Genome Wide Association Studies for Temperament in New Zealand Dairy Cattle

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in Animal Breeding and Genetics

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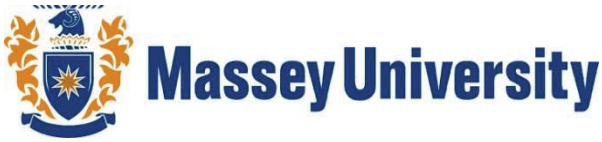

Fei Yi Chan

2012

DEDICATION

To my parents

ABSTRACT

The aim of this thesis was to identify genomic regions associated with cow temperament in New Zealand dairy cattle. The data set used was provided by the Livestock Improvement Corporation, and contains estimated breeding values (EBV) for temperament of 3140 bulls of three breeds; Holstein-Friesian (HF), Jersey (JE) and Crossbreed (XB) born between 1994 and 2006. Biallelic genotype data were also provided containing 700,000 single nucleotide polymorphism (SNP) markers. Estimated breeding values for cow temperament were adjusted (Adj-EBV_{temp}) for age, percentage of North American Holstein genes (NAH%), percentage of New Zealand Holstein-Friesian (HF%) and percentage of Jersey (JE%). Using PLINK, the association between individual markers and temperament was investigated. PLINK was also used to produce output with genomic corrected p-values (GC) which adjusts for inflation based on the median chi-square statistic. Suggestive and genome-wide significance thresholds were set at $-\log_{10}$ ($1e^{-4}$) and $-\log_{10}$ ($5e^{-5}$) respectively. From literature review, the average estimate of heritability of temperament was 0.17, and average genetic correlation with milk yield was 0.165. JE bulls had the highest average EBV for temperament (0.0352 ± 0.239) followed by XB (0.0079 ± 0.217) and HF bulls (-0.0402 ± 0.256). PLINK analysis show BTA 4 to contain genome-wide significant genomic regions across all three breeds for Adj-EBV_{temp}. Further investigation on individual chromosomes provided no further information on significant genomic regions affecting temperament. A closer look at many of these regions show they are in domains known as "Junk DNA". Results suggest that although genomic selection for temperament in dairy cattle is plausible, genetic gain via direct selection for this trait will be slow and desired effects may not be immediate.

Keywords: Temperament, dairy cattle, genomic selection, quantitative trait loci (QTL).

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"...but those who hope in the LORD
will renew their strength.
They will soar on wings like eagles;
they will run and not grow weary,
they will walk and not be faint." Isaiah 40:31

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The Lord is my strength and my shield; my heart trusts in Him, and He helps me. My heart leaps for joy, and with my song I praise Him. Psalm 28:7

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LIST OF ABBREVIATIONS

Abbreviations

AEU	Animal Evaluation Unit
AI	Artificial Insemination
BLUP	Best Linear Unbiased Predictor
BTA	*Bos taurus* Autosome
BW	Breeding Worth
DNA	Deoxyribose Nucleic Acid
EBV	Estimated Breeding Value
EV	Economic Value
GBV	Genomic Breeding Value
GC	Genomic Corrected p-value
GWAS	Genome Wide Association Studies
HF	Holstein-Friesian
JE	Jersey
LD	Linkage Disequilibrium
LIC	Livestock Improvement Corporation
LW	Lactation Worth
MAS	Marker Assisted Selection
PW	Production Worth
QTL	Quantitative Trait Loci
SNP	Single Nucleotide Polymorphism
TE	Transposable Element
TOP	Traits Other than Production
XB	Crossbreed (Holstein-Friesian x Jersey, a.k.a. KiwiCross)

CHAPTER ONE

INTRODUCTION

The dairy industry is one of the primary economic drivers in New Zealand. The dairy industry for the 2010-2011 season was comprised of 4.5 million cows distributed across 11, 735 herds. The average dairy farm had an average herd size of 386 cows and an effective area of 140 hectares, with an average stocking rate of 2.76 cows per hectare. The breed structure of the national herd was 40.0% Holstein-Friesian (**HF**), 12.4% Jersey (**JE**), 38.9% Holstein-Friesian cross with Jersey (**XB**), 0.8% Ayrshire and the remaining 7.9% were of other breeds.

Genetic improvement has been known to be an effective method for achieving permanent progress in animal production efficiency. The New Zealand dairy industry understands this and has been working determinedly to achieve this goal over the last few decades. One of the important elements of achieving this is through careful planning and the formation of a comprehensive breeding programme.

The breeding goal of the dairy industry is to identify animals whose progeny have the potential to be highly effective in converting feed into profit (AEU, 2010). Currently, the breeding objective is comprised of seven traits considered to have the most economic impact. These are milk fat, milk protein, milk volume, fertility, somatic cell count, feed conversion efficiency and longevity (or residual survival). Animals are then selected based on an economic index called breeding worth (**BW**) that is calculated as the sum product of the estimated breeding values (**EBV**) for these seven traits, each multiplied by its corresponding economic value (**EV**). Genetic evaluation to obtain EBVs is based on herd testing and reproduction and survival records. The primary objective of this process is to obtain data on production, such as milk volume, milk fat and milk protein levels, as well as reproduction records such as pregnancy rate at 21 and 42 days after planned start of mating. This information allows comparisons to be made between individuals, on a within- and between herd, and within- and between breeds basis (Harris et al., 1984).

Currently, EBVs are obtained for milk production (volume, fat and protein), live weight, fertility, longevity and somatic cell score. EBVs are also available for non-production traits, and a list is specified by the New Zealand Animal Evaluation Unit along with the scoring method for these traits. Livestock Improvement Corporation additionally calculates BV for gestation length and feed intake. The dairy industry has also created a system whereby farm information can be linked to the national database for the purpose of genetic evaluation. There is also increased focus in recording reproductive and heath traits to be included in traditional herd testing records. Technological improvements combined with revolutionary methods of genetic evaluation now allow breeding values to be estimated with higher degrees of accuracy and reliability; this same advance has also lead to the estimation of breeding values for new traits such as mastitis and lameness.

In the 2010/2011 season, 71.7% of the total national herd as well as 70.4% of the total number of cows were herd tested. The herd test averages for that season revealed the following statistics.

Table 1.1: New Zealand dairy industry herd test averages (per cow) for milk volume, milk fat, milk protein, milk solids, number of days in milk and somatic cell count for the 2010-2011 season.

Average Values	
Milk (litres)	4101
Milk fat (kg) (%)	194 4.73
Milk protein (kg) (%)	154 3.75
Milk solids (kg) (%)	348 8.48
Days in milk Herd test (days) Production (days)	 229 274
Somatic cell count (000 cells/ml)	232

Currently, temperament is not yet included in the breeding objective, and as a result there has been no conscious selection for temperament. However, evidence exists showing that temperament acts as a good predictor of longevity (Larroque & Ducrocq, 2001; Berry et al., 2005). Longevity plays an important part in the selection objective of New Zealand's dairy. A large number of different predictors can be used to define longevity apart from direct longevity, which is only measurable at the end of the animal's life (Mark, 2004).

High culling rates are therefore of concern dairy farmers, especially those wanting to expand their herds. There are two forms of culling; voluntary culling and involuntary culling. Voluntary culling occurs when a farmer makes a conscious decision to remove a healthy, fertile cow due to poor milk production. Involuntary culling, on the other hand, occurs when farmers are forced to remove a highly productive and profitable cow due to illnesses, fertility issues or death (Weigel et al., 2003).

One of the main reasons for involuntary culling of cattle by farmers is temperament. Easily agitated cows often cause handling issues, which can be dangerous for both the animals and handlers involved.

Involuntary culling by farmers often lead to a loss in profit, as well as slower rates in genetic gain. If fewer animals are culled for temperament, there is a higher chance for these individuals to stay in the herd longer, which corresponds to an increase in herd survival rate. This also means that there is a higher chance for a greater increase in genetic gain and profit for the farm involved (Larroque & Ducrocq, 2001).

Cows considered to have a "bad temperament" can cause severe problems such as inducing higher labour costs, injuries to both handlers and the individual animal, or even cause fatal accidents. Farmers have been demanding cattle with "good temperament", or those that are docile. Easy and safe handling has now become a vital factor to consider when purchasing livestock.

Selecting for good temperament in dairy cattle will enable cattle to withstand environmental stressors encountered in grazing systems. This can help improve the animal's welfare as well as productivity, and can benefit farmers as well by ensuring

safety when handling livestock (Phocas et al., 2006). Any information pertinent to the discovery of genes influencing temperament can therefore be of benefit to breeding programmes, as they can aid in the identification and selection for animals with temperament better suited to their environment (Broucek et al., 2008).

Traditionally, genetic selection had been carried out using progeny testing. This method has been highly successful, and resulted in moderately high accuracy and reliability. However, this method takes a long time to implement, and the generation interval for both bulls and cows are high (approximately 6 and 7 years respectively).

Even though the idea of using DNA markers to improve genetic gain has been present for many years, it has been severely limited due to the lack of necessary technology (Hayes et al., 2011). The use of genomic selection can decrease the generation interval, with the caveat that reliability will also decrease. However, with increased improvement in genetic technology in the past decade, the notion of genetic gain via genomic selection has gained popularity, and is now more achievable than ever.

Genomic selection is essentially a form of marker assisted selection (**MAS**), and requires information from a large number of single nucleotide polymorphisms (**SNP**). In whole genome association studies (**WGAS**), animals are genotyped for a large number of SNP, which are then tested for an effect on a specific trait of interest (Hayes et al., 2011). Fortunately for cattle, this information is freely available through the bovine genome database, which was released in October 2004 (Hayes et al., 2011). The availability of this information combined with other new and improved technology (such as computer packages which allows fast and accurate analyses of genotype information) has opened up new and exciting possibilities for better and more accurate ways of genetic selection in the dairy industry.

The objective of this study is to identify genes affecting temperament in the New Zealand dairy cattle using genomic selection. This study will look at using both genotypic and phenotypic information of dairy bulls of several breeds, provided by the Livestock Improvement Corporation Ltd, Hamilton, New Zealand.

CHAPTER TWO

LITERATURE REVIEW

2.1. Breeding Programme for New Zealand dairy cattle

New Zealand has a well-designed breeding programme for the genetic improvement of dairy cattle to ensure that the best animals are selected for breeding to increase genetic gain. The breeding programme involves of a number of steps that should be carried out sequentially to ensure that all the requirements in each step are fulfilled. If earlier steps are not dealt with adequately, they will need to be revisited and revised, until all steps are mutually compatible (Harris et al., 1984). Figure 2.1 provides a basic outline of the steps involved in designing a successful breeding programme.

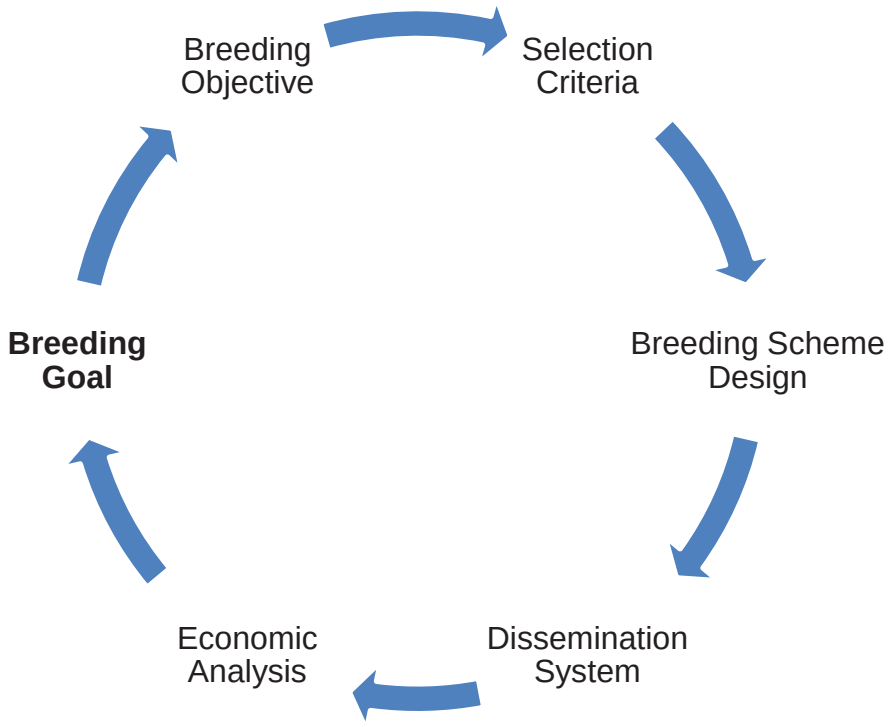

Figure 2.1: A step by step approach towards designing an effective breeding programme for New Zealand dairy cattle (Lopez-Villalobos & Garrick, 2005).

2.1.1 Breeding Goal

Defining the breeding goal is the first step towards designing a breeding programme. New Zealand Animal Evaluation Limited (**NZAEL**) was set up with the primary purpose of planning and managing the national breeding objective, which is to:

"Identify animals whose progeny will be the most efficient converters of feed into farmer profit." (NZAEL, 2012)

New Zealand is unique in that it is one of very few dairy industries in the world with a well-defined breeding goal, as well as being able to communicate this goal clearly to all dairy farmers in the nation (Lopez-Villalobos & Garrick, 2005). With the breeding goal in place, the breeding objective can next be developed.

2.1.2 Breeding Objective

Developing the breeding objective is the next step. This involves identifying a list of traits that have the highest potential to influence the breeding goal, and then quantifying the EV of each of these traits. This results in functions of BVs and EVs, which are the core of the breeding objective.

The current EVs for the traits in the breeding objective are shown in Table 2.1 below. The EV for each trait is representative of the expected income per unit of genetic change coming from breeding replacements. The model includes a wide range of income streams, as well as cost streams associated with maintaining and growing the animals. Predictions of future milk component prices are also taken into account (AEU, 2010).

The EVs are combined with the BVs to calculate the breeding worth (**BW**) of an animal in the form of:

Breeding Worth =	Milk fat BV	x	EV Milk fat	+
	Protein BV	x	EV Protein	+
	Milk BV	x	EV Milk	+

Live weight BV	x	EV Live weight	+
Somatic cell BV	x	EV Somatic cell	+
Fertility BV	x	EV Fertility	+
Residual survival BV	x	EV Residual survival	

Table 2.1: Economic values of traits important to the breeding objective of the New Zealand dairy industry. Figures provided are currently in use (NZAEL, 2012).

Trait	Economic Value
Milk fat ($/kg)	1.920
Milk protein ($/kg)	8.685
Milk volume ($/kg)	-0.094
Live weight ($/kg)	-1.480
Somatic cell score ($/score)	-31.460
Fertility ($/%)	3.118
Residual survival ($/day)	0.048

Currently, temperament is not considered to be economically important, and as a result of this, no economic value exists for this trait.

2.1.3 Selection Criteria

In this step, the breeding program has to create a system to identify animals with the highest genetic merit for the breeding objective. Hazel (1943) defined the selection criteria as those traits that can be measured on the animals, and can be used as predictors of the traits included in the breeding objective. Traits included in the selection criteria may be the same or different from traits in the breeding objective. If traits are different, the selection criterion is known as an indicator trait; in this case, it must be strongly linked to traits in the breeding objective. Indicators traits are commonly used as they are often easier or cheaper to measure than the objective trait itself.

The New Zealand dairy industry, through the New Zealand Animal Evaluation Limited, has a well advanced system for the genetic evaluation of dairy cattle. There is a centralised data base in which herd-testing results are stored from about 74% of the lactating cows in each production season (Livestock Improvement, 2004). Since 1996, the national genetic evaluation system has been conducted across breeds using single trait animal models with best linear unbiased procedures (Harris et al., 1996). This system allows the simultaneous evaluation of cows and sires using all known relationships, and is conducted with a common base for all breeds and crosses. This common base, or genetic base, is set by the Evaluation unit consisting of a group of 30, 000 cows born in 1995. During each evaluation, the average breeding values for these cows are set to zero for all traits to form a reference point for comparison with current cows (NZAEL, 2010). The purpose of the evaluation system is the identification of the most efficient dairy animals to convert feed into farm profit, regardless of breed.

Based on the breeding objective as shown above, it can be seen that emphasis is placed on seven traits, most of which are production and reproduction traits. These are milk yield in terms of volume, fat and protein; live weight; fertility; longevity; and somatic cell score.

There are also a number of traits not included in the selection objective list, but is a part of the genetic evaluation. These are for milk fat percentage, milk protein

percentage, calving difficulty, body condition score and traits other than production (**TOP**) (NZAEL, 2012).

Although TOP traits are not included in the breeding objective, the evaluation system still measures and estimates the BV for each of these traits. According to Ahlborn-Breier (1988), the purpose of this is to "contribute to the overall value of any animal, cow or bull." As such, there are many traits measured either by the farmers themselves or certified inspectors. Data collection is in the form of a linear assessment scale, and is widely accepted across the dairy industry. Scoring is also kept fairly constant throughout the nation. The low end of the scale (i.e. a score of 1) is used to describe the extreme negative or most undesirable for any trait, and the high end of the scale (score of 9) is used to describe the most positive or most desirable for the same trait.

Table 2.2: Traits other than production recorded by the animal evaluation system and the linear scoring criteria for each trait, based on Ahlborn-Breier (1988).

Information supplied by farmer	Scoring (1-9)
Shed temperament	Vicious (1) - Placid (9)
Milking adaptability	Slowly (1) - Quickly (9)
Milking speed	Slow (1) - Fast (9)
Overall opinion	Undesirable (1) - Desirable (9)
Information supplied by inspector	
Stature	<105cm (1) - >140cm (9)
Weight	<250kg (1) - >600kg (9)
Capacity	Frail (1) - Robust (9)
Rump angle	Pins high (1) - Pins low/slopey (9)
Rump width	Narrow (1) - Wide (9)
Legs	Straight (1) - Sickled/curved (9)
Udder support	Weak (1) - Strong (9)
Front udder	Loose (1) - Strong (9)
Rear udder	Low (1) - High (9)
Front teat placement	Wide (1) - Close (9)
Rear teat placement	Wide (1) - Close (9)
Udder overall	Undesirable (1) - Desirable (9)
Dairy conformation	Undesirable (1) - Desirable (9)

Additionally, any other characteristics not described in the list can be recorded as the farmer's or inspector's comments. Examples of this include the fate of the

animal (culled, died or sold); disease problems such as bloat, mastitis or facial eczema; reproductive problems such as calving difficulty or being empty; conformation problems in the feet (lameness), udder or teat; and other problems such as sickness (Ahlborn-Breier, 1988).

Table 2.3 shows results from the 2010 genetic evaluation, and highlights both production and non-production traits measured for three breeds (HF, JE and XB), as well as the national average, maximum and minimum values for these traits.

Table 2.3: Average breed breeding worth (BW) and estimated breeding values (EBV) for production and non-production traits of the Holstein-Friesian (HF), Jersey (JE) and crossbreed HFxJE (XB) cows. Figure is taken from the NZAEL (2012).

	HF			JE			XB		
	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum
Production Traits									
Breeding Worth	36.6	-313.8	266.9	59.9	-320.0	262.0	107.5	-285.3	318.9
Milk fat EBV	12.3	-30.5	53.8	-2.0	-69.1	35.4	12.3	-32.0	44.8
Protein EBV	20.2	-22.7	46.4	-9.7	-59.3	16.9	8.1	-17.1	32.6
Milk volume EBV	729	-443	1834	-622	-1865	279	6.2	-870	962
Live weight EBV	54.7	-13.1	117.4	-57.9	-109.0	-8.1	-6.5	-70.1	62.2
Fertility BV	-3.2	-22.9	12.8	1.8	-12.5	10.1	1.8	-11.5	11.7
Somatic cell EBV	0.04	-0.92	1.58	0.05	-0.85	1.12	0.04	-0.98	1.1
Residual survival EBV	-30	-495	410	-10.2	-576	543	-4	-325	323
Total longevity	-20.4	-827	503	35	-891	554	151	-669	453
Calving difficulty EBV	3.2	-3.5	15.9	-2.9	-6.6	3.4	-0.69	-4.5	3.8
Gestation length	-1.22	-10.7	7.4	-1.01	-11.1	8.7	-2.5	-12.7	11.2
Body condition score	-0.03	-0.55	0.46	-0.13	-0.60	0.22	0.01	-0.32	0.38
Non-Production Traits									
Adaptability to milking	0.05	-1.02	1.20	0.03	-1.02	1.25	0.02	-0.71	0.59
Shed temperament	0.05	-1.21	1.26	0.06	-1.05	1.30	0.02	-0.69	0.58
Milking speed	-0.02	-1.58	1.34	0.07	-1.58	0.97	0.05	-0.92	0.74
Overall opinion	0.14	-0.89	1.11	0.01	-0.85	0.71	0.07	-0.57	0.68
Stature	1.10	-0.64	2.81	-1.02	-2.15	0.11	-0.17	-1.72	1.18
Capacity	0.09	-0.87	1.10	-0.03	-1.28	0.91	0.15	-0.69	1.05
Rump angle	-0.01	-1.01	0.80	-0.09	-0.94	0.71	-0.03	-0.90	0.73
Rump width	0.44	-0.51	1.48	-0.30	-1.30	0.54	0.01	-0.99	0.65
Legs	-0.05	-0.51	0.38	0.10	-0.22	0.41	0.07	-0.21	0.34
Udder support	0.35	-1.03	1.66	0.01	-1.19	1.14	-0.00	-1.07	0.79
Front udder	0.19	-1.20	1.39	0.17	-0.99	1.25	0.02	-1.02	0.89
Rear udder	0.29	-1.23	1.82	0.18	-1.01	1.25	0.03	-0.96	0.93

Front teat placement	0.16	-1.16	1.00	0.10	-0.70	0.85	0.00	-0.73	0.86
Rear teat placement	0.37	-0.96	1.69	-0.09	-0.95	0.91	0.01	-1.10	1.23
Udder overall	0.35	-1.56	1.65	0.17	-1.12	1.38	0.06	-1.01	0.84
Dairy conformation	0.27	-0.79	1.22	-0.00	-0.93	0.82	0.13	-0.56	1.06
Based on	**2384 Sires**			**1365 Sires**			**505 Sires**		

EBVs obtained from the genetic evaluation were chosen because they are related to traits included in the breeding objective. These EBVs are then combined into different economic indexes used for different purposes.

