THE UNIVERSITY of ADELAIDE

GENOMIC SELECTION FOR HIGH QUALITY BEEF PRODUCTION

A Thesis Presented for the Degree of

Doctor of Philosophy

By

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Abstract

This thesis focuses on the implementation of genomic selection within Wagyu, a breed of cattle that is highly desired due to its propensity to accumulate marbling. Initial focus of the thesis was to investigate using genomics to breed purebred Wagyu, producing crossbreds with improved marbling performance. However, the thesis had to undergo a change in direction due to unforeseen delays in obtaining crossbred genotype and phenotype data. The experimental chapters, therefore, focus on scenarios within the core nucleus breeding herd while the literature review considers the influence of crossbreeding heavily.

Chapter two considered a comparison between pedigree and genomics with relationship matrices built from 10,549 and 4,940 individuals respectively. Animal models for multiple traits found genomics resulted in more accurate breeding values. This was evident through higher breeding value standard deviations and lower mean breeding value standard error. Additionally objective carcass measures were more heritable than subjective measurements (Meat Image Japan (MIJ) vs. AUS-MEAT grading) and highly correlated to their equivalent AUS-MEAT counterparts. This is consistent with findings from the meta-anlaysis in the literature review.

Chapter three investigated how differing SNP densities describe genomic relationships across the Wagyu population herein, utilising masked subsets from a 30K base SNP density and HD SNP data. It was demonstrated that small SNP subsets of 2,500-5,000 were sufficient. Imputation was used to impute these subsets to a ~30K density, producing a genomic relationship matrix (GRM) with highly correlated elements to a GRM built using all 30K SNP data. Imputation to a high density SNP platform (770K) improved the description of relationships further by better describing highly related animals.

Given imputation requires well-formed reference populations, Chapter four compared four published methods to select animals to form a reference population for imputation to whole genome sequence. Methods investigated used relationship matrices or haplotype libraries. The MCG method, which utilises a genomic relationship matrix to select animals highly related to the target population but distantly related to other selected candidates, accounted for the most genetic variance in the population relative to the other methods when 100 animals were selected. This method was then used to select 70 animals to be sent for whole genome sequencing.

Chapter 5 planned to compare genetic parameters estimated from imputed whole genome sequence data to those from the commercial 30K SNP chip. Due to delays in obtaining sequencing data, a back-up set of 770K genotypes that accounted for a similar proportion of genetic variance as the original 70 animals selected by MCG (Chapter 4), was used to build an HD genomic relationship matrix to compare trait heritabilities and animal selection decisions. Animal models were used, as in Chapter 2, finding HD genotype arrays resulted in improved prediction accuracy through increased spread of breeding values and higher heritability estimates across traits.

With Wagyu product worth an exceptional premium and with multiplier effects of genetic gain from the nucleus to daughter herds, marginal gains in accuracy are of high value. This supports that investment in higher density genotyping, including sequencing, and objective marbling assessment.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

I give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

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Chapter 1 : Literature Review

This thesis covers a number of aspects relating to the implementation of genomic selection within a Wagyu beef seedstock program. The primary focus is placed on producing and maintaining high quality beef, particularly marbling. Topics of research and discussion include contrasting measurement of specific traits and their relationship to other economically important traits, comparing cost effective ways to obtain high density genotypes and the benefits these higher density panels have on additive variance estimates and animal selection decisions.

1.1 Introduction to Marbling (Physiology, Evaluation and Major Genes of Interest)

The aim of this literature review is to provide background and context to the experimental results demonstrated in this thesis. Chapter 1 presents the literature review in two distinct sections. The first, introduces Wagyu and marbling with physiology and genes related to this key trait discussed. This section culminates with a meta-analysis that presents weighted and un-weighted heritability estimates for traits, calculated from the published literature. The second section was written to capture the initial planned direction for the thesis, breeding purebreds that produce better crossbred Wagyu. A review of genomic selection is presented in Appendix 1.

The initial focus of the thesis was to explore genomic selection in the context of a purebred Wagyu breeding program and expand into the potential use of crossbreed Wagyu data. That is, using crossbred data to breed better pure-breds for crossbred production. Wagyu are well suited for this purpose having a genetic pre-disposition to display extreme marbling characteristics under the correct management conditions. This characteristic has made them highly desirable, but not easily accessible due to price of product. Using Wagyu sires in

crossbred systems has been rapidly gaining momentum as a way to amplify the amount of marbled product available for consumers, sold at a premium yet still accessible price (i.e. restaurants serve Wagyu at a range of price points).

However, throughout the course of candidature a change in direction had to be made due to unforeseen delays in obtaining crossbred phenotype and genotype data. The focus in experimental chapters was hence shifted towards the core nucleus breeding program. This crossbred scenario is still referenced throughout the thesis as the focus shifted from breeding better crossbreds to a full-blood nucleus scenario and is the subject of future work for the herd analysed herein.

1.1.1 Wagyu background

Australian beef production is commodity based with potential for cuts of saleable meat to vary greatly in eating quality, a factor that consumers desire to be consistent across meat purchases. Marbling, which is defined as the accumulation of triacylglycerol in muscle tissue primarily occurring within adipocytes between muscle fibre bundles (Harper and Pethick 2004), is an integral component within breeding objectives of Australian beef producers. The initial interest in marbling was from producers and exporters targeting high value export markets however it is becoming increasing sought after in the domestic sector due to consumer associations with improved beef quality. Wagyu offer an entry point into these markets having a propensity to produce highly marbled beef (Gotoh *et al.* 2009).

Wagyu is a general term used to describe beef breeds native to Japan and literally translates to 'Japanese cattle'. Wagyu cattle consist of four *Bos taurus* beef breeds known as the Japanese Black, Japanese Brown, Japanese Shorthorn and Japanese Polled. These breeds were developed by crossing native Japanese cattle with foreign breeds, e.g. Simmental, Ayrshire, Brown Swiss, Devon and Hanwoo, approximately 90 years ago to generate cattle suitable for

draft work. However this crossbreeding produced large bodied, slow moving draft animals with poor meat quality resulting in the discontinuation of the practice and the initiation of interbreeding for improved meat quality to get the modern Japanese breeds today (Gotoh *et al.* 2014). The Japanese Black is the predominant breed accounting for 97% of Wagyu in Japan (Hirooka 2014; 1,663,000 head; Motoyama *et al.* 2016). The other three breeds are considered to be minor regional breeds (Hirooka 2014).

Wagyu are typically bred in Japan by the crossing of three major bloodlines, identified and developed due to geographical isolation in Japan. These are the Tajima bloodline (Hyogo Prefecture), Kedaka bloodline (Tottori Prefecture) and the Itozakura bloodline (Shimane Prefecture) (Motoyama *et al.* 2016). Japanese Black cattle all have pedigrees consisting of these three bloodlines to some degree and the specific crossing of these bloodlines is followed by Wagyu breeders outside of Japan today (Dr Joe Grose; Seedstock Wagyu Breeder, *Personal communication*)

The Japanese black is what is commonly referred to as 'Wagyu' in herds outside Japan, with grey skin, black muzzles and hooves, tame demeanour and a brownish-black coat. The breed is known for its marbling capabilities and high intramuscular fat (IMF) content (Motoyama *et al.* 2016) which improves texture, juiciness and therefore overall palatability of beef cuts (Iida *et al.* 2015).

The Australian beef industry is comprised of 27.4 million head of cattle as of 2014-15, producing 2.34 million tonnes (carcass weight) of beef and veal valued at $14.3 billion in 2015-2016 (Meat and Livestock Australia 2016). Wagyu have been bred in Australia since their importation from Japan via the United States of America during the 1990s (Maeda *et al.* 2014). Currently it is estimated that Wagyu, and Wagyu infused stock, account for 1-2% of the national beef herd with 80-90% of its production being exported (Australian Wagyu

Association 2015). In 2013, Wagyu globally accounted for about 2%, 2%, 4% and 8% of beef carcass production in the US, Brazil, China and Argentina respectively (Motoyama *et al.* 2016)

1.1.2 Physiology of Marbling

Strong evidence within the literature suggests that several forms of stem cells, identified as pluripotent stem cells, exist within the skeletal muscle of mammals acting as a replenishable pool with cells from other body parts. These pluripotent stem cells are thought to lie dormant within muscle tissue until an external stimulus induces them to differentiate towards a specific lineage of cells. Differentiation towards the adipocyte lineage results in the accumulation of adipocytes within muscle fibre bundles resulting in the visible white flecks of fat known as marbling (Harper and Pethick 2004).

Marbling is an important factor in determining meat quality characteristics especially meat texture (tenderness), juiciness and flavour (Thompson 2004). The shear force value, a measurement of toughness (opposite of tenderness), in the Longissimus muscle has been shown in Wagyu to decrease after approximately 20 months of age, concurrent with the increased attainment of intramuscular fat demonstrating marbling's role in tenderisation (Nishimura *et al.* 1999). The structure, composition and amount of intramuscular connective tissue within skeletal muscle contribute to meat texture. As the animal grows, collagen crosslinks become more stable which increases the structural integrity of intramuscular connective tissue, contributing to the toughening of meat. Intramuscular fat deposits between muscle fibre bundles cause remodelling of these connective tissues by disrupting their structure integrity thereby reducing the mechanical strength and contributing to beef tenderization (Nishimura 2010). There are a quite a few known characteristics, both animal and environmental, that influence the expression of marbling such as age and time on feed, muscle chronology, gender, nutrition and gene effects (Harper and Pethick 2004).

The conclusion that intramuscular fat is late developing is a common assumption from animal developmental studies (Vernon 1980) and the order of development for fat depots is usually reported as abdominal, intermuscular (between muscles), subcutaneous and then intramuscular (within muscles). However fat is deposited within in the body at a greater rate than lean tissues and so the concentration of intramuscular fat will ultimately increase as the animal ages. Therefore the visible intramuscular fat, i.e. the expression of the commercial marbling trait, and actual IMF% (percentage intramuscular fat) are late maturing however the deposition of fat within intramuscular adipocytes is not late maturing (Cianzio *et al.* 1982; Pethick *et al.* 2004). The rate of intramuscular fat accretion between hot carcass weights of 200kg and 400kg is approximately 0.47% per 10kg of HCW for British breeds (Duckett *et al.* 1993) or 0.56% units for F1 Wagyu (Aoki *et al.* 2001). A study involving Wagyu x Holsteins found that IMF content did not increase above carcass weights of around 420kg, implying that IMF has a 'maximum value', most likely due to declining feed intake as the animals approach their mature weight (Aoki *et al.* 2001). However the sooner the animal reaches its maximal potential for muscle and fat growth i.e. approaches "muscle maturity", the sooner marbling will be expressed at commercial levels. This means that faster growth throughout the animal's life will result in the expression of marbling at an earlier age. In addition, longer feeding regimes allow the cattle to acquire higher intramuscular fat levels since there is time for the animal to reach muscle maturity with additional time following for the muscle to accrue fat. Shorter feeding regimes pose a greater risk of failing to meet market marbling requirements, particularly if animals in these regimes are slower to reach muscling maturity (Pethick *et al.* 2004).

Marbling is of most interest in muscles that have a relatively high commercial value, such as the striploin and tenderloin, however it is also expressed within other skeletal muscles such as the gluteal group etc., but to different extents (Brackebusch *et al.* 1991). Gender also plays

a role in determining marbling with heifers expressing higher marbling than steers at a given slaughter weight and time on feed (Slanger *et al.* 1985; Jones *et al.* 1990).

Nutrition and diet composition influences the expression of marbling. Diets with an increased energy density, due to a high percentage of concentrates in the ration, drive greater rates of fat synthesis and therefore marbling (Prior *et al.* 1977; Pethick *et al.* 2004). Diets that contain metabolic modifiers such as hormonal growth promotions may decrease the rate of intramuscular fat deposition due to its promoting effect on muscle growth. Vitamin A levels in the diet and corresponding serum Vitamin A levels in the animal have also been associated with marbling ability (Naruse *et al.* 1994; see below)

1.1.3 Why are "Wagyu" able to produce highly marbled beef?

The specific details about why Wagyu have a high marbling phenotype, above and beyond the marbling displayed by other cattle breeds, is not well known. It has been demonstrated in rats that adipocytes store retinoid (such as retinol, retinal and retinoic acid which collectively are known as Vitamin A) as well as synthesize and secrete retinoid-binding protein (Tsutsumi *et al.* 1992) necessary for the movement of retinol across membranes. It is hypothesised that Japanese cattle may have a genetic predisposition to increase intramuscular fat content for storage of vitamin A during periods when dietary levels are low. This hypothesis is plausible given the Japanese climate which is characterised as temperate and humid with very marked changes of the four seasons resulting in cold winters. Cold winters are especially prevalent in the remote hilly and mountainous areas where the ancestors of the Japanese Black were raised for draft use. These native ancestral cattle would've usually been kept in barns over the winter in these areas due to a lack of green forage (high in beta carotene, the precursor for vitamin A) caused by heavy snow cover. This poor environment may have driven Japanese native cattle to develop mechanisms against vitamin A shortages, such as increasing adipocyte

deposition within muscle, resulting in an enhanced genetic potential to marble driven by long term natural and artificial selection (Hirooka 2014).

Torii *et al.* (1996) reported total adipogenic activity increased with improved marbling score and that adipogenic activity was negatively correlated with serum retinol concentration in Wagyu and Wagyu x Holstein carcasses. This implied that retinol level in blood during the finishing period influences the deposition of intramuscular fat. Similarly, Gorocica-Buenfil *et al.* (2007) reported marbling scores of Angus cross steers increasing by approximately one-third of a grade when steers were fed vitamin A restricted diets. Kato *et al.* (2011) first reported the negative genetic correlation between serum vitamin A concentration and marbling score occurring during the later stages of finishing. In fact, vitamin A restriction in the finishing diets of Wagyu is widely practiced in feedlot operations within (Hirooka 2014) and outside of Japan to achieve higher marbling carcasses. One explanation for the inverse correlation between marbling and serum vitamin A concentration is that retinoic acid, a metabolite of vitamin A, may regulate adipogenic differentiation and thereby inhibit terminal differentiation of intramuscular adipocytes (Oka *et al.* 1998).

This relationship between vitamin A status and marbling has been described in other breeds (Kruk *et al.* 2004) indicating that this is a general relationship among cattle. In fact some studies into vitamin A status and fat deposition in other breeds were motivated by seeing Japanese and Korean production systems (Siebert *et al.* 2006; Kruk *et al.* 2008). This suggests that maybe low vitamin A resulting in higher marbling is a result of the production system rather than serving a genetic basis for marbling propensity in Wagyu.

1.1.4 Marbling and Subcutaneous Fat deposition in Wagyu

Gotoh *et al.* (2009) investigated the IMF content in the longissimus muscle of carcasses from 24 month old cattle, all raised under standard conditions, and found that the Japanese Black

animals had an IMF% of 23.3% which was substantially higher than 4.4%, 4.7% and 0.6% and reported for Angus, Holstein Friesians and Belgian Blue respectively. This agrees with previous knowledge about Wagyu's known predisposition to marble and IMF% has been reported in Wagyu to sometimes exceed 50% in the longissimus muscle (Motoyama *et al.* 2016). However, Wagyu seemingly also have the benefit of increased marbling without a steep accompanying increase in external or subcutaneous fat (Pitchford *et al.* 2002). Gotoh *et al* (2009) also reported that for every 1% increase in IMF, an increase of 3.0, 4.3, 7.9 and 10.7 kg of subcutaneous fat was observed in Japanese Black, Holstein Friesian, German Angus and Belgian Blue respectively. Oyama (2011) supports these results, reporting a low and even negative genetic correlation (-0.06) between marbling and subcutaneous fat thickness in Japanese black cattle as does McEwin (2016; -0.05) whereas a moderately positive correlation (0.44) has been reported for British/European breeds (Gregory *et al.* 1995). The breeds do not differ in their mechanisms of postnatal fat accretion however, but rather in their efficiency of accretion of IMF (Gotoh *et al.* 2009).

1.1.5 Evaluation of Marbling and Marbling Fineness

Marbling fineness is described as the distribution and size of fat flecks within the meat, often measured within the rib-eye at the 12-13th rib cross-section in Australian evaluation systems (AUS-MEAT Limited 2005) or at the 6-7th rib cross-section in Japanese evaluation systems (Japan Meat Grading Association 2000). A fine marbling particle has been described as a particle ranging in size/area from 0.01 to 0.5 cm^2 (Maeda *et al.* 2014).

Australian meat quality evaluation schemes include the Australian Meat Industry classification system as well the Meat Standard Australia (MSA) which requires members of the supply chain to obtain MSA certification as part of the programs aim to guarantee excellent eating quality beef (AUS-MEAT Limited 2005). The AUS-MEAT marbling score evaluates the degree of

marbling on a scale of 0-9 and is used on all breeds in Australia. Due to the limited range in the marbling scale, AUS-MEAT may lack the range of values necessary to accurately evaluate the highly marbled Wagyu carcasses produced in Australia. In addition, the AUS-MEAT scale does not take into account distribution or fineness of marbling in the ribeye. The MSA marbling scale differs in that it provides an indication of marbling distribution as well as fat fleck size or fineness. The scale ranges from scores of 100 to 1100 in increments of ten, providing a finer description of marbling in the ribeye however still may not be adequate to describe highly marbled Wagyu carcasses. Both evaluation systems require an official grader to subjectively score (AUS-MEAT Limited 2005).

Wagyu carcasses are assessed in Japan on the basis of the beef marbling standard (BMS) which indicates the amount of marbling in the rib-eye on a scale of 1-12 (Japan Meat Grading Association 2000). Cameron *et al.* (1994) reported the IMF% within each BMS finding a mean IMF% ranging between 3-7.4% for a BMS of 1 and 29.9-38.1% for a BMS of 12. More recently the content of crude fat within the rib-eye at a given BMS has been increasing. Meat with a BMS of 12 contained 29% intramuscular fat in 1988, however that same fat content extracted from carcasses in 2004 was graded into a BMS of 5 (Horii *et al.* 2009). This variation in consistency between BMS and IMF% is important considering the BMS is used as a measure of determining eating quality and that an increase in IMF% has a corresponding increase in the sensory qualities of the meat such as tenderness and juiciness (Okumura *et al.* 2007; Iida *et al.* 2015).

To address the issue regarding the subjective measurement of the evaluation systems, many objective systems were developed but only one has been commercialised in Japan. A computerised image analysis system has been developed as a new method for objectively assessing fat within the rib-eye of beef carcasses (Kuchida *et al.* 1997a, 1997b). Marbling traits

that can be assessed with the image analysis system include percentage marbling area, marbling coarseness, marbling fineness as well as average luminance of exposed lean within the rib-eye (Nakahashi *et al.* 2008; Osawa *et al.* 2008).

Highly marbled meat attained through numerous small marbling flecks is highly desired by Japanese consumers (Motoyama *et al.* 2016). The view that more finely marbled meat is more desirable is agreed upon by packers and consumers in western societies, such as the US (Vierck *et al.* 2017). Marbling coarseness is higher in crossbred Wagyu (Wagyu x Holstein) compared to full-blood animals and is higher in carcasses from heifers than those from steers (Kuchida *et al.* 2002). There is a lack of understanding around the physiology of marbling fineness and its effect on eating quality. One study reported that coarser marbled beef was juicer and more flavourful than medium textured or fine marbled beef (Vierck *et al.* 2017) which is in disagreement with current consumer views. This study used striploins assessed under USDA grading and took samples that had been categorized into quality grades “choice” and “select”. These particular quality grades serve those consumers who were looking for a more economical beef product and would not include beef with marbling equivalent to that of a top Wagyu carcass. Kato *et al.* (2017) found that finer marbled beef was more palatable in consumer taste trials of highly marbled Waygu (BMS 6 or 7) It is possible that as marbling reaches higher levels that fineness plays a role in determining sensory characteristics.

Improving fineness is not as simple as increasing the number of small marbling fleck sizes. Bottema *et al.* (2020) demonstrated that marbling is actually a single connected entity in beef striploins using 3D image analysis. That is, marbling represents a single structure rather than being isolated flecks of fat, appearing to be deposited along an existing internal network (such as the vascular system). This finding suggests that it is the shape of this internal network that results in the 2D trait “marbling fineness”. As marbling increased in the samples, the diameter

of the interconnected structure increased yet the shape of the structure itself does not change.

1.1.6 Major and Minor Genes influencing marbling

A major gene known to affect carcass fatness within beef is GDF8, more commonly known as myostatin, which is responsible for the double muscling phenotype in cattle (Harper and Pethick 2004). The GDF8 gene is a growth regulator for muscle development and mutations that affect its function generally result in increased muscle mass (McPherron *et al.* 1997). Additionally it has been demonstrated that double muscled animals have fewer deposits, or 'islands', of adipocytes in their *longissimus dorsi* muscle that also exhibit slower growth patterns and are smaller in size compared to wild-type cattle of the same age and finishing period (Wegner *et al.* 1998).

Significant associations exist between DNA markers CSSM34 and ETH10 on chromosome 5 and marbling score. CSSM34 is associated with RARG (retinoic acid receptor gamma), a known factor in adipocyte growth and differentiation, and ETH10 is associated with RDH5 (retinol dehydrogenase 5), a catalyst for the interconversion of retinol and retinoic acid (Barendse 2002). This does make sense considering an animal's serum vitamin A level has been directly linked its marbling performance. Additionally thyroid and steroid hormones, e.g. thyroxine, retinol and estrogen, bind to nuclear receptors, such as RARG (Barendse 2002). Retinoic acid receptor gamma, in turn, then binds to specific sequences of DNA in the nucleus, increasing the rate of transcription from the gene to which it bound. These receptors are important elements in the growth and differentiation of tissues (Solomin *et al.* 1998).

The TG5 (thyroglobulin 5' leader sequence) single nucleotide polymorphism (SNP) has also been associated with marbling variation in cattle. The TG gene encodes a protein that plays an indirect role in the regulation of metabolic rate. These TG5 SNP is located within the 5'

untranslated region of the TG gene, rather than within the coding region, which could indicate that it is involved in the regulation of the gene's activity. None of the genes immediately surrounding TG5 are obvious candidates for marbling with the next closest gene likely to have an effect on fat being DGAT1 (Diacylglycerol O-Acyltransferase; see below), approximately 15Mb away on the human map (Barendse *et al.* 2004).

Michal *et al.* (2006) reported that fatty acid binding protein (FABP4) could be associated with marbling. FABP4 is expressed in adipose tissue and plays an important role in lipid metabolism and homeostasis. Their data indicated that FABP4 falls into a QTL interval for marbling reported in three different populations on bovine chromosome 14. In Hanwoo cattle, genetic variants of FABP4 have also shown associations with marbling (Lee *et al.* 2010; Shin *et al.* 2012). However a more recent study found no association between FABP4 and marbling, instead suggesting that Fatty acid desaturase 2 (FADS2) would be a useful genetic marker for improving marbling in beef cattle (Matsumoto *et al.* 2014). Hudson *et al.* (2015) perhaps proposed an explanation for this demonstrating that fat metabolism genes such as FABP4, but also THRSP, CIDEC and ACACA, diverge in expression quite late in postnatal development, with divergent expression between high marbling and low marbling animals appearing at approximately 20 months of age. Michal *et al.* (2006) did not disclose age of animals included in their study, but it is possible that if their animals were older than 20 months than that would explain why they were able to find an association between marbling and FABP4. Matsumoto *et al.* (2014) used animals ranging from 20-29 months of age and found no association.

There are multiple other genes that have been reported be associated with marbling as well; WNT1-inducible-signaling pathway protein 2 (WISP-2 also named CCn5), GADD45A, PIAS3, CCRN4l, DIRAS3, POU5F1, HOXA9, ATP2A2, PIM1, AKIRIN2, EDG1, RPL27A and MYBPC1 (Sadkowski *et al.* 2014; Sukegawa *et al.* 2014; Hudson *et al.* 2015; Tong *et al.* 2015). Inter-

allelic interactions between some of these genes have been reported suggesting that for effective marker-assisted selection to improve marbling, these interactions need consideration (Sukegawa *et al.* 2014).

Clearly marbling is highly polygenic indicating that no single gene or small group of genes is causative. Additionally it has been shown that certain genes associated with marbling are differentially expressed at different time points in postnatal development. The Myostatin gene described earlier is a major gene influencing the degree of marbling in other breeds, but perhaps is not particularly relevant in the Wagyu breed which is mostly homozygous for the wildtype. This does make marker-assisted selection for improved marbling difficult but not impossible. A suite of SNPs may need to be included in breeding programs to account for large proportions of marbling variance in addition to selecting the most appropriate SNPs, for example those that can better predict marbling performance at a young age.

1.2 Genetic parameters of traits

Genetic variability consists of differences found between species, between breeds, differences due to crossing of breeds and differences between lines or individual animals within breeds. It is this variation between animals within a breed that is used to estimate the heritability of and genetic correlations between traits (De Smet *et al.* 2004).

To gain insight into the range of genetic parameters for traits in Wagyu a meta-analysis of published reports was conducted. Heritabilities can be reported as both un-weighted and weighted heritabilities when they are being compared by authors. Unweighted heritabilities were calculated as follows:

(1) Unweighted $h^2 = \frac{\sum_{i=1}^{N} h_i^2}{N}$

Where N is the number of estimates reported for the trait and h_i^2 describes the *i*-th heritability for the trait.

Weighted heritabilities were estimated such that, each individual heritability was weighted by its accompanying standard error, as detailed by Koots *et al.* (1994). The weighted heritability estimate was calculated as follows:

(2) Weighted h^2 = $$\frac{\sum_{i=1}^{N} \frac{h_i^2}{(SE_i)^2}}{\sum_{i=1}^{N} \frac{1}{(SE_i)^2}}$$

Herein, all reported standard errors were treated equally, regardless of the methodology used in their calculation. Where authors reported standard errors as a range, the largest standard errors were assigned to each heritability estimate. Where authors reported no standard errors, heritability estimates were excluded from the calculation of weighted means meaning the number of reports included in the weighted average was always equal to or less then the unweighted average, though missing standard errors were uncommon.

For genetic correlations, weighted averages were not calculated due to the limited availability of published estimates between all traits. Unweighted averages, where presented, were calculated using a simple average.

The traits of interest in the meta-analysis included carcass traits and MIJ camera traits. The strategy of searching for published studies aimed to locate all studies in English. However, papers in other languages were included if their abstracts/tables were in English and contained enough detail to be sure the paper met the inclusion criteria. Articles on these topics were identified using Google Scholar, Pub Med and Web of Science. Key concepts used for searching were “Wagyu” and “genetic parameters”. Hand searching of reference lists was performed to identify any other relevant studies for inclusion.

Studies were only included if they reported heritabilities on any of the traits of interest in Full-Blood Wagyu estimated from pedigree data. For example, reports on Angus or crossbred Wagyu would have been ineligible for inclusion.

The final list of traits to include was determined by looking at the common traits reported across all papers, after they were grouped into "themes" i.e. carcass traits, MIJ camera traits or fatty acid traits. It was possible for papers to fit more than one theme depending on the scope of their investigation.

1.2.1 Carcass Traits

Carcass traits in Wagyu have been reported to range from being lowly to highly heritable depending on the trait. Oyama (2011) reported heritabilities of carcass traits, for specifically the Japanese Black, graded under Japan meat grading association (JMGA) guidelines, sourced from 18 published studies. Oyama (2011) found unweighted and weighted heritabilities for carcass weight, rib-eye area, subcutaneous fat depth and marbling score to be 0.48, 0.46, 0.39 and 0.55 and 0.46, 0.49, 0.32 and 0.21 respectively (Table 1.1).

The heritability of similar carcass traits, estimated under the AUS-MEAT grading system, has been published for Japanese Wagyu in Australia yielding similar results to Japanese publications (Table 1.1). These studies were designed so that they could be compared to Japanese studies by grading at the 5-6th rib-eye cross-section (Maeda *et al.* 2014; Zhang *et al.* 2015). In other breeds, using a different grading system, heritabilities for carcass weight, rib-eye area, subcutaneous fat thickness and marbling score have been reported as moderately to highly heritable (0.23, 0.22, 0.25 and 0.48 respectively) consistent with the range of heritabilities for specifically Wagyu described in Table 1.1 (Gregory *et al.* 1995).

Calculated unweighted heritabilities are similar to weighted averages with the exception of marbling score graded under the JMGA method. The weighted mean is substantially lower

than the unweighted mean due to the inclusion of a low heritability estimate with a low standard error (Oyama 2011).

Table 1.1: Range of Direct heritabilities (h2) and number of reports referenced (N) for carcass traits of Japanese Black cattle assessed using Japan meat grading association (JMGA) and Australian meat industry classification system (AUS-MEAT) grading methodology at the 6-7th and 5-6th rib cross-sections respectively.

Trait	**Measurement method**	**N**	**Range of h^2**	**Unweighted mean h^2**	**Weighted mean h^2**
Carcass Weight	JMGA	18	0.23-0.78	0.48	0.46
	AUS-MEAT	2	0.47-0.59	0.53	0.48
Rib Eye Area	JMGA	18	0.37-0.45	0.46	0.49
	AUS-MEAT	1	0.59	-	-
Subcutaneous fat thickness*	JMGA	18	0.07-0.59	0.39	0.32
	AUS-MEAT	2	0.25-0.84	0.55	0.43
Marbling Score	JMGA	11	0.16-0.74	0.55	0.21
	AUS-MEAT	2	0.23-0.54	0.38	0.43

Oyama (2011), Maeda *et al. (2014),* Zhang *et al. (2015)*
*Subcutaneous fat thickness for AUS-MEAT grading refers specifically to the P8 fat depth measurement (mm)

Heritability estimates in Wagyu have been published recently, with 50K SNP genotype data utilising models that included the additive polygenic effect and chosen SNPS or just the polygenic effect alone. Estimated heritabilities for the carcass traits listed above ranged from 0.40 to 0.84, with the proportion of variance attributable to the SNPs increasing as the number of SNP effects that fit increased (Watanabe *et al.* 2014).

