, as might be expected *AB* and *AC* were among the less common genotypes with frequencies of approximately 6% and 10%, respectively. These proportions were similar across the four farms, with this suggesting that in Hereford herds in New Zealand, natural or breeding-mediated factors had led to selection away from variant *A*.

Phenotypically, the *AB* and *AC* cows tended to explore larger areas in a slighter wider range of elevations, while displaying straighter trajectories (see Tables 2.5 and S2). From a genetic viewpoint, variant *A* was associated with elevation range, home range and movement tortuosity (Table 2.4); hence selection for more *A* in herds could result in changes to grazing patterns. It could then be further hypothesized that selecting for *A* at the expense of cattle with *B* (which had the largest daily movement tortuosity), would lead to differences in collective grazing personalities. Taking the above-mentioned example from Pauler et al. [143], selecting towards *A* could therefore increase the proportion of the 'Highland cattle-like' grazing pattern. However, to confirm the changes of grazing patterns for entire herds (i.e., collective grazing personality *sensu* Moreno García et al. [109]), the genetic associations and trends towards association with phenotypic behaviours reported here need to be further investigated in larger populations and with better balance introduced into the design to insure all the possible genotypes were evenly represented. It must also be acknowledged that other variants might be found as cattle of differing breed and larger herds are studied.

2.4.3 Genotype-phenotype effects on grazing patterns

The most important results arising from this study are probably the discovery of genetic effects over consistently displayed grazing patterns in cattle and its potential for selecting individuals and designing herds based on desired grazing behaviours. The potential for genetic associations were investigated for simple behaviours (e.g., daily horizontal distance travelled, daily elevation gain and daily home range) as well as with a variable (daily movement tortuosity) that was constructed from the daily horizontal distance travelled and the daily home range. Associations were also investigated with so-called 'relative' behaviours, which express the grazing behaviour of an individual cow relative to the average behaviour of the herd. While associations were revealed with the simple GP-behaviours, no associations were found with relative behaviours. This latter approach attempts to fairly compare cows tracked under different conditions (e.g., on different farms and for different years), albeit the need to have an unbiased comparison was addressed in this study by correcting the mixed models with explanatory factors.

Interestingly, our results revealed trends for association and associations between *GRM5* variation and horizontal distance travelled and home range, respectively (Tables S2 and 2.5). Furthermore, we discovered stronger genetic association with daily movement tortuosity (Table 2.5), in part confirming the validity of the genetic effects on horizontal distance travelled and home range. Previous studies of *GRM5* genetic associations with indexes of terrain use in cattle have yielded contradicting results with Bailey et al. [41] describing associations, but Pierce et al. [57] failing to find associations. We hypothesize that one reason for the failure to detect genetic associations by Pierce et al. [57] could be their use of created or synthetic indexes that integrated two or more simple GP-behaviours, but in a normalized and averaged manner that rank individual cows according to the behaviours measured. This would be consistent with the lack of associations with the relative behaviours reported in the present study. It is unknown whether the simple behaviours chosen by Pierce et al. [57] (with or without normalization and centring) would have shown any signs towards genetic association. In this study, no association was revealed between *GRM5* variation and the 85th quantile of daily slope, which is consistent with Pierce et al. [57]. Overall, it could be concluded that the reporting of trends toward genetic association (i.e., when $P < 0.1$) for simple variables is required to investigate and better understand potentially stronger associations with constructed variables that denote behavioural patterns, such as those observed in cattle grazing personality.

This study revealed *GRM5* exon 5 associations with daily home range and daily movement tortuosity (Table 2.5), which were age-dependent and that implied variation in grazing patterns among genotypes. Homozygous *BB* cows displayed the smallest daily home range and the most tortuous trajectories, while *AB* and *AC* cows had among the largest home ranges and straighter trajectories. Such observations applied well for four-year-old and older cows, but were not obvious for younger cows where *BB* individuals displayed similar home ranges and movement tortuosity to the *BC* and *CC* genotypes (Table 2.5). Behaviour changes during an animal's lifetime are known as personality 'development' and may occur for a variety of intrinsic reasons including genetic and epigenetic regulation, neurological and hormonal effects, among others [156] and may be affected by external reasons such as the social environment [109], for example. In dairy cattle, Neave et al. [150] studied the behavioural reactivity to novelty over the maturation of individuals from 1 month of age to 30 months. The experiment involved two longitudinal observations of ≈ 32 female Holstein cattle each, where individuals were evaluated with three personality tests (i.e., response to a novel environment, a novel human and a novel object), which were conducted on consecutive days at four defined times of development (i.e., pre-weaning, post-weaning, puberty and first lactation). Some behaviours were consistent between the pre-weaning and post-weaning stages, as well as between puberty and the first lactation; but the consistency was absent when behaviours were compared before and after cows' sexual maturity (i.e., behaviours measured either in pre-weaning or post-weaning were inconsistent to measurements conducted later on over lactation). The authors concluded that personality traits became more consistent after sexual maturation and pointed out the need for studies beyond the first lactation [150]. Because the phenotypic expression of the *BB* genotype seems to be expressed in four-year-old and older cows in this study, it suggests that grazing personality is still developing in younger cattle, and supports the notion that an animal's maturity affects grazing personality.

Regardless *GRM5* genotype, in our study, older cows displayed smaller home ranges and reached steeper terrain than younger cows (see section Cow age-class as main factor). This suggests that younger cows were able to graze larger areas displaying untwisted trajectories because they use gentler terrain, which contradicts the effects of cow age on the use of steep and rugged terrain previously reported [39, 157].

Are there potential opportunities in selecting cattle based on *GRM5* genotypes? With the information collected, one cannot be certain about the impact of selecting based on *GRM5* variation, but an estimate of the size of the effect can be ascertained from the differences in the marginal means in the GLMMs. Within a given cow age-class, differences in the marginal means for genotypes with the lowest and highest values of home range and movement tortuosity were 19% and 23%, respectively (section Cow age-class as main factor). For home range, the *BB* cows had the lowest marginal mean while the highest was for *AB* cows. Inversely, the lowest marginal mean of movement tortuosity was modelled for the *AB* genotype and the highest for *BB* (i.e., same genotypes but in opposite ends), which is supported by the negative correlation between the two GP-behaviours. We speculate that such differences could be higher if cows with genotype *AA* were well represented in cattle herds and therefore could be included in the comparisons. Even with these results, a change of roughly 20% in daily home range and approximately 23% of movement tortuosity over the explored area could make a notable difference in rangeland use, ecological functioning and eventually in cattle production. Further research is encouraged to elucidate the benefits of applying grazing personality in cattle selection programmes.

2.5 Conclusion

Our study revealed the association of glutamate metabotropic receptor 5 gene (*GRM5*) variation with home range and movement tortuosity that could possibly be used in cattle breeding programmes to improve rangeland utilisation and grazing distribution. There appeared to be a genetically determined trade-off between the daily home range and daily movement tortuosity. Our research also showed a widespread association between cow age-classes and most behaviours of grazing personality with two interesting implications. Firstly, grazing personality development occurs beyond a cow reaching her sexual maturity and it appears to stabilise in 4-year-old cows. Secondly, cows of younger age classes grazed larger but gentler areas, while displayed straighter trajectories than their counterparts older cows. In this study, three novel sequence variants of *GRM5* exon 5 were revealed, and these had different frequencies in the Hereford cattle. The asymmetric occurrence of *GRM5* variation offers the opportunity to shape the grazing patterns of beef cattle through selection.

2.6 Supplementary material

Fig. S1. Residuals versus fitted values (Part A). Plots of scaled residuals versus fitted values of linear mixed models of six (A-F) grazing personality behaviour. Residual outliers are values beyond ±3.

Fig. S2. Residuals versus fitted values (Part B). Plots of scaled residuals versus fitted values of linear mixed models of five (A-E) grazing personality behaviour. Residual outliers are values beyond ±3.

Table S1. Akaike Information Criterion (AIC) for linear mixed models of grazing personality behaviours (GP-behaviours) fitted with twelve combinations of random factors.

Table S2. Associations between genotypes of the glutamate metabotropic receptor 5 gene (*GRM5*) and grazing personality behaviours (GP-behaviours).

Chapter 3

Key grazing behaviours of beef cattle identify specific genotypes of the glutamate metabotropic receptor 5 gene (*GRM5*)

3.1 Introduction

Grazing personalities of foraging animals were defined as 'suites of traits of different nature (e.g., behavioural, cognitive, physiological, and morphological), which are correlated and often concatenated, to result in specific grazing patterns displayed consistently across contexts and over time' [109]. In this context, the consistent expression of distinctive grazing personalities might be at least in part underpinned by specific grazing genes [109]. The social and biophysical environments as well as the animal's experiences and emotional states likely also affect grazing behaviours at the individual and collective level.

The social and biophysical environments are strong drivers of grazing behaviours in herbivores [158, 33, 159], which are further shaped by the cognitive condition of animals. Yet, behavioural genes and their expression are also intrinsic determinants of behaviours that are passed inter-generationally. Meta-analyses of animal personality in wild and domestic animal populations provides estimates of such inter-generational genetic effects. For example, van Oers and Sinn, [6] reported animal personality trait heritability ranging from 0.24 in domestic populations to 0.36 in wild populations after a metaanalysis of 75 studies of animal personality. Similarly, Dochtermann et al. [54] targeted publications on animal personality with estimates of repeatability and heritability of animal behaviours. These authors concluded that despite the often moderate to low heritability of behaviour, repeated behaviours pertaining to animal personalities had much higher heritability with an estimated 52% of its variation explained by genetic variation. In cattle while grazing behaviours has been attributed to animal personality [160], its genetic basis seems to be poorly understood.

Studies by Bailey et al. [41] and Pierce et al. [57] pioneered the use of whole genome screening of free-range management cattle to attempt to identify genetic regions associated with terrain use indexes. These were derived from key grazing behaviours, such as a cow's movement relative to elevation, slope, and distance to water sources. While these studies reported promising genetic associations that suggested potential quantitative trait loci (QTL) and candidate genes, the sample size in the Bailey et al. [41] study was small ($n = 87$) suggesting the need for a larger investigation. Pierce et al. [57] had a larger sample size ($n = 321$), but their results were not consistent with Bailey et al. [41], and they reported only weak associations, possibly because of still only having a moderate sample size, and because of the heterogeneity of their grazing data.

Moreno García et al. [161] targeted the glutamate metabotropic receptor 5 gene (*GRM5*) studying variation in the exon 5 region and reported genetic associations between genotypes and grazing behaviours such as home range and movement tortuosity, and with a trend towards association with elevation range and horizontal distance travelled. Their findings, together with the early study of Bailey et al. [41], and studies that have associated *GRM5* expression with activity levels and exploratory behaviours in animal models [111, 112, 113], support the relevance of *GRM5* as a predictor of grazing personality behaviours in beef cattle.

Moreno García's et al. [161] analyses were performed with a mensurative approach and without control over the representation of *GRM5* genotypes in the sampled cattle. This forced the authors to exclude from analysis a rare genotype present in just 1% of the cattle investigated and apply their modelling to an unbalanced dataset of five *GRM5* genotypes, where two genotypes accounted for 71% of the sampled cows. Haixiang et al. [162] describe the problems encountered by classification algorithms when dealing with unbalanced datasets, and among other solutions discuss 'dataset under sampling' (i.e., randomly discarding cases of the majority classes) to obtain better balanced datasets that equally represent all classes under investigation. The use of a more balanced dataset might revealing hitherto hidden differences that would otherwise be undetected. Accordingly, in this study, a *quasi*-manipulative experiment design was set by under sampling to investigate bovine *GRM5* genotypes and grazing behaviours in the beef cattle.