An economic index is essentially the function of EBVs of a number of traits combined with their respective economic values (see Table 2.1). Also called an economic evaluation, it serves to measure an animal's ability to convert feed into profit via breeding replacements, its lifetime production and/or production in the current season. These measures are known as breeding worth, production worth (**PW**) and lactation worth (**LW**) respectively.

BW was developed to compare and determine individual animals on net farm profitability for breeding replacements. Records are based primarily on the performances of close relatives. Currently, seven traits are considered to be of most importance to the breeding objective; these are milk fat, milk protein, milk volume, live weight, fertility, somatic cell count and residual survival (Mark, 2004; AEU, 2010). A high reliability can be achieved if there are extensive records on daughter performance, and BW is useful in deciding which cows to retain calves from.

PW is a measure of the cow's ability to convert feed into profit over her lifetime, and it does not extend to her offspring. High reliabilities can be achieved with records based on the individual's herd testing history. PW is useful when making purchasing and culling decisions.

The final index, LW, is a measure of the cow's ability to convert feed into profit for the current season only. As a result of this, the LW early in the season has to be estimated from limited information, which means that this measure is expected to change significantly during the course of the season. It is not used for important decision making, because external factors can have an effect on the index in one season which may not be present in the future. For example, temporary lameness means that the individual's production for the current season is low, but the same scenario may not apply next season.

2.1.4 Breeding Scheme

The following step is designing a suitable breeding programme that allows breeders to select for animals with the highest genetic merit for the objective. This step is crucial because it dictates the amount of genetic gain that can be potentially achieved, and has a major influence on the cost-effectiveness of the breeding programme (Garrick, 1993; Lopez-Villalobos & Garrick, 2005).

The common selection scheme currently used in the New Zealand dairy industry is the sire proving scheme (SPS). In this system, sires are evaluated in specific SPS herds, while daughters are scored to provide sire evaluations. The genetic merit of bulls selected for breeding is predicted based on progeny performance. Hence, the use of progeny testing is widespread in the dairy industry. This is because for any given animal to be widely used for breeding purposes, its EBV must first achieve sufficient reliability. Progeny testing is the best way of ensuring that the BV of the animal is reliable. In the case of bulls used in the dairy industry, young sires are selected from planned matings of sires and dams with the highest BV. These sires are then tested based on the milking performances of their daughters to determine EBV. The New Zealand dairy industry follows the four pathways of selection, as proposed by (Rendel & Robertson, 1950).

Table 2.4: The four pathways of selection used in the New Zealand dairy cattle breeding programme (Lopez-Villalobos & Garrick, 2005).

		Accuracy		Generation	
Pathway	Selection %	*i*	rTi	Interval, L	*i* x rTi
Sire of bulls	0.030	2.270	0.880	6.00	1.998
Sire of cows	0.100	1.750	0.880	6.50	1.540
Dams of bulls	0.003	3.040	0.610	4.00	1.854
Dams of cows	0.950	0.110	0.610	5.30	0.067
Total				21.75	5.459
				ΔG/year = 0.251 x 65 = $16.315	

Key: *selection %* = proportion of animals selected from the entire national cattle population; *i* = selection intensity; r_{Ti} = accuracy of selection; *L* = generation interval, in years; *i* x r_{Ti} = selection differential, described as a function of the selection intensity multiplied by the accuracy of selection. ΔG/year = genetic gain per year. Genetic standard deviation for BW assumed to be 65.

Genetic gain is calculated using the formula proposed by Rendel and Robertson (1950):

$$\Delta G = \frac{[(i_{cc}\, x\, r_{cc})+(i_{cb}\, x\, r_{cb})+(i_{bc} x\, r_{bc})+(i_{bb}\, x\, r_{bb})]\, \sigma g}{L_{cc}+L_{cb}+L_{bc}+L_{bb}}$$

where σ_g denotes the genetic standard deviation of the aggregate genotype for all the animals in the population; *i* is the selection intensity; *r* is the accuracy of selection; and *L* is the generation interval, defined as the age of parents when progeny is born. The subscripts *cc*, *cb*, *bc* and *bb* represent the four pathways of selection, i.e. cows to breed cows, cows to breed bulls, bulls to breed cows, and bulls to breed bulls respectively.

When the relevant genetic parameters are combined with their respective current economic values, it is possible to estimate the theoretical annual rate of genetic gain measured in profit per unit of feed consumed (in New Zealand, this is $/4.5tDM).

Genomic selection can also be used to improve genetic gain. Although this idea is relatively new, it has gained in popularity for several reasons. According to the formula by Rendel & Robertson above, it can be seen that changes in genetic gain is affected by four components: the selection intensity, accuracy of selection, genetic standard deviation and generation interval. A change in any one of these components

causes a subsequent positive or negative change in genetic gain. For example, increasing the generation interval will cause the rate of genetic gain to decrease.

Genomic selection has a beneficial role in improving genetic gain for several reasons. Firstly, it has the ability to reduce the generation interval by at least half in dairy cattle (Hayes et al., 2009). This simply means that there is potential for genetic gain to double each year, thus allowing the industry to achieve its goal in a shorter time span. Schaeffer (2006) also suggests that genomic selection can allow for the potential dams to be genotyped and later selected based on their EBV; this in turn means that the accuracy of selection for dam is increased, causing a correlated increase in genetic gain. Further, the accuracy of selection for sires can also be improved by the same logic. Genomic selection also offers an additional advantage of lowering inbreeding rates, which is important in any industry because it lowers incidences of unwanted diseases and/or recessive alleles (Hayes et al., 2009).

A more detailed discussion describing genomic selection is and its functions are provided in section 2.6: Genomic Selection below.

2.1.5 Dissemination System

Once individuals with the highest genetic merit have been identified, the next step is to transfer the genes of these individuals to the commercial population (Lopez-Villalobos & Garrick, 2005). There are two main components to consider when deciding on the best dissemination system; mass and magnitude. Harris et al. (1984) explain that it is important to consider the number of improved animals the system is trying to produce (mass), as well as the genetic improvement expected from using these high merit animals (magnitude). The benefits of using a particular dissemination system are therefore a function of mass multiplied by magnitude, where the relative costs involved are also considered as a primary concern. Commercial population size as well as the total costs and efficiencies of available biotechnologies available should also be taken into consideration when planning the dissemination system (Lopez-Villalobos & Garrick, 2005).

Currently, the most efficient method for doing this is by artificial insemination (**AI**). Although other options are available (e.g. multiple ovulation embryo transfer,

cloning and sexed semen), AI is still first choice for the simple reason that it is easy to implement and is cost effective (Harris et al., 1984; Lopez-Villalobos & Garrick, 2005). It also has the added benefit of being able to improve accuracy by improving sire evaluation using multi-herd progeny tests, as well as increase the selection intensity among sires that have been progeny tested (Harris et al., 1984; Kinghorn, 1992). In smaller flocks or herds, the use of AI will also provide and later on maintain a good mix of genes and hybrid vigour (Hammond, 1992).

In New Zealand, high pregnancy rates achieved via AI as well as generally low culling rates also mean that it is possible for desired genes to be quickly established in a population, and for almost every cow replacement to be the progeny of a high merit bull (Lopez-Villalobos & Garrick, 2005).

Crossbreeding offers many advantages, with the exploitation of heterosis being the primary reason farmers choose to crossbreed. The availability of crossbreeds also allows farmers the freedom and flexibility to design more complex mating plans, and achieve personal goals. However, most choose to use more systematic mating plans, such as the 2-breed rotational crossbreeding (Lopez-Villalobos & Garrick, 2005).

Crossbreeding allows for many benefits. One of these is the ability for breed effects to be averaged. In this case, the intermediate genotype of two separate breeds is considered to be ideal. Swan & Kinghorn (1992) offers the example of weight gain in cattle; rapidly growing individuals are ideal for the purpose of high live weight and carcass weight, but at the same time the foetus must be of a reasonable size so as to not cause dystocia. In this example, it makes sense for farmers to breed for small calves at birth, but with a fast enough growth rates to meet the target weight at weaning. The benefit of average breed effects also extends to traits that are negatively correlated across breeds with multiplicative expression of economic values.

As mentioned above, heterosis is one of the main benefits of crossbreeding. It allows farmers and breeders to exploit the better of two breeds to produce offspring that are better performers than the average of their parent breeds. Heterosis can be measured as:

$$\% \text{ heterosis} = \frac{(\text{mean of } F_1) - (\text{mean of parental breeds})}{\text{mean of parental breeds}} \text{ x } 100$$

There are also problems concerned with crossbreeding, and these need to be carefully considered and addressed before crossbreeding is applied in any system. Crossbreeding programmes can become complicated because there is often the need to maintain purebred and crossbred animals at the same time, but within different management groups. This is generally highly intensive and requires more management compared to systems using purebreds only. There are also extra costs involved in using imported crossbred stock (Swan and Kinghorn, 1992).

2.1.6 Economic Analysis

One of the most effective ways of deciding the appropriate breeding programme to use in any industry is to run a systems analysis, or economic analysis to compare the costs and benefits of each programme. Harris et al. (1984) provides a detailed modelling of the entire system. Although the example provided is for use in the broiler chicken industry, the steps are general enough for it to be extrapolated and used in the dairy industry, as seen in Lopez-Villalobos & Garrick (2005).

In Harris et al. (1984), the model for broilers was developed using a series of iterations followed by a comprehensive comparison of alternative programmes. For the dairy industry, the same steps are used, with very few changes. When defining the model, it is assumed that the dairy industry does not have sole control over the breeding programme; instead, it is the collective decision of dairy farmers all around the nation, who are more or less in sync with both economic and genetic aspects of genetic material available to them (Lopez-Villalobos & Garrick, 2005).

Harris et al. (1984) provides a list which generally describes the steps needed in order to obtain the best model for describing the system. These steps comprise of:

1. Predicting necessary details in each step of the breeding programme (Figure 2.1).
2. Searching literature for relevant theories and/or methodology pertinent to the design at hand.
3. Extending the theories and/or methodology as necessary for completion of the model.
4. Deriving algebra appropriate to the model.

5. Developing appropriate computer simulation procedures using appropriate computer packages if necessary.
6. Execution of the programme to produce numerical comparisons of each design, searching for the one deemed to be most valuable.

Rendel et al. (1996) expanded on the above list to produce a list relevant to the dairy industry. A more specific method of modelling economic changes is provided.

1. For each selection pathway, calculate the expected change in genetic merit.
2. As a result of any changes in genetic merit, calculate changes occurring in on-farm product flows.
3. Calculate changes in revenues and costs resulting from product flows, without semen and/or technology costs, but including current discounts.
4. Separately calculate changes in semen and/or technology costs, including current discounts.
5. Calculate the final value of change as (3) – (4).

As with any modelling design, there are advantages and disadvantages associated with thorough economic analysis. Firstly, there is difficulty in defining a variable useful for measuring the overall effectiveness of breeding programmes. As such, a number of variables exist with such potential. For example, calculating the rate of genetic gain achieved; economic benefits (for farm productivity, milk processing and product commercialization); profit for companies employing artificial breeding (as a measure of semen revenue less the cost of breeding scheme); and profit for commercial farmers (Lopez-Villalobos & Garrick, 2005).

Caution must also be taken when selecting a breeding programme, due to the vacillating nature of the industry. For example, changes in economic or marketing and changes in technology invariably means that it is necessary to constantly adjust models in order to make them compatible and relevant (Harris et al., 1984).

The breeding programme has many steps involved, and careful planning is required in order for the programme to be executed successfully. The brief description of each step provided above must be carefully taken into consideration; planning a

breeding programme is therefore not an easy task. A large number of people and organizations are often involved in decision making, with each participant representing a different facet of the industry (for example the multiplier breeder and the commercial producer). As a result of this, the importance of each component in the breeding programme will be different, and a compromise must be reached in order to develop a design that satisfies the requirements and expectations of everyone involved. By doing so, it is possible to improve the efficiency of animal production, which would benefit both the developers and other segments of the industry, as well as public consumers of the products (Harris et al., 1984).

2.2 Traits Other than Production

While production traits are an important economic factor in the New Zealand dairy industry, there are a large number of non-production traits (traits other than production, **TOP**) which also have an effect on the efficiency and profitability of dairy farms. In recent years, TOP has received more attention, as researchers discover their significance and potential in altering the economic status of the industry. This focus on TOP offers several advantages, such as improvement in facilitating sire selection; providing a more objective assessment of animals in the herd; and ease of use, which therefore means that it is of more value in animal breeding when it comes to maximizing farm income. It is also compatible with electronic data processing, and it is easily understood and accepted by both cattle breeders and commercial farmers (Ahlborn-Breier, 1988). All these advantages show that there are obvious benefits for animal selection to be based on TOP to a reasonable extent.

Ahlborn-Breier (1988) and Cue et al. (1996) categorizes TOP into two broad categories. These are for traits with information supplied either by the farmer (e.g. shed temperament; milking adaptability and speed; and overall opinion) or a qualified inspector (stature; weight; capacity; rump angle and width; legs; udder support; front and rear udder; front and rear teat placement; overall udder; and dairy conformation). Visscher et al. (1994) improved on this by categorizing traits into either production, type or workability traits. Temperament falls under “workability”, as does milking speed.

Both production and non-production traits undergo the same evaluation system here in New Zealand, though the relative emphasis placed on each trait will differ according to the perceived value of the trait. For example, traits such as milk composition, longevity and udder health are considered important, and are in fact major components of the selection objective, but little attention is given to other traits such as stature, teat placement or temperament.

2.3 Temperament

2.3.1 Definition

Temperament is an important trait and has many implications in animal husbandry (Boissy & Boissou, 1995). There have been a large number of studies carried out to investigate the effects of temperament on animal production. These studies cover a wide range of animals, from goats (Lyons et al., 1988; Lyons, 1989) to horses (Visser et al., 2001) as well as beef (Muller & von Keyerlingk, 2006; Olmos & Turner, 2008) and dairy cattle (Kilgour 1975; Welp et al., 2004). However, many difficulties still arise in defining temperament, despite the wide range of studies that have been conducted on this subject.

Quick reviews of about a dozen papers have revealed just as many definitions. These different definitions can be categorized into three general groups. Firstly, temperament is simply described as the animal's physical expression of behaviour. This is described as fearfulness, nervousness or skittishness in the animal (Burrow, 1997; Boissy et al., 2005; Hafez et al., 1969). A second description states that temperament is simply the individual differences in the response of an animal to human handling or an environmental change or challenge (Boissy & Boissou, 1995; Boissy et al., 2005; Muller & von Keyserlingk, 2006; Nkrumah et al., 2007; Welp et al., 2004). Following that, any physical, hormonal or behavioural changes that results have also been used to describe temperament (Kilgour, 1975; Lyons, 1989). The appeal of using physical characteristics is simply that any changes can be measured easily, and are therefore convenient indicators of temperament.

Across all these different definitions, however, is a basic recurring theme; temperament has been shown to be relatively stable and is consistent in each

individual over time (Lyons et al., 1988; Muller & von Keyserlingk, 2006; Olmos & Turner, 2008; Visser et al., 2001).

2.3.2 Temperament Measures and Ratings

The primary difficulty in measuring temperament is that no consensus has been reached as of yet regarding the manner in which this trait can be measured and recognized in farm animals, despite the scores of studies conducted on this topic (Boissy & Bouissou, 1995). Lyons (1989) provide further support by stating that while directly recorded measures can provide valuable and reliable information, they also fail to capture many other aspects of behaviour that are vital for describing temperament differences in individuals. The difficulties encountered in finding a simple yet effective definition for temperament is also present in measuring or rating temperament. As a result of this, a multitude of possible ways of measuring temperament has been proposed.

While the accuracy of these methods may be questionable, their effectiveness can be guaranteed to a certain extent. Grandin et al. (1995), Cue et al. (1996), Ahlborn-Breier (1988) and Dickson et al. (1967) used a numerical scale to rate temperament in dairy cattle. Lyons (1989) used a rating system based on seven behavioural items (Table 2.5), which was similar to a method used by Feaver et al. (1986). Flight speed (Burrow & Dillon, 1997; Petherick et al., 2002; Nkrumah et al., 2007) and vigilance (Welp et al., 2004) have also been proposed and used as a means of measuring temperament in dairy cattle.

Table 2.5: Temperament rating system as proposed by Lyons (1989) based on seven behavioural items.

Item	Behavioural definition
Excitable	Reacts strongly to a change in the environment.
Tense	Shows restraint in movement and posture; carries body stiffly alert.
Watchful	Looks readily at a change in environment.
Apprehensive	Seems to be anxious about everything; fears and avoids any kind of risk.
Confident	Behaves in a positive, assured manner; not restrained or tentative.
Friendly toward people	Initiates proximity and/or contact with people.
Fearful of people	Retreats readily from people.

For the purpose of this thesis, the definition provided by the AEU to describe temperament will be used. It is described as how placid or vicious the animal is in the farm dairy, while being handled and milked. It is scored based on a linear scale of 1 to 9, where 1 represents a vicious individual, and 9 representing a placid individual (Ahlborn-Breier, 1988).

2.3.3 Factors Affecting Temperament

There are many factors affecting temperament; some have a genetic basis (for example sire effects, age and sex effects) while others are environmental. It is possible to reduce environmental factors but conscious selection during breeding will be needed to alter temperament genetically. Stress and fear are important issues (Boissy et al., 2005) and reduced stress has the potential to improve an animal's ability to adapt to various farm environments. An animal's temperament may also be related to past experiences; for example a cow may have had a particularly bad experience in the milking shed, and is therefore difficult to handle in subsequent milkings even though the stressor has been eliminated. Conversely, habituation or maturational changes are

also reasons why a previously ill-tempered cow is now mild-tempered (Lyons et al., 1988).

2.3.4 Heritability of Temperament

The process of domestication has changed not only an animal's physical appearance, but also its behavioural characteristics towards those more suited to human requirements (Broucek et al., 2008). Domestication has provided the basis of genetic change, whether or not they were deliberate to begin with. Therefore, in order for a permanent change in behaviour and/or physical traits to exist, these traits must first have a heritable genetic component.

Heritability of traits plays a major role in genetic improvement in breeding programmes, although some traits have a lower heritability than others. The heritability of temperament has not received much study, but any values that exist show that this trait has low to moderate levels of heritability (Table 2.6).

Alternatively, indirect selection for a trait via a different trait (indicator trait) which has a strong genetic correlation has also been popular. Temperament has been proven to have a favourable genetic correlation with milk yield; dairy cows with a calm temperament show increased milk production (Lanier et al., 2000; Berry et al., 2004; Core et al., 2009). Temperament is also strongly negatively correlated with somatic cell count and for other diseases, with nervous cows being more vulnerable to higher levels of disease occurrence (Berry et al., 2004; Schutz & Pajor, 2001).

Despite all this, the use of indirect selection may be rather weak. Also, in order for indirect selection to be worthwhile, there is a need for a very strong genetic correlation with high heritability, lower recording costs or the advantage of being easily measurable earlier in life (Schutz and Pajor, 2001).

A large number of studies completed some 30 to 40 years ago (Shrode & Hammack, 1971; Hearnshaw & Morris, 1984; Fordyce et al., 1988) all conclusively prove that temperament is definitely heritable. The only challenge now is deciding whether temperament has the potential to be included in the breeding objective. Care must be taken to ensure that selection for improved temperament is not done at the

expense of positive changes that can otherwise be made in other more economically important traits (Morris et al., 1994; Konig et al., 2006).

Another important question to consider is whether the selection for calmer animals will induce responses in other traits, either positively or negatively. Examples include things like growth, reproductive performance, calving ease and/or maternal behaviour (Phocas et al., 2006). More studies on these areas are needed before temperament can be considered as a beneficial trait worthy of being included in the breeding objective.

Table 2.6: Trait definition(s) and heritability estimates for temperament and genetic correlation estimates between temperament and milk yield as found by different studies over the last 50 years.