Unweighted averages of genetic correlations between carcass traits in Wagyu populations have been summarised by Oyama (2011; Table 1.2). Moderately positive average correlations were reported between carcass weight and ribeye area and ribeye area and marble score (0.44 and 0.43 respectively). The preceding correlation is slightly higher than what has been published in an Australian Wagyu herd which is different to the one utilised in experimental

chapters herein (0.38; McEwin 2016). However this correlation is still in agreement with what is to be expected. The latter correlation (0.43) is a unique characteristic of Wagyu as previous estimates in other breeds have been zero or lowly negative (Gregory *et al.* 1995). McEwin (2016) demonstrated that the moderately positive correlation in Wagyu was perhaps explained by the excessive amount of marbling, exhibited by the breed, pushing the muscle fibre bundles apart and thereby increasing the ribeye area measurement. Lean muscle area in the rib-eye was estimated by removing the estimated intramuscular fat percentages and reported a negative correlation more similar to what would be observed in other breeds. As discussed previously, another unique Wagyu characteristic is the apparent lack of, or slightly negative, genetic correlation between marble score and subcutaneous fat depth. The average correlation, as calculated by Oyama (2011), is presented as -0.06 (Table 1.2) whereas in other breeds it is moderately positive (0.44; Gregory *et al.* 1995).

Table 1.2: Unweighted Genetic correlations among carcass traits (above diagonal) and the number of reports included in unweighted average (below diagonal) in Japanese Black Cattle (Oyama, 2011).

Trait		**1**	**2**	**3**	**4**
1	Carcass Weight		0.44	0.31	0.15
2	Rib eye Area	5		0.02	0.43
3	Subcutaneous Fat thickness	5	5		-0.06
4	Marble Score	6	6	6	

1.2.2 Camera Image traits

Image analysis methodology presents as an objective way to assess carcass quality characteristics such as marbling traits, meat colour/brightness and muscle symmetry. A description of the image analysis traits presented in Table 1.2 can be found in Maeda *et al.* (2014) with the exception of the ratio of minor to major rib-eye axis trait which describes the symmetry of rib-eye muscle when observed at the rib-eye cross-section.

Weighted heritabilities for image analysis carcass traits were calculated as being highly heritable (greater than 0.40) with the exception of coarseness index for the largest marbling particle which is lowly heritable (0.12; Table 1.3). This is in contrast to the higher heritabilities of percentage marbling area and marbling coarseness index (0.52 and 0.42 respectively) which could suggest that an extremely large marbling particle might be distributed in the muscle at random (Osawa *et al.* 2008). Where unweighted and weighted means are both estimated, the weighted mean differed little from the unweighted mean, although this might be due to the small spread of studies included.

Japanese Black Wagyu is the predominant breed utilising image analysis with few publications available to include in a weighted analysis of heritabilities. In addition not all studies encompassed all traits analysed here, or provided sufficient information and that is why some calculations are missing from Table 1.2. Certainly, more studies which include a larger spread of breeds and traits would be valuable as only shared traits among studies that incorporated one breed were included here. However the high heritability of the majority of traits certainly suggests that image analysis traits may be useful in the design of breeding programs and could be an alternative to the current subjective grading systems.

Table 1.3: Range of Direct Heritabilities (h2) for image analysis traits and the number of reports referenced (N) in Japanese Black Cattle.

Trait	**N**	**Range of h^2**	**Unweighted mean h^2**	**Weighted mean h^2**
Camera rib-eye muscle area	3	0.44-0.62	0.50	0.49
% Marbling area	4	0.37-0.59	0.52	0.52
Marbling coarseness index	4	0.31-0.47	0.38	0.42
Coarseness index of largest marbling particle	4	0.05-0.20	0.11	0.12
Marbling fineness index	2	0.50-0.55	0.53	0.51
Average luminance of exposed lean	2	0.40-0.57	0.49	0.47
Ratio of minor to major rib-eye axis	2	0.08-0.32	0.2	-

Osawa *et al. (2004; 2008) , Maeda et al. (2014), Zhang et al. (2015).*

Osawa *et al.* (2008) describes best the genetic correlations between some of the image analysis traits above. Camera rib-eye muscle area is lowly-moderately correlated with percentage marbling area, overall marbling coarseness and coarseness of maximum marbling particle (0.36, 0.39 and 0.24 respectively; Osawa *et al.* 2008). Similarly marbling percentage and coarseness of maximum marbling particle are lowly-moderately correlated (0.29) which could present as more evidence for coarser marbling particles to be distributed in the muscle at random. Unsurprisingly, marbling percentage had a high genetic correlation with overall coarseness and overall coarseness was highly correlated to coarseness of maximum marbling particle (0.69 and 0.85 respectively; Osawa *et al.* 2008).

In general image analysis traits have high genetic correlations with their carcass trait counterparts i.e. Camera rib-eye muscle area and rib-eye muscle area (0.97) and marbling percentage and marble score (0.97; Osawa *et al.* 2008), with similar values reported by Zhang *et al.* (2015). This suggests that the image analysis traits would be an appropriate, objective substitute for the current subjective methodologies. In addition these traits have low negative correlations (-0.03 to -0.21) with subcutaneous fat thickness, which is unsurprising in Wagyu for reasons described previously, as well as low to moderate correlations with carcass weight (0.17-0.35; Osawa *et al.* 2008). Therefore improvement in marbling characteristics will not hinder gains in carcass weight as well as not result in an accompanying increase in subcutaneous fat, improving yield potential.

Osawa *et al.* (2008) reported an undesirably moderately-strong correlation between marble score and overall marbling coarseness (0.66) suggesting that improvement in marbling based on marble score could be accompanied by an increase in coarser marbling particles. Therefore breeding programs would have to potentially mitigate this by incorporating assessment of

marling fineness into their breeding objectives which should be plausible given its high heritability (see above; Table 1.3).

1.2.3 Conclusion

Marbling is quickly becoming an integral component within breeding objectives of Australian beef producers due to its association with meat eating quality, specifically tenderness, juiciness and palatability. Wagyu appear as an attractive breed to meet rising demand for higher marbled beef due to their genetic predisposition to produce high degrees of marbling. Currently there is a demand for finer marbled beef and subjective grading programs are not able to capture this variation in marbling fineness. This is important to capture due to a strong genetic correlation between marbling and marbling coarseness (0.66). This has led to the development of camera imaging technology (objective marbling assessment) to describe marbling fineness and coarseness indexes. Heritabilities of these new novel traits in Wagyu have been reported as moderately to highly heritable, although a range exists, which suggests they could be implemented into successful breeding programs. Delving into the genes responsible for marbling, no genes of major effect (or causal genes) have been reported; rather many genes seem to contribute a small effect each on phenotype. While traditional breeding programs have made head-way in improving the amount of marbling in retail cuts, there is suggestion that the pairing of 'high-tech' phenotypes (Camera imaging and fatty acid analysis) with genomic selection (Appendix 1) presents as an exciting future opportunity worth exploring.

1.3 Within breed selection is sufficient to improve terminal crossbred beef marbling: A review of reciprocal recurrent genomic selection

The development of a genetic evaluation program for beef cattle in Australia started as the National Beef Recording Scheme (NBRS) in the late 1970's and became BREEDPLAN in 1985 (Graser and Hammond 1985). The purpose of BREEDPLAN is to quantitatively evaluate an individual's genetic merit before they are selected as breeding stock on a breed by breed basis. This is achieved using recorded phenotypes and knowledge of an animal's pedigree.

Traditionally, only phenotypes from stud recorded, purebred relatives have been able to be included in genetic modelling, due to pedigrees not commonly extending between breeds across nucleus herds. However, with the development of genomic selection (Meuwissen *et al.* 2001), it has become increasingly easier by replacing pedigree relationships with those estimated from single nucleotide polymorphism (SNP) data. This allows access to much larger pools of data which could in turn improve the accuracy of genomic predictions (Hayes and Goddard, 2008), that is assuming marker density is sufficient to capture linkage disequilibrium (LD) among breeds (De Roos *et al.* 2009).

Many studies struggle to find an advantage of using multi-breed evaluations. Prediction accuracies for certain traits can be improved but often the effects are neutral or slightly decreased (Hayes *et al.* 2009; Erbe *et al.* 2012; Moghaddar *et al.* 2014; Brito *et al.* 2017). Degree of relatedness between populations is key, with closely related populations offering more benefit than distant relations (Habier *et al.* 2010). First cross (F_1) data is a good example of this, whereby cross-breed phenotypes are obtained on animals who are sired by individuals within the nucleus herd. Assuming the correlation between the purebred and crossbred trait (r_{pc}) is high, F_1 data can easily be utilised in the nucleus through a single-trait GBLUP (genomic best linear unbiased prediction; VanRaden, 2008). For instances where r_{pc} is low, i.e. the

purebred and crossbred traits are considered different traits, than a multi-trait GBLUP model can be used to incorporate cross-bred data into purebred evaluations (Olson *et al.* 2012).

A lot of emphasis in cattle breeding is placed on breeding better purebreds, yet the commercial product is often the result of cross-breeding to capitalise on heterosis and breed complementarity. A particular beef cross that achieves this is Japanese Black Wagyu x Angus, Angus dams provide high weaning rates and growth while Wagyu sires provide high marbling (intramuscular fat) attributes. In addition, obtaining large numbers of Wagyu cows is prohibitively expensive while Angus dams are more accessible.

Breeding better purebreds does result in improved performance in cross-bred progeny (Banks 1995) but there is often phenotype unpredictability associated with cross-breeding. This is particularly the case for marbling in F_1 Wagyu (Brethour, 1995), which is undesirable considering these animals are on feed for over 300 days. The question then is, for a system such as F_1 Wagyu production, is there breeding systems available, utilising cross-bred information, that breed better purebreds, for the purpose of improved (and consistent) cross-bred performance. To what extent is there genetic (sire) by genetic (dam breed) interactions in highly marbled crossbreds?

Reciprocal recurrent selection (RRS) describes the selection of purebreds to maximise crossbred performance utilising both additive and non-additive genetic variance (Comstock *et al.* 1949). In other words, there is potential to breed for increased heterosis as well as utilise additive variance. This approach was developed in maize populations and has been expanded to the poultry and pig industries with varying success (Wei & Van der Steen, 1991). As pedigree recording is not common practice in commercial beef herds, RRS has not yet been applied in this industry. Genomic selection may make the utilisation of RRS in beef cattle plausible, especially in Wagyu with their very high price point. Several publications have proposed model

considerations for reciprocal recurrent genomic selection (RRGS) for application in the pig and poultry industries (Dekkers 2007; Ibáñẽz-Escriche *et al.* 2009; Zeng *et al.* 2013; Vitezica *et al.* 2016; Xiang, Christensen, Vitezica, & Legarra, 2016).

The aim of this paper is to review proposed approaches to RRGS and to evaluate their usefulness for a potential application in beef cattle. This knowledge is collated with literature regarding the relative importance of additive and non-additive variance in animal performance and breeding programs. This is achieved by focussing on a suggested two-way, terminal cross scenario between the Japanese Wagyu and Angus breeds that will have a primary focus on meat quality attributes such as marbling.

1.3.1 Importance of additive vs. non-additive genetic effects

Genetic variance (σ_G^2) can be partitioned into three components; additive (σ_A^2), dominant (σ_D^2) and epistatic (σ_I^2) gene action with the epistatic component able to be partitioned further (Falconer and Mackay 1996). Dominant and epistatic variance components are collectively referred to as non-additive genetic variance and have implications in inbreeding and heterosis.

Traditional selection of livestock has focused purely on the additive genetic component of an individual's genotype using an animal model with numerator relationship matrix based on pedigree (Henderson 1976) or SNP (VanRaden 2008). This additive component is expressed as an estimated breeding value (EBV) or genomic estimated breeding value (GEBV) when estimated from pedigree or genotype information respectively. Breeding values are determined using the statistical method known as best linear unbiased prediction selection (BLUP; Henderson 1984), termed GBLUP when genomic information is considered. Estimated breeding values have been effective for increasing economically important traits of agricultural species where pedigree is accurately recorded on each individual (Banks 1995; Parnell 2015) or genotypes are available (VanRaden 2008).

The importance of considering additive and non-additive genetic effects in breeding programs can loosely be considered under two criteria;

1) What traits are under selection pressure (fitness vs. production) and,
2) What is the basic breeding program design (purebred production vs crossbred production).

Non-additive genetic variance is more influential for fitness traits and forms part of the genetic mechanism underpinning heterosis, although the exact mechanism is not known (East 1908; Shull 1908; Ford 1945; Davenport 1908). Maize is perhaps one of the most well-known agricultural crops to exploit heterosis for commercial hybrid production, having laid the foundation for exploitation of breeding hybrids in other agricultural crops (Virmani and Edwards 1983; Mühleisen *et al.* 2013; Zhao *et al.* 2013). As well as heavily exploiting heterosis, crop yield is of high economic importance which is biologically a fitness trait for seed production.

Maize is a naturally cross pollinating crop with separate male and female inflorescence making artificial hybridisation and self-pollination (or "selfing") for seed production relatively easy. The inbred-hybrid concept is widely used in maize production for the production of commercial hybrids. Here, inbred, homozygous, elite parental lines are developed through many generations of selfing and then a single cross between two lines is used to produce the heterozygous, high vigour, commercial hybrid. Where maize breeding exploits this further is that while the hybrid progeny are heterozygous at an individual plant level, they are homogeneous at the population level. This means commercial hybrid progeny exhibit uniform performance assuming uniform environmental effects (Hallauer *et al.* 2010).

Inbred lines are developed for use in hybrid production and this is where their commercial value lies. A specific line may excel for several traits but unless they produce excellent hybrid

combinations, they are unlikely to make it into commercial production. Combining ability of lines was a general concept for classifying inbred lines relative to their hybrid progeny performance. Sprague and Tatum (1942) describe general combining ability (GCA) and specific combining ability (SCA) which relates to genes having largely additive and non-additive effects respectively (Reif *et al.* 2007).

A similar mechanism to SCA has been described in animal breeding as "nicking", a term used to describe matings which produce unexpectedly superior offspring, which has been attributed to non-additive genetic effects in early literature (Seath and Lush 1940). Early comparison of dairy bulls based on average performance of daughter-dam groups give inconclusive evidence for nicking, in fact differences between groups could have easily been due to chance or environmental influences (Heizer *et al.* 1938; Johnson *et al.* 1940; Seath and Lush 1940). With more modern methods which look at including the pedigree relationship matrix (animal model; Meyer 1989) or genomic relationship matrix (VanRaden 2008) in the analysis of trait data, several studies have further partitioned the genetic variance to include dominance and/or epistatic variance in livestock (Tempelman and Burnside 1989; van der Werf and De Boer 1989; Miglior *et al.* 1995; Rodriguez-Almeida *et al.* 1995; Palucci *et al.* 2007) reporting significant although relatively small non-additive variance. It is important to distinguish here between effects seen between breeds (heterosis) and at an individual level (dominance effects). The latter is harder to implement in animal breeding programs due to the difficulty of estimation. It is much easier to utilise individual level dominance in programs such as maize due to using inbred lines.

Hill, Goddard & Visscher (2008) evaluated the evidence from a number of empirical studies of genetic variance components and reported that additive genetic variance typically accounts for over half the total genetic variance for a trait. Certainly traits of economic importance in

Wagyu, such as Carcass Weight, Rib-Eye Area, Subcutaneous Fat thickness and Marbling (marble score), have heritabilities (σ_A^2/σ_P^2) of approximately 0.50 (Oyama 2011), indicating that σ_A^2 alone accounts for 50% of the total phenotypic variance. This means dominance, epistasis and all environmental variance contribute no more than σ_A^2 collectively. For example, in an F_1 population of pigs, dominance variation accounted for a marginal proportion of the total genetic variance in litter size, 13% (Vitezica *et al.* 2016). In addition, Hill, Goddard & Visscher (2008) reported theoretical models which predicted high proportions of additive genetic variance, even in the presence of non-additive gene action, due to the likelihood of most alleles being at extreme frequencies i.e. 1 or 0. This certainly suggests that dominance variance is smaller than additive, however large dominance effects could still exist.

In pigs, dominance and epistasis have been reported to account for approximately 6 and 9% of the phenotypic variance for daily weight gain in pigs in respectively, although only the dominance variance was significantly different from 0 ($P<0.05$; Su *et al.* 2012). Additionally, reliability of genomic breeding values increased by 1% when dominant and epistatic terms were included in the model (Su *et al.* 2012). Sun *et al.* (2014) and Aliloo *et al.* (2016) reported dominance variance accounted for 5 and 7% of total variance for milk yield traits in Holsteins and Jerseys respectively. For these yield traits, inclusion of both additive and dominance variance described the data better, and increased prediction accuracy, compared to just including additive variance (Sun *et al.* 2014), although this wasn't consistent for all traits (Aliloo *et al.* 2016). In Broiler chickens, for seven feed related traits, dominance accounted for approximately 10-13% of phenotypic variance across traits and 29 – 58% of the total genetic variance (Li *et al.* 2017).

Using a high density SNP chip array and a dominance relationship matrix, Bolormaa *et al.* (2015) reported significant ($P < 0.001$) proportions of phenotypic variance attributable to

dominance effects for post weaning live weight (11%), intramuscular fat (10%) and retail beef yield (18%) in admixed cattle populations. Dominance was also significant (P <0.01) for feedlot exit live weight accounting for 7.0% of phenotypic variance. In contrast to Su *et al.* (2012), Sun *et al.* (2014) and Aliloo *et al.* (2016) but in agreement with Li *et al.* (2017), Bolormaa *et al.* (2015) reported no improvement in the accuracy of genomic breeding values by including dominance variance terms in the model and attributed this to the more distant relationship of the cattle populations examined. For example Su *et al.* (2012) utilised a more closely related pig population. Xiang et al. (2016) reported similar predictive ability for crossbred pigs with and without accounting for dominance for total number of piglets born, as did Jiang *et al.* (2017) for 8 complex traits in dairy. Additionally, significant effects of heterozygosity are often associated with significant effects of dominance variance for traits (Bolormaa *et al.* 2015; Li *et al.* 2017); but this is not always the case i.e. for intramuscular fat or retail beef yield (Bolormaa *et al.* 2015). Clearly estimates of dominance here are small (although significant for some traits) and the literature appears conflicting as to whether inclusion of dominance in the model improves predictive ability compared to additive variance alone.

1.3.2 Pure-line selection for crossbred production

Breeding for improved pure-line or purebred performance through the use of EBVs (additive genetic variance) has demonstrated commercial production advantages where superior genetic sires are mated over commercial females (Banks 1995). Using sires with high breeding values for growth resulted in a 1.6-8.4 g/day increase in post weaning growth rate of crossbred progeny per kg increase in sire growth EBV for Australian Sheep flocks (Hall *et al.* 1997; Hegarty *et al.* 2006). Measured another way, post-weaning weight of crossbred lambs has been shown to increase 0.1-0.9 kg per kg increase in sire growth EBV (Hall *et al.* 1992; Fogarty *et al.* 1997; Hall *et al.* 1997; Hegarty *et al.* 2006). Other traits influenced cross-bred post-

weaning weight as well with an additional half a kg being achieved in crossbred lambs per mm increase in sire EBV for post weaning eye muscle depth (Hegarty *et al.* 2006). The proportion of sire EBV observed in crossbred progeny did not differ significantly from the expected value of 0.5 in the studies discussed above (Hall *et al.* 1992; Fogarty *et al.* 1997; Hall *et al.* 1997). That is, genetically superior sires do transfer 50% of their superiority to their cross-bred progeny.

Similar results have been observed in crossbred cattle where Brahman dams were mated to Angus, Hereford, Shorthorn, Belmont Red and Santa Gertrudis sires in a multi-breed evaluation study (Newman *et al.* 2002). Regressions of cross-bred calf performance on sire EBV were all found to be significantly different from zero for the weight-related and carcass traits investigated. Four hundred day weight (400W) of cross-bred progeny increased by 0.5kg per kg increase in the 400W sire EBV, and did not differ significantly from the expected 0.5 kg/kg (Newman *et al.* 2002). In contrast, regressions of crossbred intramuscular fat (IMF) on sire IMF EBV and crossbred P8 (subcutaneous fat depth) on sire P8 EBV were less than the expected 0.5.

The regression coefficient $b_{x,EBV}$ between a trait x of cross breed and the EBV of purebred sire is calculated as;

$$b_{x,EBV} = 0.5\ x\ r_{pc}\frac{\sqrt{h^2}\times\sigma_p}{\sigma_{EBV}}$$

Where h^2 is the heritability, σ_p is the variation of the trait in crossbreds, σ_{EBV} is the additive genetic (EBV) standard deviation in the purebred and r_{pc} the genetic correlation between the purebred and crossbreds for the same trait. Regressions less than 0.5 could be due to the genetic correlation between purebreds and crossbreds (r_{pc}) being less than 1, the trait having

a lower heritability (h^2) in crossbreds or the trait having lower variance (σ_p) in crossbreds (Hebart *et al.* 2020).

If the genetic correlation between traits, the heritability and the variation in sire EBV are known, or assumed to be known, and are likely to remain constant, it is the variation in carcass traits, such as that caused by scale effects, that is likely to have the biggest impact on the regression coefficient (Hebart *et al.* 2020). Sire EBVs are computed based on a standard 400kg carcass however the mean carcass weight in the crossbred study of Newman *et al.* (2002) was 259kg. This lower carcass weight could indicate lower variation in crossbred IMF and P8 fat and hence explain the lower than expected regression coefficient.

Purebred-crossbred genetic correlations (r_{pc}) have been estimated in cattle (Newman *et al.* 2002). Using elite EBV sires results in increased cross-bred progeny performance. However, there has been little discussion about the possibility of sires re-ranking based on usefulness as crossbred progenitors; at least where additive genetic variance is concerned. Purebred-crossbred genetic correlations of traits are less unified for weight traits (400W and Carcass Weight; r_{pc}=0.48 each) than carcass quality traits such as retail beef yield, IMF and P8; r_{pc} of 0.83, 0.95 and 1.00 respectively (Newman *et al.* 2002). In general, r_{pc} decreases with increasing dominance level or increasing genetic disparity (gene frequency difference) between parental populations. Conversely, traits which have highly positive r_{pc} are often an indicator of greater importance of additive genetic effects for that trait (Wei and Van der Steen 1991). Heterosis does influence growth and hence 400 day weight and carcass weight and is not nearly as important for fat traits such as IMF (Pitchford *et al.* 2017), however both trait groups are highly heritable being governed largely by additive genes and so consistently high r_{pc} might have been expected across the traits. Environment may have been a contributing factor leading to low r_{pc}. Performance data on Angus, Hereford and Shorthorn is

typically measured in southern Australian climates while crossbreds were reared, and some finished, in a sub-tropical environment. (Newman *et al.* 2002). Additonally r_{pc} is often confounded with genotype by environment effects. However the less unified r_{pc} could be suggestive that producers may encounter some re-ranking of sires for weight related traits when selecting bulls for use over Brahman cows, though this isn't expected to be the case for marbling (Newman *et al.* 2002).

In summary, sires being genetically evaluated within their own populations (breed) is an effective strategy to improve cross-breed performance. Under this frame work, only additive genetic variance is utilised and any heterotic effects expressed in crossbred progeny are simply an expression of the genetic distance between those two breeds. Theoretically one could purposely use genetically distant breeds to capitalise on heterosis as well as utilise the additive genetic variance. This has practical applications in that sires could potentially re-rank based on the potential of their cross-bred offspring.

1.3.3 Reciprocal recurrent selection

Not all crosses are equal regarding heterosis response in progeny performance (Long 1980). For example heterosis in cattle is greatest when *Bos indicus* and *Bos taurus* breeds are crossed i.e. Brahman x Angus matings produce greater heterosis expression in progeny than Brahman crossed with a tropical composite e.g. Belmont Red (Newman *et al.* 2002). However an element of breed complementarity needs to be considered and this is where reciprocal recurrent selection can be useful.

Reciprocal recurrent selection (RRS) describes the selection of purebreds to maximise crossbred performance utilising additive and non-additive genetic variance (general and specific combing abilities respectively) (Comstock *et al.* 1949). This is in contrast to pure-line selection (described above) which can only use additive variance. Reciprocal recurrent

selection was developed for maize breeding (Comstock *et al.* 1949) where performance of inbred lines in hybrid combinations is paramount. Reciprocal recurrent selection procedures have improved commercial hybrid performance in maize for yield characteristics as well as some other agronomic parameters (Penny and Eberhart 1971; Keeratinijakal and Lamkey 1993; Moll *et al.* 1994). For example, improved heterosis response in line crosses has been observed indicating greater utilisation of non-additive genetic effects (Moll *et al.* 1994).

Most commercial poultry birds (layers and broilers) result from the crossing of three or four pure lines (Hunton 1990) and some seed-stock breeders are using RRS, or variations on RRS, as a means to select purebreds for crossbred performance. Selection response for an egg production trait from first egg to 40 weeks of age was evaluated using pure-line (within-line) selection versus RRS. In both breeding systems responses to selection were significantly greater than zero but not significantly different to each other (Calhoon and Bohren 1974). Where improvement in the rate of egg production is concerned, pure-line selection and RRS were again equal in response (Saadeh *et al.* 1968) despite it being expected that RRS would be advantageous over pure-line selection. However, this certainly appears to be the case for litter size and feed efficiency in pigs, with RRS found to be advantageous over pure-line selection (reviewed by Wei and Van der Steen (1991)).

Selection of broilers for 10-week body weights using RRS was shown to improve the trait favourably however combining ability was not improved and therefore improved performance was likely due to an accumulation of favourable additive alleles (Griesbach 1962). In pigs, the usefulness of RRS for selection response in weight traits is generally less or equivalent to pure-line selection (Wei and Van der Steen 1991).

The application of RRS has not been tested in beef and this is due to pedigrees not being recorded in crossbred commercial herds. Additionally, the reproductive rate of cattle is slow

and so complex crossbreeding schemes are not common. Pure-line selection is successful at improving cross-bred performance, and crossbred data can be utilised to improve purebred predictions. How this data is used depends on the corresponding r_{pc}. With genomic information able to replace pedigree and Wagyu constituting a high value market, any small additional improvements to crossbred performance through RRGS could see significant financial advantages.

1.3.4 Genomic selection of purebreds for crossbred performance

Genomic selection for purebred production has been adopted in purebred breeding schemes, particularly dairy (VanRaden 2008; Goddard *et al.* 2010; Jiang *et al.* 2017). Recent literature has proposed selecting purebreds for crossbred performance using genomic selection (Dekkers, 2007; Ibánẽz-Escriche *et al.* 2009; Zeng *et al.* 2013). This is possible in multi-breed populations when using sufficient SNP densities to ensure the consistency of LD between the populations (De Roos *et al.* 2008; Lu *et al.* 2012; Porto-Neto *et al.* 2014). Studies in this area can be largely categorized into 3 groups; those that account for additive gene action, those that account for additive and dominant gene action and those that account for breed specific allele effects (breed x additive interactions). As genomic selection traditionally accounts for additive gene action, the two latter categories will be the primary focus of discussion regarding selection for crossbred performance.

Breed specific allele effects

Marker assisted selection for crossbred performance was proposed by Dekkers (2007) as a method for overcoming the limitations of combined crossbred and purebred selection (Wei & Van der Steen, 1991; Lo *et al.* 1993). Combined crossbred and purebred selection refers to the utilisation of phenotypic data from commercial crossbred relatives for selection of purebreds, but is limited in that improved crossbred performance is accompanied with increased

inbreeding in the parental lines (Bijma *et al.* 2001). This can only be countered by extensive pedigree recording. Dekkers (2007) demonstrated through simulation that using marker-EBVs, i.e. the sum of marker effects, derived from crossbred phenotypes, to select a terminal sire line, resulted in increased rates of response in crossbreds and reduced parental line inbreeding. This was relative to the accuracy of the marker-EBV. Implementation of this methodology using SNP markers is a good strategy, however the best results for selection of purebreds mean training on crossbred data. As crossbreds represent a mixture of specific marker-QTL associations that exist within the parental breeds, knowledge of breed-specific effects i.e. from which parental line the marker allele/haplotype was inherited, are essential to be able to use these markers/haplotypes to aid selection of purebred parents (Dekkers 2007).

Ibánẽz-Escriche *et al.* (2009) investigated the importance of a breed specific SNP allele model (BSAM) further, constructing a simulation that compared a classical genomic selection model with breed-specific allele effects to an across breed SNP genotype model (ASGM) where SNP effects were assumed the same across the parental breeds. Considering SNP number (500 vs 2000), parental breed relatedness and number of training records (1000 vs 4000), breed specific marker effects outperformed across parental breed effects when the lesser density of SNPs (500) was utilised, breeds were distant or unrelated and when the larger level of training records was utilised. Regardless of this trend, the largest difference in accuracy of purebred breeding values trained on F_1 crossbred data, between the two models, was 4%. Genotype simulation was for one chromosome, 1M in length where trait phenotype was known to be influenced by 30 QTL with a moderate heritability of 0.30 (Ibánẽz-Escriche *et al.* 2009). Where 10 chromosomes, 1000 QTL and 10,000 SNPs were considered a BSAM model performed equal to or worse than an additive model (GS BayesC) regarding cumulative response to selection in the crossbreds after 20 generations (Zeng *et al.* 2013). Duenk *et al.* (2019) utilised

a genomic relationship matrix that accounts for the breed-of-origin of alleles, demonstrating that accounting for breed of origin is beneficial for improving prediction accuracy. However this was only the case when r_{pc} for the trait was lower 0.8. Traits with more unified r_{pc} (0.98) did not benefit.