On the basis that grazing genes and their expression precede the development of individual and collective grazing personalities [109] in the following study it was hypothesized that a combination of consistent grazing behaviours might reveal specific *GRM5* genotypes in beef cattle under free-range management. It was further hypothesized that genetic variation of *GRM5* would result in differences among cows for simple grazing personality behaviours. The aims were therefore to identify a combination of grazing behaviours that assist in discriminating *GRM5* genotypes in beef cattle and to assess the behavioural differences that might exist between different *GRM5* genotypes. A further aim was to ascertain if temporal consistency is needed for selecting grazing behaviours that assist in the distinction of *GRM5* genotypes.

3.2 Materials and methods

The Lincoln University Animal Ethics Committee approved all procedures involving animal handling and sampling (AEC 201816, AEC 201816 extension and AEC 202002).

The investigation was conducted using a selected subsample of the cattle described in the previous chapter and Moreno García et al. [109], to create a *quasi*-manipulative experiment where *GRM5* genotype was the treatment, farm was a block effect and individual cows of four and five years of age (i.e., cow age class 2 in previous chapter and in Moreno García et al. [161] were the experimental unit [163]). We combined the under-resampling method (the random discard of samples from the major classes) and the exclusion of possible outlier individuals (detected with the 'aq.plot()' function of the R package 'mvoutlier' [164, 165]) to build a balanced dataset. This was expected to improve the accuracy of discriminant models instead of using unbalanced data, which can compromise the performance of classification algorithms [162].

Two separate datasets were selected, a fully balanced training dataset and a 'testing' set. The training dataset included four cows for each *GRM5* genotype present at greater than a 5% threshold (i.e., AB, AC, BB, BC, and CC) from each of the four farms (n = 80 cows in total) and thus evenly represented the five common *GRM5* genotypes with 16 cows per genotype. The testing dataset included the same five *GRM5* genotypes, but with unbalanced representation (i.e., AB, AC, BB, BC, and CC; with n = 7, 16, 13, 16, and 16 cows respectively). These cows were still present on all four farms (n = 68).

The training dataset was purposely balanced to evenly represent the *GRM5* genetic variation as well as the four farms involved in the original study. However, in the testing dataset, there was unbalanced representation of the five genotypes on all four farms, with fewer cows that were AB and BB, than were AC, BC and CC (i.e., $\approx$ 11%, $\approx$ 23%, $\approx$ 19%, $\approx$ 23% and $\approx$ 23%, respectively).

Age-based variation in the data was minimized by selecting only cows of 4-5 years of age, which were expected to display the 'stable grazing behaviours' of mature animals [109].

3.2.1 Study sites and cattle

For a detailed description of the methods, refer to the previous chapter and Moreno García et al. [161]. Briefly, the study involved four private farms located in the steep and rugged hill country terrain of Canterbury, New Zealand. The cows studied were randomly selected from existing commercial herds. The location of the grazing cows was at elevations ranging between 200 and 1,000 metres (m) above sea level in relatively large and undeveloped paddocks of an average size of 34.5 hectares (ha). All the paddocks had at least one water supply (natural springs and streams were sometimes present).

The cows were tracked with home-made GPS units in tracking collars (modified i-gotU GT-600 loggers; Mobile Action) deployed over winter months (April-August) of 2019 and 2020 seasons. From each cow deployment, a trajectory including free-range grazing was created with the 'adehabitatLT' R package [120]. GPS outliers were excluded based on turning angles and the speed of consecutive geolocations [121] and trajectory parameters were recalculated. Geolocations were annotated for elevation, slope and aspect using the 'raster' R package [124] and derived rasters (3D Analyst toolbox; ArcMap$^{\text{TM}}$, [123]) from digital elevation models of New Zealand (16 m resolution; the shuttle radar topography mission, Land Information New Zealand [122]).

3.2.2 Grazing behaviours

The trajectories of the cows were used to calculate variables related to cattle grazing patterns and grazing distribution in free-range systems as descriptors of grazing personality behaviours (Table 3.1). The broad array of 35 variables were chosen based on their use in previous studies [81, 166, 126, 167, 158, 142, 168]. Over the 15 consecutive days of GPS-based, the individual cow measurements were aggregated into daily mean values and coefficient of variation (CV) using the 'summary()' function of 'dplyr' R package [125]. Variables that were not normally distributed were excluded from further analysis. Table 3.1 presents a summary of the calculations and data transformation used.

Table 3.1. List of grazing personality behaviours with abbreviations, units, data transformations and description of calculations.

Grazing personality behaviours	Abbreviations	Units	Transformation	Description
Daily horizontal distance travelled	dist_ho	m/d	Square root	Distance calculated as the sum of distances between consecutive GPS[1] data points per day using two dimensions (i.e., Easting and Northing) of the UTM[2] projection
Daily vertical distance travelled	dist_ve	m/d	Log	Distance calculated as the sum of the absolute difference in elevation (i.e., dimension z) between consecutive GPS data points per day using a DEM[3]
Daily elevation range	ele_range	m		Range of elevation computed as the difference between the daily maximum and minimum elevation
Daily elevation gain	ele_gain	m/d	Square root	Sum of positive changes of elevation between consecutive GPS data points as depicted from a DEM
Daily mean elevation centred per farm	ele_mean_farm	m		For any given cow of a farm, the mean elevation across GPS data points per day as depicted from a DEM minus the mean elevation for that farm calculated across all days and cows included in the analysis
Daily elevation 85th quantile centred per farm	ele85_farm	m		For any given cow of a farm, the 85th quantile of elevation across GPS data points per day as depicted from a DEM minus the mean elevation for that farm calculated across all days and cows included in the analysis
Daily elevation 15th quantile centred per farm	ele15_farm	m		For any given cow of a farm, the 15th quantile of elevation across GPS data points per day as depicted from a DEM minus the mean elevation for that farm calculated across all days and cows included in the analysis
Daily slope mean	slope_mean	°	Square	Mean slope across GPS data points per day as depicted from a DEM
Daily slope maximum	slope_max	°	Cube root	Maximum slope registered in any given day across GPS data points as depicted from a DEM
Daily slope 85th quantile	slope85	°	Cube root	85th quantile of the slope across GPS data points per day as depicted from a DEM
Daily slope 15th quantile	slope15	°	Cube root	15th quantile of the slope across GPS data points per day as depicted from a DEM
Daily home range	hr_mcp	ha/d	Log	Explored area estimated by calculating the minimum convex polygon depicted from all GPS data points per day using the R package 'adehabitatHR'
Daily movement tortuosity	sp_tortuosity	m/ha	Log	Movement tortuosity using the spatial search pattern estimated as the ratio between daily horizontal distance and daily home range

Continuation of Table 3.1 List of GP-behaviours...

Grazing personality behaviours	Abbreviations	Units	Transformation	Description
Adjusted daily horizontal distance travelled	adj_dist_ho	m/d		In any given day, the cow's horizontal distance minus the minimum horizontal distance recorded in the herd plus 3500 (i.e., roughly the mean daily horizontal distance for all cows and days)
Adjusted daily elevation mean	adj_ele_mean	m	Square	In any given day, the cow's mean elevation minus the minimum elevation recorded for the same day in the herd plus 350 (i.e., roughly the mean daily elevation for all cows and days)
Relative elevation range	rel_ele_range	0-1 scale	Cube root	In any given day, theratio between the cows' elevation range and the elevation range of the herd
Relative elevation mean	rel_ele_mean	0-1 scale	Cube root	In any given day, ratio between the cows' mean elevation minus the minimum elevation of the herd and, the elevation range of the herd
Relative slope range	rel_slope_range	0-1 scale	Log	In any given day, ratio between the cows' slope range (i.e., maximum minus minimum slope) the slope range of the herd
CV[4] of daily horizontal distance travelled	dist_ho_cv	0-1 scale		Coefficient of variation of dist_ho
CV of daily vertical distance travelled	dist_ve_cv	0-1 scale	Log	Coefficient of variation of dist_ve
CV of daily elevation range	ele_range_cv	0-1 scale	Log	Coefficient of variation of ele_range
CV of daily elevation gain	ele_gain_cv	0-1 scale	Log	Coefficient of variation of ele_gain
CV of daily elevation 85th quantile centred per farm	ele85_farm_cv	0-1 scale	Log	Coefficient of variation of ele85_farm
CV of daily elevation 15th quantile centred per farm	ele15_farm_cv	0-1 scale	Log	Coefficient of variation of ele85_farm
CV of daily slope mean	slope_mean_cv	0-1 scale	Log	Coefficient of variation of slope_mean

Continuation of Table 3.1 List of GP-behaviours...

Grazing personality behaviours	Abbreviations	Units	Transformation	Description
CV of daily slope maximum	slope_max_cv	0-1 scale	Log	Coefficient of variation of slope_max
CV of daily slope 85th quantile	slope85_cv	0-1 scale	Log	Coefficient of variation of slope85
CV of daily slope 15th quantile	slope15_cv	0-1 scale	Log	Coefficient of variation of slope15
CV of daily home range	hr_mcp_cv	0-1 scale	Log	Coefficient of variation of hr_mcp
CV of daily movement tortuosity	sp_tortuosity_cv	0-1 scale	Log	Coefficient of variation of sp_tortuosity
CV of adjusted daily horizontal distance travelled	adj_dist_ho_cv	0-1 scale	Log	Coefficient of variation of adj_dist_ho
CV of adjusted daily elevation mean	adj_ele_mean_cv	0-1 scale	Cube root	Coefficient of variation of adj_ele_mean
CV of relative elevation range	rel_ele_range_cv	0-1 scale	Log	Coefficient of variation of rel_ele_range
CV of relative elevation mean	rel_ele_mean_cv	0-1 scale	Cube root	Coefficient of variation of rel_ele_mean
CV of relative slope range	rel_slope_range_cv	0-1 scale	Log	Coefficient of variation of rel_slope_range

[1] GPS: Global Positioning System fixes recorded with i-gotU GT-600, Mobile Action.
[2] UTM: Universal Transverse Mercator.
[3] DEM: Digital Elevation Model with a 16 m x 16 m spatial resolution.
[4] CV: Coefficient of variation.

The analysis included days with more than a 75% fix rate for a frequency set at 5 min (i.e., at least 216 out of a maximum of 288 data points per day). Any days that included collar deployment, deliberate herding and stock movement, and general animal handling were excluded, so the data only represented periods of 'free-range' grazing for the cows. The grazing days were recorded in hill and high country grasslands, which were labelled as such when the median daily slope of the herd was greater than 8 degrees (°) (i.e. rolling or steeper slope classes in New Zealand grasslands [127]). Finally, only cow deployments with seven or more days of behavioural data were used for analysis, as this was deemed sufficient to represent consistent behaviours.

3.2.3 Statistical analysis

Statistical analyses were conducted with R [131]. For data wrangling, several functions of the following R packages were used, including 'Reshape' [169], 'dplyr' [125], and 'data.table' [170]. Skewness, kurtosis, and normality of grazing behaviours per *GRM5* genotype were evaluated with histograms against corresponding normal distribution curves and with Q-Q plots. When needed, data transformations were applied to better-fit raw values into normal distributions. The Shapiro-Wilk test of normality was performed using 'stat.desc()' function from the 'pastecs' R package [171].