Reference	Trait definition	No. of animals	Method	Heritability	Genetic correlation with milk yield
O'Bleness et al. (1960)	-	661	Regression of daughter on dam	0.40	0.08
Dickson et al. (1970)	Temperament score	1017	Paternal half-sib correlation	0.53	0.05
	Dominance value	921	Paternal half-sib correlation	0.07	-0.02
Thompson et al. (1981)	Disposition	8977	REML, sire model	0.06	
Agyemang et al. (1982)	No trouble	5601	Henderson I, sire model	0.07	
	Slight trouble	5601	Henderson I, sire model	0.06	
Baehr (1983)	Feeding frequency	102	Paternal half-sib correlation	0.61	
	Resting period	102	Paternal half-sib correlation	0.23	
Sullivan and Burnside (1988)	Ease of handling	18178	Sire model	0.12	-0.15
	Milking behaviour	18178	Sire model	0.16	-0.17
	Aggressiveness at feeding	18178	Sire model	0.11	0.23
Foster et al. (1988)	Disposition	43428	REML, sire model	0.08	
Lawstuen et al. (1988)	Temperament score	9546	REML, sire model	0.12	0.12
Erf et al. (1992)	Trouble-free workability	5353	REML, threshold model	0.11	0.25
	Overall satisfaction	5653	REML, threshold model	0.08	0.69
Visscher and Goddard (1994)	Temperament score	14596	REML, sire model	0.22	0.11
	Likeability	14596	REML, sire model	0.18	0.50
Cue et al. (1996)	-	2099	REML, sire model	0.14	0.03

Sewalem et al. (2002)	Milking temperament	656839	Animal model	0.08	0.23
Berry et al. (2004)	-	2224	Multivariate analysis	0.11	0.44
Phocas et al. (2006)	Response to human handling	2781	REML, sire model	0.18	0.07
Broucek et al. (2008)	Response to human handling				0.19
AEU (2010)	-			0.14	
Sewalem et al. (2011)	Milking behaviour			0.13	
	Ease of handling				
	Aggressiveness at feeding				
Berry and Kearney (2012)	-			0.13	

2.3.5 Importance of Temperament

Visscher & Goddard (1994) have found in dairy cattle that fear of humans is weakly negatively correlated to milk production. Although the correlation is small, it still plays a role in reducing overall farm profit, which can be detrimental to farms, especially those which rely on every bit of income they can gain to survive.

Boissy et al. (2005) suggests that genetically selecting dairy cows for reduced fear could be implemented and may be beneficial, since there are no apparent adverse effects on desirable productive traits, and has the potential to improve other adaptive behavioural traits. Also, temperament may be useful as either indicator traits in genetic evaluations, or because this trait may have a potential direct economic value (Nkrumah et al., 2007).

2.3.6 Challenges of Extrapolating Temperament Measures

As with any given species of animal, there is animal variation within a herd of dairy cattle. This means that differences in temperament between dairy cattle are to be expected, and are most definitely unavoidable. Breed differences for temperament have been observed in several breeds of cattle (Fordyce et al., 1985; Vanderwert et al., 1985). For example Morris et al. (1994) discovered that Angus cattle are highly reactive to human handling, compared to Herefords, while found that Simmentals and Limousin are even more difficult to handle than Angus cattle (Gauly et al., 2001; Le Neindre et al., 1995). Even though these examples are of beef cattle, it is reasonable to extrapolate from them and assume these differences exist in dairy cattle also.

In addition to that, behavioural traits are often measured subjectively, which means that the residual component of any results obtained will increase (Konig et al., 2006). All these factors highlight to the need for the development of methods suitable for measuring temperament, as well as the need to validate test situations and their biological significance for the species involved (Grandin et al., 1995; Boissy & Bouissou, 1995).

The implications of these findings are that dairy cattle breeders have to be aware that research results of temperament for one breed are not necessarily true for other breeds, or even other populations of the same breed. Researchers themselves also have to be wary when extrapolating data.

2.4 Genetic analysis of temperament using genetic markers

2.4.1 Genetic Markers

Genomics is the study of the entire genome sequence of an individual in order to understand their function and interaction, as well as their evolutionary history (Hartl, 2011). As an extension of studies in molecular genetics, it allows researchers to investigate how certain genes interact to affect specific traits in an individual. The genome of an individual contains all the necessary information needed to maintain life in an individual; this biological information is encoded in the individual's DNA, which are divided into units known as genes (NCBI Resources, 2004). Differences in the genetic makeup of individuals or species can be represented by genetic markers. All gene markers have a specific position within a chromosome, called loci. Study of these markers and their effects on a trait (or multiple traits) is possible and can provide valuable insight to different fractions of the community, for example the dairy industry.

According to Collard et al. (2005), there are three major kinds of genetic markers available: (1) morphological (or classical or visible) markers: these are markers which themselves act as phenotypic traits; (2) biochemical markers: these include enzyme variants known as isozymes; and (3) DNA or molecular markers: these are able to reveal the precise sites where variation occurs in DNA. Of these, DNA or molecular markers are of most importance for the purpose of this thesis.

Genetic markers located close to the gene of interest (or are tightly linked) are known as gene tags. Although tightly liked, these tags do not affect the phenotype of the trait in question. They are merely useful because they help identify genes affecting traits of interest in any given individual. All gene markers have a specific position within a chromosome, called loci. The same marker will be found on the same loci of a chromosome in any individual within the same species.

DNA markers are also more widely used due to their availability. These markers come from different kinds of DNA mutations, for example substitution or point mutations, insertions or deletions, or errors occurring during replication of tandemly repeated DNA. One of the major advantages of using DNA markers is that they are unlimited in number, as well as being virtually unaffected by environmental factors, and/or the developmental stage of the individual. This means that regardless of where the individual is from, or at what age it is when testing is performed, it is almost guaranteed that any results obtained using DNA markers will be accurate (Collard et al., 2005).

2.4.2 Linkage Maps and QTL Maps

Also known as a genetic map, a linkage map shows the order of genes in a chromosome in which distances between adjacent genes are proportional to the recombination rates between them (Hartl, 2011). Linkage maps are an important component of marker assisted selection. The use of MAS involves utilizing the presence or absence of a marker to either substitute or assist in phenotypic selection. QTL maps, on the other hand, aim to identify at least one genomic region (quantitative trait locus, **QTL**) contributing to variations in a given trait. These maps form confidence intervals for genomic regions, as well as estimate their effects on the trait. Details of QTL mapping is provided in section 2.4.3 below.

There are three main steps involved in constructing a linkage map; these are (1) producing a mapping population; (2) identifying polymorphisms; and (3) linkage analysis of markers. Linkage maps are constructed via the analysis of segregating markers.

Paterson (1996) describes a linkage map as being a “road map” for the chromosomes of a given individual, derived from two different parents. They provide an indication of the position and relative distance between markers along a chromosome, and are vital in identifying chromosomal locations which contain genes and genomic regions associated with a given trait.

Linkage maps are created based on the principle of crossing over of genes and markers (or chromosome recombination) during meiosis, enabling their analysis in the

progeny (Paterson, 1996). It is common knowledge that genes or markers that are closer together or are tightly linked will be inherited together in the progeny, at a higher frequency than genes or markers that are further apart.

To construct the mapping population, parents are first selected. These individuals are chosen based on differences for one or more traits of interest. The number of individuals chosen can vary, but larger populations will be required for high-resolution mapping. It is important to remember that these maps must be phenotypically evaluated; in other words, physical data of the trait of interest must be collected before mapping is carried out. To optimize mapping population output, several different populations of the same species may be utilized, with each population possessing its own unique advantages and disadvantages (Paterson, 1996; Collard et al., 2005).

The next step in constructing a linkage map is identifying DNA markers that show differences between parents. These are also called polymorphic markers, and it is critical to ensure sufficient differences or polymorphisms exist between parents before a linkage map is constructed (Young, 1994). When deciding which DNA markers to use, it pays to consider two factors: the availability of markers as well as the appropriateness of particular markers for any given species. Once identification of polymorphic markers has been made, they can then be screened across the mapping population. Inclusion of both the parents (and if possible, the F1 hybrid) is crucial. This process is called population genotyping.

The final step in linkage map construction involves data coding for each DNA marker available for each individual of a population. Linkage analysis using computer programmes are also carried out at this stage, and missing marker data are filled using values extrapolated from these programmes. In the dairy industry, linkage analysis is used as part of the grand-daughter design in progeny testing.

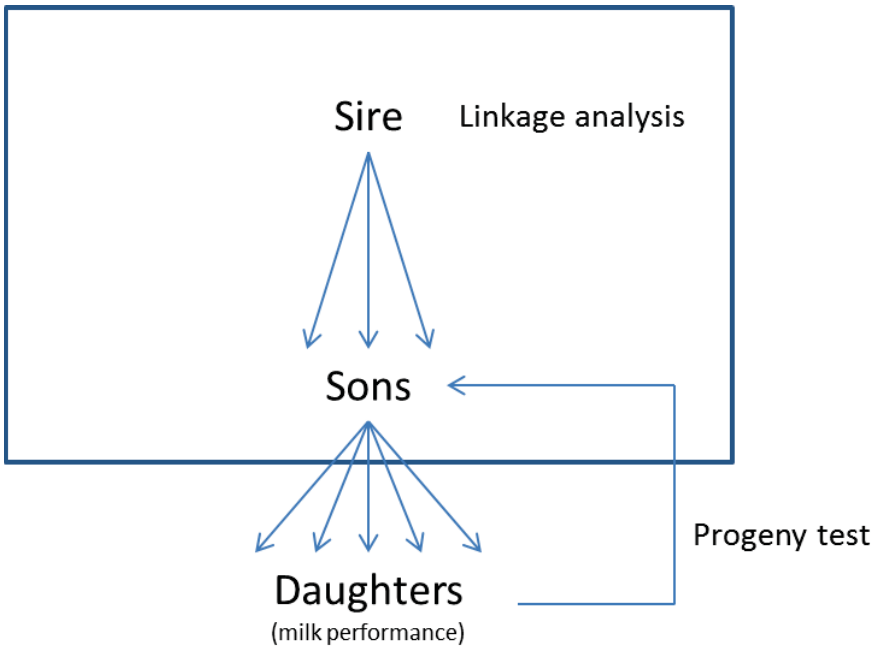

Figure 2.2: The grand-daughter design. Linkage analysis is performed in half-sib families, where each family consists of a sire and a large number of sons (half-sibs). The milking performances of each son is analysed as part of progeny testing (Georges et al., 1995).

In the grand-daughter design, cow and herd data are collected frequently, and the EBV for each individual is calculated using BLUP with an individual animal model (Georges et al., 1995). Before the onset of the grand-daughter design, a similar design known simply as the daughter design was used. Although this method was useful, Weller et al. (1990) proposed the alternative grand-daughter design because it offers several advantages that would benefit the system more. One of the advantages of this method is that it takes advantage of pedigree information provided to allow QTL mapping (Georges et al., 1995).

Even though the magnitude of QTL effects measured using the grand-daughter design amounts to only half that of the daughter design, the number of progeny used for analysis of quantitative traits can be far greater. It is also shown that for many dairy cattle populations, the grand-daughter design allows for genotyping to be possible with less than half as many individuals and yet still provide higher power than is achievable with the daughter design (Weller et al., 1990).

2.4.3 QTL Analysis

The principle of QTL analysis is based on the concept of detecting associations between phenotypes and marker genotypes. The mapping population is partitioned into different genetic groups using markers, and they are grouped based on the presence or absence of a particular marker locus. This process will help determine if a significant difference is found in the trait measured between different groups. If a significant difference is detected between phenotypic means, it could mean that the marker locus used to partition the population is linked to a genomic region controlling the trait of interest. However, this is also highly dependent on the marker system and the type of population used.

There are three methods used to detect genomic regions; (1) single marker analysis; (2) simple interval mapping; and (3) composite interval mapping. Of these, single marker analysis is the easiest genomic region detection method. It includes the use of *t*-tests, analysis of variance and linear regression. To detect genomic regions, there is no set number of DNA markers required; a relatively sparse framework of evenly spaced markers (between 100 and 200 markers) can be adequate, but more markers will be needed for species with larger genomes.

There are many factors which can influence detection of genomic regions. The most important factors are genetic properties of QTLs controlling traits, environmental effects, population size and experimental error (Asins, 2002; Collard et al., 2005).

Firstly, detection is only possible for genomic regions with relatively large phenotypic effects. Those with small effects may fall below the significant detection threshold, and therefore discounted (Collard et al., 2005). What this means is that the full effect of genomic regions affecting a trait may not be taken into account, thus decreasing the accuracy of further evaluations. Haley & Andersson (1997) states that population size is one of the most important factors affecting detection of genomic regions. Larger populations are generally more useful because they will yield more accurate results, as it will increase the likelihood of detecting genomic regions with smaller effects. In terms of experimental error, mistakes made during marker genotyping and/or phenotypic evaluations are common. It is therefore crucial to

ensure that these errors are minimized, so that more accurate and more reliable QTL maps can be produced (Collard et al., 2005).

2.4.4 Single Nucleotide Polymorphisms

A single nucleotide polymorphism (**SNP**) is defined as the change in a single base of the DNA sequence, usually with an alternative of two possible nucleotides (Vignal et al., 2002; Emara & Kim, 2003; Khlestkina & Salina, 2006). SNPs are biallelic in practice, and are only taken into consideration if the allele frequency is 1% or greater (Vignal et al., 2002; Khlestkina & Salina, 2006). SNPs within a coding region can either be synonymous or non-synonymous; this simply means that they either cause no change, or does cause changes in amino acids, respectively (Emara & Kim, 2003).

SNPs are discovered by comparing locus specific sequences which are generated from different chromosomes. The easiest way for SNP discovery is to directly sequence genomic PCR (polymerase chain reaction) products in different individuals. However, this can be expensive if carried out on a large scale. This is because there is a need for locus specific primers, which are limited only to regions containing sequence data (Vignal et al., 2002). Emara & Kim (2003) also states that the optimal approach for SNP discovery is to carry out a comparative analysis of DNA sequences between individuals to identify variations in any given gene. Other suggested methods include amplification by PCR, RFLP (restriction length polymorphism), SSCP (single stranded conformation polymorphism), or by melting temperature statistics.

When searching for SNPs, it is important to make sure that false results are avoided. This is usually in the form of false positives, which can occur because of the alignment of sequences from repeated loci. Non-detection errors should also be avoided as much as possible; this is usually when heterozygotes are genotyped as homozygotes, or vice versa.

To genotype SNPs, microsatellite markers are used. The standard procedure involves PCR and size determination, carried out using an acrylamide gel electrophoresis. The key features to note in this process (apart from direct

hybridization) are a two-step separation: (1) generation of allele specific molecular reaction products; (2) separating and detecting allele specific products (Vignal et al., 2002). A diagrammatic representation of this process is provided in Figure 2.4 below. The details of these techniques are beyond the scope of my study; refer to Vignal et al. (2002) for further information.

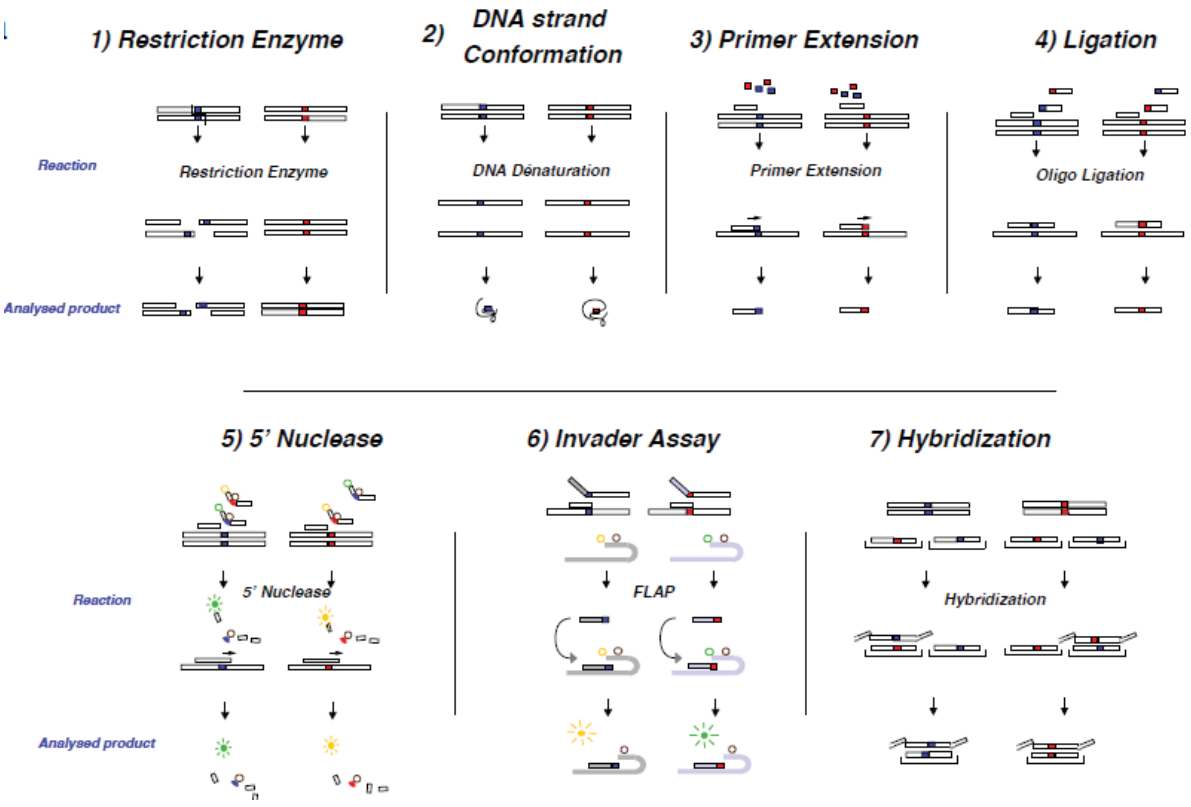

Figure 2.3: Principal molecular reactions used to generate allele specific signals (Figure is taken from Vignal et al., 2002).

2.5 Genome Wide Association Studies (GWAS)

Genome wide association studies (GWAS) estimates the regression coefficient of a trait on each of the SNP genotypes, and the results are then incorporated into predicting breeding values (Hayes & Goddard, 2010). Manhattan plots are most generally used to display GWAS results visually; they are basically scatter plots showing locations across the chromosomes of an individual's genome (displayed on the horizontal x-axis) where statistically significant differences between genetic

variants exist. This is usually displayed as a significant value (p-value) with a $\log_{10}$ transformation along the vertical y-axis. Dots located higher along the y-axis signify stronger genetic association.

2.5.1 Candidate Gene Discovery

Computer programmes specially designed for GWAS are now freely available via internet. A basic association analysis is used to explore the relationship between a SNP marker and the trait in question; outputs from the programme include the position of the marker as well as a p-value indicating significance. Once these associations have been drawn and QTL locations identified, it is possible to investigate nearby genes in order to discover potential candidate genes affecting the trait (Boldt, 2008). To do this, the Bovine Genome Database (http://www.ncbi.nlm.nih.gov/projects/genome/guide/cow/) proves to be a particularly useful tool. Using this database, genes on a chromosome can be viewed using either the marker name, gene name or symbol, sequence accession number, or chromosomal coordinates (Bovine Genome Database, Build 3.1).

To search for candidate genes using marker name, gene name or symbol, or sequence accession number, the database provides a search window which allows users to select the organism and enter the search term involved. Maps and regions will be displayed, and it is possible to further manipulate the options to obtain the information desired. Each map also has a link which allows users to download the data for personal use, in the form of a tab-delimited text file. To search using chromosomal coordinates, the database provides a page with all the chromosomes available for the organism. By selecting the chromosome, users can then enter the coordinates in the search box (either exact coordinates, e.g. 61551076 or values such as 61551K). Again, maps and regions are displayed, and users are able to further explore the data by downloading each map as a tab-delimited text file (Bovine Genome Database, Build 3.1).

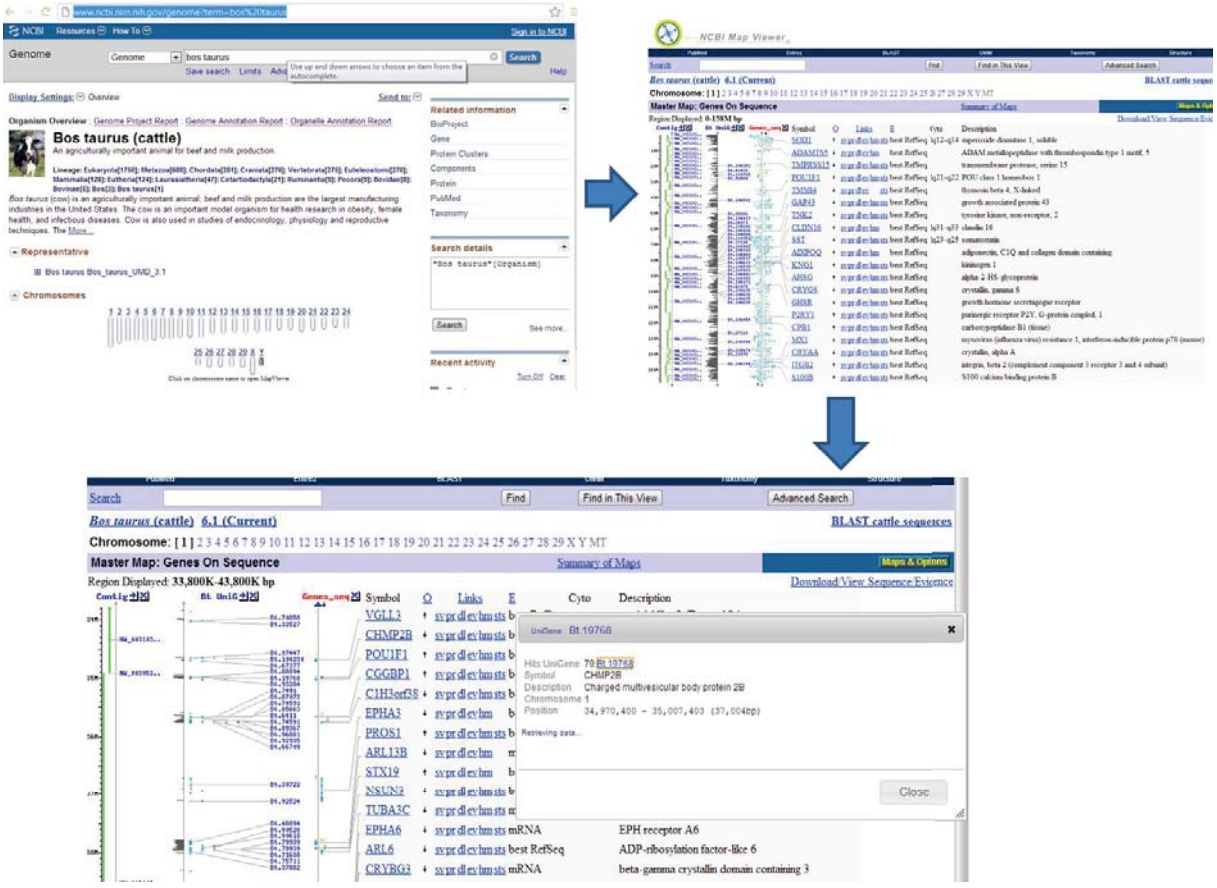

Figure 2.4: Exploring candidate genes via the Bovine Genome Database using the chromosomal coordinate method. Coordinates are located on their respective chromosomes, and each region displayed provides information of gene or genes found in these locations.