Models that include dominance

Kinghorn *et al.* (2010) built further on the work done by Ibánẽz-Escriche *et al.* (2009) by exploiting within-locus dominance effects as well as additive effects, for selection within purebred parental lines for crossbred performance in a two-way cross. Denoted reciprocal recurrent genomic selection (RRGS), a "genomic key" (a set of weightings to calculate GEBVs using genotype) was derived for each separate parental line from crossbred phenotypes and their gametotypes contributed by each parental line for each crossbred individual (similar to BSAM; Ibánẽz-Escriche *et al.* 2009). In simulation, RRGS resulted in a substantial increase in genetic merit, but at the expense of genetic merit in the purebred lines, which decreased over generations of selection in purebreds. Another scenario was investigated herein similar to ASGM (Ibánẽz-Escriche *et al.* 2009), where crossbred genotypes and phenotypes were used to make one genomic key for use across both parental lines. This method utilises diploid information (genotypes) compared to haploid (gamete gametotypes). Responses in crossbreds were still favourable and almost comparable with RRGS however genetic merit of the purebreds was able to be maintained and slightly increased over 40 generations of selection. This was true when genomic and phenotypic information from the crossbreds was updated each generation as alleles. The drive in both RRGS and ASGM is such that allele frequencies within pure lines can drift to opposing extreme or fixed values, leading to increased heterosis expression (Kinghorn *et al.* 2010).

The practicality of either RRGS or ASGM method is dependent on situation. If the aim is purely crossbred performance then RRGS will achieve the highest genetic merit. If parental line performance is desired to at the very least be maintained, then ASGM may be more suitable although one limitation is that crossbred progeny will need to be genotyped every year. Phenotype collection would be a non-issue, relating back to the crossbred Wagyu example herein; all progeny of the two-way cross would be slaughtered making phenotypes of each generation available. Another benefit of ASGM is that phasing of alleles/haplotypes etc. is not required.

Zeng *et al.* (2013) compared a BSAM model to an additive and dominance model. Their dominance model is potentially advantageous. Breed of origin must be known or inferred for BSAM, however no such knowledge is need for the dominance model. This is a different approach to Kinghorn *et al.* (2010) as BSAM was investigated in conjunction with dominance deviations, rather than compared separately. In the absence of overdominance the dominance model was favoured over the additive model and where no dominance was present the models were equivalent. The dominance model outperformed BSAM in all cases; however this advantage may decrease as the disparity in LD, between breeds, increases. While the additive and dominance variances at the QTL may be consistent between breeds, the SNP effects between breeds may differ due to differences in LD (Zeng *et al.* 2013).

A limitation in the approach of Zeng *et al.* (2013) is that SNP effects were estimated once and then used for 20 generations of purebred selection for crossbred performance. This is rarely done in practice as retraining is usually carried out after each generation of selection; with retraining the advantage of the dominance model is expected to decline relative to the additive or BSAM models (Zeng *et al.* 2013).

The studies discussed thus far investigating purebred selection for crossbred performance have been simulation studies. As the simulations move to more realistic scenarios accuracy estimates or responses to selection are shown to drop. For example, Ibáñẽz-Escriche *et al.* (2009) conducted a more realistic simulation scenario where breeds were closely related, comparing the BSAM and ASGM models utilising 20,000 segregating SNPs from the crossbred population over 10 chromosomes. A 25% drop in accuracy from the single chromosome simulation was observed (Ibáñẽz-Escriche *et al.* 2009). Similar tendencies have been observed when moving from a simple to a more realistic simulation regarding levels of response to selection (Kinghorn *et al.* 2010; Zeng *et al.* 2013). Additionally due caution is warranted as some results presented herein will likely not translate to performance under real conditions particularly because the ability to perfectly estimate QTL effects and trace the inheritance of crossbred alleles back to parental lines is assumed (Ibáñẽz-Escriche *et al.* 2009; Kinghorn *et al.* 2010). This demonstrates the requirement for such models, as described above, to be investigated in the field using real data.

Purebred selection for crossbred performance with real data

Studies that investigate purebred selection for crossbred performance using real data are limited and have only been published in recent years with a large focus on pig production. Vitezica *et al.* (2016) ran a GBLUP analysis to estimate genetic parameters (additive and dominant) for an F_1 pig population based on approximately 7500 SNP genotypes, that was inclusive of the parental lines as well as the crossbreds. A multivariate model that included purebred and F_1 performance for litter size was used. The theory presented demonstrated the estimation of variance components under a genomic model with additive and non-additive inheritance. In particular three variance components were described i.e. the additive genetic variance due to alleles from population 1, the additive genetic variance due to alleles from

population 2 and the dominance genetic variance from the F_1 population, that have interpretation value in terms of variances of breeding values (general combining abilities) and of dominance deviations (specific combining ability). Genomic correlations between the parental lines and crossbreds were presented. Additive genomic correlations between parental lines were 0.78 and ranged from 0.60-0.83 between the parental lines and F_1s. This indicates that the additive effects of SNPs are similar between lines and between crossbreds. The dominance correlations were lower regardless of population (0.47-0.54) indicating dominance effects differ between the populations. This allows for selection of a specific pair of parents to produce superior F_1 individuals in a GBLUP evaluation framework i.e. matings with the highest specific combining ability (heterosis) can be predicted. This was assuming SNP effects were independent of the origin of alleles and that allele frequencies differed between the parental populations (Vitezica *et al.* 2016).

Esfandyari *et al.* (2016) used a dominance model for genomic prediction of crossbred performance based on purebred landrace and Yorkshire litter size data. They found prediction accuracy of GEBVs for cross-bred performance was highest when both additive and dominance effects were accounted for in the prediction model (Esfandyari *et al.* 2016) which is in agreement with previously discussed simulation studies (Ibánẽz-Escriche *et al.* 2009; Zeng *et al.* 2013). Esfandyari *et al.* (2016) used a dominance relationship matrix to estimate SNP dominance effects. However, simply accounting for heterozygosity of individuals may be simpler for evaluating crossbred performance using dominance variation as done in a tropically adapted composite population (Pitchford *et al.* 2017). While this study was not a genomic reciprocal recurrent selection program, including heterozygosity as a fixed effect, prevented bias in GBLUP estimates from heterosis in the composite population. Heterozygosity effects reflect heterosis and/or dominance effects (animals with higher

heterozygosity were bigger and conceived faster) and are much simpler to calculate (Pitchford *et al.* 2017).

The importance of breed-specific allele effects inherited by F_1 pigs, from Large White or Landrace parental lines for litter size and gestation length was investigated (Lopes *et al.* 2017). Here the prediction accuracies of GEBVS for a traditional genomic selection model were compared with those obtained from a model that accounts for breed-specific effects, trained on purebred and crossbred data. For both traits, estimates of breed-specific additive genetic variance were only slightly larger for alleles inherited from the Large White population in the F_1s, although standard errors were expectedly large due to dataset size. Additionally the highest accuracies for predicting crossbred performance were observed when training was done on crossbred data; prediction accuracies between the traditional GS model and the model accounting for breed-specific effects were similar. This is consistent with other studies where training on crossbred data (Moghaddar *et al.* 2014), or data that comprised both parental breeds (Ibáñẽz-Escriche *et al.* 2009; Esfandyari *et al.* 2016) resulted in equal or higher prediction accuracies for crossbred performance.

A genomic selection model accounting for breed-specific allele effects is expected to be advantageous when crossbred populations are larger and the parental breeds are more distantly related (Ibáñẽz-Escriche *et al.* 2009). In pigs, the F_1 cross between two dam lines or 'dam breeds' is done to produce F_1 dams to mate to terminal sire lines. Crossing separate dam (maternal) lines, as in Vitezica *et al.* (2016) and Lopes *et al.* (2017), likely doesn't involve distantly related breeds, which could potentially be achieved by crossing with a terminal sire boar for pork production. Therefore the examples with pig data here, although suggestive, are not a good representation of the terminal cross Wagyu x Angus beef scenario where it might be expected that breed-specific effects to be of greater importance.

The pig examples here, as they are producing maternal lines, would place emphasis on reproduction, where non-additive genetic effects are more important. In the F_1 Wagyu scenario, emphasis would be largely on production traits relating to meat quality (marbling) and production efficiency (yield and feed use efficiency). Although there is some evidence for non-additive effects influencing production traits, the effect is often small and inclusion of such effects does not improve the prediction accuracy of individuals breeding values. It is likely that for the traits of interest,in a terminal Wagyu x Angus cross, that additive genetic effects, being far more abundant and important, particularly for marbling will suffice to improve F_1 performance through selection of purebreds.

1.3.5 Summary of Reciprocal Recurrent Genomic Selection

Complex crossbreeding schemes, such as reciprocal recurrent selection, are not popular in commercial beef herds. This is largely due to the requirement to keep pedigrees in order to feed progeny information back into purebred breeding programs, as well as low reproductive rates. Genomic selection technology presents a way to replace traditional pedigree recording, making selection for purebreds for crossbred performance possible, assuming crossbreds are both phenotyped and genotyped. Additionally, the financial incentive that Wagyu offer provides an opportunity where even small improvements in cross-bred performance are sought after. In most studies discussed, non-additive genetic variance accounts for small proportions of total variance in production traits. This non-additive variance is more influential in reproduction traits although nowhere near to the degree of additive gene action. Including dominance in prediction models varies in its effectiveness to improve predictive ability. Simulation studies show that including dominance in RRGS models may be advantageous to accounting for breed-specific allele effects where breed origin of alleles is difficult to determine or cannot be inferred. Additionally, accounting for breed-specific-allele effects is

only benefical where r_{pc} for a trait is low. Advantages gained by including dominance in the model, over purely additive variance, faded as more realistic simulations were conducted. Studies with real data in pigs confirmed the advantage of accounting for dominance, although emphasis in these studies was for maternal reproduction. For a Wagyu x Angus breeding program, emphasis would be on production traits, namely marbling, so accounting for dominance will not be as advantageous and selection on additive variance will likely suffice. This is supported by earlier reciprocal recurrent selection studies in livestock species where response to selection was generally no different to that achieved using pure-line selection.

Chapter 2 : Genetic Parameters for Economically important traits in an Australian herd of Japanese Black Wagyu

2.1 Introduction

There are multiple methods to assess marbling in Australia which have been discussed previously in detail (1.1.5). One method is AUS-MEAT (AUS-MEAT Limited 2005), which subjectively assesses marbling using visual scoring systems. This method lacks the precision and range to accurately record high marbling phenotypes in Wagyu where there are a large proportion of carcasses at the maximum score (9). Furthermore, it only crudely assesses marbling fleck size. It is likely that objective measures will be more accurate and repeatable than visual scores. An example is the Meat Image Japan (MIJ) camera which measures both marbling and fineness.

Genetic evaluation is traditionally carried out with the use of pedigrees to record ancestry. However pedigree based relationships are not able to capture Mendelian segregation amongst relatives (Visscher 2009), particularly full siblings. This is described in more detail in the following chapter (Chapter 3.1). The described relationships between animals are combined with recorded phenotypes to produce Estimated Breeding Values (EBVs), an estimate of an animal's genetic merit. Information from more distant relatives, especially relationships between ancestors, are often ignored in pedigree BLUP as they tend to fall outside the known pedigree. Genomic selection (Meuwissen *et al.* 2001) is an approach that exploits thousands of SNP (single nucleotide polymorphisms) markers that are in linkage disequilibrium (LD) with QTL associated with traits of interest to produce Genomic Estimated

Breeding Values (GEBVs; Appendix 1). With the use of genomics, distant relationships can be detected (even if small) and phenotypes used if genotyping is widely carried out. The debate is not whether to use exclusively genomics or exclusively pedigree, estimates are generally similar between the two. However, pedigree can be difficult to collect, incorrectly recorded or not recorded at all. This is particularly the case for slaughter data, with slaughtered animals mostly being commercially bred and, therefore, difficult for seedstock breeders to obtain with good management groups and pedigree records.

Three previous studies have estimated and compared genetic parameters of AUS-MEAT and MIJ camera traits in Australian Wagyu cattle, two using pedigree (Maeda *et al.* 2014; Zhang *et al.* 2015) and one utilising genomics through single step BLUP (Zhang and Banks 2019). While not yet accredited as such, users of the MIJ camera, such as the Australian Wagyu Association, believe it is more accurate than AUS-MEAT scoring, especially at high marbling levels. However, the relationship between marbling and other live measure traits such as weight/growth traits (Birth-weight; BWT, and yearling weight; 400_WT), ultrasound measurements from 12 month old animals and daily feed intake (DFI) have not been widely reported in Australian Wagyu.

The aim of this chapter is using genomic information to 1/ Estimate the heritability of image marbling traits relative to AUS-MEAT score as an indication of value of objective measure to increase genetic gain and 2/ Assess the genetic relationships between marbling and other economically important traits in a breeding population of Wagyu.

2.2 Materials and Methods

2.2.1 Genotype and Pedigree Data

Genotype information on 4,940 Full-Blood Wagyu individuals and a subset of 1091 with carcass information was utilised for this study. These animals were genotyped with the 30K GGP-LD (NeoGen Australasia: GeneSeek Genomic Profiler Low-Density) SNP chip. Animals were not excluded on SNP call rate, however SNPs were excluded if they possessed ≥ 5% missing data (95% call rate) and/or a minor allele frequency (MAF) less than 0.05. This meant 20,955 of 29,547 SNPs were retained for the analysis. Homozygous genotypes were coded as -1 and 1, with heterozygous genotypes as 0. After genotype cleaning a genomic relationship matrix (GRM) was constructed as per VanRaden's first method (VanRaden 2008);

$$\boldsymbol{G} = \frac{\boldsymbol{Z}\boldsymbol{Z}'}{2\sum_{i=1}^{n} p_i(1-p_i)}$$

Where **Z** denotes a centred matrix of allele effects with a mean of zero, p_i is the frequency of the second (minor) allele at locus i and division by $\mathbf{2}\sum p_i(1-p_i)$ scales the **G** matrix to be similar in magnitude (diagonal elements average 1) to the numerator relationship matrix constructed from genealogy (VanRaden 2008).

For comparison, a pedigree relationship matrix was also constructed using the R-package *pedigreemm* (Version 0.0.3; Bates and Vazquez 2014) with the pedigree consisting of 10,549 animals.

2.2.2 Phenotype Data

A subset of phenotype data was provided; collected on individuals born from 2009 to 2017 for 14 different traits including live weight and ultrasound measurements as well as and carcass measures (AUS-MEAT and MIJ). The AUS-MEAT traits analysed here were hot standard carcass weight (HSCW), AUS-MEAT P8 site fat depth (P8_FAT) and AUS-MEAT marble score (A_MARB).

Ultrasound traits measured on 12 month old animals describing intramuscular fat (U_IMF), P8 site fat depth (U_P8) and eye muscle area (U_EMA) as well as measurements on birth weight (BW), 400 day weight (400_WT) and average daily feed intake (DFI) were also utilised (Table 2.1).

MIJ traits included were image eye muscle area (I_EMA), image percentage marbling (I_MARB), image coarseness of marbling (I_COARSE), percentage image marbling minus largest marbling particle (I_MARB2) and fineness of image marbling index (I_FINE). I_FINE is described as the total circumference of marbling particles (mm) divided by the square root of the rib-eye area. Further definitions and methodology for MIJ camera traits are presented by Kuchida *et al.* (2006), Kato *et al.* (2014) and Maeda *et al.* (2014). These traits were measured at the 5^{th}-6^{th} ribbing site.

Table 2.1: Summary Statistics and number of records in the subset provided for 14 traits measured in an Australian Japanese Wagyu Herd from 2011 to 2018.

Trait	Units	No. records	Mean	Min	Max	SD
BWT	kg	2252	29.6	14.6	46.0	4.5
400_WT	kg	2990	256.1	95.5	478.0	55.2
U_IMF	%	3072	6.2	2.2	8.3	1.3
U_EMA	cm^2	3070	58.4	25	94	11.1
U_P8	mm	3073	5.9	0	18	2.7
DFI	kg/day	1462	9.6	2.5	16.5	2.2
HSCW	kg	1091	437.8	302.0	618.5	46.8
P8_FAT	mm	1091	18.0	5	44	7.1
I_EMA	cm^2	1079	45.4	25.0	82.2	7.8
I_MARB	%	1079	29.1	13.5	60.3	6.6
I_COARSE	%	1079	29.1	13.7	57.1	5.8
I_FINE	mm/cm^2	1079	55.0	23.7	89.5	11.1
I_MARB2	%	1079	27.1	12.8	51.5	6.0
A_MARB	Score 0-9	1091	7.7	2	9	1.4

Pedigree information was available on all animals that had phenotypic records. For the carcass progeny records, 79 sires and 1129 dams with an average of 14 progeny/sire and 1.5 progeny/dam. The number of progeny per sire ranged from 1 to 53 (Figure 2.1)

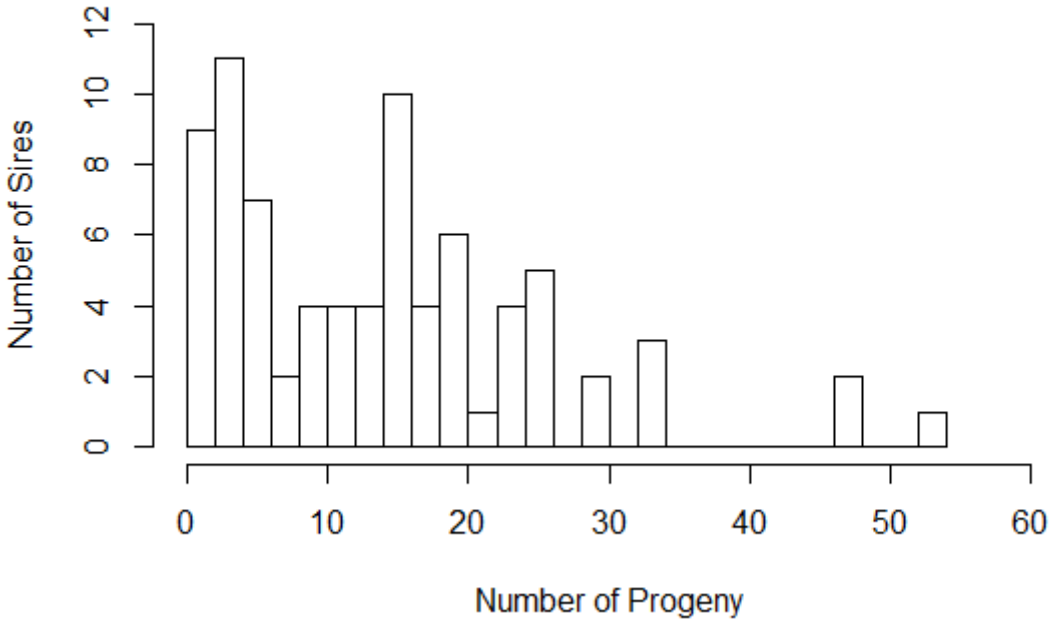

Figure 2.1: Distribution of number of progeny per sire for 1091 Full-Blood Wagyu carcass records.

2.2.3 Model Development and Statistical Analysis

Datum was first analysed as a series of univariate models with a general linear mixed model using ASReml-R 4.0 (Butler *et al.* 2017) such that;

$$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Zu} + \boldsymbol{e}$$

Where, $\boldsymbol{b}$ is the vector of fixed effects, $\boldsymbol{u}$ is the vector of random effects (with $\boldsymbol{X}$ and $\boldsymbol{Z}$ their respective design matrices) and $\boldsymbol{e}$ is the vector of residual variance. All traits included fixed

effects of dam age (Maiden; < 2 year of age, Mature; 3-9 years or Old; > 10 years), heterozygosity (calculated as the proportion of heterozygous genotypes, Figure 2.2), Sex (Heifer, Bull or Steer, except for BWT which just had two levels i.e. Heifer or Bull) and contemporary group based on a predefined age slice that grouped animals born within the same year and calving period. A birth date co-variate was also fitted nested within management group. Management groups were defined as:

- Birth weight management group for BWT (12 levels);
- The combination of 400 day weight management group and 400 weight date for 400_WT (40 levels);
- The combination of 200 weight management and feed test date and feed test pen for DFI (21 levels);
- 200 weight management for ultrasound traits (16 levels) and;
- The combination of 200 weight management, Kill management, Kill date for carcass traits (23 levels). Carcass traits were not adjusted for HSCW.

Comparison of 200 and 400 day management groupings showed that animals were kept in the same management groups formed at weaning as those when scanned as yearlings. Given this, 200 day management group was utilised for ultrasound/carcass traits over 400 day management as it had the greatest number of complete records.

No maternal effects were added in the model. This could be considered an omission for BWT and 400_WT. A subsequent analysis found maternal effects not significant for BWT and of diminishing importance for 400_WT using the same dataset herein.

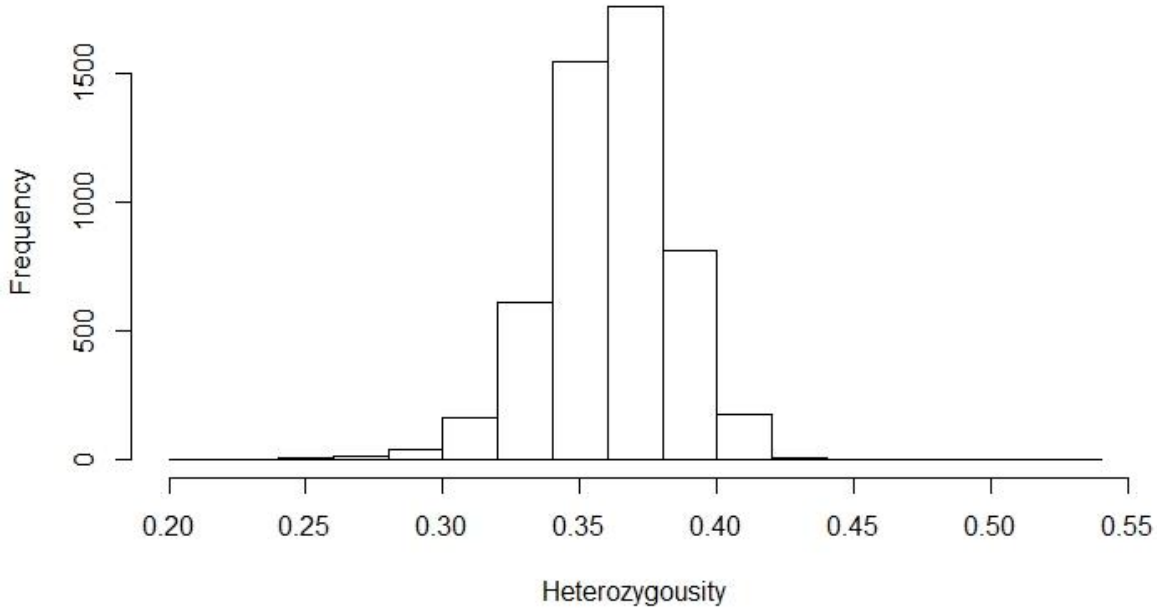

Figure 2.2: Distribution of Heterozygosity values for 4940 Full-Blood Wagyu genotypes, genotyped with GGP-LD 30K SNP chip.

Graser *et al.* (2005) described the genetic evaluation system (BREEDPLAN) currently used in Australia. The models used herein follow similar concepts to that adopted by BREEDPLAN with some key differences noted. BREEDPLAN currently accounts for random maternal genetic and random permanent maternal environmental variances (only where repeated records are present) in their modelling which is not done herein. BREEDPLAN also does not estimate age of dam or age of animals within the model, rather utilising phenotypes that have been pre-adjusted with specific adjustment factors. Contemporary group definitions are similar between BREEDPLAN and the models herein, except for age slice. BREEDPLAN subdivides animals into age slices of 45 or 60 days depending on the trait, beginning with the oldest animal in a contemporary group. Herein, we applied age slice using fixed dates chosen relative to the distribution of calving across years.

Having identified the appropriate models from the univariate analysis, a bi-variate analysis between all traits was conducted incorporating the univariate models. Let $\boldsymbol{y} = (\boldsymbol{y_1'}, \boldsymbol{y_2'})'$, be the combined vector of data between two traits. The mixed model for the bivariate analysis is given by;

$$\boldsymbol{y} = \boldsymbol{X^*b} + \boldsymbol{Z^*u} + \boldsymbol{e}$$

Where $\boldsymbol{b} = (\boldsymbol{b_1'}, \boldsymbol{b_2'})'$is the 2*m* x 1 vector of fixed effects with $\boldsymbol{X^*} = \boldsymbol{I_2} \otimes \boldsymbol{X}$ the associated design matrix; $\boldsymbol{u} = (\boldsymbol{u_1'}, \boldsymbol{u_2'})'$ is the 2*n* x 1 vector of random effects with $\boldsymbol{Z^*} = \boldsymbol{I_2} \otimes \boldsymbol{Z}$ the associated design matrix and $\boldsymbol{e} = (\boldsymbol{e_1'}, \boldsymbol{e_2'})'$ the vector of residual variance ordered as for the data vector. The variance assumptions for the random effects are;

$$var(\boldsymbol{u}) = var\begin{pmatrix}\boldsymbol{u_1} \\ \boldsymbol{u_2}\end{pmatrix} = \begin{bmatrix}\sigma_1^2 & \\ \sigma_{12} & \sigma_2^2\end{bmatrix} \bigotimes \boldsymbol{G}$$

where σ_1^2 is the variance at trait 1, σ_2^2 is the variance at trait 2 and σ_{12} is the covariance between the two traits.

Univariate and bi-variate models were fitted for all traits utilising a genomic relationship matrix with heritabilities, genetic and phenotypic correlations calculated. Univariate models utilising the **A** matrix constructed from pedigree were only run for HSCW, I_MARB and 400_WT. Models were fitted that attempted to estimate random effects of both **A** and **G** simultaneously (analogous to single step) but surprisingly, there was no additional variance estimated in **G** beyond that described by **A**.

2.3 Results

The majority of traits were moderate to highly heritable (>0.23, Table 2.2), with the exception of ultrasound intramuscular fat (U_IMF, 0.15). Marbling associated traits such as carcass I_MARB, I_MARB2 and AUS_MARB were highly heritable (0.68, 0.67 and 0.50 respectively) and were substantially higher than the equivalent ultrasound measure. The calculated I_FINE index was less heritable (0.44). Image loin eye muscle area (I_EMA) was also more heritable than its live measure counterpart U_EMA (0.38 compared to 0.23) while the opposite was true for P8_FAT (0.40 for U_P8 vs. 0.24 for P8_FAT). HSCW was the most heritable of the weight traits (0.56) and DFI was moderately heritable (0.29).

Table 2.2: Variance components, heritabilities and their standard errors and standard deviation of estimated breeding values from genomic univariate analysis.

Trait	σ_A	σ_P^2	σ_{EBV}	h^2	se
BWT	2.74	16.3	1.91	0.46	0.03
400_WT	17.8	920	12.12	0.34	0.03
U_IMF	0.55	1.96	0.32	0.15	0.03
U_EMA	2.62	29.9	1.74	0.23	0.03
U_P8	1.13	3.25	0.83	0.40	0.03
DFI	0.76	2.05	0.47	0.29	0.05
HSCW	32.9	1928	20.8	0.56	0.05
P8_FAT	2.94	36.3	1.46	0.24	0.06
A_MARB	0.97	1.88	0.70	0.50	0.05
I_EMA	4.55	54.9	2.53	0.38	0.06
I_MARB	5.21	40.1	4.17	0.68	0.05
I_COARSE	0.042	0.0034	0.026	0.53	0.05
I_FINE	6.19	87.2	4.52	0.44	0.06
I_MARB2	0.047	0.0033	0.038	0.67	0.05

A pedigree analysis was conducted for comparison for three selected traits. The estimated genetic standard deviation (σ_A) from pedigree was similar to or greater than estimates from genomics. Heritabilities are difficult to compare directly due to the scale effect of the matrix on the variance components so σ_{EBV} is presented. The standard deviation of the EBVs (σ_{EBV}) were greater for all traits in the genomic analysis. The same pattern was observed in the standard errors for the individual EBVs estimated from either method ($se_{pedigree}$ and $se_{genomic}$, Table 2.4). On average standard error for the predicted EBVs was lower when using genomics than when utilising pedigree. In addition, standard error estimates on heritabilities were lower within the genomic prediction compared to pedigree given the same phenotype information.

Table 2.3: Variance components, heritabilities and their standard errors and standard deviation of estimated breeding values (EBVs) from pedigree univariate analysis.

Trait	σ_A	σ_P^2	σ_{EBV}	h^2	se
400_WT	22.2	988	10.4	0.50	0.05
HSCW	32.5	1998	12.6	0.53	0.10
I_MARB	5.58	42.7	3.14	0.73	0.10

Table 2.4: Mean standard errors (se) of estimated breeding values (EBVs) reported from genomic and pedigree univariate analysis.

Trait	$se_{pedigree}$	$se_{genomic}$
400_WT	16.0	11.7
HSCW	27.0	23.6
I_MARB	4.4	3.5

All measures of carcass marbling were uncorrelated with carcass weight (Table 2.5). P8 fat (0.30) and I_EMA (0.33) were more highly correlated with HSCW. This is in contrast to ultrasound fat measurements (U_IMF and U_P8) which were moderately negatively genetically correlated with HSCW (-0.39 and -0.20 respectively). As expected, traits that

indicate weight or muscling (I_EMA, U_EMA, BWT and 400_WT) have moderate to strong genetic correlations to HSCW (0.33 to 0.71) as well as between themselves (0.16 to 0.68).

While measured at almost a year apart and at very different weights and stages of maturity, ultrasound traits generally had strong genetic correlations to their equivalent carcass measurements; 0.53 between U_P8/P8_FAT and 0.62 between U_IMF/I_MARB respectively. The exception was U_EMA which was only moderately correlated to I_EMA (0.29). There was also a high degree of relationship between marbling traits with I_MARB with genetic correlations of 0.64, 0.77 and 0.96 between I_COARSE, I_FINE and A_MARB respectively. Phenotypic correlations amongst these traits were lower, reported as 0.55, 0.66 and 0.77 respectively.

As expected, feed intake (DFI) was positively correlated with weight and muscling traits such as HSCW, I_EMA, U_EMA, BWT and 400_WT (0.41 to 0.79). However, feed intake was lowly correlated with both subcutaneous and intramuscular fat depots.