Linear discriminant analyses (LDA) were performed with the R packages 'MASS' [172] and 'DiscriMiner' [173] in a backward stepwise iteration that started with all grazing behaviours. Initially grazing behaviours were selected based on pooled discriminant scores and on the discriminant accuracy rate achieved by each model. Variables were further selected to avoid multi-collinearity with the variance inflation factors (VIF) threshold of < 10 calculated with the R package 'car' [174]. Homogeneity of covariance was assessed with Box's M-test ('heplots::boxM()' R function [175]) and a final model applying quadratic discriminant analysis (QDA) was built with 'MASS' and the 'DiscriMiner' packages.

Multivariate analyses of variance (MANOVA) were performed with the R package 'PERMANOVA' [176] to graphically identify variable redundancies and importance in the MANOVA map, and to assess the amount of total variation explained by the selected combination of variables. Test of multivariate normality per genotype was performed using 'byf.shapiro()' function from the R package 'RVAide-Memoire' [177].

Two-way analyses of variance (ANOVA analyses were performed with *GRM5* genotypes or with *GRM5* variants' presence/absence as main effects and with farm_id as covariate (block effect) using the 'aov()' function of R. Equality of variance among *GRM5* genotypes was tested with the 'leveneTest()' function from the R package 'car' [174].

Inter-class correlation coefficients (ICCs) for each variable were calculated with the training dataset using the R package 'psych' [178, 179]. For all ICC, LDA, QDA and MANOVA analyses, transformed data was used when needed (Table 3.1) and missing values were imputed using the 'imputeMFA()' function ('missMDA' R package [180]).

3.3 Results

3.3.1 Discriminant model for *GRM5* genotypes

Based on the mean value and the coefficient of variation (CV) of several grazing behaviours calculated from 15-days repeated measurements, 35 grazing behaviours variables were assessed as candidates for a discriminant model of the *GRM5* genotypes. Several iterations of linear discriminant analyses were run with the training dataset (n = 80, four individuals per genotype [n = 5, i.e., *AB*, *AB*, *BB*, *BC*, and *CC*] and per farm [n = 4]). The relevance of each behavioural variable was assessed using their pooled linear discriminant scores. Furthermore, the misclassification rates obtained with each combination of variables in the corresponding discriminant models were compared (data not presented) and variables either were kept (increased accuracy) or discarded (diminished accuracy) from the model. The combination of variables was then fitted into regression models to assess their multi-collinearity with the variance inflation factor (VIF). Thus, a first quadratic discriminant model (QDM) was built with the combination of eleven grazing behaviours with highest discriminant scores and that showed non-collinearity (see top of Table 3.2). A second model was generated with a selection of high-scored variables, which were excluded from the first model because of multi-collinearity (bottom of the Table 3.2).

Table 3.2. List of selected grazing personality behaviours (GP-behaviours) used in two quadratic discriminant models (QDMs) of *GRM5*[1] variation and their associated descriptive statistics

Grazing personality behaviours[2]	VIF[3]	LDA[4] pooled scores	ICC2[5] (Mean CV[6])	MANOVA[7] per genotype (*P*-value)	ANOVA[8] per genotype (*P*-value)	ANOVA[8] per GRM5 variant (presence/absence) (*P*-value)		
						A	*B*	*C*
slope_mean	10.59	4.66	0.34	0.657	0.463	0.394	0.337	0.406
rel_ele_mean	6.16	3.89	0.26	0.272	0.120	0.241	*0.089*	0.398
rel_ele_mean_cv	3.34	3.42	(-0.71)[5]	0.223	0.132	0.314	0.489	0.144
ele_range	5.82	3.08	0.31	0.183	**0.004**	0.102	**0.004**	**0.001**
ele_gain	4.23	2.98	0.45	*0.086*	*0.053*	*0.063*	*0.054*	*0.064*
rel_ele_range_cv	2.19	2.76	(-1.06)[5]	**0.036**	*0.057*	**0.003**	0.564	0.300
ele85_farm_cv	3.94	2.61	(-0.74)[5]	0.577	0.253	0.309	0.112	0.113
slope15	8.17	2.58	0.29	0.486	0.135	0.240	0.493	0.471
sp_tortuosity	3.23	1.66	0.30	0.341	0.207	*0.063*	0.695	0.488
ele85_farm	3.43	1.41	0.25	0.210	0.239	0.476	*0.058*	*0.058*
slope_mean_cv	2.17	0.86	(-1.55)[5]	0.957	0.887	0.513	0.630	0.588
dist_ho	8.73	3.49	0.65	0.837	0.542	0.310	0.135	0.220
slope_mean	7.01	3.26	0.34	0.657	See above			
slope_max	5.65	3.15	0.22	0.417	0.403	0.699	0.206	*0.051*
sp_tortuosity	9.38	2.90	0.30	0.341	See above			
rel_ele_mean_cv	2.39	2.83	(-0.71)[5]	0.223	See above			

Continuation of Table 3.2. List of selected grazing personality behaviours.

Grazing personality behaviours[1]	VIF[2]	LDA[3] pooled scores	ICC2[4] (Mean CV[5])	MANOVA[6] per genotype (P-value)	ANOVA[7] per genotype (P-value)	ANOVA[8] per GRM5 variant (presence/absence) (P-value)		
						A	*B*	*C*
hr_mcp	9.14	2.68	0.16	0.251	0.251	0.386	0.160	*0.068*
adj_dist_ho	2.56	2.39	0.35	**0.009**	**0.002**	**0.026**	*0.057*	**0.003**
rel_ele_range_cv	2.15	2.28	(-1.06)[5]	0.036	See above			
ele85_farm_cv	2.90	1.96	(-0.74)[5]	0.577	See above			
ele_range	3.12	1.55	0.31	0.183	See above			
slope_mean_cv	2.21	1.12	(-1.55)[5]	0.957	See above			

[1] *GRM5* = glutamate metabotropic receptor 5 gene exon five region with five genotypes (i.e., *AB*, *AC*, *BB*, *BC* and *CC*) and three variant sequences (i.e., *A*, *B* and *C*).

[2] See GP-behaviours abbreviations and details in Table 3.1. Bold indicate exclusive variable for the discriminant model.

[3] VIF = Variance inflation factor.

[4] LDA pooled scores = Sum of the four absolute linear discriminant scores in the final selection of variables.

[5] ICC2 = Inter-class correlation coefficient in two-way random-effects model, where cows and GPS-tracking collars were randomly allocated.

[6] Mean CV = Mean of the coefficient of variation of a GP-behaviour across all cows.

[7, 8] (MANOVA) and ANOVA = P-values of (multivariate) analysis of variance between genotypes and variants, respectively. Variants' presence/absence comparisons. $P < 0.1$ in italic and $P < 0.05$ in bold.

The first discriminant model exclusively included variables related to elevation (elevation gain [ele_gain], the 85th quantile of elevation centred per farm [ele85_farm], relative elevation mean [rel_ele_mean]) and the 15th quantile of slope [slope15]) (Table 3.2). The second model exclusively included horizontal distance travelled (dist_ho), home range (hr_mcp), maximum slope (slope_max) and the adjusted horizontal distance travelled. Both models shared seven behavioural variables: mean slope (slope_mean) and slope_mean_CV, elevation range (ele_range), CV of the relative elevation range (rel_ele_range_CV), rel_ele_mean_CV, ele85_farm_CV and movement tortuosity (sp_tortuosity)(Table 3.2).

Other variables with high discriminant scores but not used in either model were vertical distance travelled (dist_ve), mean elevation centred per farm (ele_mean_farm), 15th quantile of elevation centred per farm (ele15_farm), 85th quantile of slope (slope85), adjusted elevation (adj_ele_mean), relative elevation range (rel_ele_range) and relative slope range (rel_slope_range). Most CV variables had low discriminant scores and were excluded from further analysis, except those included in both discriminant models (i.e., CV of relative mean elevation [rel_ele_mean_cv], relative elevation range [rel_ele_range_cv], 85th quantile of elevation centred per farm [ele85_farm_cv] and mean slope [slope_mean_cv]).

The first model had VIF below 6.5 for most variables (9 out of 11), except slope15 (VIF = 8.2) and slope_mean (VIF = 10.6), which were considered acceptable and without multi-collinearity (Table 3.2). The combination of variables selected (Table 3.2, top) showed heteroscedasticity as per the Box's M-test ($P < 0.001$) and therefore, a quadratic discriminant analysis (QDA) was preferred over a linear discriminant analysis (LDA) because QDA assumes different variance matrix for each dependable variable. This model achieved 86% accuracy to ascertain genotype (*GRM5* genotype AB = 69%, AC and CC = 87%, BB and BC = 94%) with the training data, which dropped to 46% when used for predicting the cow's genotype from cows not used in training the model. Thus, true classifications for cows of the testing dataset were AB = 43%, AC = 50%, BB = 54%, BC = 56% and, CC = 31%. The *GRM5* genotype mean discriminant scores of the first model are presented at the top of Table 3.3.

Table 3.3. Mean discriminant scores[1] of glutamate metabotropic receptor 5 gene (*GRM5*) genotypes for two quadratic discriminant models[2] (QDMs)

Grazing personality	*GRM5* genotype				
behaviours[3]	*AB*	*AC*	*BB*	*BC*	*CC*
slope_mean	-0.1512	0.3386	-0.0311	-0.0289	-0.1274
rel_ele_mean[4]	-0.2191	0.4792	0.0241	-0.1923	-0.0918
rel_ele_range_cv	-0.4999	-0.3079	0.2075	0.1304	0.4698
ele_range	-0.1339	0.4217	-0.4278	0.0608	0.0792
ele_gain	-0.1274	0.5912	-0.3583	-0.0130	-0.0925
rel_ele_mean_cv	-0.1582	-0.0859	-0.1979	0.5272	-0.0852
ele85_farm_cv	0.1484	-0.3569	0.1854	0.0072	0.0159
slope15	-0.1446	0.3750	0.0031	0.0063	-0.2398
sp_tortuosity	0.1399	0.3051	0.0378	-0.0769	-0.4058
ele85_farm	-0.2858	0.4805	-0.2281	-0.0045	0.0379
slope_mean_cv	0.0668	0.0702	0.0486	-0.0118	-0.1737
dist_ho	-0.0366	0.2287	-0.1877	-0.0467	0.0423
slope_mean	-0.1512	0.3386	-0.0311	-0.0289	-0.1274
slope_max	-0.3518	0.2472	-0.1590	0.1721	0.0915
sp_tortuosity	0.1399	0.3051	0.0378	-0.0769	-0.4058
rel_ele_mean_cv	-0.1582	-0.0859	-0.1979	0.5272	-0.0852
hr_mcp	-0.1893	-0.0499	-0.3095	0.1124	0.4363
adj_dist_ho	-0.4296	-0.1473	-0.3275	0.2542	0.6502
rel_ele_range_cv	-0.4999	-0.3079	0.2075	0.1304	0.4698
ele85_farm_cv	0.1484	-0.3569	0.1854	0.0072	0.0159
ele_range	-0.1339	0.4217	-0.4278	0.0608	0.0792
slope_mean_cv	0.0668	0.0702	0.0486	-0.0118	-0.1737

[1] Mean discriminant scores of QDMs per *GRM5* genotype for the corresponding grazing personality behaviours (GP-behaviours). QDMs built with scaled data (i.e., centred by their mean using the R function 'scale()').

[2] The top of the table lists GP-behaviours used in the 'elevation discriminant model'. The bottom of the table list GP-behaviours of the 'exploration discriminant model'.