The use of GWAS generally presents both computational and statistical challenges. The primary challenge is in the form of increased multiple-testing burden, i.e. highly significant results are expected by chance, thus making it difficult to distinguish between signal from noise, especially when a large number of tests (hundreds of thousands) are performed. In addition to that, the sheer sizes of the data sets also present a computational and statistical burden, because many existing analysis software programmes were not originally designed to handle WGAS. Large data sets can often also result in an increased potential for bias to affect results. A final (but by no means the least) challenge is what is known as non-random genotyping failure. This is arguably the most insidious source of confounding in WGAS. It is simply the case where an individual's SNP genotype is incorrectly called, or not called

at all. Worse still, false-positives can often occur, especially when the failure is non-random for both genotype *and* phenotype.

Computer packages such as PLINK (a whole genome analysis toolset) was therefore designed in order to facilitate analyses of whole-genome data. This is carried out in a number of ways: it offers an easier way to manage data, and also has a range of new analyses methods which take full advantage of whole-genome coverage (Purcell et al., 2007). Normal screening procedures (such as overall genotyping rate and Hardy-Weinberg equilibrium) will often fail to detect biased SNPs. PLINK is able to provide a closer look at patterns of genotyping failure, in respect to both genotype and phenotype, thus providing a solution which eliminates bias. In short, the PLINK GWAS tool set was designed to: (1) provide a way for managing large GWAS data sets; (2) assess confounding caused by stratification and non-random genotyping failure, as well as producing a wide range of other summary statistics; (3) efficiently perform standard association tests; and (4) provide a way for common SNP panels to be assayed for rare variation, thus providing a method of mapping that will be a better performer when the multiple rare variant model holds (Purcell et al., 2007).

In addition to PLINK, there are two other software programmes which can be linked to PLINK to provide a more efficient and more interactive analyses of the data set. These two programmes are called gPLINK and Haploview. gPLINK is a Java-based graphical user interphase. The purpose of gPLINK is to offer project management, to track PLINK analyses and facilitates the integration of PLINK with Haploview. Haploview is a software offering tools for tasks such as tabulating, filtering, sorting, merging and visualizing PLINK GWAS output.

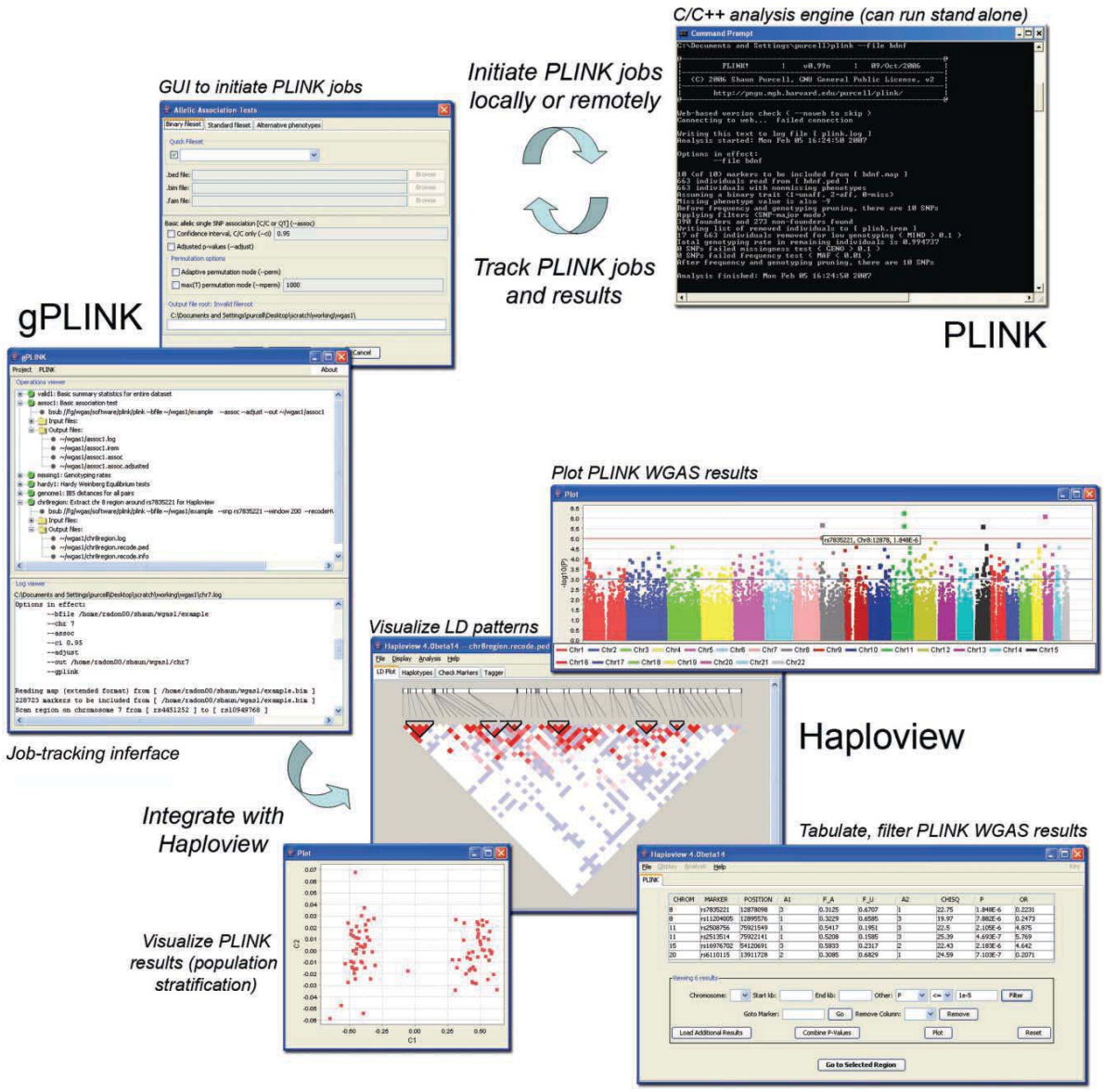

Figure 2.5: The integration of PLINK, gPLINK and Haploview. PLINK serves as the primary C/C++ engine, which runs as a stand-alone tool or in conjunction with gPLINK. These tools are easily configured, and both the whole-genome data and PLINK analyses can reside on a remote server but all can be initiated and viewed locally (i.e. on an individual's laptop or desk top). Figure is taken from Purcell et al. (2007).

Some of the types of analyses performed by PLINK is described in some detail in Purcell et al. (2007). Several mathematical and statistical models are also provided as examples.

2.5.2 Estimation of Genomic Breeding Value (GBV)

Calculating GBV is relatively simple in theory. Results from statistical associations of SNP with phenotypes are used as a predictor for BV of candidates of a new generation or from a different population (Dekkers, 2010). The method for calculating GBV in New Zealand is similar to that used in Australia and the United States. Hayes et al. (2009) provides a detailed description of this method, which will be included briefly in here.

The first step involves genotyping individuals who have been phenotyped for the trait in question, or for individuals who have progeny with phenotypes for large numbers of SNP markers across a genome (Dekkers, 2010). Animals that have been progeny tested are genotyped using the Illumina Bovine SNP50^{TM} chip. These animals can be both cows and/or bulls. The samples are then screened for any missing genotypes (Hayes et al., 2009).

Typically, samples containing more than 10% missing genotypes are removed. Strict regulations mean that only SNP which meets the following criteria are included: there must be less than 10% of missing genotypes across samples; the minimum allele frequency must be more than 2.5%; and the deviation of observed genotype frequencies from expected frequencies (as calculated from allele frequencies) must be less than 600. These criteria were chosen to ensure that SNP with high rates of genotyping errors and SNP with very low frequency are excluded from the final data set. This is because such data will be very poorly estimated, and can have significant impact in the derivation of the final prediction equation.

Parentage checking is also performed, to remove any genotypes that are deemed incompatible with the pedigree. To impute missing genotypes, Hayes et al. (2009) provides the following steps: using the programme Bovine Genome Build 3.1, SNP are ordered according to chromosome position. These genotypes are then analysed, chromosome by chromosome, in fastPHASE, software used for haplotype reconstruction and estimation of missing genotypes from a population data. This software then attempts to fill in all missing genotypes. Comparisons of imputed genotypes with known genotypes are then carried out to evaluate the accuracy of this method.

Secondly, data results from the genotyping process are used to estimate statistical associations of individual SNP with the phenotype for the trait in question. The resulting estimates are then used to predict BV for selection candidates (Dekkers, 2010). Generally, the prediction equation used can be developed by fitting the following model to phenotypes of the individuals making up the population:

$$y_i = m + \Sigma_j X_{ij} b_j + e_i$$

where y_i denotes the phenotype of individual *i*, m represents fixed effects, Σ_j is the summation of all genotyped SNP, X_{ij} represents the number of copies of allele "1" versus allele "0", b_j is the effect of allele substitution and e_i is the random residual error (Dekkers, 2010).

To derive the prediction equations, several options are provided. BLUP and a Bayesian approach (BayesA) were employed by Australia. BayesA is a method which uses prior knowledge that a large number of SNP have potential effects on a trait, with only a few having moderate to large effects. In New Zealand, a wider range of methods were used; BLUP, BayesA, BayesB (where it is possible for some SNP to have zero effect), least angle regression, and a Bayesian regression (Hayes et al., 2009; Meuwissen et al., 2001). A pedigree based polygenic component (additive BV) was also included in the estimation of GBV.

Estimation of GBV reliabilities are carried out through direct inversion of a set of mixed model equations. As previously mentioned, a SNP based genetic relationship matrix is used in place of the average relationship matrix commonly used in MME (Habier et al., 2007; Hayes et al., 2009). For the calculation of the final GBV, the parental average BV from pedigree information is combined with BV from genomic information, via selection index theory (Hayes et al., 2009).

To determine the accuracy of genomic selection, the phenotypic records used to estimate SNP effects can be used. According to Hayes et al. (2009) and Pryce et al. (2011), there are four major parameters affecting the accuracy of final GBV: the level of linkage disequilibrium (**LD**) between markers and QTL; the size of the reference population from which the SNP effects are estimated; the heritability of the trait or the reliability of BV if deregressed BV is used; and the distribution of QTL effects. Linkage disequilibrium describes the non-random association of two or more loci

within a chromosome. Accuracy will increase with the number of phenotypic records available, as there will be more observations available per SNP allele. In the case where a large number of QTL have only small effects contributing to the variation of the trait, larger numbers of phenotypic records will be required to increase accuracy in estimating these effects.

Because the accuracy of GBV can be difficult to ascertain, Dekkers (2010) suggests using a "validation" step as part of the predictive method. This step involves splitting the data set into training and validation sets. The prediction model is first used on the training set. The prediction model is then applied on the validation set to compute estimated GBV. Resulting GBV from both data sets are then correlated to determine the accuracy of predicted GBV.

2.6 Implementation of Genomic Selection

In recent years, traditional methods of animal selection and breeding has been slowly replaced with more efficient methods directly utilizing DNA profiles of the individuals involved. Most of breeding programs for dairy cattle have systems of genetic evaluation based on phenotypic and pedigree information. With the onset of the genomic era, accuracy of EBV can be further improved with the use of low cost assays in the form of SNP (Hayes & Goddard, 2010).

A reference population of individuals containing both genotypic and phenotypic information is crucial for the implementation of genomic selection. This reference population will be used to derive the prediction equations for predicting GBV (as shown above), which is later used to select individuals with marker genotypes but have no trait records (Hayes & Goddard, 2010). The number of SNPs required to do this is dependent on the extent of LD in the target species; lower LD indicates the need for a larger number of SNP to be included so that at least one SNP will be found in LD with each QTL.

Goddard & Hayes (2009) illustrate this method using pigs as an example. The benefit of using genomic selection is that the method can be applied to any species of animal.

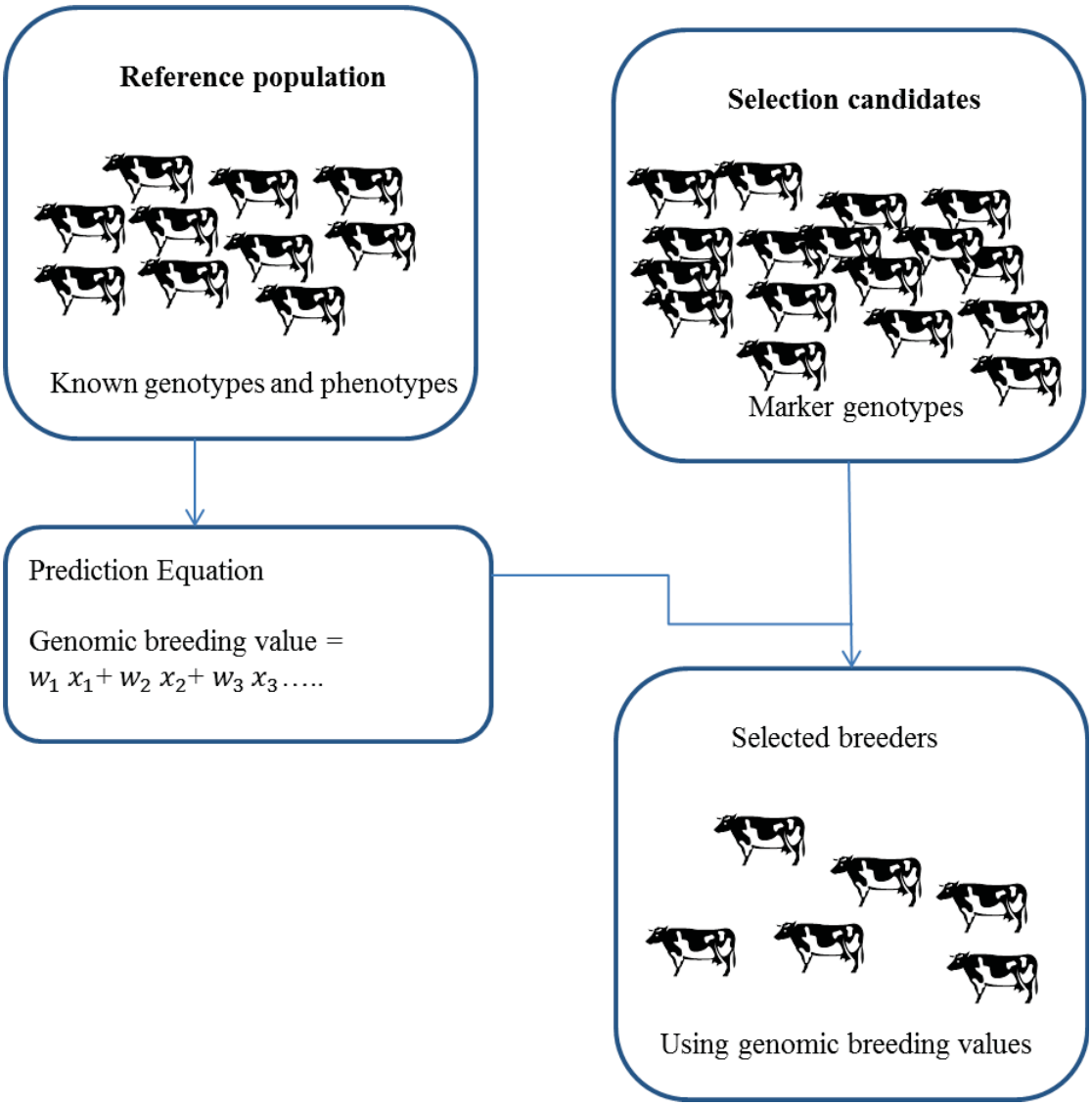

Figure 2.6: Implementation of genomic selection in the dairy industry. Figure is modified from Goddard & Hayes (2009).

Genomic selection offers many advantages. It has the potential to double the rate of genetic gain, through selecting and breeding bulls at a younger age (2 years old rather than 5 years old) (Schaeffer, 2006). There is also evidence that costs can be reduced by eliminating the need for progeny testing (Schaeffer, 2006). The accuracy of GBV is lower compared to the accuracy of estimated BV obtained from progeny test (Hayes et al., 2009). Additional benefits include a reduction of generation interval by at least half (Khatkar et al., 2004; Hayes et al., 2009); potentially large genetic gains on the maternal side via genotyping and selecting potential dams based on GBV (Khatkar et al., 2004; Schaeffer, 2006); and a decreased risk of inbreeding compared

to non-marker BLUP using pedigree and phenotypic information only, especially for low heritability traits (Daetwyler et al., 2007; Hayes et al., 2009).

One limitation of using genomic selection, however, is that SNP estimations calculated from one breed or population do not produce accurate predictions for other breeds or populations (Harris et al., 2009; Hayes et al., 2009). Genomic selection relies heavily on the phase of LD between markers and QTL being similar in each population as those in the reference population. However, the chances of this occurring are very low, especially if the distances between markers and QTL are large.

Pryce et al. (2011) suggests that the use of a multi-breed reference population could potentially increase the accuracy of GBV in the breeds used. However, for SNP to have any effect in a multi-breed reference population, it must be in high LD with QTL in all breeds of the reference population. Additionally, the use of a multiple breeds can be an option for increasing the population size used to derive prediction equations in genomic selection.

Table 2.7 below show the potential genetic gain which can be achieved using the traditional selection method (i.e. progeny testing), or genomic selection, when applied to the basic four pathways of selection method used here in New Zealand.

Table 2.7: The selection percentage, selection intensity, accuracy, generation interval and overall genetic gain achieved when using genomic selection for breeding in the New Zealand dairy system. Figures are taken from Schaeffer (2006) and are adjusted to fit the dairy breeding system in New Zealand (Lopez-Villalobos & Garrick, 2005).

		Accuracy		Generation	
Pathway	Selection %	*i*	rTi	Interval, L	*i* x rTi
Sire of bulls	0.05	2.06	0.75	1.75	1.54
Sire of cows	0.20	1.40	0.75	1.75	1.05
Dams of bulls	0.02	2.42	0.75	2.00	1.82
Dams of cows	0.85	0.27	0.50	4.25	0.14
Total				9.75	4.55
					ΔG/year = 0.467 x 65 = $30.355

Key: *selection %* = proportion of animals selected from the entire national cattle population; *i* = selection intensity; r_{Ti} = accuracy of selection; *L* = generation interval, in years; *i* x r_{Ti} = selection differential, described as a function of the selection intensity multiplied by the accuracy of selection. ΔG/year = genetic gain per year. Genetic standard deviation for BW assumed to be $65.

Table 2.7 shows that selection for breeding using the genome wide strategy is more effective than the traditional progeny testing method. Using genomic selection, a higher percentage of both bulls and cows can be selected for breeding. The generation interval is also significantly smaller (a reduction of ~55%). These values support studies and figures produced by both Khatkar et al. (2004) and Hayes et al. (2009). From these examples, we can therefore safely conclude that the use of genomic selection in animal breeding is efficient, can be used by any animal breeding system, and will offer many advantages if correctly implemented.

CHAPTER THREE

MATERIALS AND METHODS

Three computer based statistical tools were used to analyse data. The Statistical Analysis System (SAS Version 9.3, SAS Institute, Cary, NC, USA) was used to generate descriptive statistics and adjust phenotypes. The second package was PLINK 1.07 (Whole Genome Association Analysis Toolset, by Purcell et al., 2009) used to run a genome wide association study test, for the identification of significant SNPs affecting temperament in dairy cattle. PLINK is available for free download at http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml. The PLINK programme is basically a whole genome association analysis toolset, and is freely available for download via any internet browser. It is designed to perform a variety of basic, large scale analyses in a manner which is effective and computationally efficient. This programme focuses primarily on the analysis of genotype and/or phenotype data. The third package used was R (R Development Core Team 2012), which was used to produce the Manhattan plots.