*Table 2.5: Genomic phenotypic (r_P , above diagonal) and genetic (r_G , below diagonal) correlations between traits**

	BWT	400_WT	U_IMF	U_EMA	U_P8	DFI	HSCW	P8_FAT	A_MARB	I_EMA	I_MARB	I_COARSE	I_FINE	I_MARB2
BWT		0.38	-0.08	0.17	-0.09	0.26	0.4	0.01	-0.04	0.18	-0.12	-0.09	0.03	-0.11
400_WT	0.68		0.02	0.5	0.14	0.43	0.5	0.08	0.00	0.11	-0.04	0.06	-0.03	-0.06
U_IMF	-0.41	-0.26		0.2	0.29	0.13	0.06	0.16	0.35	0.00	0.34	0.12	0.22	0.35
U_EMA	0.23	0.55	-0.01		0.39	0.44	0.4	0.06	0.01	0.19	-0.07	0.05	-0.02	-0.08
U_P8	-0.39	-0.11	0.39	0.25		0.2	0.08	0.29	0.03	-0.02	0.07	0.07	-0.01	0.07
DFI	0.42	0.7	-0.14	0.49	-0.01		0.52	0.09	0.1	0.21	0.06	0.14	0.02	0.05
HSCW	0.53	0.71	-0.39	0.43	-0.2	0.79		0.22	0.19	0.32	0.1	0.21	0.07	0.08
P8_FAT	0.05	0.03	-0.09	0.01	0.53	0.08	0.3		0.00	0.00	0.03	0.05	-0.03	0.03
A_MARB	-0.2	-0.04	0.63	0.05	-0.01	0.16	0.08	-0.02		0.3	0.77	0.5	0.56	0.76
I_EMA	0.16	0.17	-0.08	0.29	-0.09	0.41	0.33	-0.02	0.33		0.19	0.41	0.38	0.17
I_MARB	-0.26	-0.12	0.62	-0.12	0.05	0.10	0.02	-0.09	0.96	0.23		0.55	0.66	nc**
I_COARSE	-0.22	-0.04	0.39	0.02	0.11	0.25	0.12	-0.01	0.64	0.36	0.64		0.00	0.45
I_FINE	-0.08	-0.08	0.42	-0.06	-0.08	-0.01	-0.03	-0.16	0.83	0.36	0.77	0.15		0.7
I_MARB2	-0.25	-0.16	0.61	-0.12	0.05	0.07	0.00	-0.09	0.96	0.23	nc**	0.59	0.8	

*Standard errors range 0.01 - 0.04 for r_P and 0.02 - 0.15 for r_G; **nc = non-converged due to a value assumed close to 1

2.4 Discussion

2.4.1 Reported Heritabilities

Weighted mean heritabilities for traits of interest were calculated for three different grading systems; being Japanese Meat grading association (JMGA), AUS-MEAT and Meat Image Japan (MIJ) camera (Chapter 1, Tables 1.1 and 1.3). For HSCW and A_MARB, the literature weighted heritability calculated was 0.48 and 0.43 respectively (Table 1.1), lower than reported herein (0.56 and 0.50 respectively). For I_MARB and I_COARSE, reported heritabilities herein of 0.68 and 0.53 were, again, higher than the weighted mean heritabilities calculated (0.52 and 0.42 respectively, Table 1.3). The difference between the review presented in Chapter 1 (Table 1.1, Table 1.3) and the study herein, is the utilisation of genomic relationships. Genomic relationships are more precise than those obtained through pedigree due to taking Mendelian segregation of alleles into consideration (Visscher 2009).

Higher observed heritabilities under genomics was not the trend for every trait herein. For I_EMA and P8_fat, heritabilities of 0.38 and 0.24 were reported which is lower than the weighted heritabilities of 0.49 and 0.43 calculated prior. However, it should be noted that these particular weighted estimates are not extremely robust due to the inclusion of only 3 and 2 studies respectively. Genomic heritabilities calculated herein were within the range of previously reported estimates used in the weighted analysis (Table 1.1 and 1.3).

BWT was highly heritable (0.46, Table 2.2) although is within the range of reported estimates for Japanese Black Wagyu (0.19 to 0.61) as discussed by Oyama (2011) who calculated a weighted mean heritability of 0.28 from 8 separate published reports. Given this, the estimate herein is certainly on the higher end compared to what might be expected. For example, within Australian and New Zealand Angus, heritabilities for BWT were reported as 0.35-0.38 and 0.29 respectively (Meyer 1995; Robinson 1996). The additive variance in Meyer (1995) for

Australian Angus was reported as 5.98 kg^2 compared to 7.5 kg^2 (Table 2.2) herein with comparable phenotypic variance. Given Oyama (2011) and Meyer (1995) did not utilise genomics in their studies, this supports a more accurate description of relationships using genomics resulting in higher heritabilities for BWT. Saatchi *et al.* (2011) reported a genomic heritability of 0.42 for BWT in American Angus data which further supports the above conclusion.

Yearling weight (400_WT) has been reported in Angus as 0.24 - 0.31 (Meyer 1995; Robinson 1996) which is slightly lower than the comprehensive weighted heritability estimate of 0.33 produced by Koots *et al.* (1994). Afolayan *et al.* (2007) reported an identical estimate for 400_WT of 0.33 estimated across diverse beef breeds which aligns with the estimate produced herein of 0.34 for Wagyu.

Heritability for DFI has been reported in Wagyu as 0.34 (Hoque *et al.* 2006) which is virtually the same as 0.29 estimated herein (Table 2.2). Hoque *et al.* (2006) reported additive and phenotypic variances of 0.44 and 1.3 kg^2 respectively (giving a residual variance of 0.86) which are lower than those reported in Table 2. The residual variance for DFI herein (1.5 kg^2) is approximately double that reported by Hoque et al. (2006), suggesting the DFI model used in this analysis is not explaining all of the variance in the trait. One explanation could be the how long the animals are feed tested for. For example in the 2006 study, Wagyu bulls were feed-tested for 112-140 days, whereas animals herein were tested for a much shorter period, 63-107 days. The shorter feeding period could be providing less accurate phenotypic records for DFI, though still should be easily sufficient (Archer *et al.* 1997). Looking at the raw phenotype data the standard deviation for DFI was 1.08kg/day in the 2006 study and 2.18kg/day herein, indicating more variation.

It is not correct to assume that genomics is responsible for higher trait heritabilities. For example contemporary group definition in the subset of phenotypes provided herein is extremely well defined which could lead to improved estimates compared to the weighted analysis where the studies included used various degrees of commercial slaughter data. When genetic parameters estimated from pedigree were compared to those estimated from genomics herein, the assumption that genomics produced higher heritabilities by definition did not hold (Table 2.2 vs. Table 2.3). This is expected due to the influence of the scaling effect of **G** and/or **A** on the variances in the mixed model equation such that;

$$\boldsymbol{u} \sim (\mathbf{0}, \sigma_G^2 \boldsymbol{G}), \qquad \boldsymbol{e} \sim (\mathbf{0}, \sigma_e^2 \boldsymbol{I})$$

Therefore parameter estimates may be biased if the genomic relationship matrix elements are in a different scale than pedigree-based estimates, although adjustments can be made to allow comparison (Forni *et al.* 2011). If we take the standard errors of heritabilities estimated into account, the estimates produced from pedigree are not statistically significantly different from genomics, except for 400_WT. Genetic standard deviation (σ_A) estimated from pedigree and genomics can be compared as this value is independent of any scaling effects. 400_WT and I_MARB had greater σ_A when estimated from pedigree compared to genomics whereas σ_A was extremely similar between the two methods for HSCW. The opposite is true when comparing standard deviations of the EBVs (σ_{EBV}) from either analysis. Genomics displayed higher σ_{EBV} for 400_WT, HSCW and I_MARB (12.12kg, 20.8kg and 4.17 % respectively) as well as a lower mean EBV standard error compared to using pedigree. This demonstrates that the ability to describe Mendelian sampling within the relationship matrix leads to a greater spread of EBVs when using genomics, indicating higher prediction accuracy. This is despite pedigree estimating greater additive variation (σ_A) for the three traits. In addition, the standard errors

on heritability estimates were far lower in the genomic analysis than in pedigree which is in agreement with previous studies (Bérénos *et al.* 2014).

The weighted analysis demonstrated that MIJ camera traits are more highly heritable than their AUS-MEAT counterparts which indicates a greater accuracy of measurement. The same trend was observed herein for marbling and eye muscle area traits (Table 2.2) indicating the value of having more precise phenotypes through using objective measurement technologies rather than subjective grading.

2.4.2 Correlations between traits

Correlations were reported between all traits included in the analysis successfully, with the only exception being between I_MARB and I_MARB2 due to non-convergence. This indicates the relationship is likely close to one which was expected given I_MARB2 is the same measurement as I_MARB minus the percentage attributed to the largest marbling particle present in the image (Kuchida *et al.* 2006; Maeda *et al.* 2014). An earlier pilot study conducted using a smaller subset of the same data herein did converge with a genetic correlation of 1 between I_MARB and I_MARB2 (McEwin *et al.* 2018). Correlations were similar between I_MARB and I_MARB2 with other traits as well.

Many of the correlations between carcass traits (HSCW, P8_Fat, A_MARB) and between MIJ camera traits have been discussed previously in detail (Table 1.2 and section 1.2.2) and are comparable to the results presented in Table 2.5. Briefly, HSCW presents moderate genetic correlations to P8_FAT and Rib-eye area (I_EMA) while appearing to be lowly correlated to A_MARB indicating that selection for improved marbling does not hinder improvements to carcass weight. Additionally, the correlation between A_MARB and P8_Fat was lowly negative (-0.02; Table 2.5) which is favourable given improvements in marbling will not also increase subcutaneous fat which needs to be trimmed at excessive levels; this is in contrast to other

breeds where marble score and subcutaneous fat depth is moderately to strongly positive (0.44; Gregory *et al.* 1995). A positive genetic correlation between rib-eye area and marbling/marble score has been reported previously in Wagyu (Oyama 2011; McEwin 2016) consistent with the values between I_EMA and I_MARB/A_MARB (0.23 and 0.33 respectively, Table 2.5). Significantly lower correlations between these traits are reported in other breeds, for example in long fed Angus the correlation is closer to 0 (Torres-Vázquez *et al.* 2018).

In general, MIJ camera traits are highly genetically correlated with their equivalent carcass traits (Osawa *et al.* 2008; Zhang *et al.* 2015). For example A_MARB and I_MARB1 were strongly positively genetically correlated (0.96, Table 3). Improvements in marbling are likely to increase marbling coarseness (Osawa *et al.* 2008) although this can be mitigated by incorporating selection for fineness (genetic correlation of 0.77 between I_MARB and I_FINE and 0.15 between I_FINE and I_COARSE, Table 3).

The high genetic correlation (0.96) between A_MARB and I_MARB1 is important to note. A_MARB is a key trait in the breeding objective of Wagyu producers, however I_MARB is a more appropriate trait to select for two reasons;

1/ I_MARB is able to capture marbling variation accurately from high marbling carcasses due to not being based on a restrictive scale. The AUS-MEAT scale of 0-9 limits the differentiation of highly marbling carcasses as they will all be grouped in the highest bracket, unable to describe carcasses that marble above a score of nine (Figure 2.2)

2/ I_MARB is more highly heritable than A_MARB (0.68 versus 0.50) which indicates response to selection in A_MARB will be greater using I_MARB through indirect selection. If we consider the genetic response (R) through direct selection using the equation;

$$R = \frac{i\,\sigma_A h}{L} \approx \sigma_A h$$

Where $\sigma_A$described the genetic standard deviation for the trait and h the square root of the heritability then the response in A_MARB under direct selection is 0.69 scores per generation. However, if we consider the correlated response (CR) in A_MARB when selection pressure is on I_MARB then the correlated response to selection can be calculated as;

$$CR = \frac{r_G i\, \sigma_P h_1 h_2}{L} \approx r_G \sigma_A h$$

Where r_G describes the genetic correlation between the two traits, σ_A is the genetic standard deviation for A_MARB and h is the square root of the heritability for I_MARB. The correlated response in A_MARB is then 0.77 scores per generation. This demonstrates that it is 12% more efficient to improve A_MARB through indirect selection on I_MARB.

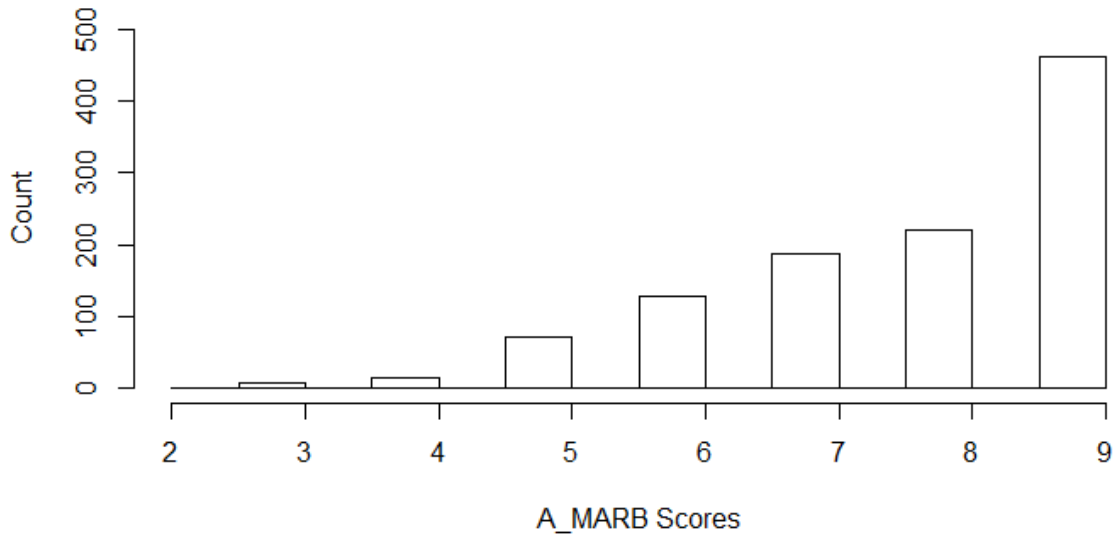

Figure 2.3: Distribution of AUS-MEAT marbling scores (A_MARB) demonstrating a large proportion of animals grouped within a high marble score of 9.

Perhaps more novel correlations for Wagyu, certainly Australian Wagyu, are the correlations between ultrasound measurements and MIJ image phenotypes. Ultrasound measures are routinely taken within most studs as an easy measure to obtain proxy carcass records on stud

stock. In general correlations between carcass marbling measures (such as extracted-IMF or marbling scores) and ultrasound measures are strongly positive around 0.7, although can be as low as 0.54 (Su *et al.* 2017; Duff *et al.* 2018). The genetic correlation between U_IMF and I_MARB herein was at the low end of reported values at 0.62 (Table 3) while the genetic correlation between U_EMA and I_EMA was even lower (0.29) although has been reported as high as 0.81 in Herefords (Su *et al.* 2017). This suggests the relationship between carcass and ultrasound measures is weaker in Wagyu than in other breeds. The studies mentioned report breeds such as Simmental, Hereford and Angus which are often short fed in the feedlot before being slaughtered in contrast to Wagyu which are commonly long fed for 300-500 days to attain high marbling. Given the long time on feed and hence an older age at slaughter, ultrasound measures, which in this dataset were taken at approximately 12 months of age, are poor predictors of carcass performance in Wagyu because of the long time between ultrasound measurement and carcass outcome. This was more the case for I_EMA than for I_MARB, which is likely due to high expressions of marbling "inflating" size of the rib-eye at slaughter (McEwin 2016), and not highly expressed during live ultrasound scanning of a 12 month old bull. U_EMA and U_IMF in this study demonstrated little genetic co-variance (-0.01), while I_EMA and I_MARB/AMARB were moderately correlated (0.23 and 0.33 respectively) demonstrating the influence marbling has on rib-eye measurements after long feed periods in Wagyu.

BWT and 400WT were both highly genetically correlated with HSCW (0.53 and 0.71) which were both higher than expected given previous reports of 0.26-0.29 and 0.56 by previous studies (Gregory *et al.* 1995; Torres-Vázquez *et al.* 2018). The genetic correlation between BWT and 400_WT (0.68) were also higher than previously reported estimates suggesting a correlation closer to 0.53 (Torres-Vázquez *et al.* 2018). As with HSCW, moderate to high genetic correlations of 0.16-0.55 were reported between BWT/400_WT and muscling

measures (I_REA and U_EMA) as well as moderately negative to low correlations (-0.26-0.05) to carcass fat measures (IMARB/P8_Fat). The moderate negative correlation (-0.26) is interesting between BWT and I_MARB as previously reported estimates in long fed Angus are near 0 also (Torres-Vázquez *et al.* 2018). Additionally the genetic correlation is close to zero between HSCW and I_MARB herein. However, given BWT was strongly correlated to HSCW, another key trait under selection, it is likely that as HSCW increases, BWT will increase, as will then marbling. Used correctly, the relationship between marbling and BWT could be used to counter the traditionally antagonistic relationship with HSCW that leads to calving difficulties and dystocia. The complexity of this multi-trait discussion highlights the importance of using a selection index with appropriate economic weights on traits.

DFI was moderately to strongly genetically correlated with growth and muscling traits (0.41-0.79) consistent with previous published estimates (Robinson and Oddy 2004; Torres-Vázquez *et al.* 2018) but generally low correlations to fat traits (-0.01-0.16) which were expected to be more moderate to strong(Robinson and Oddy 2004). I_COARSE was an exception to this trend having a moderate genetic correlation of 0.25 with DFI, suggesting higher daily feed intakes could lead to coarser marbling particles being formed but not necessarily higher marbling percentages overall. Given the low correlation to fat traits, selection for high IMF should not impact on feed intake. This is perhaps due to IMF and P8_Fat not being highly correlated, therefore high IMF in Wagyu is coming more from improved fat distribution rather than more fat deposition, unlike in other long fed datasets (Robinson and Oddy 2004; Torres-Vázquez *et al.* 2018). Clearly daily feed intake is more important for growth/weight improvement than fat acquisition which might be expected; by definition animals that eat more will be heavier.

Residual feed intake was proposed as an alternate measure of feed efficiency by Koch *et al.* (1963) and describes the difference between actual feed intake and the expected feed intake

to facilitate body weight maintenance and growth. Given the genetic correlation between DFI and 400_WT was high (0.70) this suggests that up to 51% of the genetic variance in DFI is independent of growth so there may be an opportunity to improve residual feed intake. Lines *et al.* (2018) suggested that this variation is likely to be associated with fatness and so any improvement would need to not negatively impact on marbling. Robinson and Oddy (2004) demonstrated that the genetic correlation between RFI and subcutaneous fat (Rib and P8) was strong (0.48-72) whereas the genetic correlation to IMF% was much more moderate (0.22), suggesting selection for improved RFI will impact Rib and P8 fat deposits more so than marbling. The genetic correlation between subcutaneous fat deposits and IMF% in their study was 0.45-0.48 which is considerably higher than what is reported herein (-0.09). Given the low correlation between fat depots in this Wagyu population, it is likely to improve RFI without negatively impacting on marbling performance.

2.5 Conclusion

Traits of economic importance were found to be moderately and highly heritable. In general, traits estimated using genomic information achieved higher heritability estimates when compared to those estimated from pedigree demonstrating the potential benefits of genomic selection within a Wagyu breeding program. Extensive genetic correlations were conducted and newer novel MIJ camera measures for marbling were highly correlated to their equivalent AUS-MEAT counterparts, and more heritable, supporting their adoption into breeding programs for faster genetic gain. Genetic correlations between economically important traits such as HSCW, I_MARB, P8_FAT and DFI were very favourable for Wagyu compared to other breeds. These results demonstrate the currently genomic methodology and traits under investigation are suitable for pursuing genetic improvement of the Wagyu breed.

Chapter 3 : Impact of SNP Density on Genomic Relationship Matrix Values

3.1 Introduction

Genomic selection is the selection of breeding stock on the basis of their genetic merit (genomic estimated breeding values) predicted from genome wide markers known as single nucleotide polymorphisms or SNPs (Meuwissen *et al.* 2001). It is advantageous over pedigree based selection when traits are hard to measure, sex-limited or appear later in life (Dekkers 2004) due to removing the need for progeny testing. Such traits include carcass and meat quality measurements, important to the breeding objective for Wagyu. Genomic selection is especially advantageous where pedigree testing is logistically complex, for example in commercial herds, where multiple sire mating and lack of dam pedigree knowledge is common.

Breeding values are estimated based on an individual's own performance (if available) and the performance of known relatives, captured through a relationship matrix. A genomic relationship matrix (**G**) is more precise than one obtained through pedigree (**A**) as it takes into consideration Mendelian segregation of alleles, characterising relationships with more accuracy (Visscher 2009). **A** is built on the idea of identity by descent (IBD) by tracing the flow of genes down a pedigree whereas **G**, which utilises genomic markers, involves assumptions of identity by state (IBS). This means markers are in linkage disequilibrium with genes controlling phenotypes, and these genes behave similarly across the whole population for relationships within and extended beyond the pedigree (Tier *et al.* 2015). Off-diagonal elements of **G** denote relationships of an individual compared to all others. When compared

to others which are unrelated, the expectation is zero with a small level of variation possible. Positive values indicate a direct relationship between animals e.g. approximately 0.5 for full-sib and parent offspring relationships. Note that in **A** these sibling, parent relationships would denote a value of 0.5 exactly. Negative values (where the expectation would be 0) are observed due to genotypic sampling and a lack of shared alleles or haplotypes between individuals. That is negative values show the unrelatedness between individuals. Diagonal elements of **G**, denote relatedness of an individual with itself. Diagonal elements of **A** are equal to $1+F_j$ (inbreeding co-efficient of individual j) so can range from 1-2, whereas in **G** the diagonal elements average 1 and have a wider range. In general elements of **G** are approximately equivalent to those obtained through **A**, however are highly dependent on allele frequencies and coding (Strandén and Christensen 2011; Tier *et al.* 2015).

The adoption of genomic technology has become widespread and is already being incorporated into the breeding programs of cattle and sheep breeds in Australia (Swan *et al.* 2012). The largest limitation to the uptake of this technology has been the cost of procuring a genotype, which is correlated to the number of SNPs in a chosen panel. Smaller arrays are cheaper and therefore could be more readily adopted by industry. Given the effective population size (N_e) of Japanese Black Wagyu is small (Nomura *et al.* 2001) the extent of whole-genome linkage disequilibrium is likely higher such that lower density panels are able to effectively capture total additive genetic variance.

One argument for using high density SNP chip arrays e.g. 770K, is that it can potentially increase the accuracy of genomic selection by capturing and describing greater population variation. As higher density chips are developed, re-genotyping previously genotyped animals or even new animals becomes an expensive undertaking. Imputation from a lower to higher SNP density offers a solution to increasing the level of high density genotype information

available. This means a proportion of the population, influential parents for example, can be genotyped with a high density, high cost, SNP chip array and the remainder (including commercial animals) genotyped using a more cost effective, lower density panel and imputed upwards. A certain level of accuracy of imputation would be required to ensure good data is being used in breeding value estimation.

The objective of this study was to investigate the impact of varying SNP densities on relationships within and between animals in a genomic relationship matrix. This was achieved by exploring several 'ad-hoc' SNP selection methods and included looking at the value of imputation up to mid-range and higher densities in a relatively closely related population of Wagyu.

3.2 Materials and Methods

3.2.1 Genotyping

DNA Hair and semen samples were collected from 4,940 Full-Blood Wagyu and genotyped with 30K GGP-LD SNP chip (Neogen: GeneSeek Operations). One hundred and sixty five of these animals, identified as being key influential sires, were genotyped with a high density, 770K chip (Ilumina BovineHD BeadChip). Animals were not excluded on SNP call rate, however SNPs were excluded if they possessed ≥ 5% missing data (95% call rate) and/or a minor allele frequency (MAF) less than 0.05. This meant 20,955 of 29,547 and 499,171 of 777,107 SNPs were retained for the analysis. Homozygous genotypes were coded as -1 and 1, with heterozygous genotypes as 0.

3.2.2 Construction of the GRM

Genomic relationship matrices were constructed using the equations of VanRaden's first method (VanRaden 2008);

$$\boldsymbol{G} = \frac{\boldsymbol{ZZ'}}{2\sum p_i(1-p_i)}$$

Where **Z** denotes a centred matrix of allele effects with a mean of zero, p_i is the frequency of the second (minor) allele at locus i and division by $2\sum p_i(1-p_i)$ scales the **G** matrix to be analogous to the numerator relationship matrix constructed from genealogy (VanRaden 2008).

3.2.3 SNP selection

A 'base' genomic relationship matrix (GRM) was constructed from the starting 20,955 SNPs and used as the comparative "gold standard". Five SNP selection method scenarios were constructed to compare to this standard detailed in Table 3.1.

Scenario 1 involved constructing a GRM from a core manifest i.e. the SNPs in common between two commercially available chips, namely GGP-LD 30K and VersaSNP 50K (Weatherby's Scientific). There were 10,898 SNPs in common between these chips that were segregating in the Wagyu population which reduced to 9,181 being retained for analysis after cleaning for SNP call rate and MAF (described above). In Scenario 2, all available SNPs (29,869 SNPs, regardless of SNP call rate and MAF) on GGP-LD were retained to construct a GRM, briefly exploring the importance of SNP data cleaning and quality.

To determine the impact of SNP density (Scenario 3) on describing relationships between animals; 1,250, 2,500, 5,000 and 10,000 SNPs were randomly sampled from the base 20,955 SNPs at 200 repetitions per density to construct GRMs. The specific SNPs from each random

sample were retained so that specific random samples could be reconstructed to be investigated further. A further sampling method was tested (Scenario 4) where the 20,955 SNPs were placed in linkage map order by chromosome and base pair position then sampled (in order) to replicate sample densities close to those of Scenario 3. This involved selecting every second, fourth, eighth and sixteenth SNP, beginning from the first SNP, until 1,310, 2,620, 5,239 and 10,478 SNPs were sampled respectively and GRMs constructed.

Table 3.1: The Base and multiple SNP selection scenarios investigated based on SNP chip involved, selection method, minor allele frequency (MAF), SNP Call Rate, SNP Density considered and whether an additional imputation study was included.

Scenario	SNP Chip	Selection Method	MAF	SNP Call Rate	SNP Density	Reps	Impute to Base	Impute to HD
Base	GGP-LD	SNPs available	5%	95%	20,955	-	-	-
1	GGP-LD/Versa 50K	SNPs in common	5%	95%	9,181	-	Yes	-
2	GGP-LD	SNPs available	-	-	29,869	-	-	-
3	GGP-LD	Random Sample	-	-	1,250 2,500 5,000 10,000	200	Yes*	-
4	GGP-LD	Sorted Sample	-	-	1,310 2,620 5,239 10,478	-	Yes	-
5	770K	SNPs available	5%	95%	499,171	-	-	Base

*For scenario 3, only the SNP subsets from each density that formed GRMs with the highest (max) and lowest (min) correlations to the base GRM were imputed for further comparisons.

3.2.4 Imputation

Imputation in this study was conducted using FImpute 2.2 (Sargolzaei *et al.* 2011). FImpute uses an overlapping sliding window approach to identify haplotype similarities between the target and reference individuals. The algorithm assumes that all individuals are related to each other at different degrees. As such, the algorithm begins with long windows to capture haplotype similarity between close relatives, shrinking this window by a constant factor after each chromosome sweep is complete to allow for shorter haplotype similarity between more distant relatives to be taken into account (Sargolzaei *et al.* 2011).

For most scenarios all 4,940 animals were used as both the reference and target population. To investigate the impact of imputing to high density genotypes on GRM elements, all 4940 animals were imputed up to 770K (479,535 SNPs), where 165 animals were used as the reference population (Scenario 5, Table 3.1). Imputation up to 20,955 and 499,171 excluded SNPs unable to be mapped to a precise chromosome/base pair location as well as SNPs with identical chromosome and base pair positions (in this instance one SNP from these duplicate or triplicate locations was kept in the dataset). This left the final imputation densities of unique SNPs as 20,874 and 479,535 respectively.

The Scenario 3 samples from each density, with the lowest and highest correlation to the base GRM, were selected for imputation. These samples, along with samples from Scenario 1 and 4, were imputed to 20,874 and along with imputation results from Scenario 5, formed into GRMs and compared to the base. Accuracy of imputation was calculated using a correlation matrix and extracting the diagonals to give the range of correlations across all individual animals between their reference and imputed genotypes of up to 20,874 or 479,535. The specific number of SNPs correctly or incorrectly imputed was also determined for scenarios imputed to 20,874 by counting against the actual genotypes.

The lower triangle elements, including the diagonals, of the GRMs built across the five scenarios were compared to the lower triangle elements of the base GRM using a simple correlation (*r*) to determine the degree of similarity in how each matrix denoted relationships between and within individuals.

Datum was prepared and analysed using R-studio and GRMs were constructed using the package cpgen (Version 0.1; Heuer 2015).

3.3 Results

3.3.1 Scenario 1 and 2

The core manifest SNP sample (Scenario 1), comprised 9,181 SNPs, and had a correlation with the base GRM of 0.99 which increased to 1.0 (0.999) after imputation (Figure 3.5). The strength of this relationship is further illustrated below which shows a comparison of the values from each GRM (Figure 3.1). Most diagonal values were between 0.8 and 1.5. An extreme group of animals can be identified where values are >1.5 to the top of the graph indicating large inbreeding coefficients. Thinning around 0.8 (Figure 3.1) denotes the change

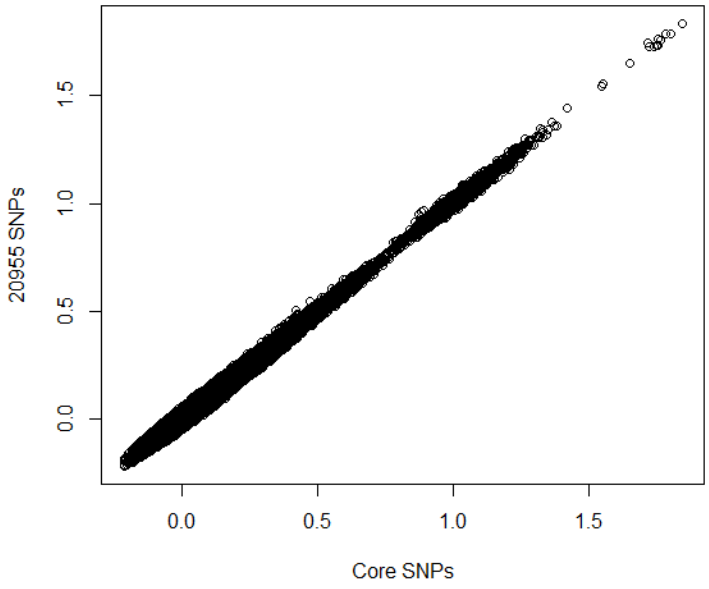

Figure 3.1: Relationship between diagonal and off diagonal elements of the lower triangle for genomic relationship matrices constructed using 20,955 (Base GRM) and 9,181 (Core) SNPs respectively.

from values describing relationships between individuals (off-diagonal elements) to values describing the diagonal elements.