[3] See GP-behaviours abbreviations and details in Table 3.1

[4] GP-behaviours exclusive for each corresponding model are in bold.

The second model had VIF values below 6 for 8 variables and no variable exceeded 10 meaning there were no issues of multi-collinearity. Heteroscedasticity was detected with the Box's M test and QDA was applied to build a discriminant model. For the training dataset, the second model achieved 87.5% accuracy, which dropped to 85% when predicting genotypes of the testing dataset. The addition (or suppression) of home range in this model did not modified the model's accuracy for the training data but increased its prediction accuracy from 81% to 85% with the testing dataset. Refer to the bottom of Table 3.3 for the mean discriminant scores of the second model.

3.3.2 Differences among *GRM5* genotypes and variants

Combined behaviours (multivariate analysis)

Plots of the MANOVA analyses are presented in Fig. 3.1A and 3.1B for the first model and in Fig. 3.1C and 3.1D for the second model. Figs. 3.1A and 3.1C shows *GRM5* genotypes ellipses of confidence regions calculated with the Bonferroni method. Grazing behaviour variables of each respective model are presented in Figs. 3.1B and 3.1D.

The first two axes of MANOVA accounted for 83.0% and 81.3% of the total behavioural variation in the first and second model, respectively. For the first model, along the axis 1 (horizontal), AC and BC were paired towards the left end (negative values), BB occupied the opposite end towards the right side (positive values), and AB and CC were located approximately in the centre. Genotypes AB, AC and BB were slightly above the origin of the axis 2 (vertical), BC was located slightly below zero and CC occupied the lowest position. Differences among genotypes on the axis 1 were mostly explained by grazing behaviours related to elevation such as elevation gain (ele_gain), elevation range (ele_range), 85th quantile of elevation centred per farm (ele85_farm), the coefficients of variation of the relative mean elevation (rel_ele_mean_cv) and of the 85th quantile of elevation (ele85_farm_cv) (Fig. 3.1B). Differences along axis 2 were largely explained by the trade-off between the CV of relative elevation range (rel_ele_range_cv) and movement tortuosity (sp_tortuosity) (Fig. 3.1B). Relative mean elevation (rel_ele_mean) and slope-related behaviours (i.e., CV of mean slope (slope_mean_cv), mean slope (slope_mean) and 15th quantile of slope (slope15) split their contribution between axes 1 and 2.

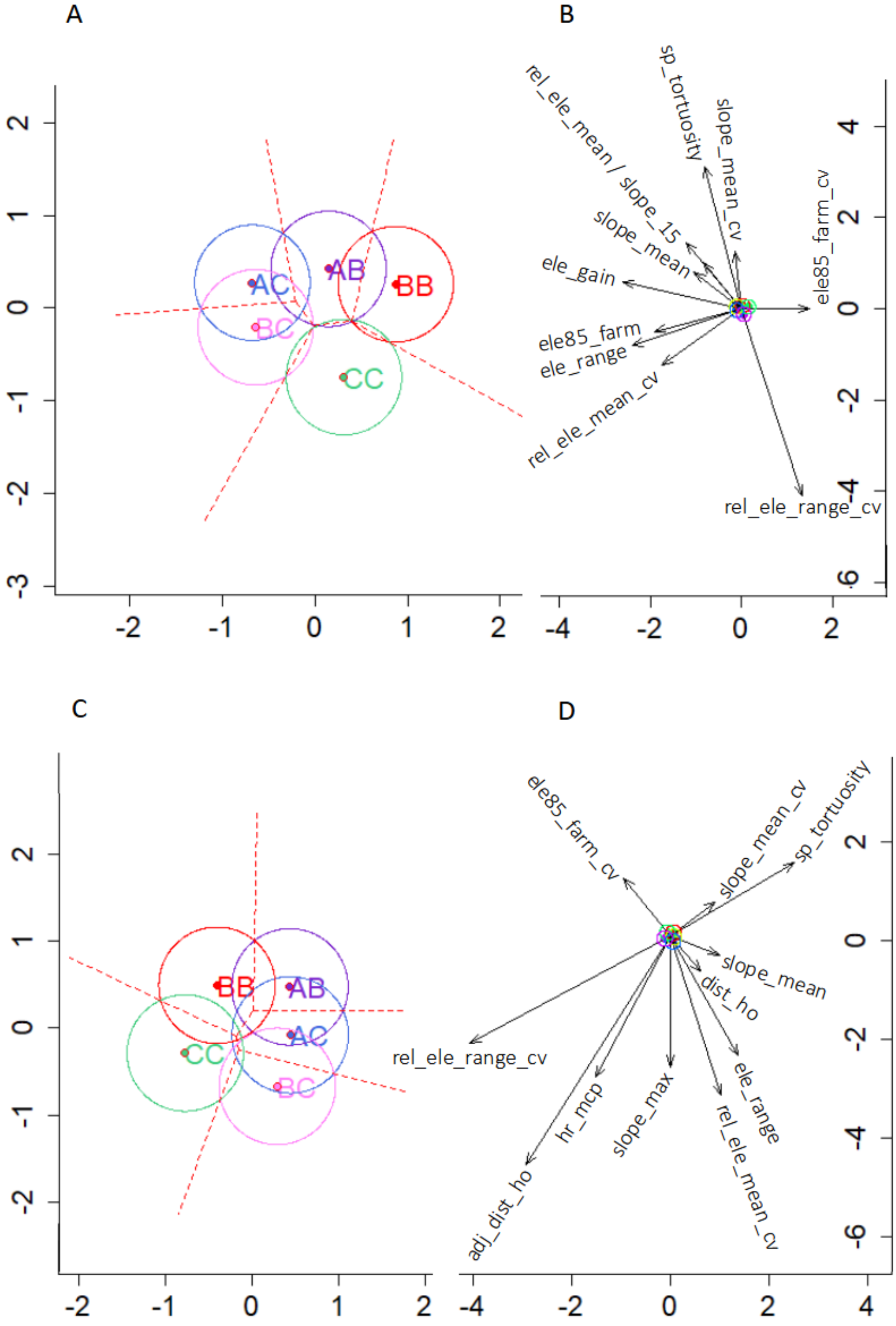

Figure 3.1. MANOVA biplots of two sets of grazing personality behaviours (GP-behaviours). Multivariate analysis of variance (MANOVA) plots of GP-behaviours from the ‘elevation discriminant model’ (Figs. 3.1A, 3.1B) and the ‘exploration discriminant model’ (Figs. 3.1C, 3.1D) with axis 1 (horizontal) and axis 2 (vertical). Figures 3.1A and 3.1C show ellipses of confidence regions (Bonferroni method) of genotypes for the glutamate metabotropic receptor 5 gene (*GRM5*). Figures 3.1C and 3.1D show selected variables used in the corresponding quadratic discriminant models. Figures coordinates were rescaled to optimal matching.

For the second model, axis 1 revealed that genotype *CC* had the lowest values at approximately -1, *BB* near -0.5 and genotypes *AB*, *AC* and *BC* with similar values around 0.5. Along axis 2, *BC* occupied the lower end, *AC* and *CC* were central, and *BB* and *AB* took the upper end.

In the ellipses of both models (Figs. 3.1A and 3.1C), *CC* had the least overlap of confidence region, sharing a relatively small area with *BB* and *BC*. *BB*'s confidence ellipse overlaps with with *AB*, *AC* and *CC* (Fig. 3.1A). In both graphs, there is relatively large overlap of confidence ellipses between *AC* and *BC*, and between *AC* and *AB*.

The MANOVA analysis of the first model resulted in a trend towards a difference for ele_gain, where the major contribution is in axis 1 and significant differences for rel_ele_range_cv corresponding to axis 2 variation (Table 3.2, top). For the second model, significant differences were detected for the adjusted horizontal distance (adj_dist_ho) and rel_ele_range_cv with contributions split in both MANOVA's axes (Table 3.2, bottom).

Individual behaviours (univariate analysis)

Two-way ANOVA analyses among the *GRM5* genotypes revealed differences and trends towards differences between *GRM5* genotypes for elevation range, elevation gain and adjusted horizontal distance travelled as well as for the CV of the relative elevation range (Table 3.2). No differences were revealed for genotype comparisons of ele85_farm (see variant comparisons below). The two-way ANOVA revealed a farm effect for all grazing behaviours except ele_gain and ele_range, but no farm effect for ele85_farm and rel_ele_range_cv. No interactions between genotypes and farms were detected.

Figure 3.2 presents box plots of selected grazing behaviours per genotype produced with the original data (unscaled and untransformed), therefore, reflects the actual measured values for each genotype. Figures 3.2A-D correspond to behaviours with differences (ANOVA $P < 0.1$,) and Figs. 3.2E-H show behaviours without detected differences (ANOVA $P > 0.1$,) in measured values.

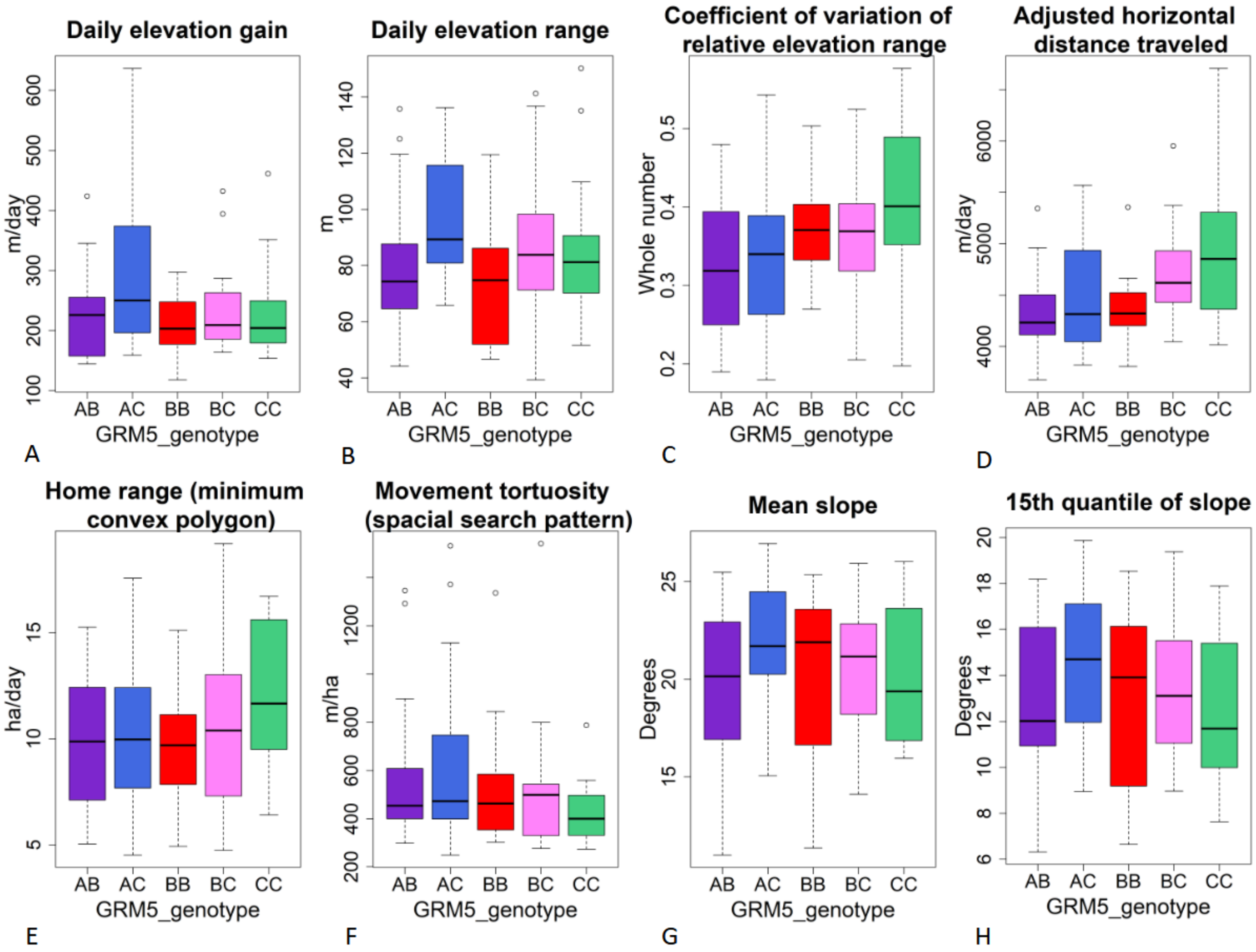

Figure 3.2. Grazing personality behaviours (A-H) box plots per genotype of the glutamate metabotropic receptor 5 gene (*GRM5*). ANOVA analyses in Table 3.2 indicated trends to differences (Fig. 3.2A) and differences (Figs. 3.2B, 3.2C and 3.2D). No further differences were detected. Boxes indicate the 50th (median line), 25th and 75th quantiles. The lower and upper whiskers indicate the smallest and largest values within 1.5x inter-quantile range. Empty circles display possible outliers.