This study looked at a set of bull temperament phenotypes adjusted for a number of variables using a multiple regression model (called Adj-EBV_{temp}). It contains phenotypes from three breeds of dairy bulls (HF, JE and XB). In the visual analysis, two threshold probability values were used; these were the suggestive and genome-wide significance. Each of these components will be discussed in more detail in the proceeding sections.

3.1 Phenotypes

The study was based on a bull population consisting of 3140 individuals of different breeds; purebred HF, purebred JE and crossbred bulls. There were also a small number of bulls of other breeds. The age group for the bulls of this data set was mixed, i.e. their birth dates range from 1994 to 2006. The sire code for each individual bull was also provided, along with percentage of North American Holstein (NAH%), percentage of New Zealand Holstein-Friesian (HF%) and percentage of Jersey (JE%) in each individual.

Estimated breeding value for temperament (EBV_{temp}) obtained by best linear unbiased prediction procedures for each bull was also provided. This data was provided by the Livestock Improvement Corporation (**LIC**) based in Hamilton, New Zealand. These breeding values were then adjusted for year of birth, NAH%, HF% and JE% with a multiple regression model using the REG procedure of SAS. This was in the form of:

$$EBV_{temp} = \beta_1 \text{ year} + \beta_2 \text{ NAH\%} + \beta_3 \text{ HF\%} + \beta_4 \text{ JE\%}$$

Residuals obtained from the model were called Adj-EBV_{temp} and used for further analysis in PLINK.

3.2 DNA Genotypes

A second set of data was also provided by LIC and contains biallelic genotype information for each of the bulls. The total number of columns provided was 1385202; the data set contains 6 preliminary columns titled family identification, animal key, sire, dam, sex code, and phenotype. The remaining 1385196 columns were biallelic genotype columns, which provide information on the alleles present at each SNP. These are called allele codes, and by default, minor alleles were coded with the number 1 while the major alleles were coded with the number 2. This data set contains 700,000 SNP markers in total.

For analysis on PLINK, a high density panel containing 700,000 markers was utilized.

3.3 Descriptive Statistics

Descriptive statistics of the phenotypic data set were obtained with the MEANS procedure of SAS. Output shows maximum and minimum values of temperament EBV for each breed, as well as the relationship between temperament EBV with each variable (year of birth, NAH%, HF% and JE %).

3.4 Genome Wide Association Study (GWAS) using PLINK

Firstly, the 700k genotype panel was converted from map and ped format to a binary format on the programme, so that consequent commands carried out will be simpler for the programme to handle. The programme is controlled via a MS-DOS command prompt, and instructions for use are found in the manual found on the website (http://pngu.mgh.harvard.edu/~purcell/plink/dist/plink-doc-1.07.pdf).

The first analysis carried out was called a basic association analysis. The output of this analysis provided information such as the regression coefficient, t-value and p-value of the trait at each SNP. For this analysis, every single individual was taken into consideration. However, due to the small number of individuals of other breeds, they were excluded from the analysis. To do this, individuals of this breed were simply omitted from the original data set. PLINK is also able to complete this process via the command to create a subset. Subsets can also be created for each of the remaining breeds (HF, JE and XB). The basic association analysis was also carried out for HF, JE and XB individually. This was done so that breed effects can be investigated more closely and also to minimize stratification in the final output.

The second analysis carried out was an extension to the original basic association analysis. An additional command to adjust the probability value produced a set of genomic corrected p-values (**GC**), which are essentially a scaled down version of the raw p-values. The purpose of this was to adjust for inflation based on the median chi-square statistic (Purcell et al., 2007). GC values were used for visual analysis.

3.5 Visual analysis

For visual analysis, a number of Manhattan plots were produced using R; plots for all breeds combined, and for each individual breed (HF, JE and XB) were produced. The R program to do the Manhattan graphs was written in April 2011 by Stephen Turner, who is an Assistant Professor at the University of Virginia, USA. The following link can be used to access the blog: http://gettinggeneticsdone.blogspot.co.nz/2011/04/annotated-manhattan-plots-and-qq-

plots.html. In addition to that, Manhattan plots showing each individual chromosome in detail were also produced. This was also performed using R.

3.6 Significance levels

Two significance levels were chosen for these plots; a point-wise significance level and a genome-wide significance level. The point-wise significance level shows the probability that an extreme deviation at a specific locus can be found by chance alone. This involves only a single test of the null hypothesis. The genome-wide significance level shows the probability that a deviation can be found somewhere in a whole genome scan, and involves looking over a large number of tests to locate results with the highest significance (Lander & Kruglyak, 1995). Point-wise p values are generally in the range of 10^{-3} to 10^{-4} for suggestive linkage, and 10^{-4} to 10^{-5} for significant linkage. To obtain the recommended genome-wide significance level of 5%, the p-value should be set at $5x10^{-5}$ (Lander & Kruglyak, 1995). For this thesis two thresholds were selected, $1x10^{-4}$ and $5x10^{-5}$. These are for the suggestive threshold and the genome-wide threshold respectively.

3.7 Significant SNP

Based on the Manhattan plots, chromosomes with noticeable peaks were investigated further. This investigation was carried out using the Bovine Genome Database (Build 3.1, http://www.ncbi.nlm.nih.gov/genome?term=bos%20taurus). It was possible to search for potential genes that are found close to or are within the range of significant markers involved. The analysis results provided by PLINK contained the exact base pair and chromosome number, which made locating them a relatively simple task.

CHAPTER FOUR

RESULTS

4.1 Basic Statistics

Descriptive statistics for the temperament EBV are shown in Tables 4.1. HF bulls had the highest BV average for temperament (0.7980), followed by JE (0.6520) and XB (0.5870) bulls. However, it is also important to note that the mean BV for HF bulls is negative (-0.0402). Reliabilities are similar across all breeds.

Table 4.1: Descriptive statistics for cow temperament estimated breeding values (EBV) of dairy bulls.

Breed	N		Mean	Minimum	Maximum	Std. Dev.
Holstein-Friesian	1664	EBV	-0.0402	-1.0200	0.7980	0.2563
		Reliability	84.486	42.300	99.000	4.404
Jersey	981	EBV	0.0352	-0.8850	0.6520	0.2385
		Reliability	84.899	69.700	99.000	4.048
Crossbreed	442	EBV	0.0079	-0.9860	0.5870	0.2174
		Reliability	83.309	39.000	99.000	4.534

Figure 4.1 provides a genetic trend of the average breeding values for each of the breeds. Of these three breeds, there is a steady increase in temperament EBV in both the HF and JE (Figure 4.1). However, XB shows a small but steady decline over the years. The regression coefficients were 0.0020, 0.0042 and -0.0049 for HF, JE and XB, respectively. However, the coefficients of determination (R^2) for HF, JE and XB were 0.0697, 0.0696 and 0.031, respectively; indicating that genetic trend of cow temperament is not significant.

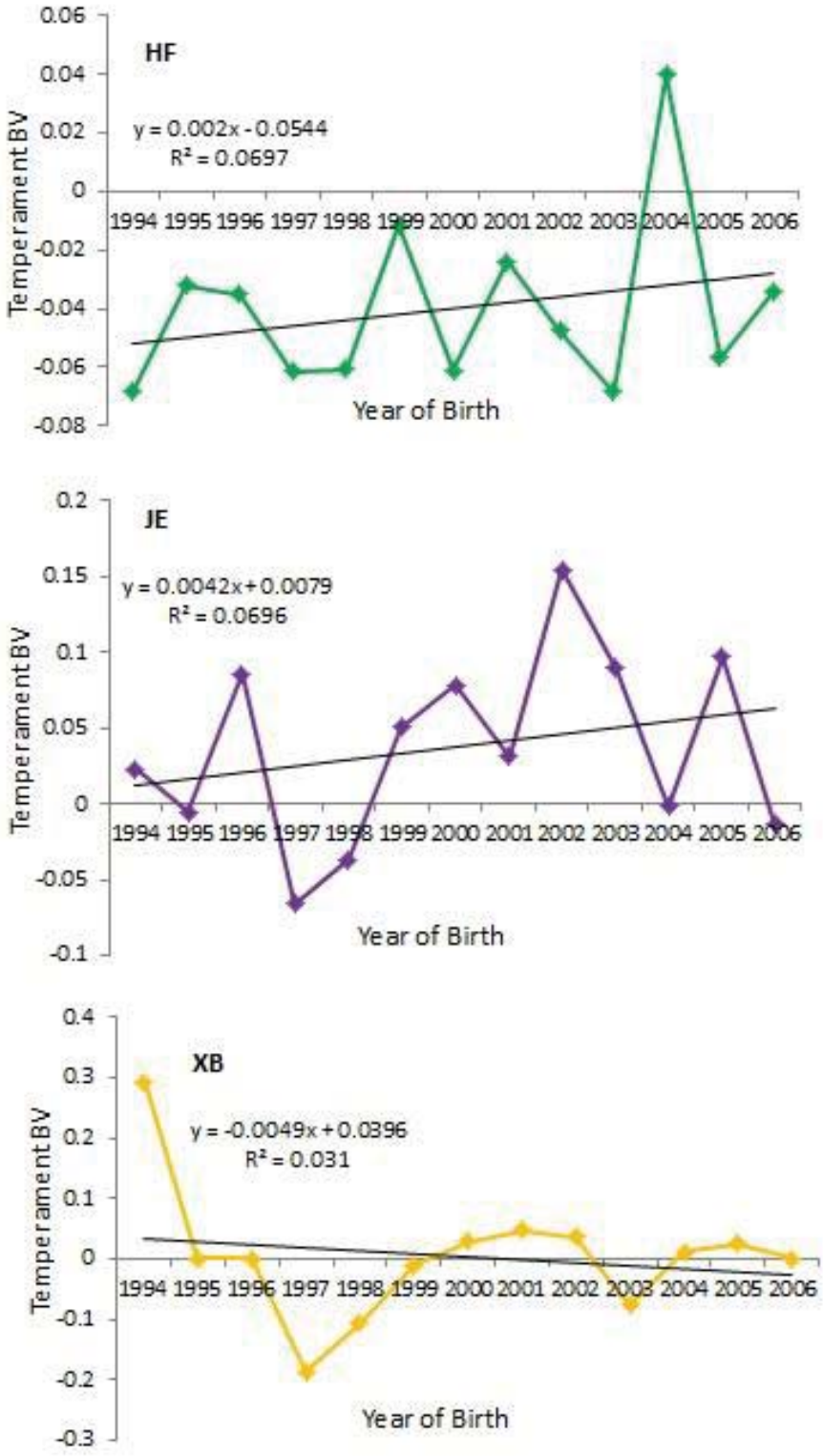

Figure 4.1: The average breeding values for temperament per year of birth (1994-2006) for HF, JE and XB bulls.

SAS output provided estimates for the effects of each variable (age, NAH%, HF% and JE%) on EBV_{temp} as well as the significance of each value. Table 4.2 shows that overall, age and HF% in an individual has a significant effect on temperament EBV, but the proportion of JE and NAH% does not.

Table 4.2: Estimates of regression coefficients of estimated breeding value for temperament on birth year and percentages of New Zealand Holstein-Friesian (HF%), Jersey (JE%) and North American Holstein (NAH%).

Effect	Estimate	Standard Error	t	Pr> |t|
Intercept	0.1469	0.07541	1.95	0.0515
Birth year	0.0035	0.00128	2.73	0.0064
HF%	-0.2294	0.07742	-2.96	0.0031
JE%	-0.1328	0.07530	-1.76	0.0778
NAH%	0.0047	0.00426	-1.11	0.2661

4.2 GWAS Analysis

Results from GWAS analyses yielded a set of Manhattan plots for each of the three breeds (HF, JE and XB) as well as for all the breeds combined. GC values were used as the scale for the y-axis in the Manhattan plots. All plots contain two significant thresholds; $1x10^{-4}$ and $5x10^{-5}$ (for suggestive significance and genome-wide significance, respectively). These are shown in Figures 4.2 and 4.3 below.

Figure 4.2 shows the Manhattan plots for all breeds combined. There are few markers above the genome-wide threshold, but peaks can be clearly seen in chromosomes 3, 6, 11, 18 and 19.

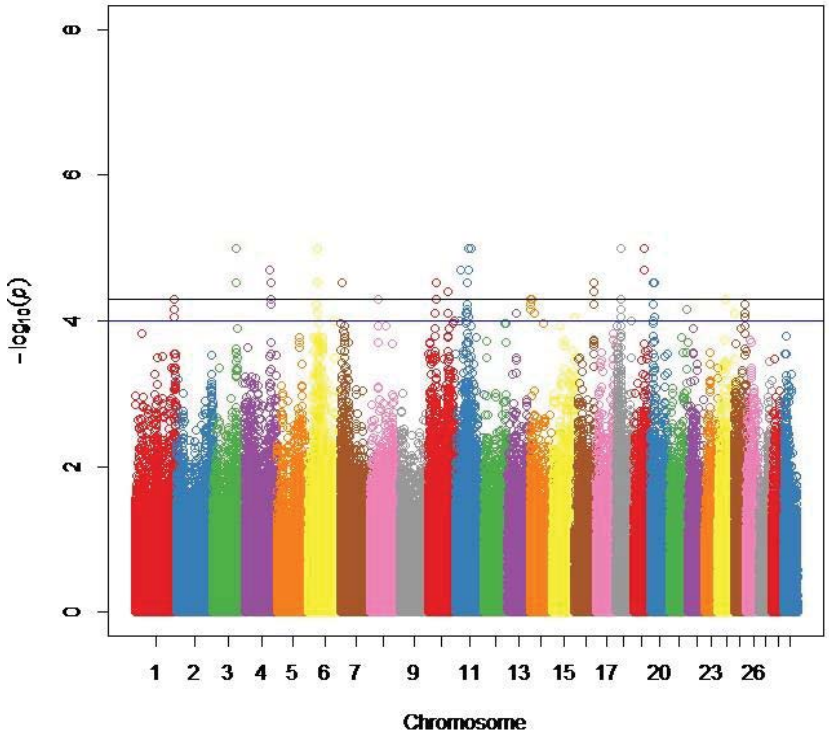

Figure 4.2: Genome-wide plots across all breeds of -$\log_{10}$(p-values) for association of SNP loci adjusted temperament EBV (Adj-EBV_{temp}) using the 700k panel. The corresponding horizontal lines indicate the suggestive significance level (blue line) and the genome-wide significance (black line).

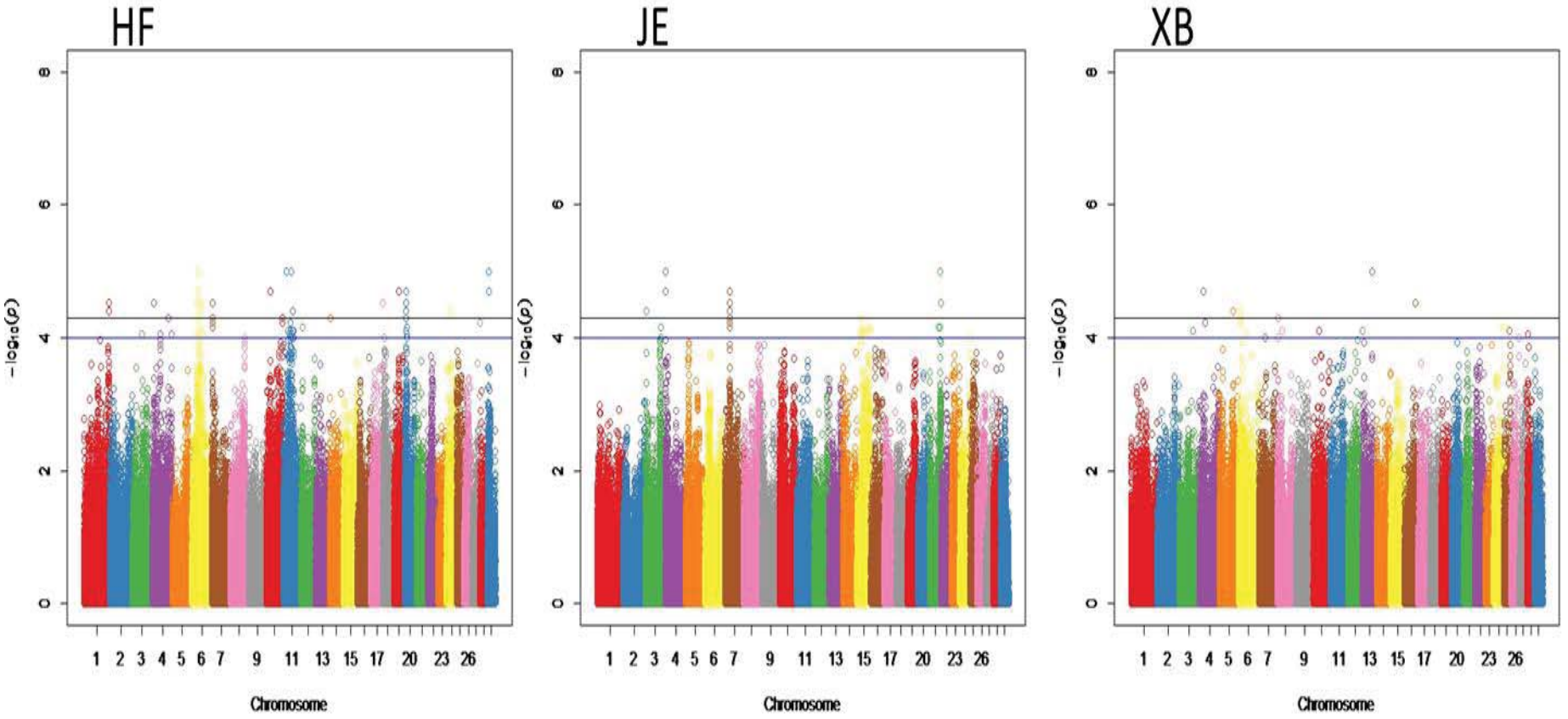

Figure 4.3: Genome-wide plots across individual breeds of -$\log_{10}$(p-values) for association of SNP loci with adjusted temperament EBV (Adj-EBV_{temp}) using the 700k panel. The corresponding horizontal lines indicate the suggestive significance level (blue line) and the genome-wide significance level (black line).

Figure 4.3 shows Manhattan plots for each individual breeds. All three breeds show a peak in chromosome 4, suggesting that there is a genomic region in chromosome 4 significantly affecting temperament in dairy cattle. There are a large number of common chromosomes with markers exceeding the suggestive threshold for all three breeds. One of the more noticeable results is that peaks in XB seem to display an intermediate pattern between those of HF and JE. For example, HF shows a strong peak in chromosome 29, while JE does not. The range of markers for XB in chromosome 29 is found to be not as high as HF, but not as low as JE either.

For each plot (all, HF, JE and XB), the individual chromosomes were also mapped out to investigate the peaks more clearly, to determine if there are any interesting patterns present. These plots are attached in the Appendix.

4.3 Significant SNP

A number of SNP markers from some of the most noticeable peaks in the Manhattan plots were investigated. For all breeds combined, BTA 3, 11 and 19 had noticeable peaks. A random selection of the top three most significant markers from each of these chromosomes was looked at using the Bovine Genome database.

Table 4.3 shows that the top most significant markers in BTA 3, 11 and 19 for all breeds seem to be random, or are simply not transcribed (i.e. not information available yet). This trend is seen for most of the top markers for temperament in all breeds; markers are either found associated with random genes, are transcribed only (but with no function assigned yet), or are not in transcribed regions.

Table 4.3: Candidate genes associated with some of the top SNP markers for all breeds (HF, JE and XB) combined.

Chr	BP	Gene	Function
3	89, 769k - 89, 773k	Complement component 8, beta polypeptide	Immune response
11	51, 753k	Not transcribed	
11	29, 132k - 59, 133k	Not transcribed	
19	41, 190k - 41, 208k	Not transcribed	

Key: Chr = chromosome number; BP = base pair position; Gene = candidate gene name; Function = candidate gene function.

Across all breeds (HF, JE and XB) individually, BTA 4 showed clear peaks indicating that it contains regions significantly affecting temperament in dairy cattle. A study conducted by Spelman et al. (1999) also found BTA 4 to be important in affecting temperament in dairy cattle in New Zealand. Based on this, further investigation was carried out on BTA 4 using the Bovine Genome database. PLINK results show that across all breeds individually, different regions were deemed to be highly significant in affecting temperament. As a result of that, a scan of the general areas around a sample of those regions was investigated to determine if any candidate genes are present that affects temperament.

A large number of these regions were not transcribed (for example at 2,000k and 11,000k); hence no candidate genes and no functions are available. Of those that were transcribed, researches still have not determined their function yet (for example at 34,000k). There were a number of regions which are found near candidate genes (for example at 49,000k and 113,000k). However, these genes seem to be random and as of yet, their function in affecting temperament is not known or recorded. For example in HF, two highly significant markers found at 49,231k and 49,233k is located within a region containing a gene known as dihydropoamide dehydrogenase, which functions in controlling glycine cleavage in the mitochondria. Similarly, in XB,

two markers found at 113,745k and 113,757k is found in a region coding for GTPase activity. The relationship between these genes and temperament is unknown.