Constructing a GRM using all available SNPs (Scenario 2, 29547 SNPs) not cleaned based on call rate or MAF, resulted in very poor correlations to the base GRM (r = 0.09, Figure 3.2). An absence of linearity can be seen in Figure 3.2 in both off diagonal (lower left cluster) and diagonal values (upper right cluster).

Additionally off-diagonal values with cleaning only based on call rate i.e. removing those SNPs with ≥ 5% missing data but not MAF, resulted in removing approximately 300 SNPs leaving 29,547 SNPs for GRM construction. The values of this GRM were also poorly correlated with the base GRM (r = 0.09) indicating that the MAF SNPs were selected at is important in data cleaning and quality more so than call rate.

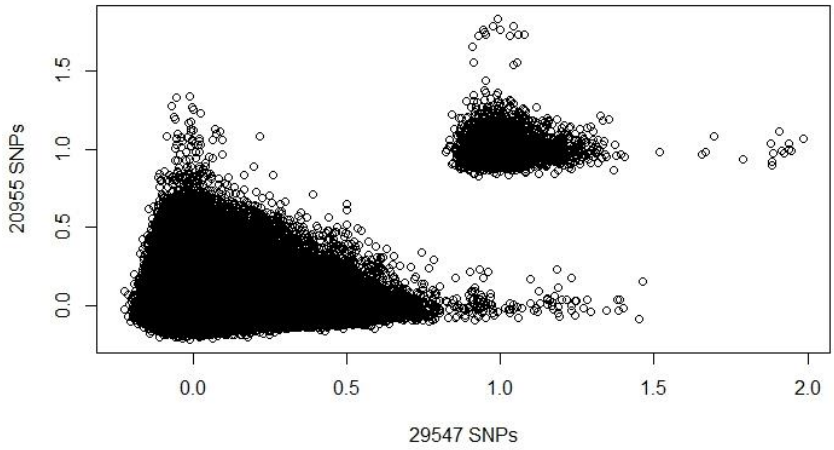

Figure 3.2: Relationship between genomic relationship values constructed using the base 20,955 SNPs and 29,547 SNPs not filtered for call rate or minor allele frequency.

3.3.2 Random and Sorted Samples (Scenario 3 and 4)

Random sampling of SNPs appeared to perform quite well with the lowest correlation to the base GRM being 0.92 obtained from a random sample at the 1250 density (Figure 3.3). The correlation to the base GRM increased with increasing SNP density such that the mean (SD)

correlation was 0.92(0.002), 0.96(0.001), 0.98(0.0004) and 0.99(0.0001) for 1,250, 2,500, 5,000 and 10,000 randomly sampled SNPs respectively. The sorted sampling performed slightly better than the random sampling with correlations of 0.93, 0.97, 0.99 and 1.0 (0.996) to the base GRM for 1,310, 2,620, 5,239 and 10,478 SNPs respectively.

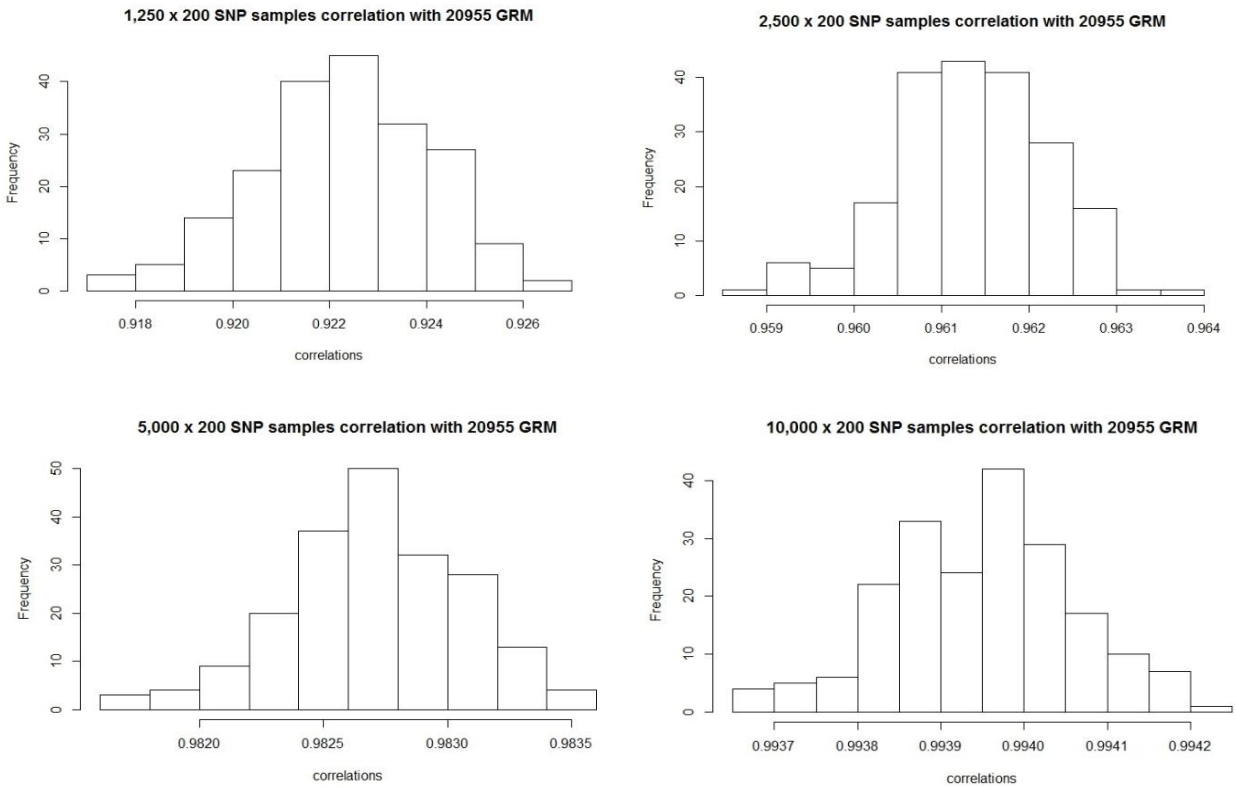

Figure 3.3: Histograms depicting the range of correlations of GRMs to the base 20,955 GRM obtained from 200 random samples of SNPs at 4 different densities (top left: 1,250; top right: 2,500; bottom left: 5,000 and bottom right: 10,000).

The off-diagonal values from the lower triangle of the base GRM were extracted along with the diagonals and plotted against the equivalent values from GRMs constructed from varying SNP densities (Figure 3.4).

As the SNP density increased, the difference between relationship values constructed in the scenario and the base GRM became smaller. This caused the linear trend to follow closer to the equivalence line indicating that the relationships described are very similar to those in the

base. More noticeably in the 1,250 max sample, more inbred individuals are identified as a tight group whereas these same relationships are more spread in the 1,250 min sample.

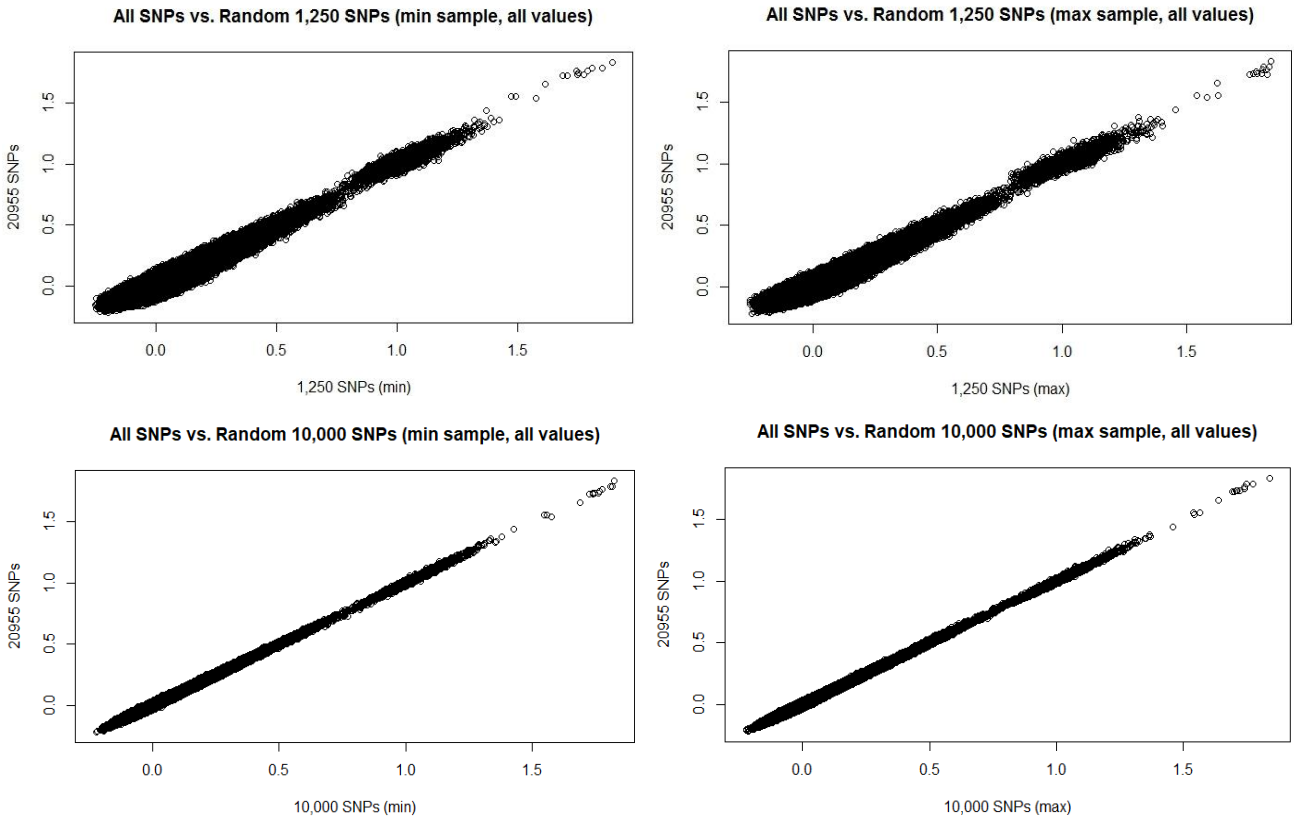

Figure 3.4: The diagonal and off-diagonal values from the 1,250 (top) and 10,000 (bottom) random samples that had the lowest (min; left) and highest (max; right) correlation with the base GRM, plotted against the diagonal and off-diagonal values of the base GRM.

Figure 3.5 shows, using the 10,000 min sample as an example, that the high correlation between the random sample and the base GRM is accompanied by a high level of agreement between the GRM elements themselves. With a mean difference of 0, there is no bias in GRM elements when using a subset or all base SNPs in GRM construction. Interestingly, there was a fanning out of values around an average measure of 0. This indicates that, while there is a high level of agreement, the biggest differences between the two GRMs are when estimating relationships that are approximately zero. That is the base GRM describes these '0'

relationships more precisely than when 10,000 SNPs are used. As the relationships compared become higher, the difference between elements decreases.

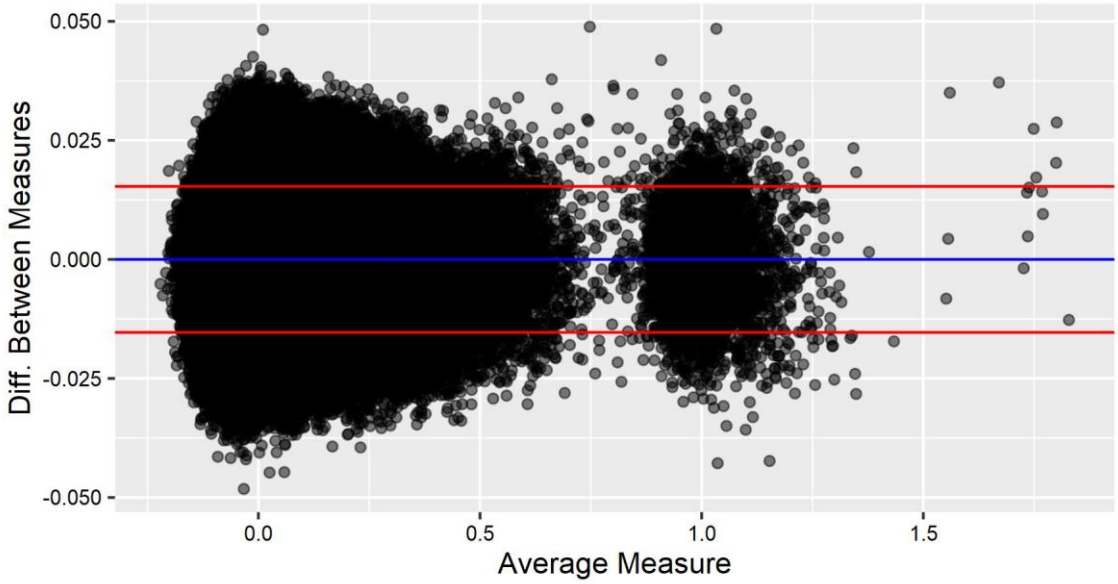

Figure 3.5: Bland Altman Plot showing the difference between genomic relationship values plotted against the average measure of values, constructed using 10,000 randomly selected SNPs compared to the base scenario with the mean (blue) and a 95% confidence interval(red) shown. *Repetition in scenario 3 that resulted in the worst correlation to the base GRM i.e. 10,000 minimum sample.*

3.3.3 Imputation

Imputation to the base SNP density had the impact of improving the correlation with the base GRM all round. Imputation of the minimum and maximum sample for the random sampling gave extremely similar correlations 0.98, 0.99, 1.0 (0.999) and 1.0 (0.999) for 1250, 2500, 5000 and 10,000 sampled SNPs respectively. The sorted SNP samples performed equally as well; all with a correlation greater than 0.99.

Again, higher starting SNP densities gave improved imputation results. The 1,250 min and max samples gave the widest range of correlations, between 0.77 and 0.98 while the 10,000 min and max samples gave correlations ranging 0.92 to 1.0 (0.999), however the distribution was more skewed towards higher correlations in the 10,000 samples (Figure 3.7).

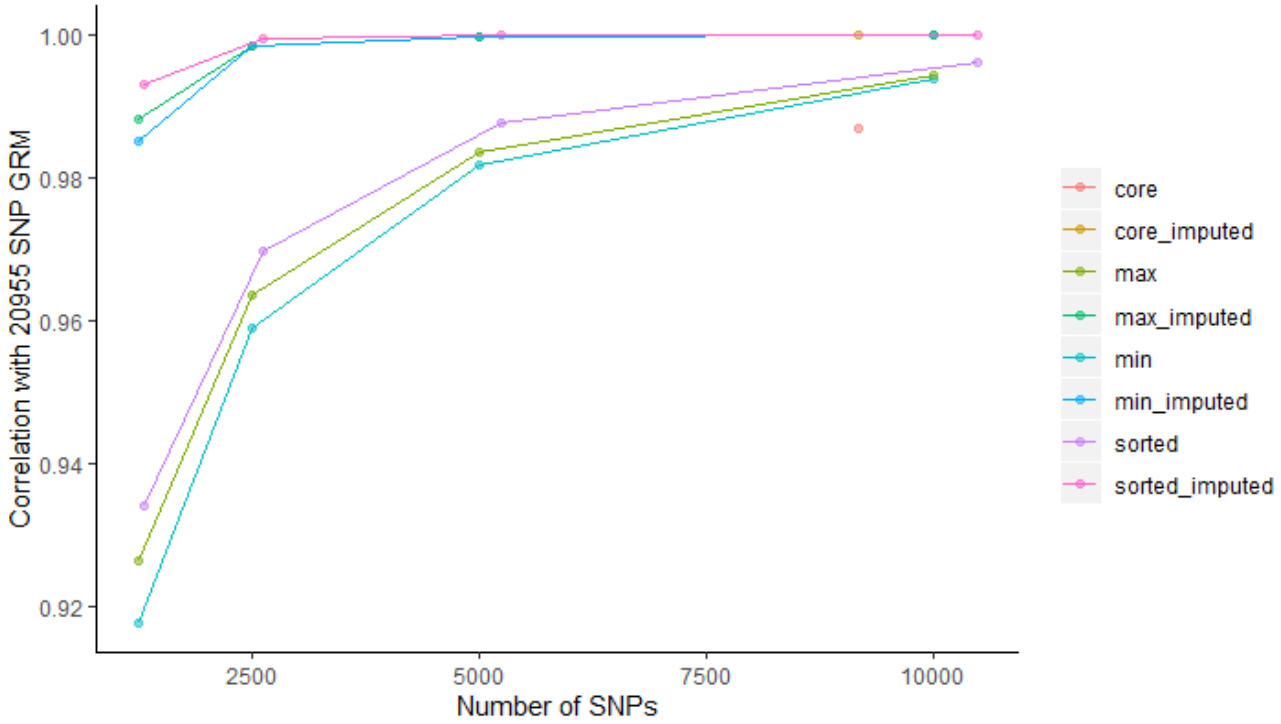

Figure 3.6: Correlation with base GRM vs. SNP density before and after imputation

In terms of the specific number of SNPs imputed incorrectly for an animal, higher starting SNP densities before imputation, unsurprisingly had more correctly imputed SNPs (Table 3.2). The sorted SNP samples gave a slight improvement in imputation accuracy compared to the random samples of approximate equivalent size.

Table 3.2: Minimum and Maximum counts of SNPs imputed to base SNP density incorrectly from random sample and sorted SNP subsets*

Random	Min Count	Max Count
10,000 min	8	1,057
10,000 max	10	1,114
5,000 min	13	1,211
5,000 max	13	1,395
2,500 min	37	1,659
2,500 max	35	1,577
1,250 min	312	3,160
1,250 max	248	2,930

Sorted	Min Count	Max Count
10,478	6	1,029
5,239	12	1,400
2,620	29	1,802
1,310	94	2,376

*Random sample SNP subsets selected for imputation are those that resulted in the highest (max) and lowest (min) correlation when formed into a GRM and compared to the base GRM i.e. 10000min and 10000max.

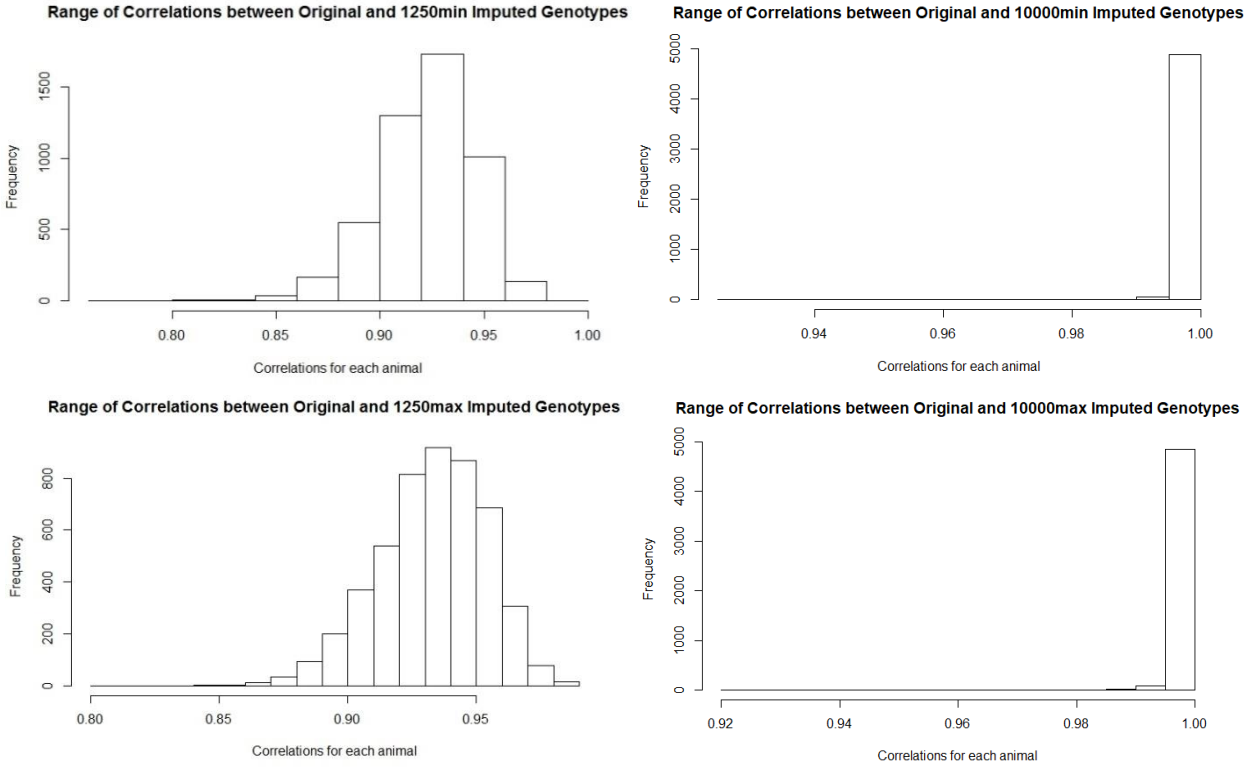

Figure 3.7: Distribution of imputation accuracy (correlations) between the reference 20,955 genotypes and imputed 20,955 genotypes for 4940 animals using random SNP samples with the best and worst correlations to the base GRM (1250; left, 10,000; right)

Figure 3.8 depicts the same random sample as Figure 3.5, however it shows the impact of imputation on specific elements in the GRM. In general, imputation had the effect of reducing the difference between elements of the GRMs compared and depicts a high level of agreement between GRM elements supporting the high correlations observed. Again the biggest differences are observed at relationships of 0, but relationships either side of this differ less than those in Figure 3.5.

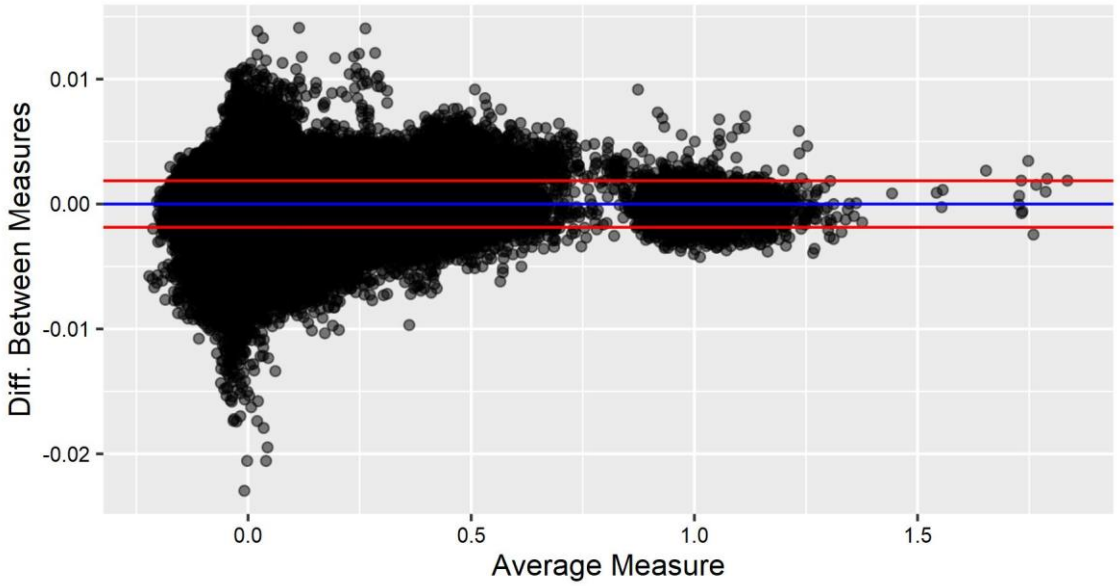

Figure 3.8: Bland Altman Plot showing the difference between genomic relationship values plotted against the average measure of values, constructed using 10,000 randomly selected SNPs imputed to base density versus the base scenario with the mean (blue) and a 95% confidence interval (red) shown.*Repetition in scenario 3 that resulted in the worst correlation to the base GRM i.e. 10,000 min sample.*

3.3.4 Imputation to High Density (770K)

The degree of similarity between GRM values calculated based on a SNP density of 20,955 and cleaned 770K data shows a strong linear relationship (Figure 3.9). There is an evident separation between the off-diagonal and diagonal values with few individuals above a self-self relationship value of 1.5 and a small proportion of animals with negative relatedness. As the SNP density increases beyond 20,955 that relationship values begin to spread out around the

equivalence line. This was most evident for the highly inbred individuals to the far right of Figure 3.9 compared to Figure 3.4.

Using 165 influential sires and dams as the reference resulted in imputation accuracies ranging from 0.84-0.94 with 0.93 being both the mean and median value.

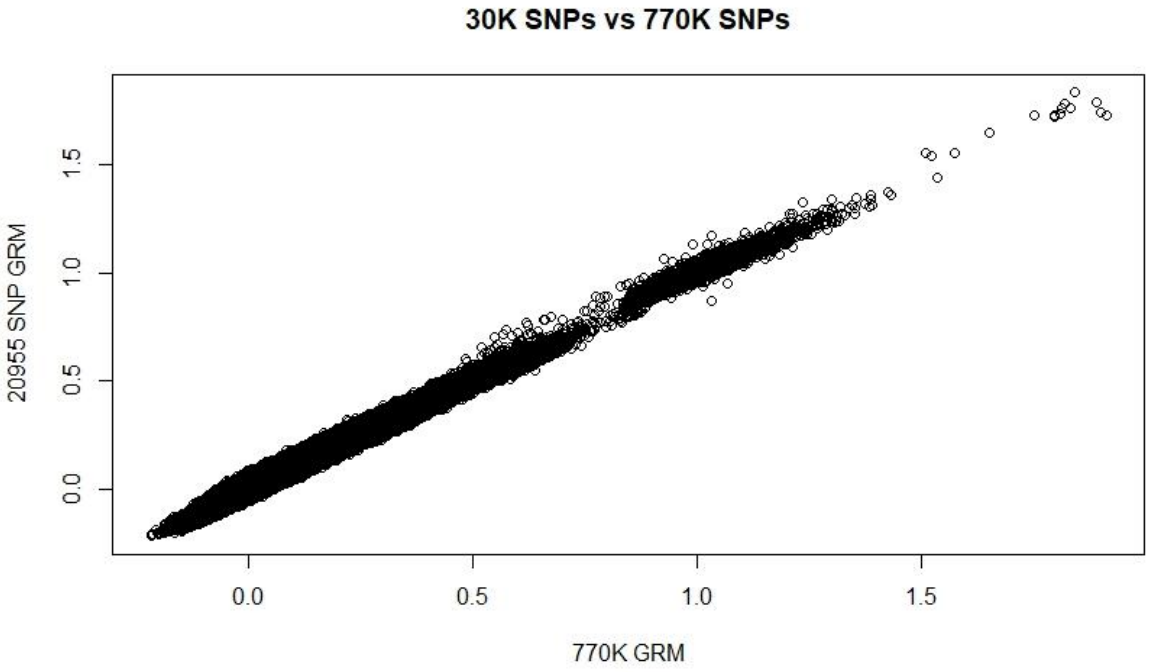

Figure 3.9: Genomic relationship values built from 4,940 30K SNP genotypes compared to 4,940 imputed 770K data.

3.4 Discussion

3.4.1 Correlation with Core Manifest (Scenario 1)

In Scenario 1 there were highly correlated relationships (0.99) can be obtained between core SNPs and the base GRM used herein. This is similar to the performance of ~10,000 SNP samples (from Scenario 3&4) having high correlations to the base GRM consistent with other studies (Rolf *et al.* 2010; Harrison *et al.* 2012; Ogawa *et al.* 2014). As the core manifest describes SNPs in common between the 30K GGP-LD chip platform and the Weatherby's Scientific 50K Versa platform, this suggests that imputation from one platform to the full SNP

set of the other may not be necessary. Rather, the overlapping SNPs between the two platforms could be retained and used to form the GRM for genomic analysis.

The success of lower density panels, such as using a subset common across commercially available chips is the ability to adequately cover regions of the bovine genome. The results here and discussion in later sections are supportive that this can be achieved with confidence in *Bos taurus* breeds such as Wagyu. Unpublished data (*personal communication*: Popplewell Composites) utilising tropically adapted composite suggests it may not be as straightforward in admixed populations. This is more likely due to specific SNPs not being informative in *Bos indicus* rather than due to being in admixture. In the Popplewell data, animals containing a high percentage of Brahman (*Bos indicus*) bloodlines had GEBVs for days to calving (measure of reproductive performance) that significantly re-ranked when the core manifest (of similar SNP density to the Wagyu population herein) was used solely to construct the GRM compared to when using GGP-LD 30K SNP chip which is designed with *Bos indicus* specific SNPs as well as taurine. It was believed that proper rankings were somewhat restored using imputation to the 30K Illumina chip from the Weatherby's platform, however the core manifest appeared to lack SNPs to properly and fully impute *Bos indicus* haplotypes in Brahman and Brahman cross cattle. However, it must be considered that another reason for the poor imputation of *Bos indicus* haplotypes could have been a lack of pure Brahman animals in the reference population used for imputation.

3.4.2 Importance of minor allele frequency (Scenario 2)

Data cleaning is considered the first and most critical step in the majority of statistical analyses. In genomic analyses, cleaning of genomic data usually includes decisions based around SNP call rate, Hardy-Weinberg equilibrium and minor allele frequency (MAF) to remove spurious genotyping results (Miyagawa *et al.* 2008). The results herein demonstrated

that MAF was more impactful on GRM elements than SNP call rate, which may be expected as MAF had a bigger impact on the number of SNPs retained compared to SNP call rate.