For elevation gain (Figs. 3.2A, 3.2B) cows with genotype *AC* displayed the highest values (250 m median ele_gain), while the other genotypes had lower and approximately similar values ($AB = 226$ m, $BB = 203$ m, $BC = 209$ m, and *CC* 204 m). For ele_range, *AC* had a median of 89 m, *AB* and *BB* displayed the lowest values of 74 m. Genotype *BC* (84 m) and *CC* (81 m) were higher, likely due to a few cows with extremely high scores (possible outliers shown with open circles).

The CV of the relative elevation range (rel_ele_range_cv, Fig. 3.2C) revealed a pattern where genotypes with *A* variant (*AB*, *AC*) had the lowest values, most with *B* variant (*BB*, and *BC*, but not *AB*) had medium values, and the homozygous *CC* displayed the highest CV of elevation range.

Adjusted horizontal distance travelled (adj_dist_ho) and home range (hr_mcp) had similar patterns where genotypes *AB*, *AC* and *BB* had about the lowest and similar medians (≈ 4300 m/d; ≈ 9.8 ha/d), *BC* was higher (4621 m/d; 10.4 ha/d) and *CC* had the highest median (4854 m/d; 11.7 ha/d). Similar patterns (but opposite in values) were revealed for movement tortuosity (sp_tortuosity)(Fig. 3.2F) where *CC* cows had the lowest tortuosity (399 m/ha) while *AB*, *AC* and *BB* had higher medium scores (453 m/ha, 472 m/ha and 462 m/ha respectively), while *BC* displayed the highest tortuosity (498 m/ha).

Figures 3.2G, 3.2H show the mean slope and the 15th quantile slope, respectively. In this case, *AB* (20.1 °, 12.0 °) and *CC* (19.4 °, 11.7 °) had the gentlest slopes, *AC* (21.7 °, 14.7 °) and *BB* (21.9 °, 13.9 °) had the steepest slopes and *BC* (21.2 °, 13.1 °) showed medium values.

3.3.3 Differences among *GRM5* variant sequences

The ANOVA results for the presence/absence of *GRM5* variants were congruent with those results presented for the genotypes in the above section. For example, ele_range were significant for variants *B* and *C*, while the three variants had trends to differences ($P < 0.1$) for ele_gain. Similarly, adj_dist_ho had differences for variants *A* and *C*, while a trend was reported for variant *B*. The variant ANOVAs also revealed differences that were not observed for the comparisons between genotypes. For example, differences and trends to differences were revealed for rel_ele_mean (variant *B*), hr_mcp (variant *C*), sp_tortuosity (variant *A*), ele85_farm (variants *B* and *C*) and slope_max (variant *C*) (Table 3.2).

Bar plots of grazing behaviour variables per *GRM5* variant sequences, based on the measured data, are presented in Fig. 3.3. For elevation-related behavioural variables such as ele_gain, ele_range and ele85_farm (Figs. 3.3A, 3.3B and 3.3C), variant *B* had the lowest values, while *A* and *C* displayed approximately similar higher values. For example, ele_gain was 264 m and 256 m for variants *A* and *C* respectively, while *B* had an elevation gain of 226 m. Similarly, the elevation range of *A* and *C* was 88 m/d and was 80 m/d for *B*. These differences between *GRM5* variant sequences might be due to the high values corresponding to the *AC* genotype (Figs. 3.2A, 3.2B and 3.2C), rather than to the contribution of the remaining genotypes, which had similar lower values (i.e., *AB* for variant *A*; and *BC*, *CC* for variant *C*).

The *C* variant cattle had the greatest home range (11 ha/d) and lower home ranges were calculated for variants *A* and *B* (10 ha/d). The movement tortuosity decreased from the highest value for variant *A* (610 m/ha) to *B* (549 m/ha), and slightly lower again for *C* (531 m/ha).

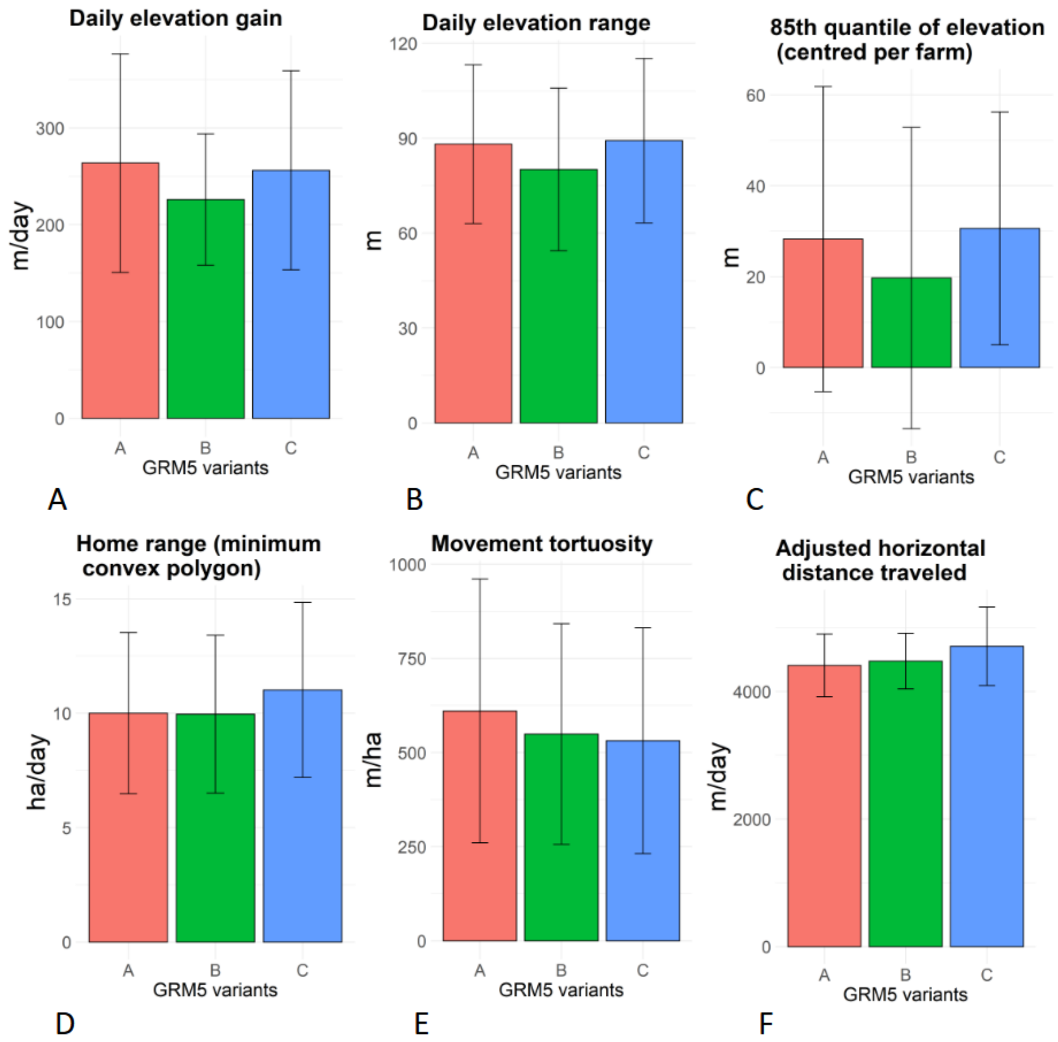

Figure 3.3. Bar plots of grazing behaviours per variant sequence of the glutamate metabotropic receptor 5 gene (*GRM5*). The error bars represent the mean values for the presence of the variant and the lower and upper whiskers indicate the standard deviation.

3.3.4 The temporal consistency of grazing behaviours

Inter-class correlation coefficients in two-way random effects model (ICC2) were calculated for grazing behaviour variables of the training data using the 15-day-periods of repeated measurements. The ICC2 ranged from a minimum of 0.16 ($P < 0.001$) for relative slope range and home range to a maximum of 0.65 ($P < 0.001$) for the horizontal distance travelled (dist_ho) (Table 3.2). dist_ho had the highest ICC2 (0.65) followed by the 15th quantile of elevation centred by farm (ele15_farm; 0.48), vertical distance travelled (dist_ve; 0.47), elevation gain (ele_gain; 0.45), and the adjusted elevation (adj_ele_mean; 0.40). Two of these grazing variables with high ICC2 were included in the discriminant models (ele_gain in the first model and dist_ho in the second one), while the others remained unselected because they caused multi-collinearity problems.

3.4 Discussion

We combined cattle relocation GPS data and satellite data to calculate 19 grazing behaviours in 15-day repeated measurements. Data was then aggregated into means with calculated CVs and tested for normality within groups (i.e., genotypes). Next 35 aggregated grazing behaviours were assessed for their suitability to discriminate between *GRM5* genotypes, and two discriminant models were built with selected behaviours. Further analyses were conducted (i.e., MANOVA, ANOVA) to detect differences between genotypes on grazing behaviours of discriminant models. Finally, the ICC was used to estimate the temporal consistency of grazing behaviours across cows.

The results revealed a selection of seven grazing behaviours used in two discriminant models plus four behaviours used in our elevation discriminant model and another four in the exploration discriminant model. Both models yielded accuracy above 86% for the training dataset, but only the latter proved high accuracy (85%) in predicting cow genotype with the testing dataset. We discuss these findings in the context of previously reported associations between *GRM5* variation and animal behaviours. We then focus our discussion on comparing the performance of the discriminant models presented here with other behavioural models in the literature.

3.4.1 Behavioural discrimination of *GRM5* genotypes

Most of the coefficients of variation (CV) for the grazing behaviours had low discriminant scores, but four of them had higher discriminant scores and were therefore included in our models. These were the CV of relative mean elevation and relative elevation range, CV of 85th quantile of elevation centred per farm, and CV of mean slope. The mean grazing behaviours used in both models included the mean slope, elevation range and movement tortuosity. The first model also utilised elevation-related behaviours (i.e., elevation gain, 85th quantile of elevation centred per farm and relative mean elevation) and therefore, it is referred to as the 'elevation discriminant model'. The second model added exploration-related behaviours (i.e., horizontal distance travelled, adjusted horizontal distance travelled and home range), so it is called the 'exploration discriminant model'.