CHAPTER FIVE

DISCUSSION

Temperament is not included in the breeding objective of the breeding program for the genetic improvement of New Zealand dairy cattle, although individual farmers have been known to make forced culling decisions on livestock based on the animals' dispositions. This simply shows the importance of temperament to each individual farmer, even if it has currently not been deemed important to the national system as a whole. The use of genomic selection is also a relatively new concept, and is currently still not widely used. Both temperament and the use of genomic selection therefore has the potential to be included in the breeding objective, for the sole purpose of animal and handler welfare, as well as a higher probability of increasing profit. The study of identifying QTL affecting temperament has also been very limited. The findings of this study will therefore be compared to any previous studies to the best of my abilities.

5.1 Importance of Temperament in New Zealand Dairy Cattle

The importance of temperament has been highlighted previously. To restate briefly, temperament is important in the New Zealand dairy system because studies have shown that it has a negative correlation with milk production (e.g. Visscher & Goddard, 1994). It is also important because highly temperamental individuals can pose a serious safety threat to handlers, as well as to other members of the herd.

In New Zealand, production animals have their merits evaluated based on EBV; hence all breeding decisions are highly dependent on the individuals' EBV superiority. This applies for seven traits currently considered to be of most use in the selection index. Temperament is not included in this list. However, the NZAEL still measures temperament as part of the animal evaluation scheme, so the importance of temperament EBV in the dairy industry as a whole is recognized. Furthermore, there is evidence that in Ireland, the economic value of temperament has been calculated (as a measure of survival) and is currently under review to be included in the nation's

breeding objective. The economic weight of temperament in Ireland is currently set at €33.69 per unit score (Berry & Kearney, 2012).

5.2 Breed Differences

Breed differences for fear behaviour in cattle has been widely known for a long time. Numerous studies on cattle behaviour support this claim; for example, Morris et al. (1994) showed that the Angus breed has higher reactions to human handling than Hereford, while Boissy et al. (2005) show that *Bos indicus* exhibit greater behaviour and physiological reactions to handling than *Bos Taurus*. Fordyce et al. (1985), Vanderwert et al. (1985), Le Neindre et al. (1995) and Nkrumah et al. (2007) all also show that behaviour differences exist between different breeds of cattle.

It is not surprising then that the same trend is seen in this study using Holstein-Friesian, Jersey and crossbred cattle. As seen in Table 4.1, the JE bulls exhibited the highest average BV for cow temperament, followed by XB and HF bulls. What this means is that overall, JE have a better disposition (i.e. "tamer") than XB or HF. Unfortunately, there have not been very many studies looking at BV for cow temperament in these breeds, even though it is measured and is an important measured trait in the animal evaluation scheme. However, it is of popular public opinion that the JE breed are considerable calmer and tamer than the HF, and most farmers and livestock owners admit to preferring JE than any other breeds.

The closest evidence available for this preference is provided by Winkelman et al. (2000). In this study, residual survival (measured in number of days) was measured for each HF, JE and XB cows as an indicator of several traits, including shed temperament. Results show that JE had the highest residual survival rate at around 138 days, while HF and XB were the same at 110 days.

5.3 Factors Affecting Temperament

A high EBV for temperament generally means that the progeny of a bull was scored for good (placid) temperament, whereas a low EBV indicates that the progeny of the bull was scored for bad temperament. In the data set used in this thesis, results show that there has been a consistent trend towards the selection of higher EBV for bull temperament between 1994 and 2006 (Figure 4.1). This is true for HF, JE and XB, even though the increase in EBV per year have been small.

Boissy et al. (2005) suggests that it is quite possible to successfully select for animals with reduced fear, since there are no obvious adverse effects on other desirable traits, such as milk production. Increasing selection for animals with high temperament BV also has the potential to improve other adaptive behavioural traits. It is therefore safe to assume that selection for dairy cows and/or bulls with high temperament BV can be safely carried out without having to worry about adversely affecting their production status.

There is also proof that favourable correlations exist between temperamental differences and milk production levels, fat production levels and shape of lactation curves in dairy cattle (Baryshnikov & Kokorina, 1959). This is also true for meat production in beef cattle (Voisinet et al., 1997). It is generally accepted that when cows are calm and comfortable in the presence of human handlers, they tend to have higher production than individuals that are nervous or highly agitated. Since conscious selection for high temperament BV has no known adverse effects, and even has the potential to increase milk production, there should not be any reason why temperament should not be included in the selection scheme in the near future.

And additionally important point to remember is that not all “bad” tempered cows and/or bulls have a low BV for temperament. As with any other species, this temporary lapse in “good” behaviour could result from a large number of factors (Breuer et al., 2000). For dairy cattle, this could be nutritional (e.g. mineral deficiency), environmental (e.g. temperature or climate change; stray voltage in the yard) or even health related (e.g. mastitis). It is important to make sure that selection for cattle with “good temperament” is not based on observation alone, as this can change from time to time; it is also important to remember that it is not reasonable to

expect cattle with high temperament BV to behave well at all times if other requirements (such as nutrition and health maintenance) are not met.

5.4 Temperament and Animal Welfare

In terms of animal welfare, selecting for mild tempered cattle can also be highly beneficial. Animal and human welfare has gained recognition in recent years, and various studies have been conducted to investigate a wide range of factors on animal welfare, for example housing and feeding. With the rising popularity of genomic selection in recent years, it makes sense to also investigate the effects of genomic selection on animal welfare. The role of genomic selection in reducing aggressiveness in pig and poultry populations was investigated in detail by Rodenburg & Turner (2012). This study focused on the effects of increased productivity on animal welfare, and also looked at ways to reduce welfare problems via genomic selection. Results from this study showed that although breeding for increased productivity in livestock has been exceedingly successful in the past 50 to 60 years, it has also resulted in negative consequences in terms of animal behaviour and welfare. This study uses broiler chickens as examples, where increased growth rates have resulted in diseases such as lameness, ascites and sudden death syndrome. If we look at the dairy cattle population, the same trend can be seen; increased milk production over the years has resulted in decreased fertility and longevity, mastitis and other associated metabolic diseases (Oltenacu & Broom, 2010).

With the increasing need and push for higher production levels, it is a common practice in various farming systems (be it dairy, beef or any other) to develop bigger and more extensive group housing systems. In New Zealand, dairy herds are not housed. However, individuals still live together as a herd, and are required to be indoors for a certain number of hours a day for milking. While welfare issues associated with temperament may not be evident while the herd is outside, it definitely is an issue and may cause severe problems during milking time. Rodenburg & Turner suggest that individuals with high performance levels may be harmful to the performance of the herd as a whole, because this animal would tend to be highly aggressive or have high levels of damaging behaviour.

While this statement was made based on observing different species (poultry), the theory is sound and may apply to other species as well. Presumably, an aggressive dairy cow has the potential to disrupt the milking pattern and habits of other members of the herd, if enough fuss is made during milking time. In pigs, aggressive behaviour can also compromise individual weight gain, meat quality and carcass grading, which is of particular significance in the beef industry as well. In the dairy industry, revenue from beef sales is also highly dependent on those characteristics.

Rodenburg & Turner (2012) and Murani et al. (2010) also reports that differences in SNP have an important role in genes mediating the hypothalamic-pituitary-adrenal (**HPA**) axis, which forms the basis of the neuroendocrine stress response pathway, and which has been associated with control of aggressiveness. This being the case, it is therefore possible to alter the functions of HPA axis towards less aggression by actively selecting for less aggressive animals. However, caution is to be exercised when putting this in practice because there is potential that altering this basic pathway may cause unwanted changes in a wide range of other biological functions as well.

5.5 Significant QTL affecting Temperament

From this study, it is seen that there are a large number of chromosomes containing regions significantly affecting temperament in dairy cattle. Of these, only BTA 4 is common across all breeds (HF, JE and XB). A number of previous studies on dairy cattle temperament provide loose support for these results. Wegenhoft (2005) and Boldt (2008) are dissertations from students in the United States, who also looked at temperament in cattle (both dairy and beef combined). Wegenhoft (2005) found that BTA 1, 4, 8, 9, 16 and 18 significantly affected temperament in two three-generation *Bos Taurus* x *Bos indicus* cattle populations. Boldt (2008), on the other hand, found BTA 3, 6, 12 and 29 to be significant.

Wegenhoft (2005) found that a genomic region on BTA 8 overlap a region on human chromosome 8, which is associated with Schizophrenia in humans. This finding is later supported by Boldt (2008). Based on this, it may be suggested that BTA 8 contains highly significant genomic regions which affect temperament in cattle.

Other studies over the last 13 years looking at the location of genomic regions affecting temperament in cattle (dairy, beef or both) have yielded slightly different results. Results from Schrooten et al. (2000) suggested that genes located in BTA 2, 3 and 23 are involved in affecting milking speed and milking behaviour. Schmutz et al. (2001) studied behaviour of beef cattle and found BTA 1, 5, 9, 11, 14 and 15 to contain significant QTL affecting cattle behaviour traits. Finally, Hiendleder et al. (2003) found BTA 5, 18 and 29 to be the most significant, with $p< 0.015$ for BTA 5 and $p< 0.012$ for BTA 18. Suggestive significance were also found in BTA 10, 20 and X/Y, where $p< 0.10$.

As results regarding the significance of BTA 10 and 20 were only suggestive, the results from this study can be used to verify and validate that of Hiendleder et al. (2003).

Of most significance is a study by Spelman et al. (1999) which found BTA 4 to significantly affect temperament in dairy cattle. This finding is important because it not only supports results from this study, but because the research by Spelman et al. (1999) is based on New Zealand dairy cattle as well, it is likely to be more relatable to the circumstances and results from this study.

5.6 Significant SNP

As mentioned in the results section above, the top 50 most significant SNP markers were found over a larger number of chromosomes. Of note are the peaks in each data set observed for each breed. It is interesting to note that XB shares several chromosomes containing significant markers in common with either HF or JE individually. This suggests that some form of genetic crossing over occurs, which is plausible considering that XB cattle in New Zealand are more often than not a cross between HF and JE cattle. For example, HF and XB share a peak in BTA 6, even though JE does not show the same pattern. The Manhattan plots for XB also show an intermediate between HF and JE, to show or provide proof that the theory of crossing over exists.

Another interesting discovery is that upon further investigation of these genomic regions on the Bovine Genome Database for each individual breed, most of

the most significant SNP markers were found to be linked to seemingly random genes. The best conclusion we can draw from this is that there is potential that pleiotropy and/or epistasis plays a major role in defining temperament, and pin-pointing the specific genes involved might prove to be an extensive task. Alternatively, these genes could simply be involved in non-production traits in general (i.e. for body confirmation, etc.) and can be helpful in explaining variation in non-production traits, but without specificity.

Due to the large number of SNP tested, it is also possible that false positives have occurred in this study. Bolormaa et al. (2010) suggest that unless strict significant thresholds are set and adhered to, false positives will be expected. The thresholds used in this study were based on Landers and Kruglyak (1995) who looked at human studies. It is quite possible that the thresholds are not completely suitable for this particular data set. An effective method to minimize the occurrence of false positives is to use a separate, independent data set to validate significant associations between the trait and SNP identified in the original data set (Bolormaa et al., 2010).

Large phenotypic effects are also generally necessary for effective QTL detection. Regions with small effects tend to fall below significance thresholds, and are therefore disregarded (Collard et al., 2005). It is highly likely that the small number of markers exceeding the thresholds in XB may be due to the size of the phenotypic effects. Larger population sizes have been found to be an effective means of detecting regions with smaller effects (Haley & Andersson, 1997); information for XB was collected for only 442 bulls, which may have affected the power or likelihood of detecting smaller effects.

Another interesting discovery is that a large number of SNP markers are found in areas of the chromosome which have been labelled as “transcribed locus” only. This is true for all three breeds. A transcribed locus is basically a locus which has been used to make RNA, but its function has not been discovered yet, and hence no name has been ascribed to them. Also known simply as junk DNA, these areas may have been left unexplored due to the relative lack of importance earlier researchers have given them. If this is true, then the next step is to investigate the full effect of them in regards to temperament. The implications of these studies are difficult to

predict, but it has potential to benefit the wider scientific and farming community in the near future.

5.7 Junk DNA

The large number of "transcribed loci" present in the results can be attributed to the phenomenon commonly known as "junk DNA". Quite simply, there are sequences found between and within gene boundaries which has seemingly no function (Brosius & Gould, 1992). Only a fraction of DNA is occupied by protein coding exons. The remaining portions are non-exonic sequences, consisting of highly repetitive elements (Makalowski, 2000). For many years, researchers have assumed that these repetitive elements have no function. Recent studies, however, have found that early researchers were quick to dismiss these regions as "junk", and that they actually have a role in genomic evolution.

Transposable elements (**TE**) are stretches in the DNA sequence that move around the genome of a cell. These elements contain all necessary instructions needed to enable themselves to leave their host DNA and splice into another spot (Biemont & Vieira, 2006). As part of the repetitive sequence of the genome (and therefore "junk"), TE and other repetitive elements have been found to play a vital role in recombination events (Makalowski, 2000). They interact with the genomic environment to increase host evolvability by serving as recombination hotspots or mechanisms for genomic shuffling (Makalowski, 2000; Makalowski, 2003). One of the most important things of note is that environmental stresses have the ability to markedly affect the way the genotype is expressed in the form of phenotypes (Biemont & Vieira, 2006).

It is possible that TEs are responsible in the expression of temperament in dairy cattle. Results show that variables such as age and NAH%, HF% and JE% had an effect on bull temperament EBV, although these effects are small.

It is also important to remember that temperament changes from time to time, and is not wholly dependent on BV. As mentioned in section 5.3 above, a whole plethora of environmental variables exist which can have a significant effect on an individual's temperament. The significance of discovering that many TEs are involved simply means that there are ample opportunities for further studies to be carried out on

these elements in order to fully understand their role and mechanism in affecting temperament in dairy cattle.

5.8 Implications

Selection for dairy cattle with good temperament has many implications. At the most basic level, it is difficult to select for temperament using selection if this trait is not heritable. However, studies have shown that temperament in cattle is heritable, albeit at a low to moderate level (Table 2.4). This simply means that while genomic selection may be a viable method, progress could be slow and it may take several years of intense selection before desired results can be seen (Boissy et al. 2005).

The selection of temperament also does not have any adverse effects on desired traits such as milk production (Boissy et al. 2005); rather, there seems to be a positive correlation between cattle with good temperament and milk production in dairy cattle (Baryshnikov & Kokorina, 1959) or meat production in beef cattle (Voisinet et al., 1997). There is also evidence that cattle with good temperament have longer residual survival (Winkelman et al. 2000) and higher feed conversion efficiency (Oikawa 1989).

Cattle welfare can also be improved by lowering risk to other herd members and/or human handlers, especially during milking time. This not only provides a safe environment for the handlers to work in, but it also ensures some form of "security" for the cattle, and aids in making sure that their milking habits are not disrupted (Boissy et al., 2005).

The use of genomic selection to select for cattle with "good" temperament is the next thing we must consider carefully. While it may be a relatively simple task to weigh up the cost and benefits of selecting for temperament and come to the conclusion that it is definitely a viable option, the same cannot be said for using genomic selection as a means of achieving this. The main problem lies in the fact that a large number of the most significant SNP affecting temperament lies in genes that has been transcribed but have no name or function ascribed to them as of yet. It is difficult to understand what role they have genetically in affecting temperament, if we cannot even ascertain if the genes act singly (i.e. have a basic dominance-recessive

effect), or if they act together (i.e. epistasis). In a way, this just means that there are ample opportunities for further research and study into these QTL, but until their roles are determined, it is difficult to implement selection for temperament using this method.

If or when genomic selection is finally proven to be a viable method for selecting temperament, and when it has been accepted and applied in the dairy industry, it is important to remember that not all "bad" tempered cattle will have a low temperament EBV. What this means is that farmers cannot expect all individuals to be placid all the time just because they "chose" cattle with high EBV for temperament; it is important to remember that a wide range of other factors also play a part in affecting temperament such as stress, environmental cues, age and habituation or maturity (Boissy & Bouissou, 1995; Boissy et al., 2005; Kilgour, 1975; Lyons et al., 1988).

5.9 Conclusions

Temperament is a complex trait, and currently is not considered as a part of the breeding objective of the New Zealand dairy cattle. However, the NZAEL and individual farmers have still identified and measured temperament as part of the animal evaluation scheme, indicating that temperament has some importance when it comes to animal selection for breeding. Breed difference is definitely a contributing factor influencing temperament BV; JE are deemed more placid than HF, and this study showed that EBV decreases as NAH percentage increases. The environment also plays an important role, as factors such as stress and nutrition can greatly influence an individual's temperament, even if the individual is supposed to have high temperament BV.

In this study, a large number of significant SNP were identified, but were not consistent across the three breed groups used. Furthermore, only one chromosome (BTA 4) was found to contain genomic regions significant to temperament across all breeds. The identification of these genomic regions with significant effects on temperament can open up further opportunities for research into this area; currently, a large number of these regions fall in sections of DNA with unknown name or function. In other words, regions previously dismissed as "junk DNA" now show some importance in affecting temperament in dairy cattle. In addition to that, the exact mechanisms in which these genes interact are also still unknown. With further advances in technology and methodology in the near future, it is possible for better and more accurate research to be carried out to determine the importance of these regions.

The implications of finding these genomic regions are that it will be of great benefit to industries employing genomic selection methods as part of their breeding programme. However, the fact that temperament has a low to medium heritability means that improvement in genetic gain via direct selection of the trait may take a very long time.