When evaluating mixed, particularly *Bos indicus*, breeds, lower MAFs to keep SNPs that belong to individual breeds may be necessary to accurately evaluate their differences (Dr Rick Tearle, *unpublished data*). There are no guidelines to decide what MAF should be used for genomic analyses. In general using a MAF cut-off of 1-5% is accepted as reasonable (e.g. Saatchi *et al.* 2011; Watanabe *et al.* 2014; Vitezica *et al.* 2016) however it appears different cleaning procedures should be trialled so that an appropriate threshold can be determined for a given dataset. For example, in the tropically adapted dataset mentioned above, noticeable differences in ranking for days to calving EBVs for high Brahman content animals were observed when a MAF cut-off of 1% was used compared to 5% during GRM construction. The lower MAF threshold had the effect of separating the indicine and taurine groups more distinctly than the higher threshold. However reducing MAF further to 0.1% had no further impact (*personal communication*: Popplewell Composites). This suggests the existence of an "optimal" MAF. Since Wagyu are wholly taurine, it is less likely that differences in MAF on a taurine built chip will have such effect and a MAF cut-off of 5% is appropriate.

3.4.3 Performance of Lower SNP Densities (Scenario 3 & 4)

Studies have shown that approximately 300-400 SNPs are sufficient to separate admixed bred animals into stable breed groups (Harrison *et al.* 2012; Judge *et al.* 2017), indicating that lower density SNP panels should be sufficient to describe relationships in a population. An analysis based tropically adapted composites (Senepol, Belmont and Bonsmara breeds), genotyped with a 54,000 SNP array, reported high correlations (0.8) between a GRM built with all available SNP data and a GRM at a density of 1,000 SNPs. Additionally correlations of 0.9, 0.95 and 0.98 were achieved for 2,500, 5,000 and 10,000 SNP GRMs respectively. Given this,

relationships between animals were generally successfully characterised using a genomic relationship matrix constructed with at least 3,000 SNPs (Harrison *et al.* 2012). This pattern is consistent with the Scenario 3 results although correlations are lower, most noticeably at the sparser densities. This is most likely a function of the degree of relatedness between individuals. The Wagyu cattle are more likely to be in a breed group with a relatively high extent of whole genome LD due to smaller effective population size, estimated as 30 up to 1990 and then sharply dropping to ~17 by 1997 (Nomura *et al.* 2001), than other *Bos taurus* breeds (N_e ~ 100) or composite populations. Indeed, a study conducted in Angus (Rolf *et al.* 2010), resulted in correlations between GRMs that were higher than Harrison *et al.* (2012) across all SNP densities but slightly lower than that seen in Wagyu in general (Ogawa *et al.* 2014). This reflects that as Ne decreases, less dense maybe sufficient.

When comparing directly to Wagyu, the results herein were almost identical with previously published estimates. Ogawa *et al.* (2014) reported correlations between all the elements of the GRM built using lower SNP density subsets and all available SNPs (38,502) were 0.92, 0.96, 0.98 and 0.99 for 1000, 2000, 4000 and 10,000 SNPs respectively. One key difference is that these SNP subsets were selected to be evenly spaced, more akin to our Scenario 4 methodology. Regardless, the random subsets herein performed just as well indicating adequate genome coverage.

In regards to sensitivity of this analysis, Ogawa *et al.* (2017) demonstrated that the method of G matrix construction, the matrix proposed by VanRaden (2008) or the later proposed modification by Yang *et al.* (2010), gave no substantial difference in GEBVs at a given SNP density for carcass weight and marble score. This indicates a robustness in using low density SNP panels across estimation methods. Additionally, Rolf *et al.* (2010) demonstrated that 2,500-10,000 SNPs (randomly chosen) was adequate for robust estimation of Average Feed

Intake and Residual Feed intake GEBVs in sires estimated from genotyped commercial steer records i.e. can adequately identify regions of the genome identical by descent between nucleus and commercial families. This is key for genomic breeding programs where phenotype collection is primarily from commercial multiplier herds.

While elements (animal relationships) of GRMS built with varying SNP densities may be highly correlated, the actual values of the relationships themselves can have a strong weighting in the estimate of genetic variance components. The Bland-Altman plots (Figure 3.5 and 3.7) presented herein demonstrate this where differences between GRM elements were greatest around values of 0, although generally building GRMs from the base or random sample subset were in high agreement.

As the number of SNPs utilised increases, the estimated residual and genetic variances gradually decrease and increase respectively (Ogawa *et al.* 2014; Ogawa *et al.* 2017). This is due to higher SNP densities having higher LD levels between the SNP marker itself and true QTL region. In Wagyu, for carcass weight approximately 97% of the genetic variance estimated from a GRM constructed with ~38K SNPs, was obtained when using 10,000 SNPs whereas only 92% of the genetic variance could be obtained for marbling (Ogawa *et al.* 2014). This result indicates that certain traits may suffer a reduced rate of genetic gain compared to other traits when smaller SNP subsets are utilised. This could be due to marbling being controlled by many QTL of relatively small effects compared to those impacting carcass weight.

In addition supposedly “cheaper” commercial SNP chips containing ~3,000-4,000 SNPs are not in production or easily available and so may not prove as a viable option to decrease genotyping costs. Another alternative for these small chip arrays could be for parentage verification of commercial animals for data inclusion in a nucleus breeding program through a traditional pedigree relationship matrix (**A**) or even single step (**H**) matrix which combines

genomic and pedigree relationships (Legarra *et al.* 2014). Rolf *et al.* (2010) demonstrated a correlation of 0.86 between **A** and **G** constructed with ~41K SNPs. This correlation was equivalent to the estimation of a GRM built with 1,500 randomly selected SNPs.

3.4.4 Imputation Performance and Impact

As newly developed SNP arrays become commercially available, it can become expensive to re-genotype animals. Imputation is a commonly suggested solution which is achieved by starting from a common SNP base before generating genotypes up to a single SNP density/array. Although correlations between GRM elements were high when comparing Scenario 3 and 4 SNP subsets and the full suite of 20,955 SNPs, imputation had the resounding effect of increasing this correlation further (>0.98) for all SNP subsets. Imputation accuracy was an important determinate of these high correlations with imputation accuracies being high; mean imputation accuracies ranging from 0.92 to 0.99 when starting with the 1,250 minimum or 10,000 maximum sample respectively. Imputation accuracies herein are on par with previously reported estimates in Wagyu of 93.4 and 97.4% when imputing from 4,000 and 10,000 SNPs to approximately 38K SNPs (Ogawa *et al.* 2014), as well as within other pure breeds (Dassonneville *et al.* 2012; Ventura *et al.* 2014).

Ogawa *et al.* (2016) demonstrated that the use of imputed data resulted in a similar level of performance compared to using all the SNPs without imputation. Correlations between GEBVs (derived from genomic relationships) obtained with imputation and those calculated using all SNPs were higher than 0.99 for HSCW and marbling in line with the correlation between GRM elements herein. This is consistent with results in Dairy (Berry and Kearney 2011). This clearly demonstrates imputation as a useful tool to capitalise on potentially cheaper genotyping options. However, denser genotypes are still required on some animals to complete

imputation. This is likely cost effective within a nucleus herd with cheaper genotyping options being more applicable to commercial operations.

3.4.5 High Density (HD) Genotyping

For the imputed high density data, imputation accuracy was calculated on average as 0.93 but ranged between 0.84 and 0.94. This was estimated from 165 animals genotyped on both 30K and 770K platforms. In general, this is consistent with results published by Aliloo *et al.* (2018) with the correlation between real and imputed genotypes around 0.76 and 0.94 for 7K and 40K SNP respectively when imputed up to a 770K panel. Given the size of the reference population utilised herein, imputation accuracies to HD are appropriate when compared to other Wagyu (Uemoto *et al.* 2015). The accuracy of imputation with low density arrays largely depends on the choice of reference population with larger reference populations generally improving accuracy. For Wagyu, accuracy of imputation to high density arrays generally doesn't improve further once 400 animals are included in the reference (Uemoto *et al.* 2015) justifying potential further HD genotyping in the future. In addition, it is also important to have a highly related reference population to the target population.

As HD SNP arrays can be imputed to with excellent accuracy, this promotes their use within breeding programs due to imputation decreasing the cost of these genotypes. Utilisation of a HD SNP array resulted in a larger proportion of the additive genetic variance being explained in Wagyu than was found for 50K and 6K SNPs respectively (Ogawa *et al.* 2017). This resulted in higher heritabilities being obtained for key traits e.g. 0.48, 0.54 and 0.56 from 6K, 50K and 770K respectively for marbling score, which gave corresponding minor increases in accuracy. Minor increases in EBV accuracy have also been reported in dairy across production and fitness traits when using an Imputed HD SNP array (Khatkar *et al.* 2012). Lu *et al.* (2016) presented opposite findings for residual feed intake beef cattle with the HD array decreasing

accuracy in purebreds compared to 50K. However, the HD array showed clear advantages when considering composite/crossbreds as it ensured LD was consistent across these populations. This could be particularly beneficial for a Wagyu breeding program where crossbred Wagyu data is more widespread and accessible than full-blood data, as was the original plan for the thesis.

Prediction accuracy was not used as a criterium for assessment herein through cross-validation. This was due to limited data-set size available to undergo cross-validation and will be completed in the future with more records. More so, the correlations between the elements of the GRM were discussed. This is elaborated on in more detail in Chapter 5, however briefly, if the data used in the analysis is the same, with the only source of change being SNP density, then this change is spread of relationships is responsible for any changes in variance components.

While benefits of high density SNP arrays appear to only be minor, there is theory to support moving to whole genome sequence (WGS) in livestock breeding would bring about further advantages. These advantages include better persistence and higher accuracies of GEBV across generations as well as more accurate GEBV across breeds. The clear advantage of using WGS data is that the actual causal mutations for economically important traits should be in the dataset.

Hayes *et al.* (2014) reviewed and summarised these advantages. Firstly, better persistence of accuracy over generations is due to a shift from focusing on large chromosome segments to causal mutations. Predictions from medium density SNP arrays focus on primarily large chromosome segments that degrade due to recombination over generations, weakening accuracy. Building the prediction equation based on the effects of causal mutations, would lead to accuracy persisting over many generations, and in more distantly related animals. This

would also be accompanied by the second advantage, which would be an increase in prediction accuracy. As current SNP arrays select SNPs with a high minor allele frequency, it is less likely that these platforms will have a SNP in linkage disequilibrium with a causal allele at low frequency. If this variation from rare alleles could be described within WGS, and used in prediction equations, significant improvements in accuracy could be yielded. Finally, for populations where assembling large reference populations is difficult (like niche/minor breeds), utilising WGS data across breeds would be appealing. While accuracy of multi-breed evaluations is close to zero with standard SNP arrays, the causative mutations which do segregate across breeds could be captured and used for prediction with sequence data. Across breed information could even benefit large populations like Holstein for hard to measure traits or for causal mutations at low frequency within Holsteins making them difficult to characterize.

3.5 Conclusion

The results of this study have demonstrated that small SNP subsets of at least 2,500 -5,000 SNPs are sufficient to describe genomic relationships across this Wagyu population. The similarity between these subsets, and the base scenario was improved with the use of imputation. In addition, animals were successfully imputed to a HD SNP platform which, when compared to the base scenario, showed an increased spread of relationship values, especially in the more inbred animals. This could have a beneficial flow on effect in genomic evaluations, leading to more accurate BLUP predictions.

While the cost of HD genotyping is rapidly decreasing, the relatively high cost is still a barrier to wide adoption in animal breeding programs. Especially as whole genome sequencing costs continue to fall. Imputation technology serves as a bridge to make the utilisation of such in depth data more accessible to the broader population of animals.

Chapter 4 : Comparison of Methods to Select Reference Candidates for Whole Genome Sequencing in an Australian Wagyu Population

4.1 Introduction

Genomic selection has been rapidly adopted by many breeding sectors following its successful introduction to the dairy industry. This is due to realised gains in prediction accuracy of genomic estimated breeding values that has increased the response to selection for key economic traits as greater proportions of genetic variation are explained and generation intervals can be decreased (Hayes *et al.* 2009a).

In genomic selection, a sufficiently dense single nucleotide polymorphism (SNP) panel that covers the entire genome is utilised, under the expectation that all quantitative trait loci (QTL) are in linkage disequilibrium with at least one SNP. This allows the prediction of QTL effects across the population over generations. For traits with few underlying QTL, lower density SNP panels may be sufficient to capture these effects, assuming close proximity of at least one SNP. However, where underlying QTL are many, denser SNP panels may be required (Hayes *et al.* 2009a). This is often the requirement for many traits in cattle breeding, such as fertility, where no QTL of major effect has been found; unlike milk fat percentage in Dairy (Grisart *et al.* 2002). As discussed in the previous chapter, denser SNP panels have been shown to increase breeding value accuracy (Khatkar *et al.* 2012; Ogawa *et al.* 2017). If there are very many QTL of minor effect contributing to variation in a desired trait, a large number of

phenotypic records will be required to achieve reasonable estimation accuracies, relative to trait heritability (Goddard 2009).

With the size of the reference population clearly having impact on the accuracy of genomic prediction in the target population, there is a clear need to identify cost-effective methods to procure more phenotypes. One solution would be to capitalise on the large numbers of phenotypes available in commercial herds using genotyping to replace often incomplete/missing pedigree data. However, this solution would be accompanied by high genotyping costs which usually only nucleus herds have means for.

In 2010, the Illumina BovineHD chip became available with 777 962 SNPs and now whole-genome sequencing is the new frontier (Georges 2014; VanRaden *et al.* 2017). However the high price of sequencing and HD chips is a barrier to their application across large numbers of animals.

Imputation can add value here. By investing in a good reference population of dense genotypes, imputation can then utilise cheaper, less dense SNP panels which reduces the overall cost of genotyping while capitalising on high density results. This was demonstrated and discussed in the previous chapter. Given this, which animals should be densely genotyped to form the reference set for imputation of sparsely genotyped animals? An ideal approach would be to select founder animals of the population, but the availability of this option is limited depending on population age (are the founders still alive/have DNA stored i.e. semen). A second approach would be to select influential animals with large numbers of effective progeny. However, this may bias certain high performing family groups by selecting relatives from a few family lines. In most cases, a set budget is implied.

The aim of this work was to identify a set of animals in a population of Japanese Wagyu, which when densely genotyped, would give the highest imputation accuracies. Strategies were

compared that fall under two categories; 1) Strategies that utilise relationship matrix data already available in routine BLUP and GBLUP analyses and 2) Strategies that take a more bioinformatics approach based on population haplotype frequency. Measures of how efficiently animals were selected, similarities between animals selected and imputation accuracies from low to mid SNP densities are discussed.

4.2 Materials and Methods

In total, five methods were trialled and compared to select candidates for whole genome sequencing in an Australian Wagyu population. The first two methods were described by Yu *et al.* (2014) denoted the MCA and MCG method. These methods select candidates for whole genome sequencing by minimising the genetic variation of the target population, relative to the selected pool, in order to improve imputation accuracy from the target density to the selected density (HD or whole genome sequence). The MCA method utilises (Wrights) numerator relationship matrix (**A**) such that;

$$\mathbf{A}_{11}^{*} = \mathbf{A}_{11} - \mathbf{A}_{12}\mathbf{A}_{22}^{-1}\mathbf{A}_{21}$$

Where the 1 subscript denotes the set of target animals and 2 subscript denotes the set of animals selected to be sequenced. The diagonal elements of $\mathbf{A}_{11}^{*}$ are the residual variances that are expected to remain if sequence data were to be obtained from the selected individuals and used to predict/impute genotypes of the target set. Animals were selected using an iterative process. An Australian Full-Blood Wagyu pedigree comprised of 10,549 individuals with a depth of up to 9 generations from the current generation was utilised to construct **A** through the R package pedigreemm (Bates and Vazquez 2014).

The second method (MCG) is akin to MCA but utilises a genomic relationship matrix (**G**) in place of **A**. **G** was constructed as per VanRaden (2008) method 1, utilising genotype

information on 5,334 individuals genotyped with 30K GGP-LD (Neogen: GeneSeek Operations) or Bovine VersaSNP 50K (Weatherbys Scientific) chips. Animals genotyped on VersaSNP 50K were imputed to 30K from the 11,484 SNPs that overlapped between the chips, due to the significantly larger reference population available (4940 vs. 394), using Fimpute 2.2 (Sargolzaei *et al.* 2011). After imputation, SNPs were retained that had a minor allele frequency greater than or equal to 0.05 before building the GRM. All genotyped animals were present in the pedigree resulting in an overlap of 5,334 animals between the numerator (**A**) and genomic (**G**) relationship matrices.

The third and fourth methods were described by Bickhart *et al.* (2016), and referred to as AHAP2 (Bickhart *et al.* 2016 modified the AHAP method presented by Druet *et al.* (2014)) and the inverse weight selection method (IWS). Both methods require the construction of a haplotype "block" library. This library was constructed utilising the 5,334 post imputation genotypes to construct **G**, using FindHap v3 (http://aipl.arsusda.gov/software/findhap/). Program settings included 4 iterations at 3 haplotype block widths (50, 75, 100 SNPs). Only the 100 SNP wide blocks were retained for analysis. Haplotype blocks, which by definition are non-overlapping, were assigned a unique ID and their frequency in the dataset was calculated. It was assumed that haplotype frequencies in this population are reflective of the Australian Industry. In total 339,824 unique haplotypes were identified with a mean haplotype frequency of 0.07% and a minimum and maximum haplotype frequency of 0.005% and 0.28% respectively. The distribution of haplotype frequencies on the log scale (Figure 4.1), clearly indicated a skewed distribution towards lower frequencies. Due to logarithmic increases in haplotype counts at lower frequencies, haplotypes with a frequency lower than 0.1% were excluded from consideration. This brought the total number of haplotypes under consideration for sampling down to 20,854 of which 588 had a haplotype frequency ≤ 5% (Common), 3,666 had a frequency ≥ 1% but < 5% (Uncommon) and 16,600 had a haplotype

frequency ≥ 0.1% but < 1% (Rare). A haplotype frequency threshold of 0.1% was chosen to allow for 1 in 1000 error in genotype calls.

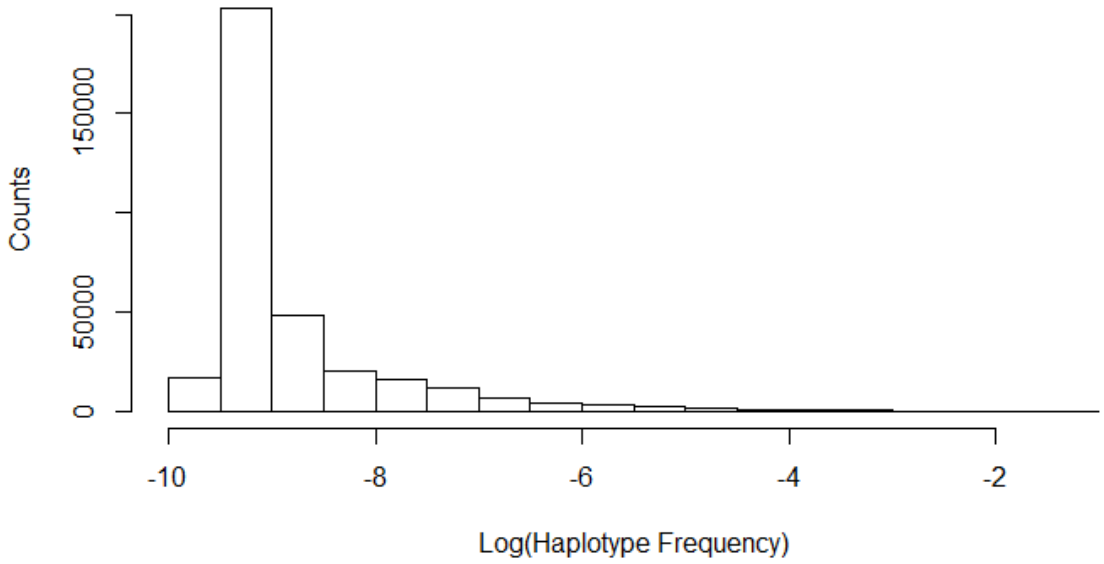

Figure 4.1: Distribution of Haplotype block frequency (log scale) of 339,824 blocks, 100 SNPs in width, estimated from a population of 5,334 genotyped Australian Wagyu.

Both the AHAP2 and IWS methods are designed to maximise the haplotype coverage from the population while minimising redundancy of haplotype sampling. Both methods choose candidates to maximise the number of haplotypes sampled per dollar invested in sequencing, achieved through a weighting system, however two separate approaches are used to achieve this.

The AHAP2 method, which is an iterative modification on the AHAP method described by Druet *et al.* (2014) utilises the following equation;

Sample weight = $\sum_{i=1}^{NHAP} f_i$ if *i*= homozygous.

The frequency of the haplotype in the population is defined by f_i as determined by FindHap, and *NHAP* is the total number of haplotypes under consideration. Only haplotypes that are

homozygous within a potential candidate are counted towards the weighting for selection. All individuals in the imputed genotype set were considered as potential candidates. After calculating the weight for all individuals, the individual with the highest weighting is selected as the sequencing priority. Once a candidate is chosen, all homozygous haplotypes that this candidate contained are removed from consideration for all remaining samples. Sample weights are then recalculated and the next sequencing candidate is selected until the desired number of candidates (n = 100) are sampled.

In reverse to the AHAP2 method, the IWS method preferentially selects candidates that carry rare frequency haplotypes. Bickhart *et al.* (2016) developed an inverted parabolic function that calculated sequencing priority (weighting) under the following equation;

Sample weight = $\sum_{i=1}^{NHAP} f_i^2 - 2f_i + 1$ if *i*= homozygous.

As f_i approaches 0, the haplotypes score approaches 1, increasing the weighting. More frequent haplotypes give an increasingly smaller weighting to the sample.

The final method is a more traditional approach that selects animals based on influence in pedigree. This was to assess a previous attempt to genotype animals that 'describe' the population. Previously 166 Full-Blood Wagyu animals were genotyped on the Illumina 770K platform. These animals were selected as influential due to having greater than 10 progeny nationwide, with effective progeny numbers of 1 to 437, mean = 47, in the pedigreed population descirbed herein. One hundred of the 166 animals were randonly chosen (**RAND**) for comparison against the other methods.

4.2.1 Calculating Imputation Accuracy

Imputation accuracy, described here as the correlation between true and imputed genotypes (r), was calculated only for the 4,940 individuals genotyped on the 30K chip by masking their

true genotypes to the ~11K overlap density. Seven rounds total of single replicate genotype imputation (Fimpute 2.2) was then carried out using 4 reference population sizes (100, 50, 25, 10) of animals selected for whole genome sequencing by MCA, MCG, IWS or AHAP2.

For MCA, out of the 100 selected animals utilising pedigree, only 75 also had genotypes and so could be used to calculate imputation accuracy. For MCG only this meant that only reference populations of 50, 25 and 10 selected candidates could be constructed. In forming the imputation reference populations of MCG selected candidates, if a candidate was selected but un-genotyped, the next available genotyped candidate was selected in their place. This means that MCG imputation reference populations were not developed using perfect ranking of candidates but can be used as an example.

For IWS and AHAP2, imputation accuracy was calculated from the top 100 animals selected if those animals were previously genotyped on the GGP-LD 30K platform. For IWS, this meant that imputation accuracy was only able to be calculated for 87 individuals as the method selected 13 animals that were genotyped on the newer Versa 50K chip.

4.3 Results

4.3.1 Overlap between chosen candidates

The degree of similarity between the MCA and MCG methods was very high with MCA selecting 70/100 individuals (Table 4.1) that were selected by MCG. Of the animals that were selected by both methods, they were ranked very similarly with a strong positive rank correlation of 0.82 (Figure 4.2). As MCA contains animals that are not available in MCG, a modified version of the MCA method was run (data not shown) where only the 5,334 genotyped animals could be chosen but still relative to the whole pedigreed population i.e. genotyped animals were selected based on their relationship to all animals in the pedigree.

This produced similar results with 73 animals being selected in common between MCA modified and MCG.

There is little overlap between the relationship matrices' methods and the haplotype methods AHAP2 and IWS (Table 4.1). For example, the specific animals themselves selected by IWS are all progeny or grand-progeny of those selected by MCG and/or MCA. There was a moderate similarity between animals selected by IWS and AHAP2. Differences are due to different emphasis weights on rare versus common haplotypes.

It is important to reiterate that all methods used the same starting population of 5,334 genotyped animals where appropriate (i.e. MCA utilised a much bigger pedigreed population). Additionally all genotyped animals were in the pedigree.

Table 4.1: The degree of overlap i.e. the number of animals selected in common, between the MCA, MCG, IWS and AHAP2 methods. The number of animals sampled by each method is displayed on the diagonal.

	MCA	**MCG**	**IWS**	**AHAP2**
MCA	100			
MCG	70	100		
IWS	5	7	100	
AHAP2	2	4	61	100

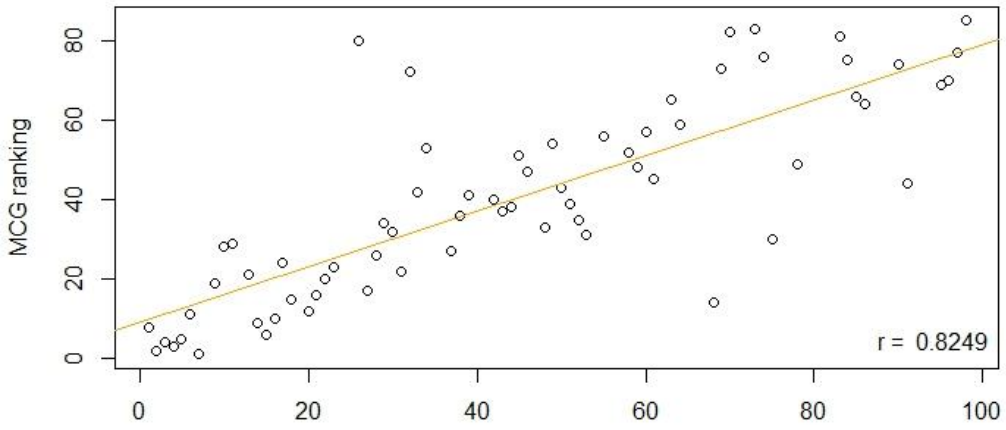

Figure 4.2: Plot of ranks of candidates selected for whole genome sequencing using the MCA or MCG methods respectively

4.3.2 Percentage of Genetic Variance Explained

The MCG method did account for slightly more genetic variance reaching 34.6% when 100 animals were selected compared to 30% accounted for using the MCA method. The first 20 selected animals accounted for 19% and 21% of the genetic variance for the MCG and MCA method respectively with each additional animal there after contributing less information (Figure 4.3). Where the number of selected candidates was low, MCA outperformed the MCG method until approximately 30 candidates where MCG became superior. IWS was superior to AHAP2 accounting for 23.3% of the genetic variance compared to 22.9% when selecting 100 candidates, although both methods accounted for significantly less genetic variance compared to methods utilising a relationship matrix. For RAND, the mean percentage of genetic variance accounted for when randomly sampling 100 of the most influential sires for 5 replicates is 29.6% (SD = 0.40, data not shown) equivalent to the MCA method. MCA modified, where only genotyped animals are available for selection relative to the whole pedigree, account for 29.3% of the genetic variance, giving very similar results to MCA.

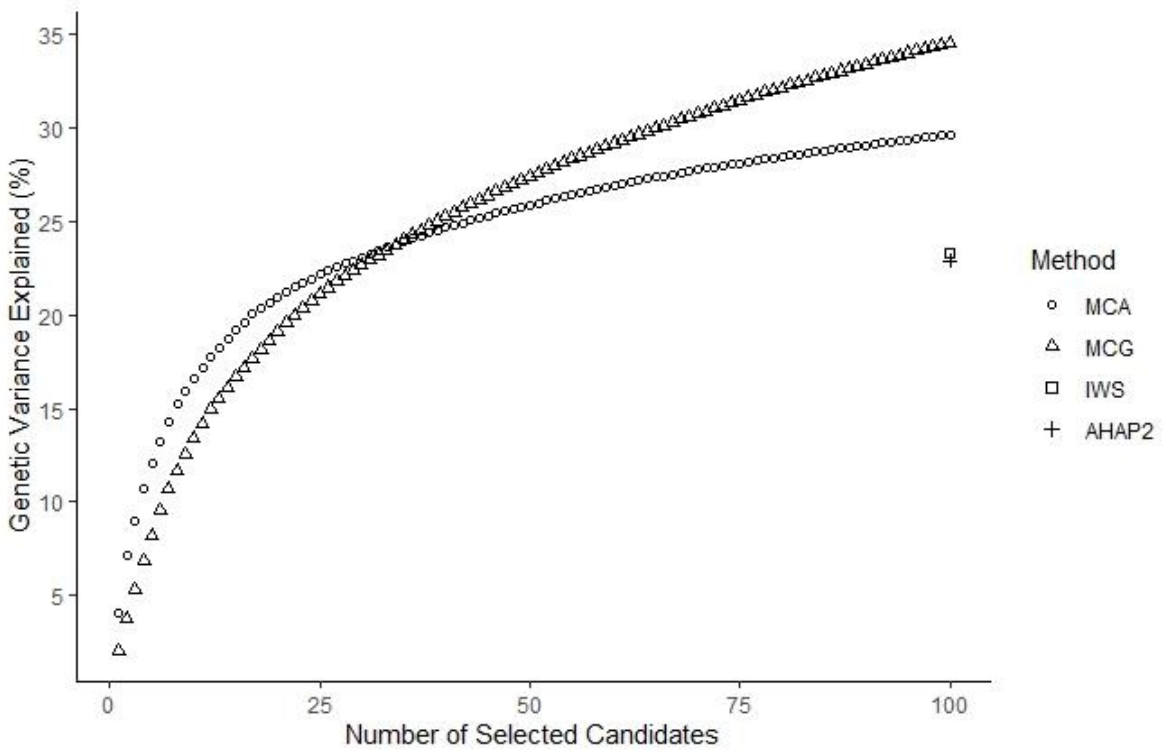

*Figure 4.3: Diagonal values of **A*** representing the percentage of genetic variance explained for each additional selected candidate for whole genome sequencing using the MCG method (top) or MCA method (bottom). The IWS and AHAP2 methods are presented as singluar dots where 100 animals have been sampled.*

4.3.3 Number of Unique Haplotypes accounted for

Haplotype blocks were categorised into common, uncommon and rare classifications based on frequency in the population. The number of haplotypes accounted for within each group was then assessed for three methods (Table 4.2). All methods were able to account for the 588 unique common haplotypes in the population and a similar number of uncommon haplotypes; approximately 3500 haplotypes out of the 3666 in the population. The three methods begin to clearly separate where rare haplotypes are considered. MCG accounted for 8175 rare haplotypes followed by IWS and AHAP2 with 6492 and 5137 respectively. This resulted in MCG accounting for the highest total number of haplotypes (12,320) compared to IWS and AHAP2.