In a paired comparison, Watanabe et al. [181] tracked the behaviour of a Holstein cow and a Japanese black cow under barn and pasture grazing conditions (respectively) using three-axis accelerometers deployed under the animal's jaw. They calculated the mean, variance, and inverted CV of the acceleration of under-jaw movement per min by aggregation of 1-s frequency measurements and for each of the three accelerometer axes. Metrics were also computed for the resultant axis, making a total of twelve variables. The authors tested several combinations of the twelve variables to build QDMs of cows' activities (i.e., eating, ruminating, and resting), which were determined by observing time-synchronized video recordings. The QDMs achieved ≈ 95% accuracy with the training dataset (the models' accuracy were not evaluated with other cows) when they included the eight means and inverse CVs variables (i.e., excluding variance variables) or all twelve variables.

In comparison, the discriminant models described here also included aggregated metrics of means and CVs from repeated measurements, but achieved ≈ 86% accuracy, which were roughly 10% less than the Watanabe's et al. [181] discriminant model. The lower accuracy of the discriminant models created here might be attributable to a larger behavioural overlap between *GRM5* genotypes than between individual cow activities, and likely explained by the larger grazing personality plasticity of *GRM5*-controlled behaviours than activity-related behaviours. Another reason for the reduced accuracy in the discriminant models created here might be because of the variables themselves, (i.e., grazing behaviours derived from GPS and satellite data *versus* accelerometer-derived data), that raise the question of whether there might be more suitable behavioural metrics to discriminate *GRM5* genotypes. Other factors that might cause decreased model accuracy are the level of control imposed by *GRM5* over grazing behaviours (i.e., to what extent does *GRM5* variation determine grazing behaviours?) and the interaction of *GRM5* with other gene(s) that might affect grazing behaviours (are there polygenic and/or pleiotropic effects on *GRM5*-controlled grazing behaviours?).

Brennan et al. [182] reported accuracies ranging from 80% to 92% for QDMs of yearling steer grazing activity using accelerometer- and GPS- derived metrics measured over three-month periods and three summer seasons (2016-2018) in native grasslands of South Dakota, USA. Grazing activity was crosschecked with direct visual observations. The authors attributed the lower accuracy achieved in their experiment to the use of longer tracking periods and the large paddocks (between 51-74 ha) compared to reports elsewhere. The accuracy performance of ≈ 86% for the models presented here that were built with the aggregation of 15 days of data are comparable to those reported by Brennan et al. [182]. The higher 96% accuracy of Watanabe's et al. [181] model correspond to much shorter measurement periods (two to four sessions of 3-4 hours) in agreement with Brennan's argument. This seems to align well with the conclusions of Dochtermann's et al. [54] meta-analysis that pointed out the decrease from a mean heritability of 0.52 to a behavioural variation of 0.14, where the latter accounted for the effect of temporal variation. In this regard, we suspect that the reason for a decreased accuracy of discriminant models using long periods of behavioural measurements might be the generalized low temporal consistency suggested by low ICCs of most GP-behaviours. For example, in our experiment, most grazing behaviours rated ICCs below 0.35. Such low ICC are indicative of 'poor' consistency [183]. Although this interpretation depends of the measurement under evaluation, ICC scores would suggest a low temporal consistency in selected grazing behaviours. Rather than questioning whether animals exhibit repeated grazing behaviours, it might be better to develop behavioural metrics that can capture the repeatability and individual differences of grazing behaviours in an animal personality sense.

The accuracy of our first model dropped to 46% when predicting genotypes of cows from the testing dataset, while the second model remained at a higher value of 85%. The loss of accuracy in the first model might question the feasibility of predicting *GRM5* genotypes of cows other than those used to build the model itself, thus suggesting strongly that an elevation discriminant model needs to be tested on other cattle. On the other hand, the exploration discriminant model that included three grazing behaviours previously reported by (Moreno García et al. [161]) that were associated with *GRM5* genotypes (i.e., home range, movement tortuosity and elevation range) yielded promising results on the ability to discriminate genotypes through grazing behaviours and to predict cow genotypes. The discriminant analyses presented do reinforce the previous findings on *GRM5* associations and highlight the possibility of hidden associations that have not been detected because of the limitations of the mensurative experiment. Future research with manipulative experiments could target predicting *GRM5* genotype of non-genotyped cows and assess the effect of *GRM5* genotype on the grazing behaviour of individual animals and collectively in herds. The use of data and new metrics derived from GPS, accelerometers [182, 181] and gyroscopes [184] as well as the annotation with external data sources (e.g. satellite- or drone- captured data) might then assist to build more robust discriminant models with higher classification accuracy.

3.4.2 Grazing behaviours linked to *GRM5*

In the elevation discriminant model, the behavioural variation among *GRM5* genotypes was primarily driven by a first axis of elevation-related behaviours (i.e., ele_gain, ele_range, ele85_farm, rel_ele_mean_cv, and ele85_farm_cv) and a second axis characterized by the movement tortuosity (sp_tortuosity) and the variability in elevation range per cow relative to the elevation range of the herd (rel_ele_range_cv) (Fig. 3.1B). Slope-related metrics (i.e., mean slope and its 15^{th} quantile) contributed to the variation in both the above-mentioned axes, with a larger effect on the second one (Fig. 3.1B). On the other hand, the axis of highest behavioural variation in the exploration discriminant model (axis 1) was primarily explained by the opposite effects of rel_ele_range_cv and sp_tortuosity (Fig. 3.1D), which resembles axis 2 of the elevation discriminant model (Fig. 3.1B). Adjusted horizontal distance travelled (adj_dist_ho) and home range (hr_mcp) had large, but split contributions to axes 1 and 2 of the exploration model. These two behaviours along with the CV of relative elevation mean (rel_ele_mean_cv), maximum slope (slope_max) and elevation range (ele_range), were the main drivers of variation in axis 2. The large and opposing effects of sp_tortuosity and rel_ele_range_cv were observed in both models (Fig. 3.1B axis 1 and Fig. 3.1D axis 2), suggesting a trade-off between these two behaviours. Furthermore, axis 2 of the exploration model showed opposite effects of sp_tortuosity towards the positive side, and hr_mcp and adj_dist_ho towards the negative end of the axis. This is in agreement with the trade-off between movement tortuosity and home range described by Moreno García et al. [161] and further supported with research by Browning et al. [126] and Pauler et al. [143].

Among the high-scoring grazing behaviours (but excluded from both discriminant models) were vertical distance travelled, 85^{th} quantile of slope, mean and 15^{th} quantile of elevation, which although relevant, might be redundant with other behaviours already included in the models. Some of the so-called relative behaviours (i.e., metrics comparing behaviours of individual cows versus behaviours of the herd) were also among the high-scored grazing behaviours not included in discriminant models. For example, adjusted daily elevation mean, relative elevation range and relative slope range. Most CV behaviours scored low in the initial discriminant analysis and might only have reduced importance in discriminating between the *GRM5* genotypes.

In the original study from where the subset of the present study was taken, Moreno García et al. [161] revealed associations between *GRM5* sequence variation and home range and movement tortuosity, as well as trends for association with elevation range and horizontal distance travelled. These discoveries were produced using a dataset derived from the movement of 303 mature cows randomly selected from existing commercial herds. In that experimental design, the genotypic frequency of *GRM5* was the result of artificial selection (as part of the management of the cattle for production purposes) and natural random effects occurring on those commercial farms. This led to an under-representation of variant A in the cattle. Perhaps unsurprisingly, given the same cows were analysed, the original study supports the findings presented here, but better-balanced dataset chosen for analysis also revealed possible linkages with elevation and the slope-related behaviours.

Bailey et al. [41] tested genotype-to-phenotype associations between the whole genome (i.e., ≈ 778 thousand SNPs single nucleotide polymorphisms) using an Illumina BovineHD BeadChip to analyse mature beef cows, and indexes of terrain use derived from cow GPS relocation data (25-112 days recorded, and 96-288 GPS fixes per day). The cattle in the study of Bailey et al. [41] included a relatively small group ($n = 87$) of lactating and non-lactating cows of various breeds (i.e., Angus, Angus x Hereford cross, Brangus, Limousin, and Simmental-crosses) under free-range grazing on five ranches in Arizona, Montana and New Mexico, USA. Terrain was purposely chosen to be diverse, including having rolling and mountainous areas with gentle to moderate slopes as well as undulating plains. The analysis of Bailey et al. [41] revealed associations between genetic variation of QTL overlapping *GRM5* and a ranking index that combined slope and elevation (called the 'rough index'). Their results support the findings described here, where axis 1 of the elevation discriminant model were mainly explained by elevation and slope behaviours (Fig. 3.1B).

Another index with reported associations to *GRM5* genotypes in the Bailey et al [41] study combined elevation, slope, and distance to water (i.e., 'rolling index'). The latter could not be calculated in the current study, because the paddocks commonly had several sources of water, rendering such calculation inappropriate. However, other proxy behaviours of grazing exploration such as the horizontal distances travelled, adjusted horizontal distance travelled and home range were main components of the exploration discriminant model (Fig. 3.1D). The importance of elevation-, slope- and exploration-related behaviours that emerged from discriminant models is consistent with the genetic associations reported by Bailey et al. [41] and they are also consistent with associations reported for *GRM5* mice models (e.g., associations to horizontal and vertical distances [113], locomotor reactivity [112, 113] and trajectory patterns [111].

3.4.3 Different grazing patterns of *GRM5* genotypes

The two-way ANOVA (Table 3.2) revealed differences and trends to differences between *GRM5* genotypes and variant sequences (presence/absence models) for GP-behaviours such as ele_gain, ele_range, rel_ele_range_cv and ele85_farm (only in variants B and C)(see Table 3.2 and Figs. 3.2 and 3.3). In contrast, ANOVA analyses failed to reveal differences for home range (data not shown) and sp_tortuosity. Moreno García et al. [161] reported differences between *GRM5* genotypes, where 4-5 years of age cows with BB genotype showed the smallest home range and the largest movement tortuosity. While the mensurative analysis revealed differences for hr_mcp and sp_tortuosity, the manipulative analysis did not, although, it highlighted the importance of both measurements to differentiate among *GRM5* genotypes.

3.4.4 A *quasi*-manipulative experiment

The original study conducted by Moreno García et al. [161] analysed grazing behaviours of cows with the proportion of *GRM5* genotypes given by the experiment conditions, i.e. mensurative experiment as suggested by Hurlbert [163]. Here, cows from the most numeric classes were excluded to build a *quasi*-manipulative experiment with a dataset that equally represents the five *GRM5* genotypes and the four farms. Our *quasi*-manipulative experiment therefore allowed *GRM5* genotype to be used as a 'treatment' and the farm factor as a covariate. Despite there being no associations between the bovine *GRM5* variation and elevation- or slope- related behaviours in Moreno García et al. [161] and Pierce et al. [57] studies, the importance of such behaviours are highlighted with the elevation and exploration discriminant models. We argue that such results support the ability of statistical algorithms to capture true positive results, when applied to balanced datasets [162, 163], and overall, the results from the elevation and exploration discriminant models are supported by research in other animal models [41, 111, 112, 113]. What-is-more, larger-scale experiments (e.g., 300 to 600 individuals) with balanced representation of *GRM5* genotypes may also improve the statistical ability to identify *GRM5*-controlled grazing behaviours.