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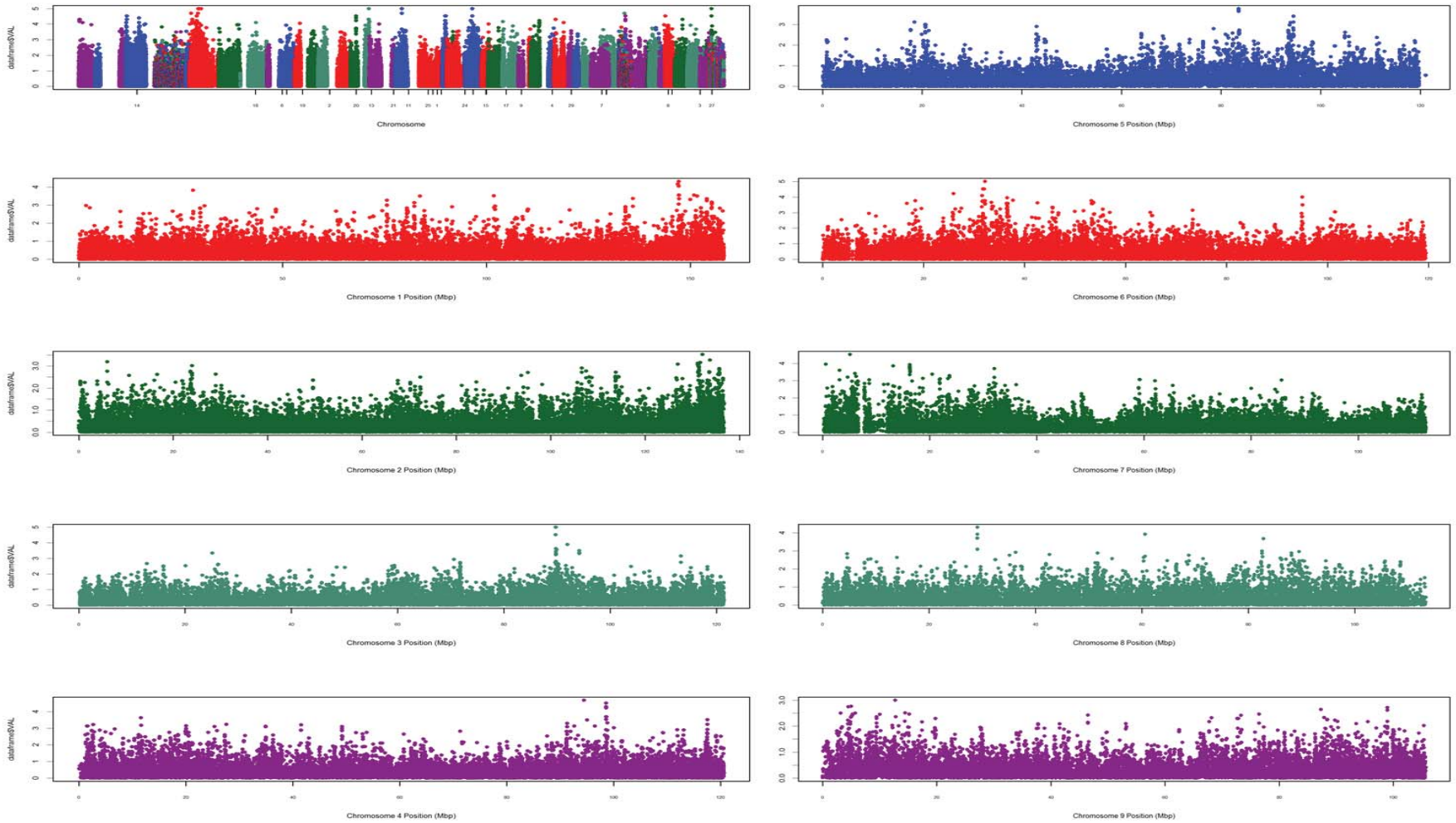
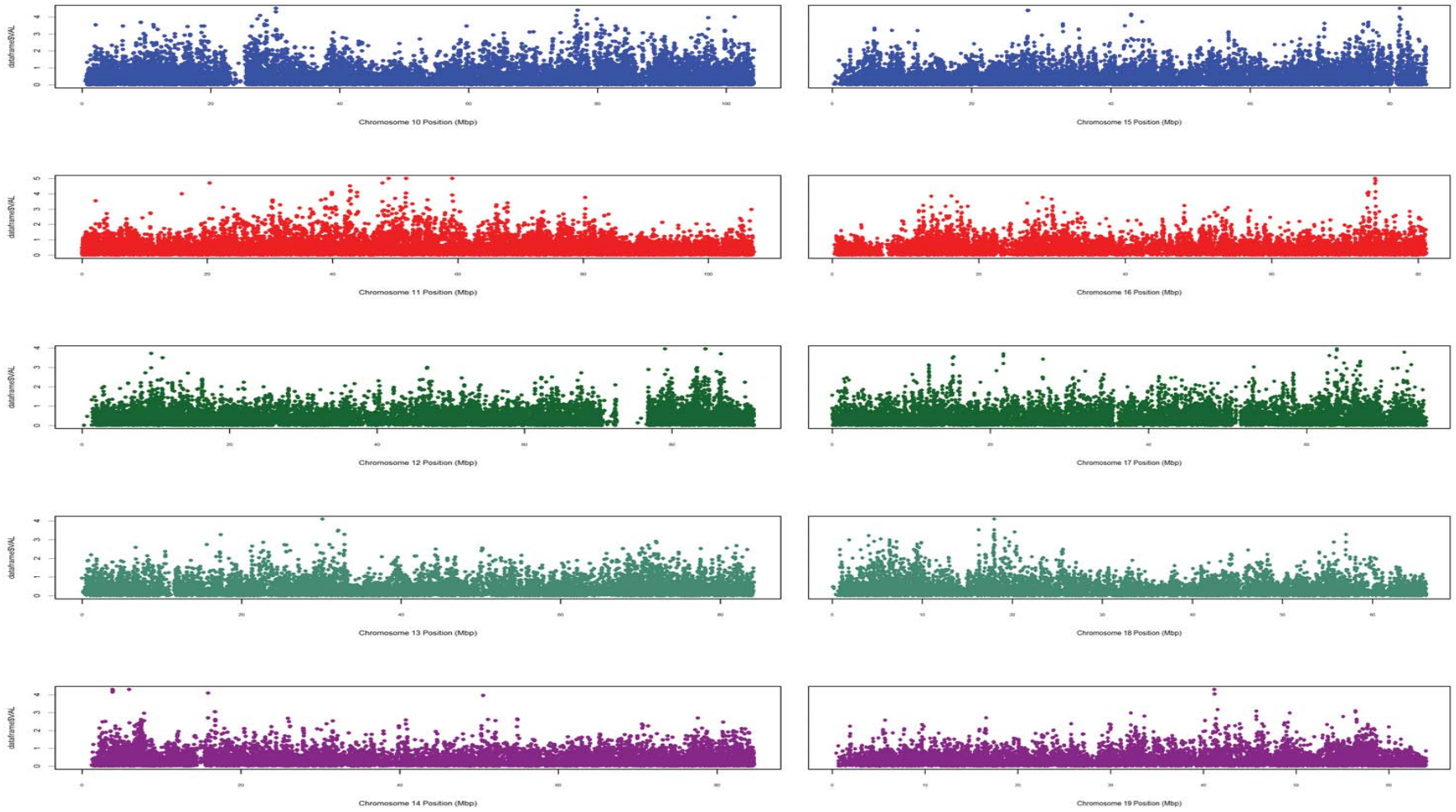
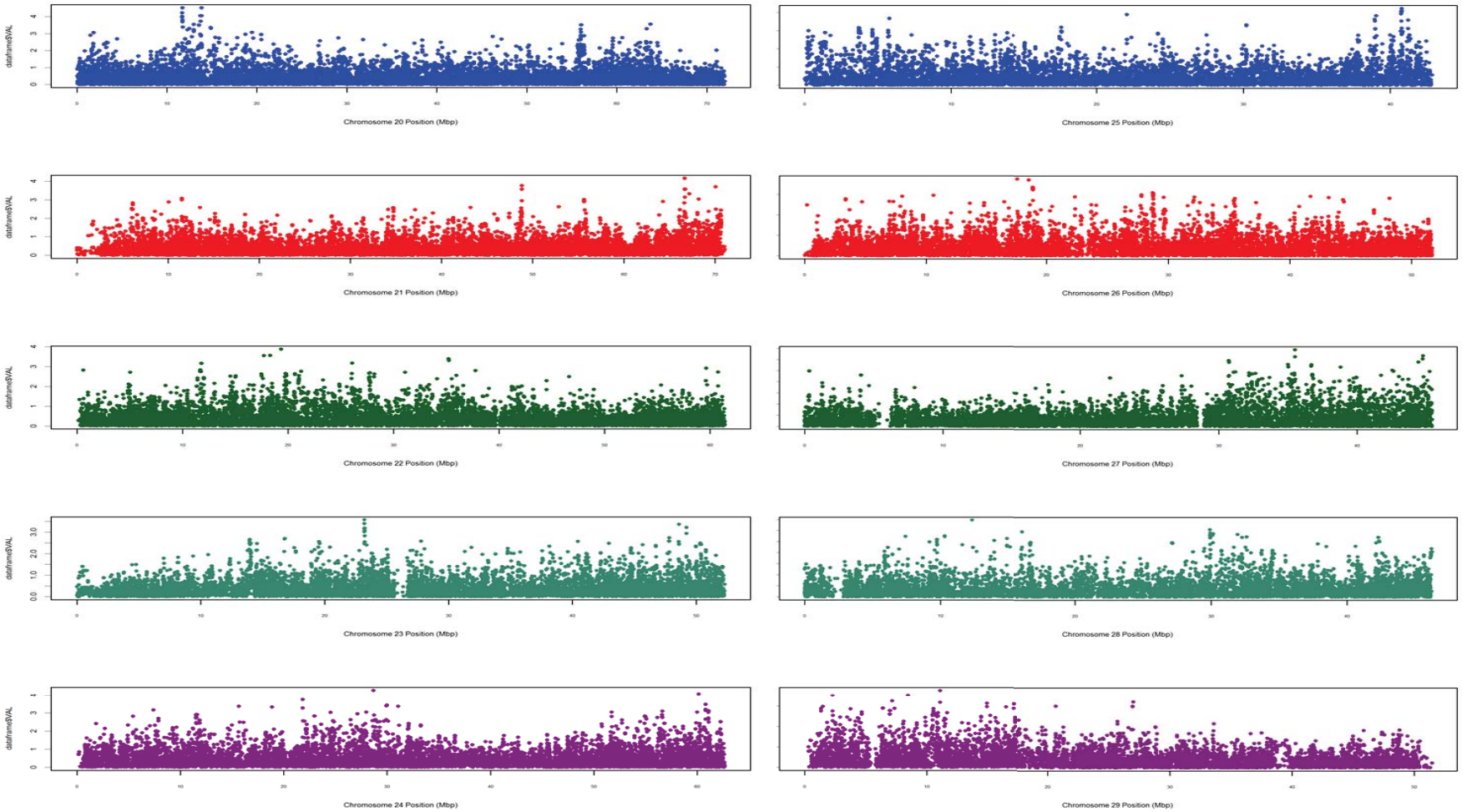
APPENDIX



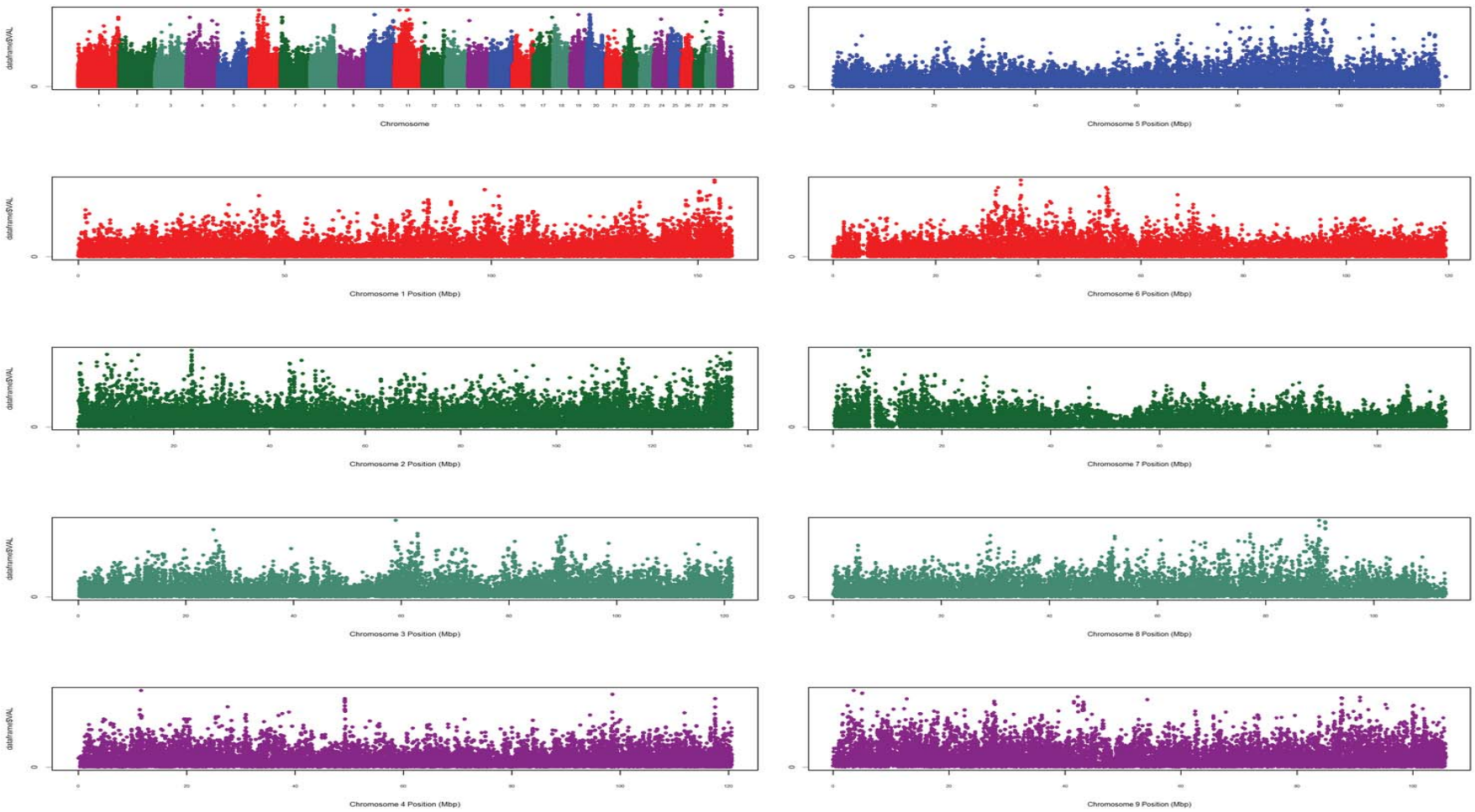
A. Individual chromosome results for adjusted bull temperament EBV (Adj-EBV$_{temp}$) for all breeds (Chromosomes 1 to 9).



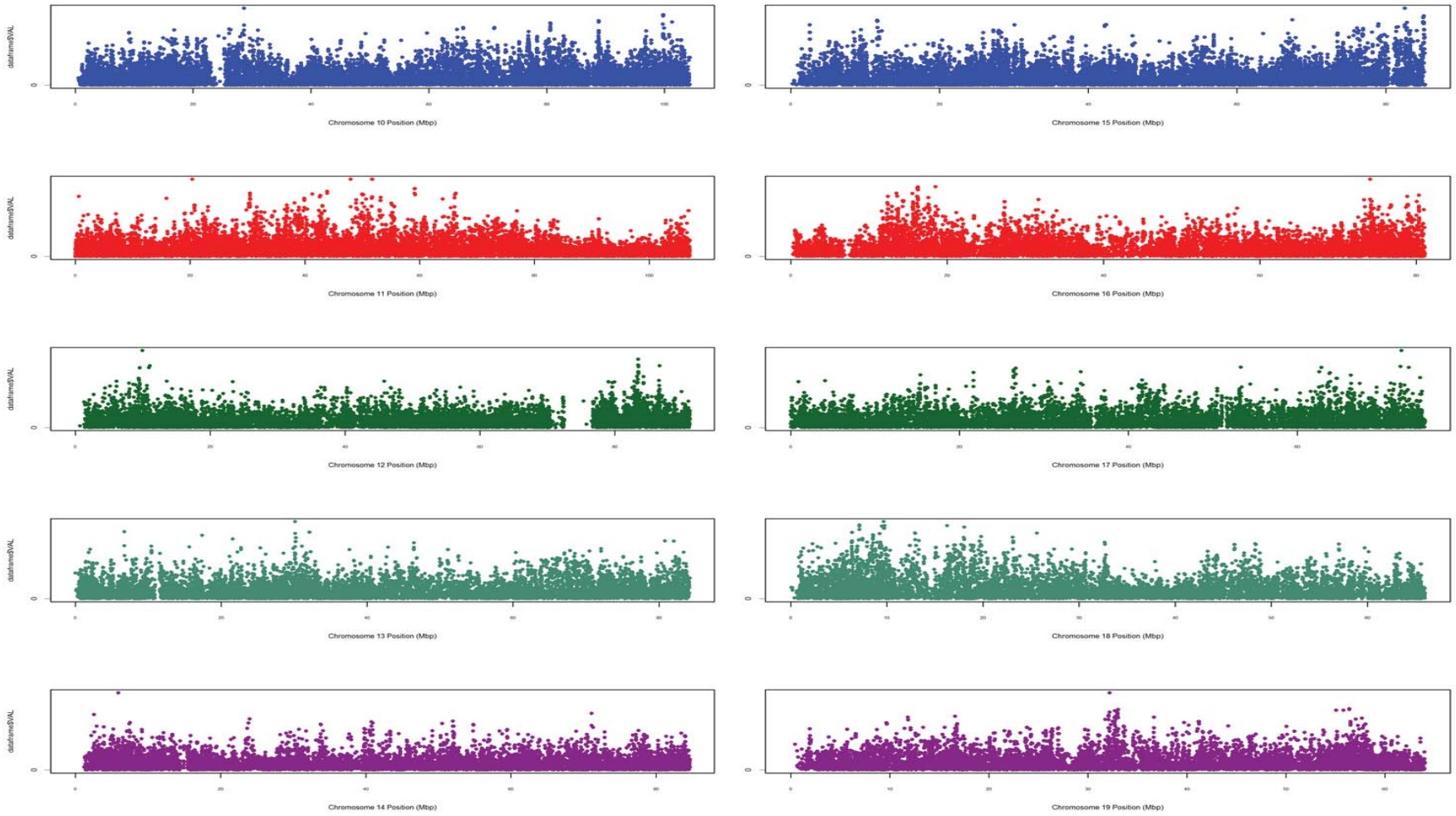
B. Individual chromosome results for adjusted bull temperament EBV (Adj-EBV$_{temp}$) for all breeds (Chromosomes 10 to 19).



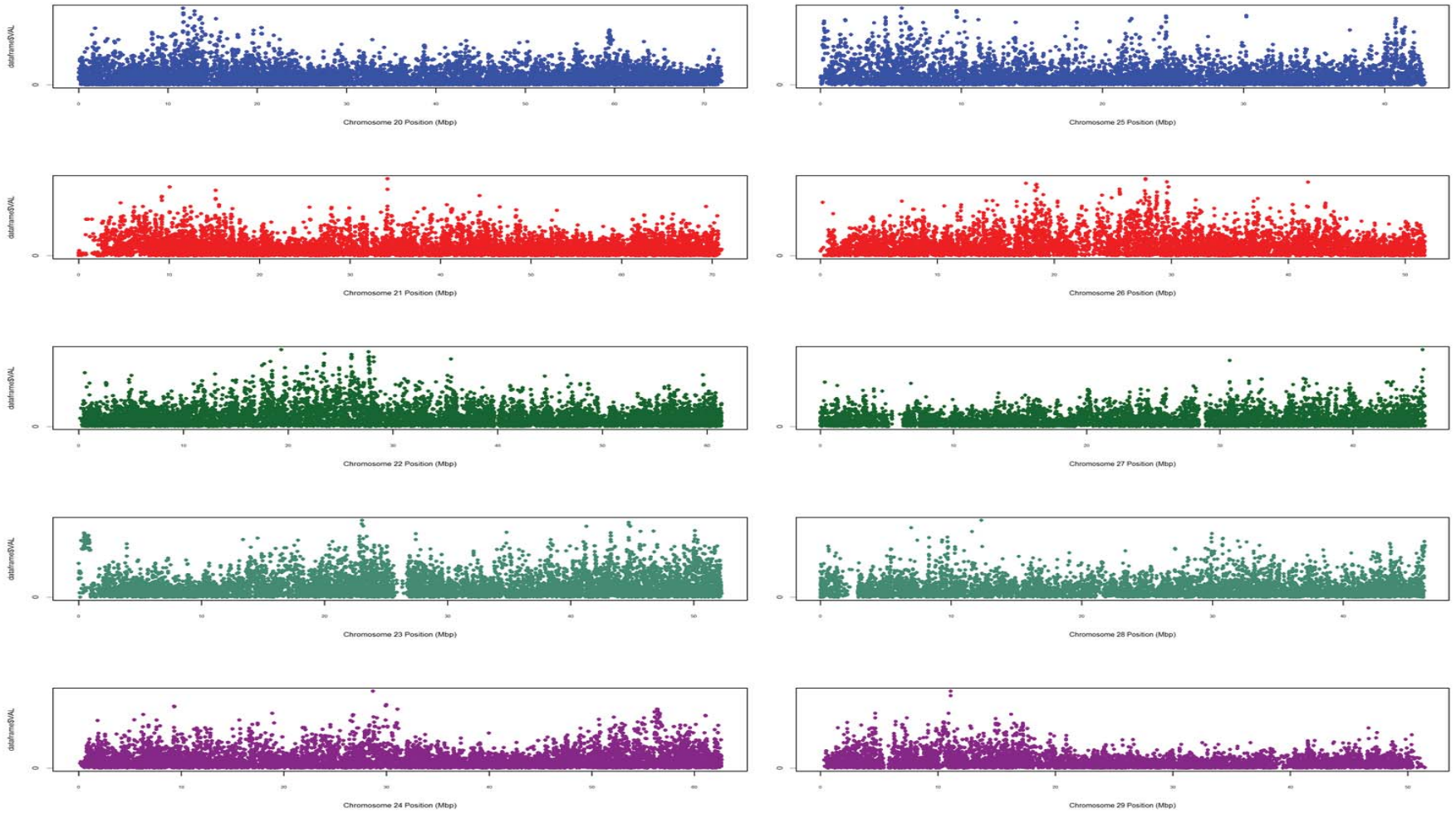
C. Individual chromosome results for adjusted bull temperament EBV (Adj-EBV$_{temp}$) for all breeds (Chromosomes 20 to 29).



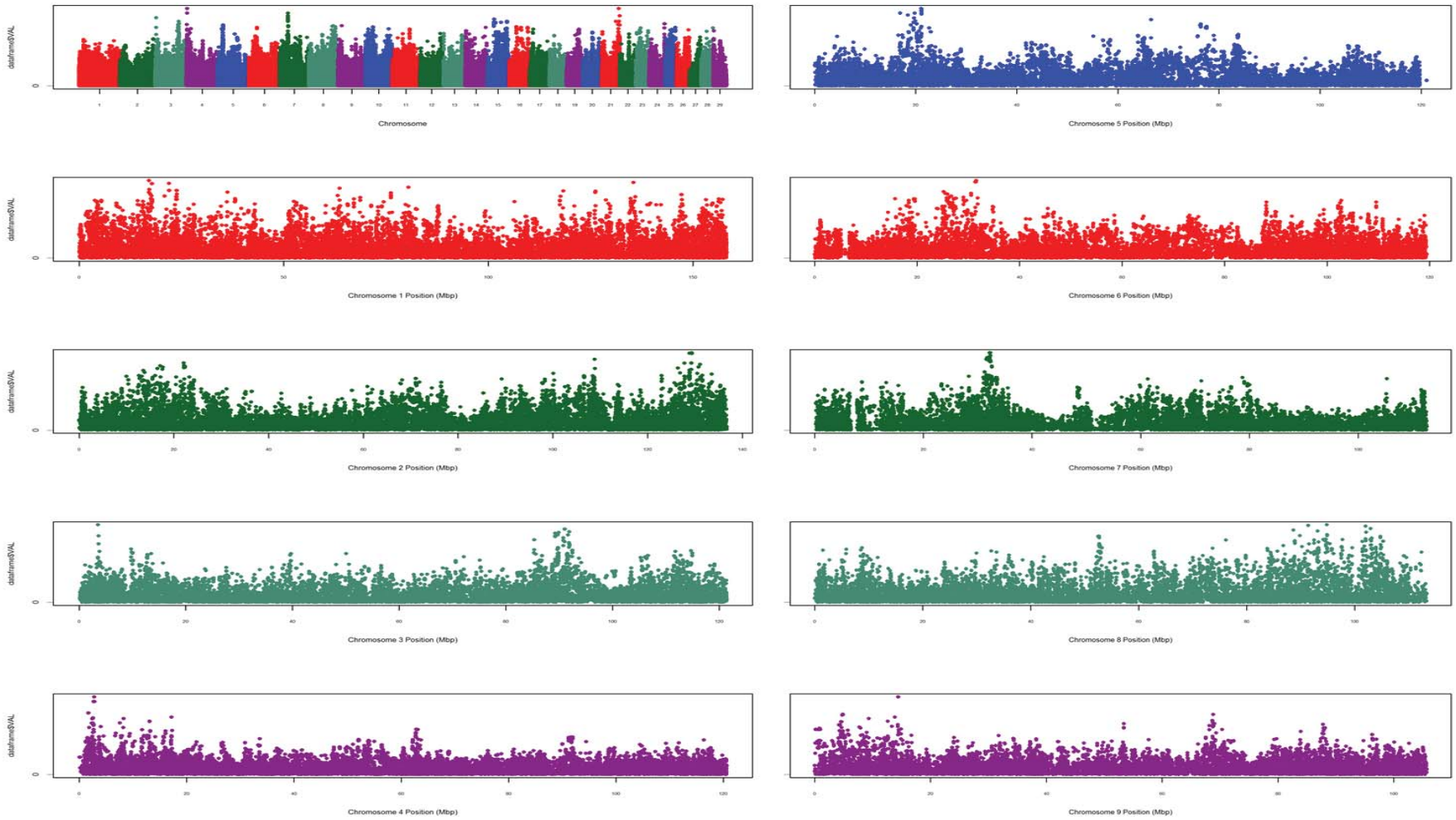
D. Individual chromosome results for adjusted bull temperament EBV (Adj-EBV_{temp}) for HF (Chromosomes 1 to 9).