Table 4.2: Number of unique haplotypes accounted for when 100 animals are selected as whole genome sequencing candidates using varying methods that utilise a relationship matrix (MCA/MCG) or haplotype library (IWS/AHAP2) respectively.

Method	Common	Uncommon	Rare	Total
	≥5%	1%- <5%	0.1% - < 1%	
MCA*	**-**	-	-	-
MCG	588	3557	8175	12320
IWS	588	3507	6492	10587
AHAP2	588	3524	5137	9249
Max #	**588**	**3666**	**16600**	**20854**

*As not all MCA selected animals were genotyped, the number of unique haplotypes accounted for cannot be estimated.

4.3.4 Imputation Accuracy

Across methods, mean imputation accuracy increased with increasing reference population size. In addition, the range of imputation values decreased with increasing reference population size. The mean imputation accuracies for MCA using 50, 25 or 10 reference animals were comparable to MCG although MCG was slightly superior (Table 4.3). Noticeably, MCA did have a much higher minimum imputation accuracy of 0.67 compared to 0.55 for MCG which indicates a narrower spread of accuracies giving more successful imputation overall. The two haplotype methods, IWS and AHAP2, gave comparable mean accuracies of 0.97 each to MCA/MCG, with the highest maximum accuracies reported although they also had the largest spread.

Table 4.3: Imputation accuracy calculated for sparse 11K genotypes imputed to 30K using differing reference populations of different sizes selected from four methods.

MCG					**MCA**				**IWS**	**AHAP2**
Ref size	100	50	25	10	100*	50	25	10	87**	100
Min	0.55	0.55	0.55	0.51	-	0.67	0.53	0.51	0.52	0.54
Mean	0.98	0.97	0.94	0.83	-	0.96	0.93	0.83	0.97	0.97
Max	0.99	0.99	0.99	0.97	-	0.99	0.99	0.99	1.00	1.00

* For MCA, out of the 100 selected animals, only 75 were genotyped and so could be used to calculate imputation accuracy. For comparisons sake, only reference populations of 50, 25 and 10 were constructed. The next available candidate was selected and so reference populations do not display perfect ranking but can be used as an example. **Only 87 animals selected by IWS had 30K genotypes to calculate imputation accuracy. The remaining 13 candidates were genotyped using the 50K Versa platform.

4.4 Discussion

4.4.1 Comparison of Relationship Matrix Methods

The methods which utilised a relationship matrix, MCA and MCG, had very high concordance between them in regard to specific candidates selected (Table 4.1). The rank correlation reported of 0.82 (Figure 4.2) is a stronger relationship than previously reported Yu *et al.* (2014). One explanation is Wagyu in Japan are known to already have a very small effective population size with only a small number animals serving as the founder population for Australia's herd today. Given this, the MCG and MCA method are more likely to select identical candidates than the population in the original study which was a Norwegian pig population pedigree with simulated genotype data (Yu *et al.* 2014).

MCA performed better where the number of selected candidates was low (Figure 4.3). This is likely due to the MCA method having access to the full pedigree of 10,549 individuals with a depth of up to 9 generations, whereas only 5,334 of these animals were available for selection under MCG. There are some population structure implications in the data behind this. The pedigree includes deeper information on original "imported" founder animals in the

population and a larger number of descendants, whereas MCG only includes genotypes on these founders and a subset of their descendants. The additional depth and breadth of pedigree appears advantageous to better inform selection decisions of early selected candidates. MCG appeared robust as the genomic relationships were able to compensate for lack of pedigree depth after a certain number of selected candidates due to more detailed relationship information regarding Mendelian sampling. When only the genotyped animals could be selected as candidates (MCA modified), it performed extremely similarly to MCA on a whole. This supports the conclusion that the pedigree used in constructing **A** is not adding any information above and beyond what **G** captures. MCG also demonstrates a steady increase in genetic variance accounted for as the number of candidates approaches 100 whereas MCA begins to level off. This can again be attributed to more variation being able to be discerned through genomic relationships which can better describe animals, particularly where relationships would be traditionally low (zero) in A and between full-sibs.

4.4.2 Comparison of Haplotype Block Methods

The methods which utilised 100 SNP wide haplotype blocks, IWS and AHAP2, had moderate concordance between the animals selected with 61/100 animals in common. In contrast, concordance between these methods and candidates selected by MCA and MCG was poor (Table e.1). An analysis of the pedigree reveals the specific animals themselves selected by IWS, in particular, are all progeny or grand-progeny of those selected by MCG/MCA. This makes sense as only homozygous haplotypes are considered in calculation of the weighting. Influential haplotypes being targeted (those accounted for by MCA/MCG) must be passed on across generations through paternal and maternal lines to be selected by IWS, and to a lesser degree the AHAP2 method.

While MCG accounted for the greatest number of haplotypes with a frequency of 0.1% or greater (12,320, Table 4.2) it did not account for the greatest number of haplotypes overall when counting haplotypes below this frequency. Candidates selected using the cut-off restrictions were compared to the unrestricted raw data to get a view of the incidental rare haplotypes that were sampled in passing. IWS, AHAP2 and MCG accounted for an additional 9842, 7221 and 2631 haplotypes respectively below a frequency of 0.1% resulting in grand-totals of 20429, 16470 and 14951 haplotypes sampled out of 339824 respectively. Given this metric, IWS was the best where total number of haplotypes are concerned. Results from Bickhart *et al.* (2016) are consistent to those above with IWS demonstrating it accounted for the greatest number of haplotypes while selecting the least number of candidates compared to AHAP2. Additionally, given a set number of candidates, IWS accounted for more haplotypes than AHAP2 which is a more comparable metric to the study herein.

A study on simulated dairy data performed by Butty *et al.* (2019) demonstrated similar findings to the study herein with IWS accounting for a greater proportion of unique haplotypes (when all incidental haplotypes are included) than a method analogous to MCG. In addition, the overlap of selected candidates was very low between these methods across varying selection densities (50 to 1200 individuals). However, IWS did not outperform MCG in terms of genetic variance accounted for (Figure 4.3). Initial thoughts in this study were that the more haplotypes accounted for, the greater the degree of genetic variance explained, but Figure 4.3 demonstrates that is clearly not the case. There could be a couple of explanations for this.

The IWS method is intentionally selecting animals that are more distantly related to others by preferentially selecting rare haplotypes. Animals that are homozygous for a rare haplotype had to receive one copy from each of the paternal and maternal lines, which to occur suggests the paternal and maternal lines were already likely related i.e. IWS selects animals from the

ends of different family branches rather than the bulk of the whole family tree. Additionally, given the haplotype blocks used aren't representative of "actual" haplotypes segregating in the population, they are merely chunks of SNPs in 100 SNP wide blocks; selection of individuals where these true haplotypes are essentially broken up could explain a loss in genetic variance accounted for. In contrast the GRM utilises all SNPs, it can capture the similarity of true haplotypes between individuals in its estimation of relationships.

Another point for consideration is that, while it could be expected that more haplotypes in the reference would yield higher imputation accuracies, IWS preferentially selected haplotypes with a low frequency. Daetwyler *et al.* (2014) demonstrated using initial data from the 1000 bulls genome project that accuracy of imputed calls was high for SNPs with a MAF > 0.1 while it decreased rapidly for rarer variant sites. Butty *et al.* (2019) demonstrated this nicely showing imputation accuracy of specific variants increases with MAF bin. Additionally, Butty *et al*. (2019) showed that reference populations selected by IWS were more effective at achieving high imputation accuracies for low MAF SNPs than other methods compared, but this advantage lessened with increasing reference population size.

The small scale study within achieved relatively high imputation accuracies overall but did not investigate the accuracies of SNPs of low MAF versus high MAF. Additionally the SNPs are filtered to only include those with a MAF ≥ 0.05 when the GRM is constructed. This effectively removed the less accurately imputed SNPs from consideration but could also be removing important genetic variation as well. As high density genotyping and sequencing costs decrease, it would be more feasible to target lower frequency haplotypes by sequencing additional candidates to improve their accuracy of imputation. Methods, such as those proposed by Ros-Freixedes *et al.* (2017) that allocate sequencing resources to specific haplotypes rather than individuals would be suitable for this purpose, in fact they propose an

adjustment to IWS to allow for this. The benefit of the method proposed by Ros-Freixedes *et al.* (2017) is that it assembles high-coverage sequence data through the accumulation of low coverage information over genome segments that are shared with many other individuals. This prevents these 'census' haplotypes from being 'over-sequenced' so that sequencing resources can then be allocated towards key-rare variants for example. A target sequencing depth (i.e. 10x) needs to be defined and the aim is to get all desired haplotypes to this target.

4.4.3 Practical Considerations

While the haplotype block methods appeared promising, their performance was inferior to relationship matrix based methods given the metrics measured herein. One-hundred animals selected under MCG accounted for the most genetic variance, accounted for the greatest number of haplotypes (above a frequency of 0.1%) and gave high imputation accuracies. But this was due to one key assumption, both the MCA and MCG method assumed that all potential selection candidates had DNA available for sequencing and in a commercial pedigree this is not always the case. This fact became partially evident in the imputation study where not all MCA selected candidates had genotypes to form the reference. This is an important consideration and both methods could be easily modified to account for this. Within an iteration, the animal that is selected is logically the one that reduces the residual genetic variance of the target population i.e. Diag($\mathbf{A}_{11}^{*}$), the most. Multiplying each candidates' impact on the residual by a simple vector of 0 (no DNA available) or 1 (DNA available) would ensure that only candidate animals with DNA are selected. This would also prevent bias when selecting sequence candidates to form the reference if you were just to remove animals with no DNA from the analysis all together. MCA clearly outperformed MCG where the number of samples selected was low and this could reflect a scenario where the sequencing budget is low. A strong depth of pedigree proved advantageous to the GRM where number of selected

candidates is low. To capitalise on depth of pedigree while utilising the detail of genomic relationships an **H** matrix could be constructed as is done for single step GBLUP (Legarra *et al.* 2009; Christensen and Lund 2010) with parameters set around DNA availability.

The relationship matrix methods also have one key advantage over haplotype methods when being applied within a breeding program. That is they utilise data that is routinely constructed within a genetic evaluation program and are therefore simple and relatively quick to implement. This is compared to constructing haplotype libraries where cut-off decisions around haplotype inclusion must be made. This decision can impact the final animals that are selected for HD genotyping or sequencing. For example, the cut-off used for IWS by Bickhart *et al.* (2016) was 4% whereas it was 0.1% herein.

In addition, the examples provided in this discussion assume selection within one population of animals and does not deeply discuss implications of across breed or crossbred populations.

4.5 Conclusion

Selection using the MCG is highly recommended as a starting point for an on-going sequencing project. Then the best method depends on the use case for the future set of sequences. If the aim is to select sequence candidates to allow for the overall imputation of the population, then it is better to select for animals carrying common haplotypes in the first instance. If the resulting sequences from the selected animals are to be used for variant discovery or annotation of deleterious variants, animals carrying novel information should be selected.

Chapter 5 : Impact of high density genotyping on genomics best linear unbiased prediction estimation and subsequent selection decisions

5.1 Introduction

Genomic selection (Meuwissen *et al.* 2001) has been widely researched and discussed in this thesis thus far, particularly regarding implications in a high quality beef breeding program. Traits of key interest are those pertaining to marbling and marbling characteristics, such as those defined by AUS-MEAT and Meat Image Japan (MIJ).

Genomic selection is facilitated through the use of SNPs that are in linkage disequilibrium with genes controlling the trait of interest. It is assumed that these genes behave in a similar manner across the whole population under selection (Tier *et al.* 2015). Relationships between animals in a population can then be calculated and when combined with phenotypic knowledge produce an estimate of genetic merit. This estimate, referred to as an estimated breeding value (EBV), informs the selection decisions for the next generation of parent stock. Chapter 3 demonstrated that the number of SNPs required to estimate relationships accurately for Wagyu was low, with only 2,500 - 5,000 SNPs required. However as the number of SNPs increased, the relationships became more accurate when compared to the base scenario (20,955 SNPs), consistent with previous studies (Rolf *et al.* 2010; Harrison *et al.* 2012; Ogawa *et al.* 2014).

Using a commercial density SNP panel (30K) the heritabilities of key traits were high (Chapter 2). For example, AUS-MEAT marble score was found to be highly heritable (AUS_MARB, 0.50) and MIJ marbling percentage was very highly heritable (I_MARB, 0.68, Table 2.2). Undesirable marbling characteristics, such as MIJ marbling coarseness, was also highly heritable (I_COARSE, 0.53) and was strongly positively genetically correlated to I_MARB (0.64, Table 2.5) indicating higher marbling is achieved through increasing the size of marbling flecks as well as the number. An index trait that increases marbling through more fine marbling particles (I_FINE) reported a very strong positive genetic correlation to I_MARB (0.77, Table 2.5) and was suggested as an alternative to I_MARB to manage marbling characteristics. Overall, key traits of interest were all moderately to highly heritable, which is desirable to make genetic progress. Given the very high economic value of high marbling animals, any small improvement could have a significant impact on profit achieved.

As discussed in Chapter 3, high density SNP panels more accurately describe relationships and increase breeding value accuracy (Khatkar *et al.* 2012; Ogawa *et al.* 2017). Procuring high-density SNP chip panels can be an expensive investment, however, through the use of imputation, a large number of high density genotypes can be acquired from cheaper, sparse genotypes. This can be achieved with accuracies ranging from 0.84 to 0.94 when imputing from 30K to 770K with a reference population of 165 individuals (Chapter 3). Important factors to consider when imputing are, that accuracy is greatest when the reference population is 1) large and 2) has strong relationships to the target. In Chapter 4, a comparison was made between four methods of selecting individuals for the reference population with the aim to allocate funds appropriately to get the best imputation results across the whole population. Methods that utilised a relationship matrix accounted for approximately 10% more genetic variance in the population with their selected candidates than methods considering haplotype blocks. However, all methods were comparable in regard to their imputation accuracies

attained. The reference population for this chapter was selected using the MCG method which selects candidates from a genomic relationship matrix that are closely related to the population but distantly related from previously chosen candidates (Chapter 4). This method is described below.

This chapter builds on the genetic parameters generated in Chapter 2 using low density SNP data and aims to supplement results, demonstrating relationships are better captured using high density SNP (through imputation, Chapter 3) due to describing greater population additive genetic variance. Particularly, changes in EBVs and subsequent animal selection decisions is discussed. To facilitate accurate imputation, selection of reference candidates was facilitated using the MCG method (Chapter 4).

5.2 Materials and Methods

5.2.1 Genotype Data

The starting genotype data for this study was the same as for Chapter 2 comprising of 4,940 GGP-LD 30K genotypes, all full-blood Australian Wagyu of which 29,869 SNPs were segregating in the population. These 4,940 individuals were imputed to a high density (HD) SNP array of 777,107 SNPs (Illumina BovineHD BeadChip). Imputation was completed using FImpute 2.2 (Sargolzaei *et al.* 2011), with 165 animals, which had been genotyped with the HD SNP array, as the reference population. SNPs were removed from the reference genotypes that were unable to be mapped to precise chromosome/base pair location as well as SNPs with identical chromosome and base pair positions; in this instance one SNP from these duplicate or triplicate locations was kept in the dataset. In addition, SNPs were excluded if they had ≥ 5% missing data (95% call rate) and/or a minor allele frequency (MAF) less than 0.05. This left the final imputation density as 479,535 SNPs which were used to construct a GRM as per

VanRaden (2008) method 1 (Section 2.2.1). Data obtained when using the 30K and 770K datasets are referred to as Low-Density (LD) and High-Density (HD) respectively.

5.2.2 Selection Reference Population for Imputation

The initial reference population was to be made up of 70 animals selected from a population of 5,334 Full-Blood Wagyu using the MCG method (Yu *et al.* 2014), described in chapter 4.2. The 70 selected animals were initially planned to undergo whole genome sequencing to form a reference population to impute the remainder of the herd to sequence. However technical difficulties with sample DNA extraction made this method unavailable so a different approach has been taken.

Due to delays in obtaining sequence data it was decided to proceed using animals with 770K genotype (HD) data to form a reference population. Given budget limitations and DNA availability, a reference population consisting solely of the top 70 animals could not be formed as they did not all have 770K genotypes. The 165 currently available animals with 770K genotypes were used instead. The preferred method discussed in Chapter 4 is the MCG method detailed by Yu *et al.* (2014) and given by the equation;

$$\mathbf{G}_{11}^{*} = \mathbf{G}_{11} - \mathbf{G}_{12}\mathbf{G}_{22}^{-1}\mathbf{G}_{21}$$

Where the 1 subscript denotes the set of target animals and 2 subscript denotes the set of animals selected to be sequenced. Diag(G_{11}*) are the residual variances that are expected to remain if sequence data were to be obtained from the selected individuals and used to predict/impute genotypes of the target set. Given this, the genetic variance accounted for by the set of 165 animals with HD genotype data can be calculated and compared to “ideal” top 70 selection. The 165 animals with HD genotypes on hand accounted for 38% of the variance in the population compared to 31% when the top 70 were selected. There was an overlap of

63 animals between the 165 used as the reference population for imputation and the top 70 originally selected by MCG.

5.2.3 Phenotype Data and Statistical Analysis

Phenotype data for this study is presented in Chapter 2 (Table 2.1). Briefly, a subset of phenotype records were made available on individuals born from 2009 to 2017 for 14 different traits spanning live weight and ultrasound measurement to carcass measures in line with Australia national AUS-MEAT evaluation system (AUS-MEAT Limited 2005) and Meat Image Japan camera image technology (Kuchida *et al.* 2006; Maeda *et al.* 2014). Records made available include 1079-1091 on carcass traits, 3073 for live ultrasound measures, 2252 for birthweight, 2990 for 400 day weight (400_WT) and 1462 for Daily feed intake (DFI).

Datum was analysed with a general linear model mixed model using ASReml-R 4.0 (Butler *et al.* 2017). Model descriptions for univariate analyses can are the same as outlined in Chapter 2.2.3. The model utilised was;

$$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Zu} + \boldsymbol{e}$$

Where, $\boldsymbol{b}$ is the vector of fixed effects, $\boldsymbol{u}$ is the vector of random effects (with $\boldsymbol{X}$ and $\boldsymbol{Z}$ their respective design matrices) and $\boldsymbol{e}$ is the vector of residual variance. All traits included fixed effects of dam age (Maiden; < 2 year of age, Mature; 3-9 years or Old; > 10 years), heterozygosity (calculated as the proportion of heterozygous genotypes, Figure 2.1), Sex (Heifer, Bull or Steer, except for BW which just had two levels i.e. Heifer or Bull) and contemporary group based on a predefined age slice that grouped animals born within the same year and calving period. A birth date co-variate was also fitted nested within management group. Management groups are described in greater detail in 2.2.3.

Genomic best linear unbiased predictions (GBLUPs or Estimated breeding values or EBVs) generated from previous analyses in Chapter 2 are compared to GBLUPs estimated from an imputed high density GRM (479,535 SNPs). Correlation between individuals EBVs from the two datasets were estimated and changes to animal rankings discussed.

5.3 Results

5.3.1 Heritability Estimation

Heritabilities were estimated for 14 traits using LD (30K) and HD (770K) genotypes. For every trait, the heritabilities estimated using HD data were equal to or higher than when LD data was utilised (Figure 1) with a mean increase of 0.03 (standard deviation = 0.02). The heritabilities most improved were MIJ percentage marbling (I_MARB), MIJ marbling fineness index (I_FINE), MIJ percentage marbling minus largest marbling particle (I_MARB2) and AUS-MEAT marble score (A_MARB) with a heritability increase of 0.05. Ultrasound eye muscle area (U_EMA) and P8 fat depth (U_P8) showed no improvement in heritability using HD data.

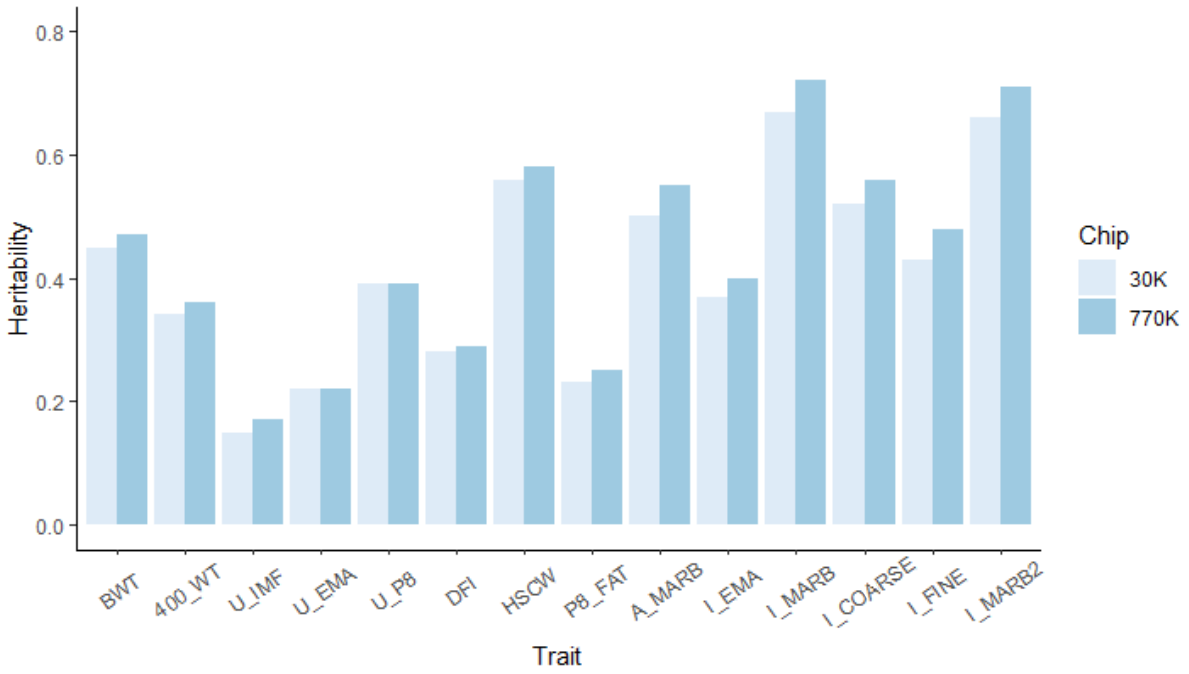

Figure 5.1: Heritability estimates for 14 traits estimated from genomic relationships constructed using Low Density (30K) and High Density (770K) genotypes on 4,940 individuals.

5.3.2 GBLUP Comparison

The minimum and maximum values obtained either using LD or HD genotype data allow the assessment of the range of BLUPS. For every trait except U_P8, utilising HD genotype data increased the range of BLUPs estimated to varying degrees (Table 5.1). This is also supported by increasing GBLUP SD when moving from LD to HD data. The correlation between BLUPs from either dataset is also reported as very high across traits with mean correlation of 0.96, with the highest correlations seen for I_MARB, I_FINE, I_MARB2 and A_MARB. U_EMA had the smallest correlation at 0.9 between BLUPs estimated from LD and HD data respectively.

Table 5.1: Minimum, Maximum and Standard Deviation (SD) values for BLUPS estimated for 14 traits using either Low Density (LD) genotype data or High Density (HD) genotype data as well as the correlation between BLUPs from the two methods for 4,490 animals.

Trait	**LD BLUPS**			**HD BLUPs**			**Correlation**
	Min	Max	SD	Min	Max	SD	
BWT	-6.74	8.49	1.91	-7.09	9.19	1.99	0.97
400_WT	-47.1	55.5	12.1	-49.6	62.0	13.3	0.95
U_IMF	-1.14	5.64	0.32	-1.10	6.86	0.34	0.97
U_EMA	-6.36	6.95	1.74	-6.29	7.84	1.78	0.90
U_P8	-3.09	3.35	0.83	-3.03	3.20	0.82	0.97
DFI	-1.80	1.84	0.47	-1.88	1.83	0.50	0.97
HSCW	-67.9	79.1	20.8	-74.7	87.9	21.8	0.94
P8_FAT	-5.97	5.26	1.46	-6.44	5.59	1.48	0.96
A_MARB	-3.74	1.98	0.70	-3.96	1.94	0.72	0.98
I_EMA	-10.0	12.2	2.53	-10.1	12.8	2.57	0.97
I_MARB	-13.6	14.4	4.17	-14.8	15.3	4.26	0.98
I_COARSE	-0.09	0.10	0.03	-0.10	0.10	0.03	0.97
I_FINE	-14.4	16.7	4.52	-15.5	16.6	4.70	0.98
I_MARB2	-0.12	0.13	0.04	-0.13	0.14	0.04	0.98

5.3.3 Animal Ranking

While there was a high correlation between animals individual BLUPs (Table 5.1), it is necessary to investigate whether changes in BLUPs impact the subsequent rankings of animals for each trait. Across most traits, greater than 30 animals are selected in common within the top 50 based on BLUPs estimated from LD or HD genotype data (Table 5.2). U_EMA is an outlier in this regard with only 19 animals in common; this is accompanied by a low rank correlation of 0.13. While most traits select similar animals in the top 50 across LD and HD genotype data, rank correlations are only moderately high, indicating some re-ranking of top animals. This re-ranking is visually evident for HSCW and I_MARB (Figure 5.2) when looking more closely at the BLUPs from LD versus HD genotype data. There was greater differences in BLUPs for HSCW than I_MARB. Their rank correlations reflect this pattern being 0.65 and 0.76 respectively. Large changes in ranking are less likely to occur in the highest ranked animals and are more evident for the lower percentile BLUPs (Figure 5.2). However there were some notable exceptions. Highlighted in blue (Figure 5.2) is one animal which ranked 22nd for HSCW (LD genotypes) that jumped to 2nd when HD genotypes were utilised for example.

Table 5.2: The number of animals in common between the Top 50 (Top 1%) selected for each trait utilising Low Density (LD) or High Density (HD) genotypes within the genetic evaluation and the Spearman Rank Correlation between the rankings of selected animals when BLUPs are estimated from either dataset.

Trait	**BWT**	**400_WT**	**U_IMF**	**U_EMA**	**U_P8**	**DFI**	**HSCW**
Overlap	38	38	34	19	39	40	35
Rank Cor	0.69	0.82	0.79	0.13	0.78	0.75	0.65
Trait	**P8_FAT**	**A_MARB**	**I_EMA**	**I_MARB**	**I_COARSE**	**I_FINE**	**I_MARB2**
Overlap	38	36	42	38	44	42	39
Rank Cor	0.73	0.39	0.76	0.76	0.75	0.77	0.68

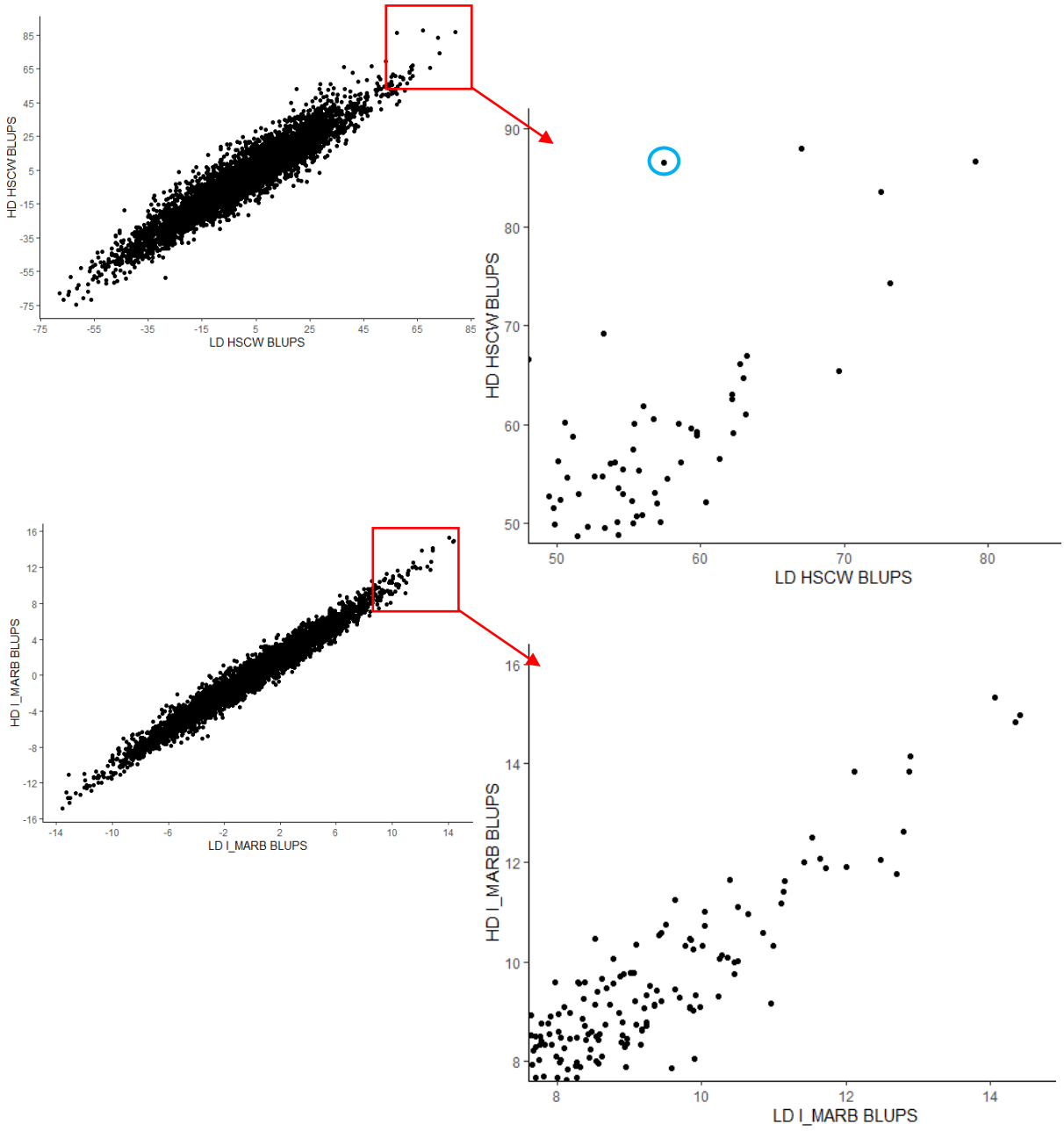

Figure 5.2: BLUP values for Hot Standard Carcass Weight (HSCW, top) and MIJ percentage marbling (I_MARB, bottom) calculated using Low Density (LD) genotypes versus High Density (HD) genotypes; zoomed in to assess changes in animal ranking for the predicted best animals.