3.5 Conclusion

We used discriminant analyses to select combinations of key grazing personality behaviours (GP-behaviours) that discriminated specific genotypes of the glutamate metabotropic receptor 5 gene *GRM5*, a potential 'grazing gene'. One quadratic discriminant model QDMs built with eleven key GP-behaviours related to elevation, slope and exploration correctly predicted the specific genotype of more than 85% of the free-grazing cows investigated in steep and rugged terrain of New Zealand. These results highlight the importance of behavioural genetics, animal personality and repeated measurement data to detect differences between individual herbivores grazing in steep and rugged terrain. The design of experiments with balanced genotypic variation might be an alternative to using more extensive experimental setups.

Chapter 4

General discussion, conclusions, and future directions

This research aimed to investigate the problem of uneven grazing distribution of beef cattle in livestock systems in hill and high-country grasslands of New Zealand.

The high-quality forage of hill and high-country grasslands growing during spring and summer has typically been utilised by grazing sheep. However, at the end of the growing season, these grasslands have often accumulated standing plant biomass, or what is colloquially known as taggy grass. This is less palatable for sheep, but if unremoved, the excessive biomass might prevent the grasslands thriving in the next spring, reducing its productivity.

Beef cattle can take advantage of the standing forage, especially in winter months after the weaning of the previous season's calf, when their nutritional requirements are relatively low. The benefits of this so-called 'pasture grooming' of beef cattle in hill and high-country grasslands are well known in New Zealand [185] and applied extensively by farmers across the country. However, the uneven distribution of cattle in steep and rugged terrain can result in the beneficial grazing or over-grazing of preferred areas, while leaving standing biomass on the rest of the land.

The main hypothesis was that large herbivores show sets of grazing behaviours that are consistent over time and across contexts, and that such behaviours differ between individuals and groups. Furthermore, it was hypothesized that consistent grazing behaviours may be genetically controlled. If correct, the selection of animals displaying an array of distinctive, yet consistent grazing behaviours could increase the utilization of less preferred areas and increase grazing 'evenness'.

In this research, I therefore investigated:

- The grazing behaviours of beef cattle in steep and rugged terrain in the South Island of New Zealand.
- Sequence variation in a potential 'grazing gene', the glutamate metabotropic receptor 5 gene (*GRM5*).

- Genotype-phenotype associations between grazing behaviours and *GRM5* variation.
- Key grazing behaviours linked to *GRM5*.

Based on a literature review (Chapter 1 [109]), a model of grazing personalities (GP-model) was developed, which proposes that cattle display grazing behaviours that are consistent over time and across contexts. These grazing personalities differ between individuals and between groups (i.e., individual and collective grazing personalities). These personalities are genetically determined, epigenetically modulated via interactions with the social and biophysical environments such as those imposed by range management, and ultimately influenced by the animals' emotional states.

Knowledge gaps in our understanding of animal personality as applied to grazing emerged from this research. For example, there is limited knowledge about sets of correlated grazing behaviours that could lead to identifying genetically associated grazing personalities in large herbivores. It is even less well understood as to how neurophysiological pathways might link genes with grazing behaviours. Genome wide association studies using QTLs have suggested possible candidate genes associated with grazing patterns, but there is a lack of studies targeting specific genes, and which could provide greater certainty about the genetic control of grazing behaviours. Other areas for future research are the investigation of possible pleiotropic effects, multi-gene control of grazing behaviour and the genomic regulation of those effects.

The GP-model added two key concepts to grazing behavioural genetics, the collective nature of foragers and the social environment of the group as modulating factor via the genetic expression of grazing personalities. However, it remains largely unknown as to whether there is genetic control over collective grazing behaviours and personalities. We still need to identify and develop reliable metrics to measure collective grazing behaviours and design studies aiming to detect behavioural differences at the collective level in large herbivores.

In Chapter 2 [161] genetic variation in *GRM5* was described, including the definition of three nucleotide sequence variants and six genotypes in the exon 5 region. These were asymmetrically represented in beef cattle herds. Upon analysis, modelling approaches revealed associations between variation in *GRM5* and home range and movement tortuosity. A trend towards association was observed for elevation range and horizontal distance travelled. Inter-genotype differences for these behaviours ranged roughly between 20% and 25% for the marginal means estimated from linear mixed models.

Using a *quasi*-manipulative experiment (Chapter 3), key grazing behaviours were identified as useful metrics to discriminate and predict cows' *GRM5* genotypes such as elevation-, slope- and exploration-related grazing behaviours. Moreover, differences and trends towards significant differences between genotypes were detected for elevation range, elevation gain, the coefficient of variation of the relative elevation range and the adjusted horizontal distance travelled.

The research here presented filled some of the above-mentioned gaps in our knowledge. For example, correlations between several grazing behaviours and even a trade-off (negative correlation) between home range and movement tortuosity were identified. These two behaviours were associated with the *GRM5* variation suggesting there is genetic control over the home range versus movement tortuosity trade-off.

While it was not directly investigated, the possible pathways by which *GRM5* affects grazing behaviours are hinted at by the literature. For example, in mouse models there is the suggestion that neurological mechanisms link *GRM5* with activity level and exploration. Cow-age class was identified as a likely factor in the size of the effect size of *GRM5* on associated behaviours. These findings should be further investigated. Finally, the research shortlisted key grazing behaviours that were associated with *GRM5* variation, and which were used to build two discriminant models with over 86% accuracy at detecting *GRM5* genotypes and one model with 85% correct genotype prediction.

There are several questions that remain unknown and that might feed the agenda of future research.

This includes the following: Poor inter-class correlation coefficients suggested low behavioural consistency. How consistent are grazing behaviours? Are there more appropriate behavioural metrics?

if cattle's daily exploration is consistent, yet different between *GRM5* genotypes, does the expenditure of energy invested in exploration vary between *GRM5* genotypes? Does the acquisition of energy ingested during grazing differs between *GRM5* genotypes, and if so, is there differences in energy balance associated with *GRM5* variation?

Are there differences in the forage harvested by grazing cattle in the hill and high-country? If so, are these differences associated with *GRM5* variation? What plant communities and forage species are being grazed by cattle with different *GRM5* genotypes? Are there differences in habitat selection?

Differences in grazing behaviours between individuals have been associated with *GRM5* variation, but: How big is the overlap of explored areas between *GRM5* genotypes? How big is the explored area exclusively used by different *GRM5* genotypes?

Beef cattle and many other large herbivores are collective foragers, does the *GRM5* genotype composition determine the collective grazing personality of the herd? Specifically, is it possible to change the exploration of beef cattle hers by changing the proportion of *GRM5* genotypes? How is this affected by range management practices (e.g., stocking rate, grazing system, paddock design, water availability)?

Finally, can we incorporate a grazing personality approach into the management of livestock systems to enhance production, the utilisation of feed, and to potentially change the use of New Zealand's steep and rugged grasslands?

This research proposed a genetic-based model of individual and collective grazing personalities for large herbivores and foragers [109]. The analysis of grazing behaviours of beef cattle measured over time and across situations suggested consistent differences between individuals associated with *GRM5* variation [161]. Linkages between bovine *GRM5* and key grazing behaviours may characterise specific genotypes and assist with their identification. Future research might elucidate and quantify the effect size and impact of behavioural genetics on grasslands ecology and livestock production. There is potential in applying the grazing personality model to New Zealand's livestock systems in steep and rugged terrain aiming to spread out the grazing distribution of beef cattle.

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Appendices

Supplementary Figures

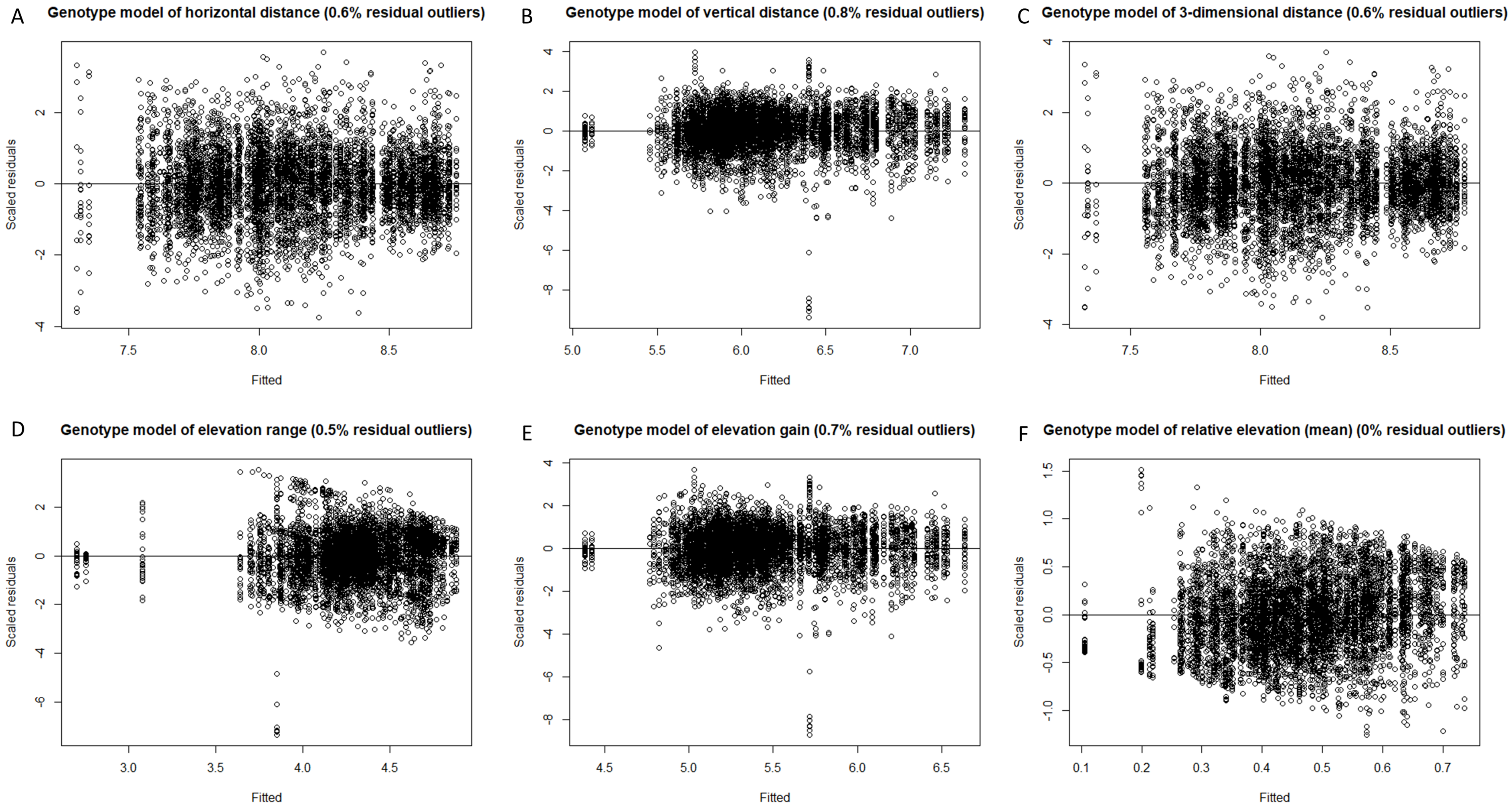

Figure S1. Residuals versus fitted values (Part A). Plots of scaled residuals versus fitted values of linear mixed models of grazing personality behaviour. Residual outliers are values beyond ±3.