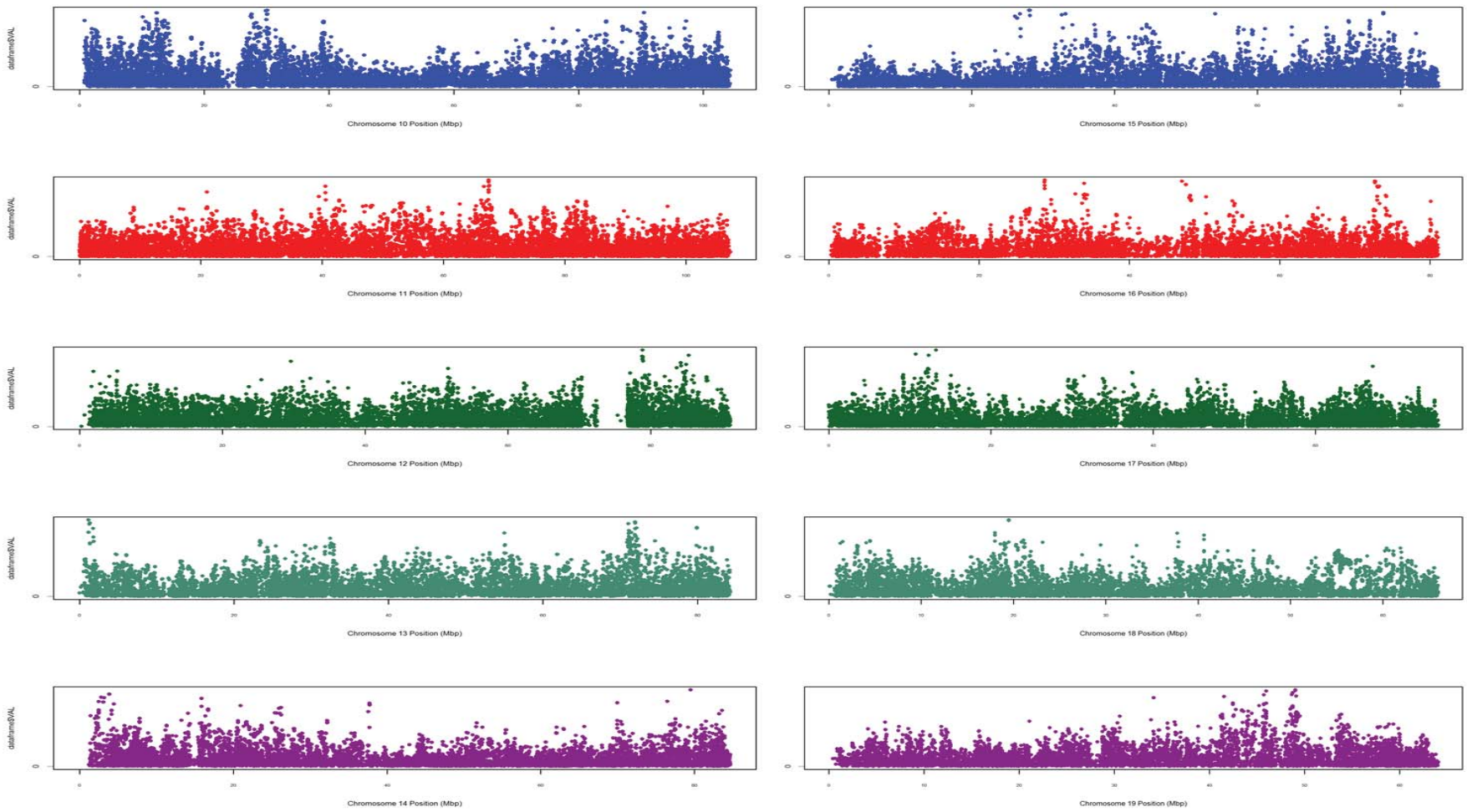
E. Individual chromosome results for adjusted bull temperament EBV (Adj-EBV_{temp}) for HF (Chromosomes 10 to 19).



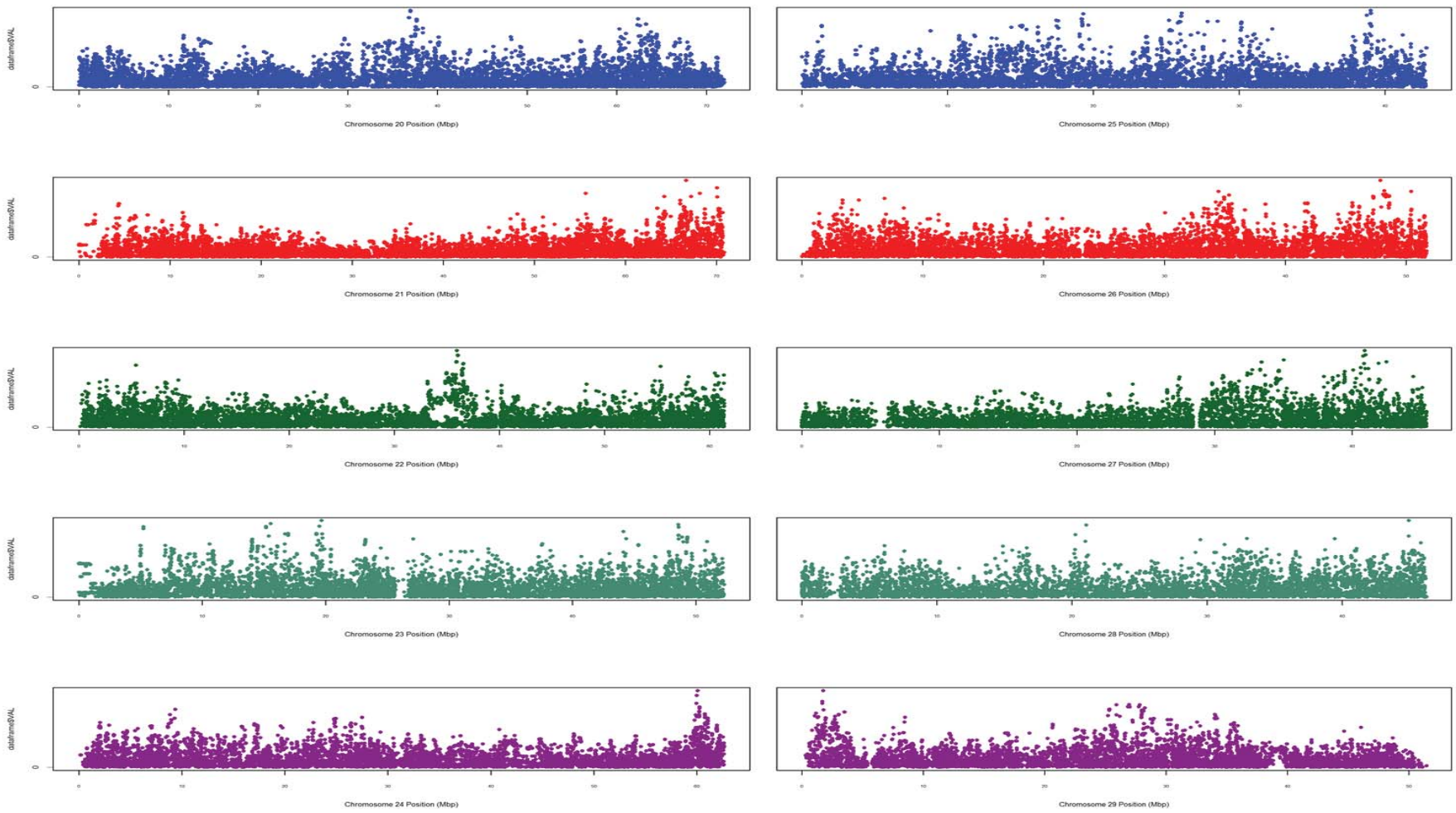
F. Individual chromosome results for adjusted bull temperament EBV (Adj-EBV_{temp}) for HF (Chromosomes 20 to 29).



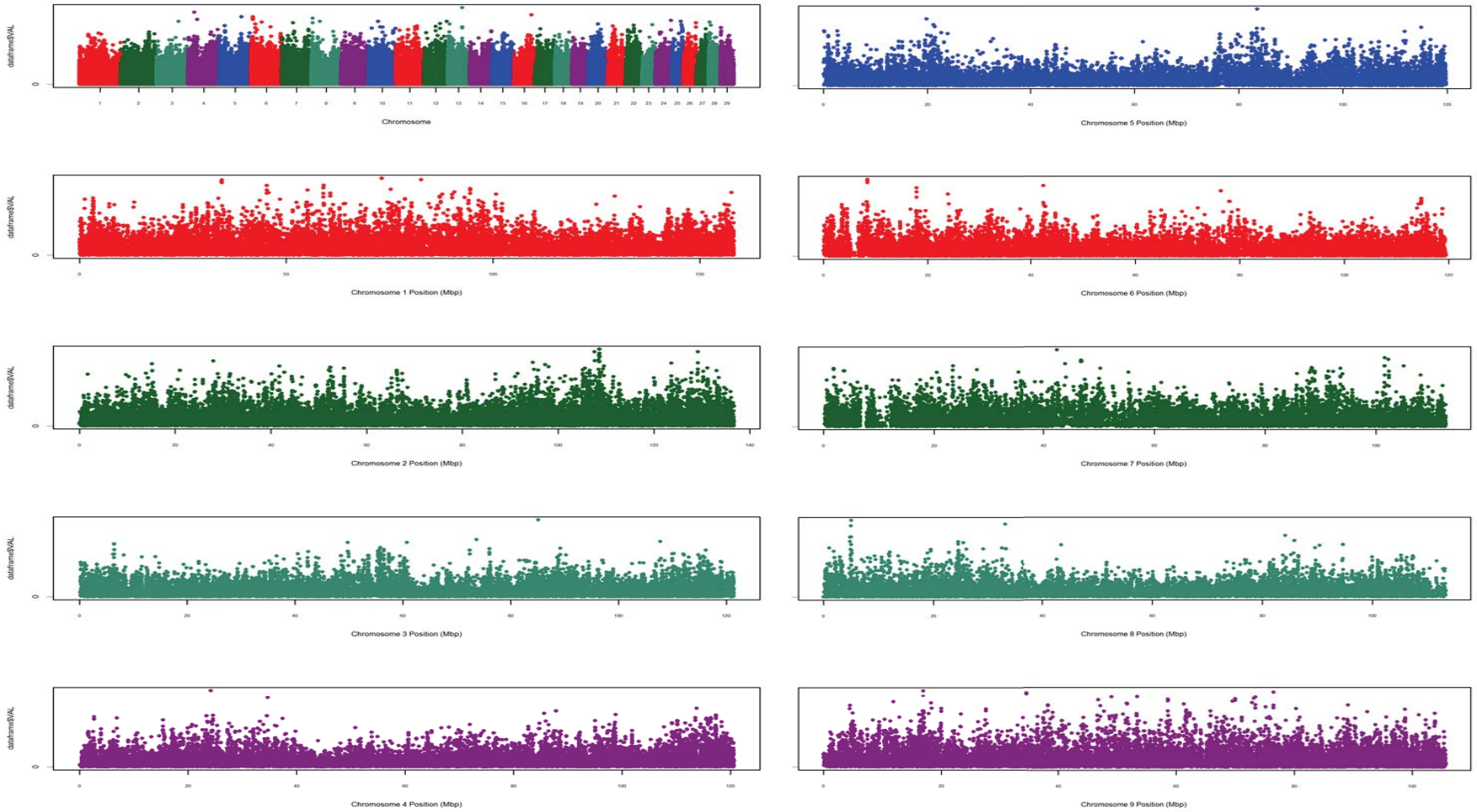
G. Individual chromosome results for adjusted bull temperament EBV (Adj-EBV$_{temp}$) for JE (Chromosomes 1 to 9).



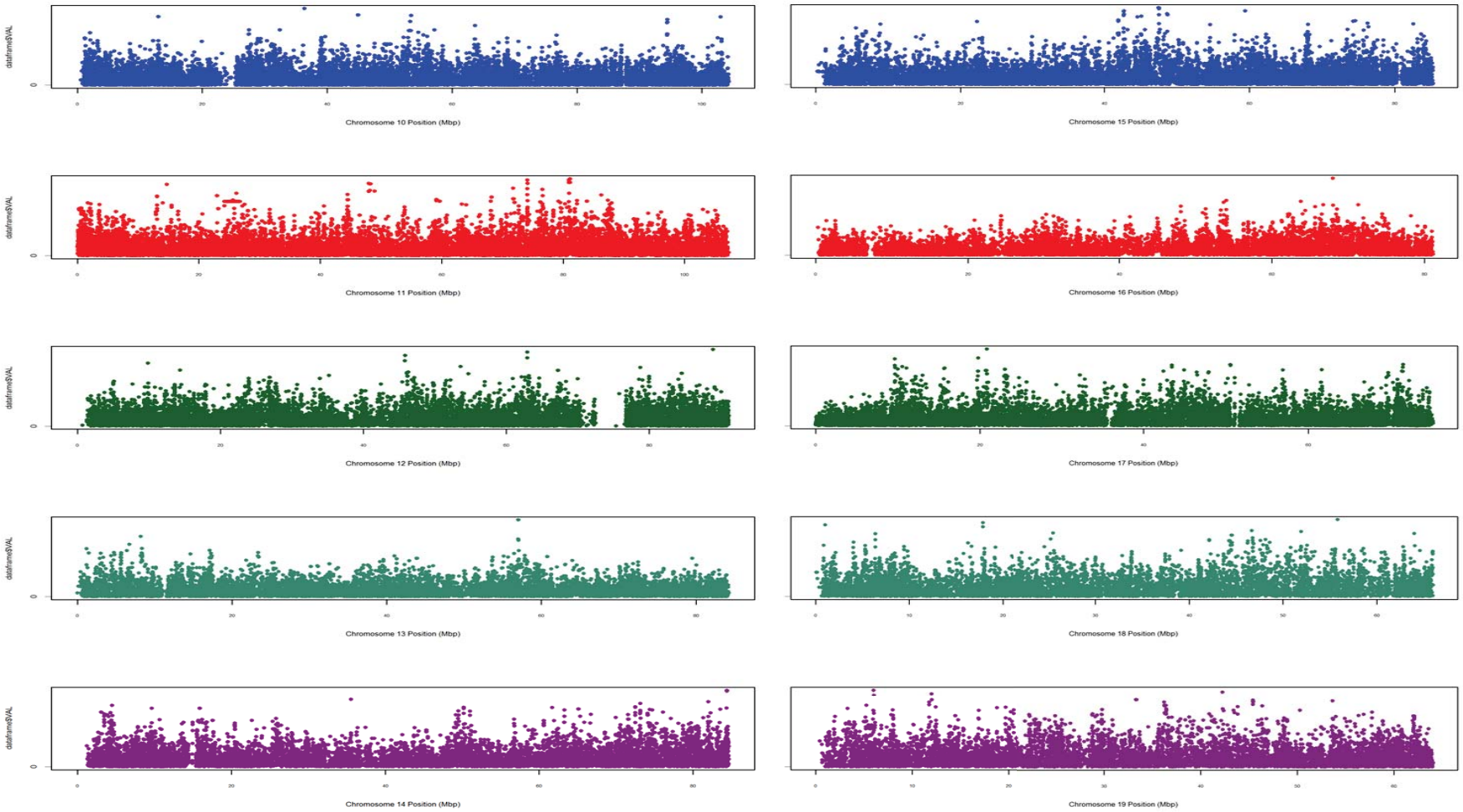
H. Individual chromosome results for adjusted bull temperament EBV (Adj-EBV$_{temp}$) for JE (Chromosomes 10 to 19).



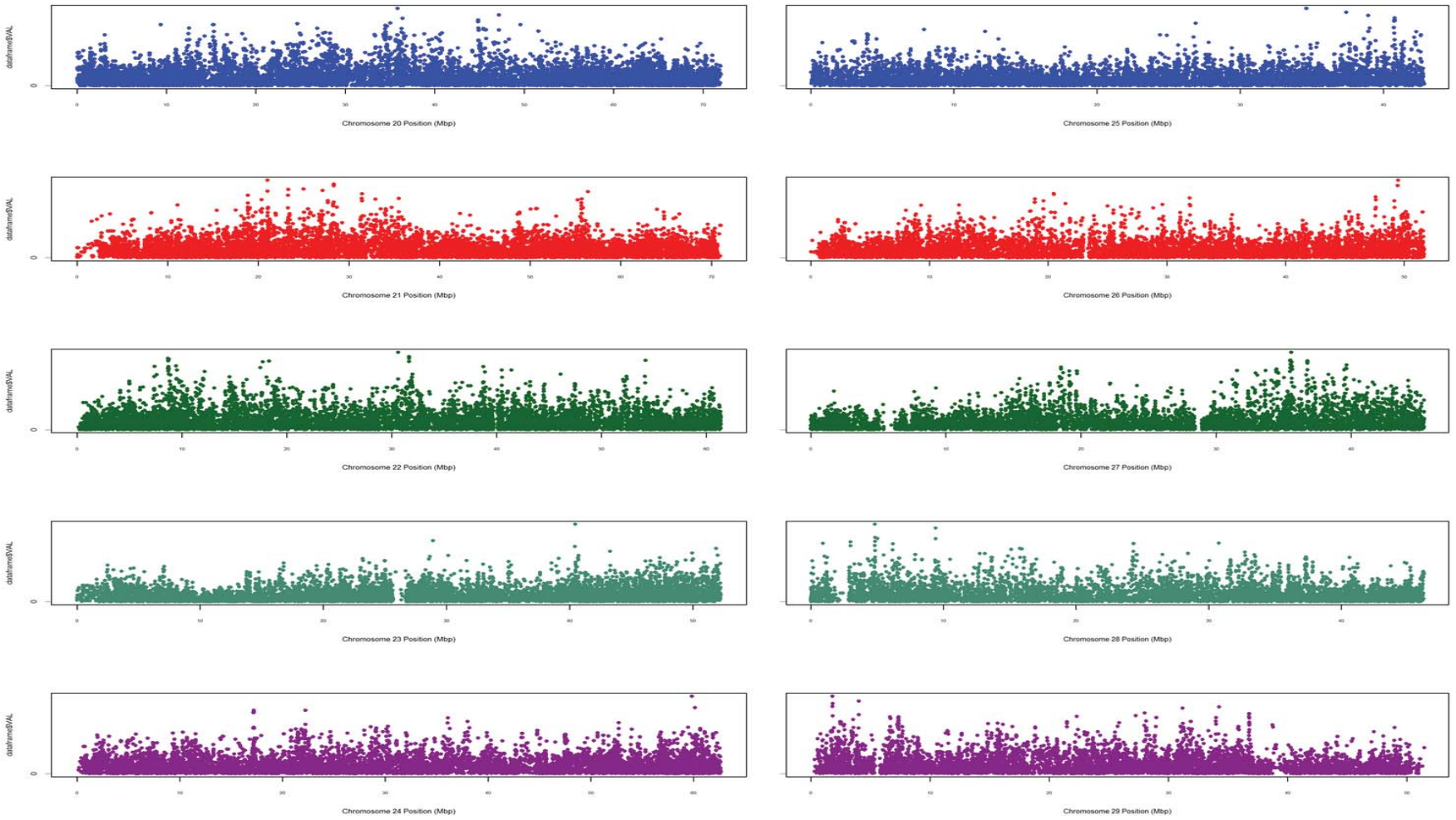
I. Individual chromosome results for adjusted bull temperament EBV (Adj-EBV_{temp}) for JE (Chromosomes 20 to 29).



J. Individual chromosome results for adjusted bull temperament EBV (Adj-EBV_{temp}) for XB (Chromosomes 1 to 9).



K. Individual chromosome results for adjusted bull temperament EBV (Adj-EBV$_{temp}$) for XB (Chromosomes 10 to 19).



L. Individual chromosome results for adjusted bull temperament EBV (Adj-EBV$_{temp}$) for XB (Chromosomes 20 to 29).