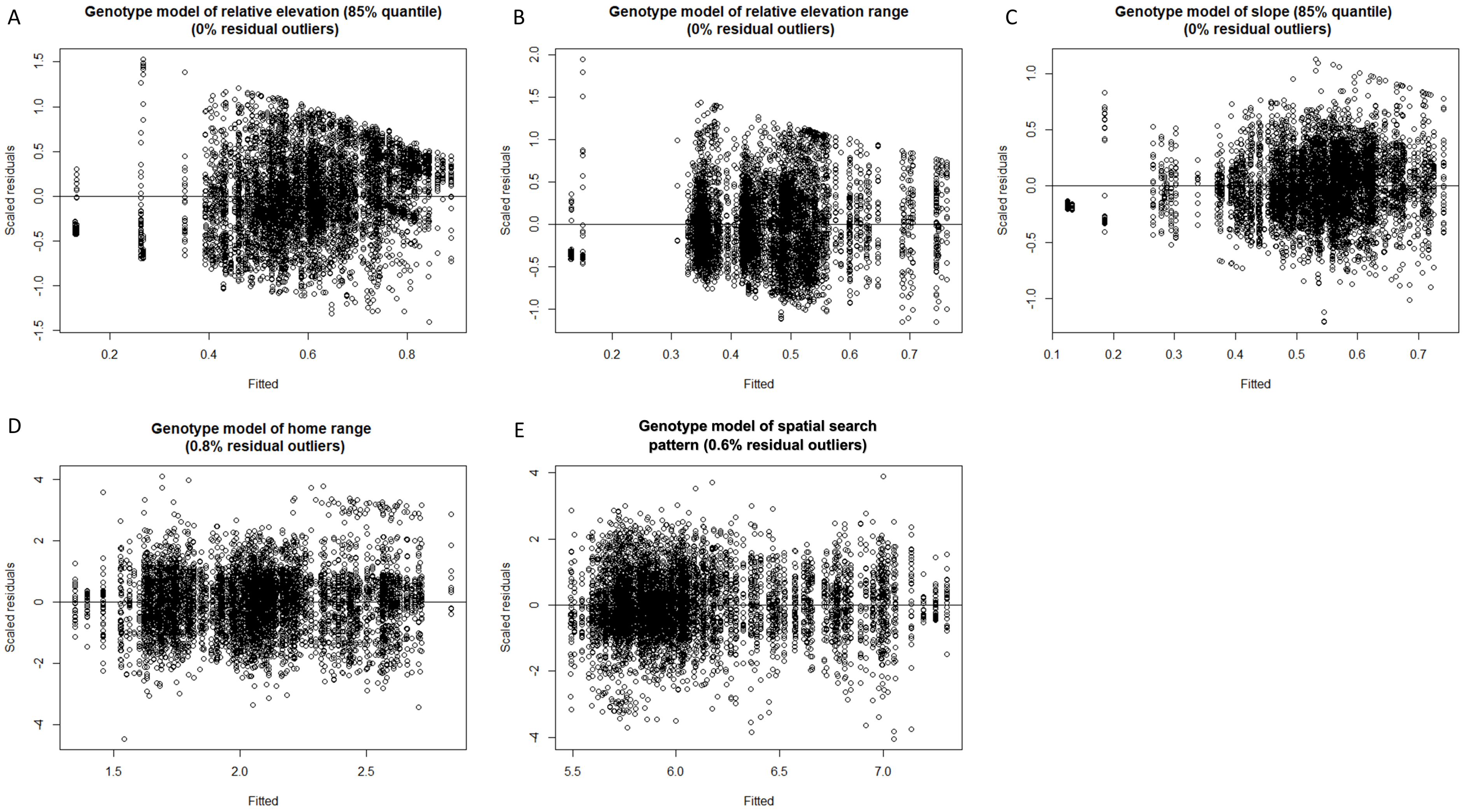

Figure S2. Residuals versus fitted values (Part B). Plots of scaled residuals versus fitted values of linear mixed models of grazing personality behaviour. Residual outliers are values beyond ±3.

Supplementary Tables

Table S1. Akaike Information Criterion (AIC) for linear mixed models of grazing personality behaviours (GP-behaviours) fitted with twelve combinations of random factors.

Random Factors[1]	Degrees of freedom[2]		dist_ho[3]	dist_ve	3D_dist	ele_range	ele_gain	Rel_ele	Rel_ele85	Rel_ele_range	slope85	hr_mcp	sp_tortuosity
	(Ga)	(Bi)	(Ga)	(Ga)	(Ga)	(Ga)	(Ga)	(Bi)	(Bi)	(Bi)	(Bi)	(Ga)	(Ga)
(1 | cow_id)	3	2	898	5051	797	9820	5880	7815	7359	7915	7906	15781	13861
(1|cow_id)+ (1|farm_id)	4	3	816	5050	717	9703	5879	7728	7269	7892	7881	15719	13863
(1|cow_id) + (1|sampling_year) + (1|farm_id)	5	4	509	4892	405	9705	5723	7730	7270	7889	7883	15697	13597
(1|cow_id) + (1|sampling_year/farm_id)	5	4	**465**[4]	4878	**366**	9693	5709	7727	7273	7802	7833	15597	13538
(1|cow_id) + (1|farm_id/sampling_year)	5	4	470	4886	371	9693	5717	7726	7270	7802	7833	15597	13546
(1|cow_id) + (1|mob_id)	4	3	478	**4757**	380	9538	5591	**7717**	**7265**	7704	**7724**	15573	**13527**
(1|cow_id) + (1|sire_id)	4	3	692	5006	593	9771	5836	7777	7322	7898	7903	15695	13795
(1|cow_id) + (1|mob_id) + (1|sire_id)	5	4	479	4758	381	**9540**	**5592**	7718	7267	**7700**	7726	**15570**	13529
(1|cow_id) + (1|mob_id) + (1|sire_id) + (1|GRM5_genotype)	6	5	481	4760	383	9542	5594	7720	7269	7702	7728	15571	13527

Continuation of Table S1. Akaike Information Criterion (AIC) for linear mixed models of grazing personality behaviours... .

Random Factors[1]	Degrees of freedom[2]		dist_ho[3]	dist_ve	3D_dist	ele_range	ele_gain	Rel_ele	Rel_ele85	Rel_ele_range	slope85	hr_mcp	sp_tortuosity
	(Ga)	(Bi)	(Ga)	(Ga)	(Ga)	(Ga)	(Ga)	(Bi)	(Bi)	(Bi)	(Bi)	(Ga)	(Ga)
(1|cow_id) + (1|farm_id) + (1|sampling_year) + (1|GRM5_genotype)	6	5	511	4894	407	9706	5724	7732	7272	7891	7885	15699	13599
(1|cow_id) + (1|sampling_year/farm_id) + (1|GRM5_genotype)	6	5	467	4880	368	9695	5711	7729	7275	7804	7835	15595	13535
(1|cow_id) + (1|sampling_year/farm_id) + (1|sire_id)	6	5	465	4878	366	9690	5709	7727	7727	7800	7835	15585	13535

[1] Random factors presented with the notation of the 'lme4r' R package for cow identity (cow_id, n = 303), farm (farm_id, n = 4), year of sampling (sampling_year, n = 2), herd per farm and per year (mob_id, n = 14), sire identity (sire_id, n = 73), *GRM5* genotype (*GRM5*_genotype, n = 5).

[2] Models set with Gaussian (Ga) or Binomial (Bi) distribution according to the distribution of each variable.

[3] See GP-behaviours abbreviations and details in Table 2.1.

[4] Bolded AIC values indicate the combination of random factors selected to fit models with fixed factors.

Table S2. Associations between bovine *GRM5* genotypes and grazing personality behaviours (GP-behaviours)

GP-behaviours[1]	Cow age class (*P*-value)[2]	*GRM5* genotype	Marginal mean[3] (standard error)	*P*-value[4]
dist_ho (m/day)	**0.043**	*AB*	3655 (±1071)	-[5]
		AC	3950 (±1154)	*0.085*
		BB	3818 (±1115)	0.319
		BC	3885 (±1131)	0.117
		CC	3842 (±1119)	0.199
dist_ve (m/day	0.692	*AB*	540 (±75)	-
		AC	583 (±79)	0.265
		BB	558 (±75)	0.614
		BC	561 (±74)	0.527
		CC	542 (±72)	0.952
3D_dist (m/day)	**0.049**	*AB*	3713 (±1089)	-
		AC	4015 (±1174)	*0.084*
		BB	3881 (±1134)	0.316
		BC	3946 (±1150)	0.120
		CC	3901 (±1137)	0.204
ele_range (m)	0.427	*AB*	72 (±9)	-
		AC	71 (±9)	0.797
		BB	65 (±8)	*0.070*
		BC	66 (±8)	*0.095*
		CC	65 (±8)	*0.064*
ele_gain (m/day)	0.699	*AB*	272 (±38)	-
		AC	291 (±39)	0.336
		BB	280 (±38)	0.645
		BC	281 (±37)	0.593
		CC	270 (±35)	0.889
rel_ele (0-1)	**0.017**	*AB*	0.40 (±0.07)	-
		AC	0.43 (±0.06)	0.606
		BB	0.46 (±0.06)	0.316
		BC	0.44 (±0.06)	0.426
		CC	0.48 (±0.06)	0.132
rel_ele85 (0-1)	**0.006**	*AB*	0.66 (±0.07)	-
		AC	0.68 (±0.06)	0.725
		BB	0.67 (±0.06)	0.835
		BC	0.66 (±0.05)	0.978
		CC	0.69 (±0.05)	0.527
rel_ele_range (0-1)	0.280	*AB*	0.50 (±0.09)	-
		AC	0.50 (±0.09)	0.854
		BB	0.48 (±0.09)	0.649
		BC	0.48 (±0.08)	0.650
		CC	0.48 (±0.08)	0.747
slope85 (0-1)	**0.020**	*AB*	0.44 (±0.09)	-
		AC	0.49 (±0.09)	0.323
		BB	0.48 (±0.09)	0.412
		BC	0.43 (±0.08)	0.718
		CC	0.43 (±0.08)	0.764
hr_mcp (ha/day)	**0.008**	*AB*	7.88 (±0.91)	-
		AC	7.82 (±0.86)	0.917
		BB	6.60 (±0.73)	**0.015**

Continuation of Table S2. Associations between bovine *GRM5* genotypes and GP-behaviours.

GP-behaviours[1]	Cow age class (*P*-value)[2]	*GRM5* genotype	Marginal mean[3] (standard error)		*P*-value[4]
		BC	7.25	(±0.75)	0.189
		CC	7.29	(±0.76)	0.223
sp_tortuosity (m/ha)	**0.008**	*AB*	540	(±75)	-
		AC	568	(±77)	0.436
		BB	665	(±90)	**0.001**
		BC	616	(±81)	**0.019**
		CC	602	(±80)	*0.053*

[1] See GP-behaviours abbreviations and details in Table 2.1.

[2] Significance level of ANOVA tests for comparison of models with and without cow age class as a fixed factor. Bold letters indicate significance ($P < 0.05$).

[3] Response marginal mean in measured units (back-transformed from the log scale as needed).

[4] Significance level for Satterthwaite's method t-tests. Italicised values indicate trend to significance ($P < 0.1$) and bolded values indicate significance ($P < 0.05$).

[5] Model intercepts set with genotype *AB* and cow age class 1