5.4 Discussion

5.4.1 Heritability estimation

Heritabilities estimated for each of the 14 traits were reported as at least equal to or higher when utilising HD data in the genomic prediction as opposed to LD data. On average, an increase in heritability of 0.03 was observed across traits, which corresponds to a mean increase in accuracy of 2% (ranging from 0% for U_EMA/U_P8 to 3.7% for I_FINE) given accuracy can be described as the square root of the heritability. This demonstrates that as the number of SNPs utilised increases, the estimated residual and genetic variances decrease and increase respectively (Ogawa *et al.* 2014; Ogawa *et al.* 2017). This is due to the HD SNP panel attaining higher levels of linkage-disequilibrium between the SNP marker and true QTL region. The G-BLUP (Genomic best linear unbiased prediction) method used to estimate breeding values herein does not directly estimate the effect of a SNP like Bayesian methods (Meuwissen *et al.* 2001; Habier *et al.* 2011; Erbe *et al.* 2012) but rather utilises a matrix of genomic relationships to estimate genetic merit. The HD genotype set has been demonstrated to still be useful in this case, while not estimating SNP effects directly, capturing SNPs in close linkage-disequilibrium to true QTL allows the estimation of relationships to be more accurate. Relationships are then estimated based on similarity of genotypes at many QTL as opposed to few, so where many QTL may have a positive association with a trait, the associated positive phenotype is explained through the relationship matrix. That is high performing animals are related to other high performing animals and so on. Certainly, Chapter 3 (Figure 3.9) demonstrated that relationships estimated from HD data had a greater spread of values than relationships estimated from LD data, while maintaining a strong linear relationship suggesting higher accuracy of relationship description.

Marbling is controlled by many QTL of relatively small effects i.e. it is highly polygenic, and this is supported by the numerous reports of varying associated genes (Barendse 2002; Barendse

et al. 2004; Michal *et al.* 2006; Matsumoto *et al.* 2014; Sadkowski *et al.* 2014; Sukegawa *et al.* 2014; Hudson *et al.* 2015; Tong *et al.* 2015). In Wagyu, 97% of the genetic variance estimated for carcass weight from a GRM constructed with ~38K SNPs, was obtained utilising 10,000 SNPs opposed to marbling where only 92% of the genetic variance could be obtained (Ogawa *et al.* 2014). This suggests higher densities are required to capture variation in marbling through relationship matrices and certainly explains why the highest improvements in heritability herein were observed for I_MARB, I_MARB2, I_FINE and A_MARB with increases of 0.05 i.e. 5%. Ogawa *et al.* (2017) reported a 2% increase in marbling score when increasing SNP density from 50K to 770K. These improvements in marbling heritability are particularly significant due to the key importance of marbling in the breeding objectives of Wagyu producers.

Accuracy is considered to be higher for traits with higher heritabilities, although the effect of heritability on accuracy becomes smaller as more phenotype information becomes available and is utilised. To consider this from a different point of view, less change in heritabilities would be expected, and by extension accuracy, where larger numbers of phenotypic records are observed. Indeed the three ultrasound traits are a good example of this. Ultrasound EMA and P8 fat (U_EMA and U_P8) reported no increases in heritability while U_IMF reported a 2% increase. These three traits have the largest number of records in the analysis (3070 to 3073, Table 2.2) In contrast the marbling traits that had the highest heritability increase had the lowest number of traits (1079 to 1079 records).

5.4.2 BLUP Comparison and Animal Ranking

In addition to increasing heritability, the range and SD of breeding values (BLUPS, Table 5.1) increased for every trait except U_P8 when HD genotype data was utilised compared to LD genotype data. The variance of EBVs becomes larger when the accuracy of prediction is higher,

with accuracy being higher for traits with higher heritabilities. Ultrasound IMF (U_IMF) displayed the largest increase in EBV spread moving from LD to HD genotype data, 17%, followed by Hot standard carcass weight (HSCW, 11%), and 400 day weight (400_WT, 9 %). Ultrasound IMF, as described above, has a large number of records and so perhaps would not have been expected to display the greatest improvement in the spread of EBVs, but critically it has the lowest heritability of any trait investigated herein (0.15 and 0.17 when using LD and HD genotypes respectively, Figure 1). This suggests that, although a large record base is present, it was not being predicted as accurately with LD data compared to using HD genotype data. Given the largest increase in EBV spread, U_IMF experienced a minimal amount of re-ranking compared to other traits with the second highest rank correlation of 0.79 (the highest being 400_WT; 0.82, Table 2) between the top 50 animals selected using either genotype dataset in the evaluation. This suggests that the LD genotype dataset was able to rank animals appropriately, given a large number of records, but that moving to higher density genotypes resulted in more accurate individual BLUP values which maintained similar rankings.

In general, for MIJ marbling traits, correlations between BLUP values from either genotype dataset are high (0.97 to 0.98, Table 1) with generally high correlations between rankings of the selected top 50 animals (0.68 to 0.76). However, given a similar number of records, similar heritability and high genetic correlations (0.96 between AUS_MARB and I_MARB; Table 2.5) to these traits, AUS_MARB experiences a significant amount of re-ranking with a rank correlation of 0.39 although having a high correlation between BLUPs estimated from either dataset itself (0.98). There is an overlap of 36 animals selected as being in the top 50 for AUS_MARB from either genotype dataset and so significant re-ranking doesn't seem plausible until the ranking method used versus the specific way the trait is recorded is investigated.

It is likely that rank correlations for all traits are under-estimated. Proper rankings should account for a "tie" where two individuals have the same value. However, BLUPs in this analysis were to 7 decimal points preventing the occurrence of ties. This is not accurate representation of how BLUPs are often portrayed. The study herein has strictly assumed a difference in BLUPs is a true difference, which is technically correct, however a breeder would look at a BLUP of +5.6567322 and +5.6477322 as equivalent and make selection decisions based on that assumption. The assumption that any difference in a BLUP is a true difference, has the largest impact on traits measured using a discrete scale such as the AUS_MARB scoring system (scores 0-9) and a lesser impact on continuously measured traits like I_MARB (%) as evident by the degree of re-ranking in AUS_MARB given similar animals are selected as superior.

Ranking herein has been compared between traits individually whereas many traits are often under selection together in a breeding program through the use of an index. A selection Index allows the consideration of multiple traits when selecting candidates by assigning weighting values to traits based on importance, often utilising dollar values surrounding economic importance. Simply put, traits in the index have their BLUPs multiplied by their respective weighting. These values are then summed together to obtain a single, combined estimate of animal's performance weighted across all traits of importance. Given some ranking changes seen within the data for a specific trait are relatively large, where the actual difference in BLUP is small, it is likely that index ranking would be quite similar across LD and HD genotype data. The index would have the effect of "levelling out" big changes in ranking that aren't true ranking changes due to big discrepancies between BLUP values from LD compared to HD data.

Knowing the implications of rankings which are calculated based on BLUPs with high significant figures, there are still examples present where true re-ranking has occurred. One specific but not unique example is highlighted in Figure 2 for HSCW where one individual rank

increases from 22nd to 2nd when HD genotype data is utilised. Given the range of EBVs for HSCW increased by 11% when HD data was utilised, it is not surprising that some significant re-ranking did occur. This supports the use of HD genotype data when making selection decisions, given a higher accuracy of prediction, as selection decisions can have longer term impacts. For example, response to selection can be altered due to the specific animals selected as replacements or for use in embryo transfer programs. Not only do selection decisions impact on a stud's genetic progress but also the progress of wider industry.

The majority of genetic progress is made in top nucleus breeding programmes which is disseminated to industry via lower tiered multiplier herds through semen and bull sales. The genetic merit of commercial herds is lagging behind that of nucleus herds due to a function of sire and dam generation interval, the rate of genetic progress in the elite herds and the genetic merit of the sires and dams themselves (Dechow and Rogers 2018). This lag describes a "delay" between selection of elite animals and realised production improvements in commercial herds. It can be lowered by reducing the generation interval of sires and dams in commercial herds and utilising EBVs with higher accuracies (Dechow and Rogers 2018). However, there is an economic trade-off between genetic improvement and longevity in that lower culling rates are favourable due to lower costs associated with maintaining herd size (De Vries 2017). This means cows, which are genetically inferior to younger heifers, are kept in the herd longer, decreasing the proportion of new genetics (i.e. replacement heifers) being brought in, thus increasing the genetic lag.

In elite breeding herds, selection decisions are based on the traits which are desirable in the market so clients can produce stock to meet market specifications (Robinson and Buhr 2005). This often means elite breeders are "looking ahead" for new price signals ensuring they are positioned to supply stud stock suitable for new emerging markets where the most profit

often lies. As markets become more targeted or change (greater spread of different traits to select on) the currently used superior stock may not be suitable either due to lack of selection for new traits or unfavourable correlations e.g. selection for yield resulted in decreased eating quality in pork below acceptable consumer levels (Lonergan *et al.* 2001; Wood *et al.* 2008). This highlights the lingering effect elite selection decisions can have on the commercial industry and why obtaining highly accurate EBVs on younger stock is important.

5.5 Conclusion

Utilising HD genotype array data has been suggested to improve accuracy herein due to increased spread of EBVs reported and higher heritability estimates across traits, which aligns with the accuracy improvements reported in the literature. This supports the investment which has been made into obtaining whole genome sequences on ancestral individuals (currently ongoing at time of writing) which will be analysed in the near future. The benefits of whole genome sequencing have been discussed in detail in previous chapters. From the results herein, it is hypothesised that the use of whole genome sequence data will provide better persistence and higher accuracies of EBVs within this Wagyu herd for selection of traits, such as marbling, due to capturing the total additive variance in the population through inclusion of actual causal mutations impacting traits. It is critical to obtain high accuracies to better inform selection decisions which will allow this Wagyu herd to achieve a high response to selection that benefits clients purchasing young bulls for their enterprises.

Chapter 6 : General Discussion

This thesis has covered a number of aspects relating to the implementation of genomic selection within a beef population of Wagyu with a focus on maintaining high quality beef, particularly marbling. Topics have included comparing and contrasting measurement of specific traits and their relationships with other economically important recorded traits, through to comparing cost effective ways to obtain high density (HD) genotyping and the benefits/implications this has on the additive variance estimated within traits and subsequent changes to animal selection decisions. The preceding chapters to this discussion have been presented in a chronological fashion allowing the connections between results to be discussed in turn, culminating with the analysis presented in Chapter 5. This discussion will re-iterate the key findings while suggesting areas of further research, particularly around the utilisation of whole genome sequencing and commercial cross-bred data.

6.1 Summary of Work

Given the high marbling capabilities of Wagyu, a relatively new breed to the Australian production system being imported in the 1990s (Maeda *et al.* 2014), it was identified that current evaluation schemes such as AUS-MEAT and MSA (AUS-MEAT Limited 2005) were not suitable to capture the variation in marbling and marbling characteristics of Wagyu cattle. Additionally, with the development of genomic selection (Meuwissen, Hayes and Goddard 2001), opportunities existed to be able to capture more additive variation associated with a trait over traditional pedigree methods. A meta-analysis conducted (Table 1.1) using weighted averages of heritability, as described by Koots *et al.* (1994), paired with the genetic parameters estimated in Chapter 2, demonstrated the value of more precise phenotypic measurement

and genomic relationships. In general, traits estimated using genomics achieved a greater spread of EBV values with less error, than when estimated from pedigree. In addition, newer novel MIJ camera measures (Kuchida *et al.* 2006; Maeda *et al.* 2014) for marbling were highly correlated to their equivalent AUS-MEAT counterparts. Results favoured newer technology over traditional methods.

The benefits of genomic selection could be further amplified with the use of HD genotyping, with heritabilities increasing by 3% on average (Figure 5.1). Although this percentage is small, given the high value received for highly marbled cuts, any small increases multiply out to have a substantial effect on profit. Four thousand and forty HD genotypes were able to be obtained accurately (mean imputation accuracy of 0.93; section 3.3.4) through imputation (Sargolzaei *et al.* 2011) using a reference population of 165 animals with HD genotypes (479,535 SNPs). Imputation was deemed a cost-effective measure to obtain HD genotypes, with the results supporting the hypothesis that whole genome sequencing would be of value to this breeding program, increasing heritability further. Re-ranking was generally minimal, although exceptions exist, when moving from low density to HD genotypes.

Phenotype and genotype data from an Australian Full-Blood Wagyu herd, specifically of Japanese Black cattle descent has resulted in the main focus of a majority of the discussions being centred on a full-blood nucleus scenario. Conclusions presented across chapters are therefore not necessarily reflective of the broader "Wagyu" industry in Australia. Australian beef production is commodity based with price/kg received at sale varying greatly due to perceived eating quality attributes, of which marbling is a determining factor. Wagyu beef has excelled in high quality markets due to a genetic predisposition to exhibit superior marbling compared to other beef breeds (Gotoh *et al.* 2009). Acquiring high numbers of full-blood Wagyu cattle is an expensive investment and so cross breeding British breeds (such as Angus)

with Wagyu bulls has been particularly common across the broader beef industry. The resulting cross has the potential to marble highly while capitalising on the growth capabilities of Angus and hybrid vigour (a.k.a. heterosis) making the cross cost-effective to produce. Production of F1 (first cross cattle) is a key market for Wagyu bull sales.

6.2 Future Work

6.2.1 Value of Whole Genome Sequencing

While the intention was to include whole genome sequences in this thesis, technical difficulties with sampling the selected animals delayed the arrival of sequence data. A different approach utilising HD genotype data had to be undertaken (Chapter 5) which demonstrated promising results. Future work will involve investigating the value of whole genome sequencing to a nucleus breeding program. Whole genome sequence data is expected to give a better persistence of accuracy over generations due to a shifting focus from large chromosome segments to causal mutations, which don't degrade due to recombination. Additionally, there is an expected increase in prediction accuracy due to the inclusion of causal alleles at frequencies too low to be included in current SNP chip arrays (Hayes *et al.* 2014).

High density genotyping also presents as a tool for managing genetic diversity. Previously high density genotyping has been used in multi-breed populations to estimate levels of LD, consistency of gametic phase between breed-groups, the presence of overlapping population structures and level of N_e (Brito *et al.* 2017). This information was used to manage and inform the optimal implementation of genomic selection in this population to ensure good accuracy of prediction in a multi-breed population. Besides facilitating multi-breed selection programs, high density genotypes could be beneficial for managing populations with small N_e that are prone to inbreeding. The ability to describe closely related animals more accurately would be beneficial to better inform mate allocation. Estimates of inbreeding obtained using pedigree

information are lower than those obtained using genomic data (Chagunda *et al.* 2018). SNP data captures Mendelian Sampling variance to revel the 'realized' homozygosity in the genome, resulting in greater variation in genomic inbreeding compared to pedigree inbreeding within a generation (Zhang *et al.* 2014; Sumreddee *et al.* 2019).

A large area of investigation has been the use of whole genome sequencing in genome wide association studies. The main focus of sequencing data moving forward will be to improve predictive abilities of breeding programs through capturing greater additive variance. This is expected to be achieved through describing relationships between individuals within the population more accurately. Such aims are compatible with the current evaluation procedure in use; that is using GBLUP models to estimate breeding values.

In the first instance, with imputed HD genotypes available, the entire population could be imputed to sequence, with imputed sequences used to build a genomic relationship matrix and estimate BLUPs. Imputation from low density to high density would preferably be done in two steps to achieve the highest imputation accuracies (Van Binsbergen *et al.* 2014). First imputation from a low density to a high density chip (as done in Chapter 3 and 5) and then to sequence. Including millions of variants in routine evaluations or on chips is difficult and computationally intensive.

A second approach involves using a subset of sequence variants, discovered in the Wagyu population, to complement existing HD SNP chip arrays. Enriching lower density SNP chips with SNPs from a high density panel for specific QTL regions has showed an increase in EBV accuracy (Saatchi and Garrick 2014). SNP subsets from sequence data have been developed using variants identified in candidate genes if the genes are known (Ortega *et al.* 2016). Subsets have also been created using results from genome wide association studies (GWAS) that utilise sequence data (Van Den Berg *et al.* 2014; Brøndum *et al.* 2015). A GWAS could be

performed on the Wagyu population herein, once imputed to sequence to identify specific QTL or QTL regions related to economically important traits. Alternatively/or additionally regions identified in literature could be utilised (Examples in Wagyu include; Nishimura *et al.* 2012; An *et al.* 2019). However, as QTL variants will likely be chosen on size of effect (significance), the benefits of this subset method may be better realised in Bayes style estimation evaluation systems, i.e. Bayes A and Bayes B (Meuwissen *et al.* 2001), Bayes C (Habier *et al.* 2011) and Bayes R (Erbe *et al.* 2012). In these methods marker effects are effectively summed to produce a breeding value. Conversely, these variants may still be useful in GBLUP if they are breed specific and help to estimate relationships between Wagyu animals better.

Sequence variants can also be selected using genomic prediction methodologies. These methodologies would be ideal as they could use currently employed methods i.e. GBLUP. Variant subsets would be chosen based on largest absolute effect or largest genetic variance contributed by the locus, where markers are chosen regardless of location. VanRaden *et al.* (2017) compared such methods, however estimated marker effects using Bayes A prediction algorithms. This could be done using GBLUP, back solving estimated BLUPs from imputed sequence data to calculate marker effects and variances.

6.2.2 Genomic Evaluation Methodology

The good performance of GBLUP can be attributed to 1/ there are many genes affecting economically important traits in livestock and 2/ linkage disequilibrium can extend over large genomic distances (Meuwissen *et al.* 2016). This thesis only evaluated GBLUP. Bayesian methods assume a prior distribution of SNP effects that may make more sense biologically than the linear assumption where SNP effects follow a normal distribution, resulting in higher accuracies (Erbe *et al.* 2012; Bolormaa *et al.* 2013).

Meuwissen and Goddard (2010) reported accuracies of prediction using sequence data that were significantly higher using Bayes B than GBLUP. For a simulated dataset considering 3 or 30 loci/Morgan, GBLUP did not appear to take full advantage of sequence data. Iheshiulor *et al.* (2016) demonstrated that SNP-BLUP (analogous to GBLUP) was as good as MixP, an estimation method similar to Bayes C, when causative QTL density in the dataset was high (132 loci/Morgan) using simulated whole-genome sequence data. MixP was superior when causative QTL was low (45 loci/Morgan) consistent with Meuwissen and Goddard (2010). These results implies that Bayes methodology is superior when traits, both lowly and moderately heritable, were controlled by few loci compared to many. Future work should investigate transitioning to Bayesian methodologies to better utilise whole genome sequence data in Wagyu and how this applies to the traits under investigation. For example, marbling is known to be controlled by many QTL of small effect.

6.2.3 Breeding Objective Suitability (traits under investigation)

Marbling fineness describes the number of fine marbling particles within a cross-section of a rib-eye. Coarser marbling particles are considered undesirable in marbled meat products (Motoyama *et al.* 2016; Vierck *et al.* 2017). This is despite a growing body of evidence demonstrating that coarser marbling particles contribute to juicer, more flavourful beef under sensory panel evaluation (Vierck *et al.* 2017; Lee *et al.* 2019). However finer marbled beef still retains a higher consumer acceptability (Lee *et al.* 2019). This suggests a balance must be struck between increasing IMF% and increasing marbling fineness. In this regard, an extensive survey to estimate appropriate economic values for marbling coarseness/fineness, including both consumers and processors, is needed so that appropriate breeding decisions can be made to produce a product that excels in sensory characteristics but remains visually appealing.

The traits utilised in Chapter 2 (and then Chapter 5) are focused strongly on growth and carcass, particularly marbling, characteristics given Wagyu are a beef producing breed. However, key traits are missing that would be considered essential in a well-rounded breeding objective for a nucleus program such as fertility and survival traits. These traits generally have low heritability and include age at first calving (<0.10 - 0.3), age at puberty (<0.10 - ≥0.60), days to calving (<0.10), scrotal circumference (0.20 – 0.80) (Cammack *et al.* 2009) and calving ease (0.01-0.22) (Cue and Hayes 1985; Eaglen and Bijma 2009; Jeyaruban *et al.* 2016). Calf survival/mortality is another trait to consider given anecdotal evidence exists (*Personal communication:* David Blackmore of Blackmore Wagyu and Scott DeBruin of Mayura Wagyu) describing Wagyu calf survival up to 3 weeks of age as highly volatile with young calves succumbing to severe scours. The reason behind this is definitely multifactorial and mortality percentages appear to vary between farms which is in agreement with the reportedly low heritabilities of mortality (0.001, Cue and Hayes 1985). Estimates of maternal as well as direct effects on calf survival would both be beneficial.

Fertility traits such as days to calving, which is the number of days between the first joining date and subsequent calving, is relatively easy to calculate under paddock mating. However under extensive artificial insemination (AI), where cows are synchronised prior to mating, this can mask variation in fertility and produce bias estimates for fertility, proportional to the number of animals used in timed AI (Oliveira *et al.* 2019). Thus, obtaining accurate fertility estimates must consider the use of AI data over traditional paddock mating. Investigating traits such as non-return rate, number of inseminations per pregnancy, days from first to successful insemination, days from calving to first insemination and age at calving, may be suitable traits to consider Wagyu female fertility with heritabilities ranging 0.01-0.1 (Setiaji and Oikawa 2019).

The suitability of the traits under-investigation, while suitable for producing high marbling Wagyu are missing key reproductive and survival traits necessary to a nucleus program. In regards to F_1 production, the traits are perfectly suitable as there is no need to apply selection pressure to reproduction if the F_1 is a terminal cross. The goal of F_1 production systems will be to produce highly marbled meat, as efficiently as possible. In this sense, selecting sires with the highest genetic marbling potential will have the desired effect in producing cross-bred progeny with higher marbling (see section 1.4). Sires with high marbling potential but lacking female reproductive fitness would be better suited for F_1 production, while a better balanced sire (albeit still strong emphasis on marbling and HSCW) would better suit the nucleus program.

6.2.4 Inclusion of F_1 data in analysis

Estimating relationships from genotypes removed a limiting barrier to genetic progress which is access to phenotype data that is well described and pedigree recorded. Using genomic relationships removes the need for pedigree recording and also, partly, the need to establish linkage sires between herds if reference populations are diverse. This has practical implications in industry, traditional pedigree evaluation has usually been conducted within breeds and not across breeds due to a lack of linkage sires making across breed evaluations difficult to establish; notable exceptions exist i.e. LAMBPLAN (Brown *et al.* 2007). The requirement for pedigree also made utilisation of commercial data difficult as parentage is rarely recorded sufficiently in these herds.

The review of reciprocal recurrent genomic selection methods (Section 1.3), where reciprocal recurrent selection refers to the selection of purebreds to breed better crossbreds utilising both additive and non-additive genetic variance (Comstock *et al.* 1949) demonstrated that RRGS methods (Dekkers, 2007; Ibánẽz-Escriche *et al.* 2009; Kinghorn *et al.* 2010; Zeng *et al.*

2013) were likely no more successful at improving crossbred performance than selecting for better purebreds using pure-line selection. Genetic evaluations of purebreds within their own populations has been sufficient to improve cross-bred progeny performance in sheep and cattle (Hall *et al.* 1992; Fogarty *et al.* 1997; Hall *et al.* 1997; Newman *et al.* 2002; Hegarty *et al.* 2006). This is due to additive variance accounting for the majority of total genetic variance (Hill *et al.* 2008). Including commercial-crossbred progeny records into the nucleus evaluation could be beneficial to better predict higher performing pure-bred bulls which is expected to result in improved F_1 performance. This is especially the case when the populations included in reference datasets are closely related and/or the access to/or provision of phenotypic records is limited (De Roos *et al.* 2009; Ibánẽz-Escriche *et al.* 2009).

Ibáñez-Escriche *et al.* (2011) reported in a pig population that the mean reliability of predicted purebred breeding values for lean meat yield, estimated in Landrace, Duroc and Pietrain pigs, was increased when crossbreed data was included using two different methods. The crossbred data included was produced from two-way (F_1; Duroc x Landrac) and three-way crosses (F_1 x Pietrain) between the three purebred breeds. Estimated heritabilities for lean meat yield were similar to those estimated in purebred data, except for the Pietrain breed which reported an improved heritability using crossbred data. This was most likely due to the inclusion of crossbred data improving the variance in lean meat yield records, compared to purebred data where the variance was quite small for Pietrain compared to other purebreds. This suggests that inclusion of crossbred data could increase the accuracy of prediction, especially for Pietrain. Ibáñez-Escriche *et al.* (2011) demonstrated that rank correlations between EBVs predicted with and without cross-bred data were highest for Duroc (0.94-0.96) followed by Landrace (0.88-0.95) and Pietrain (0.81-0.85). Duroc records maintained a moderate heritability throughout the study and included greater than 2.5x the number of records, so minimal re-ranking is expected.

Earlier studies have demonstrated that the benefit of crossbred information was largest when the genetic correlation between purebred and crossbred performance (r_{pc}) was low (Wei and van der Werf 1994; Bijma and Van Arendonk 1998). The inclusion of crossbred information was worse than purebred data only when r_{pc} > 0.8 (Wei and van der Werf 1994). A study by Newman *et al.* (2002) demonstrated that r_{pc} in cattle are moderate for weight traits such as 400 day weight and HSCW ($r_{pc} = 0.48$), yet high for carcass quality traits (retail beef yield, IMF and P8_Fat; r_{pc} of 0.83, 0.95 and 1.00 respectively). Inclusion of crossbred data may not result in improved selection for marbling of purebreds in Wagyu based on these results. The study in 2002 did not consider crosses between highly marbled Wagyu and more common Taurus breeds like Angus though. The r_{pc} for marbling between these two breeds could be expected to be less unified due to substantial variation in expression of the trait noted between the two breeds. The correlation coefficient is expected to decrease with increasing genetic disparity between parental populations. Such genetic disparity between the two breeds could explain the vastly different marbling phenotypes observed. The Australian Wagyu Association (AWA) reported a significant genetic disparity between Wagyu and 10 other beef breeds, including Angus (Teseling 2016) in the development of their cross-bred Wagyu test. The r_{pc} between traits measured in Wagyu and their cross-bred progeny needs to be assessed.

Not all commercial data is suitable for inclusion in genetic analyses, with contemporary groupings needing to be well defined in order to model additive genetic variances effectively. The crossbred data, in this case F_1 Wagyu, should be related to the population under selection to be most beneficial. The SNP chip density used to estimate relationships between the two populations (Wagyu and F_1) needs to be sufficiently high to ensure that the phase of linkage

disequilibrium between SNPs and QTL is consistent between populations, especially diverged populations (De Roos *et al.* 2008; Porto-Neto *et al.* 2014).

6.3 Conclusion

This thesis has investigated a number of aspects regarding the implementation of genomic selection within a high quality beef breeding program. The core focus has been on investigations relating to the nucleus breeding program covering extensive genetic parameter estimation for 14 different traits, with carcass quality parameters explored in depth. These parameters have been estimated at two separate SNP chip densities, with higher densities yielding generally higher heritabilities. Comparison of methods to develop appropriate reference populations to facilitate imputation resulted in high imputation accuracies being obtained. Development of the breeding program for this Wagyu herd is on-going and four key areas of future research have been identified for perusal. The first is exploring the value of whole genome sequence data and secondly the value of Bayesian methods over currently used BLUP estimation. Thirdly, to improve the breeding objective, it is recommended that fertility and survival traits be developed and included, which may centre on utilising artificial insemination records. Lastly, due to the common use of Wagyu sires to produce cross-bred progeny, modelling development work is required to determine the value of crossbred data to better predict high ranking nucleus sires, facilitated through genomic relationships.

Chapter 7: Appendix

Appendix 1: Development and Review of Genomic Selection

Appendix 1.1 Traditional pedigree based selection

Traditional breeding strategies such as outcrossing, line breeding and inbred matings are generally slow to show genetic improvement amongst breeds. This is particularly true for traits that have a low heritability which is common for fitness traits such as reproductive and maternal traits (Falconer *et al.* 1996). Most genetic improvement in the national herd using traditional breeding strategies have been made by identifying key breeding objectives driven to meet the demand of environmental and market forces.

The development of a genetic evaluation program in Australia started as the National Beef Recording Scheme (NBRS) in the late 1970's and became BREEDPLAN in 1985 (Graser and Hammond 1985). The purpose of BREEDPLAN is to quantitatively evaluate an individual's genetic merits before they are selected as breeding stock on a breed by breed basis. BREEDPLAN estimates an individual's genetic merit, for each analysed trait, as an Estimated Breeding Value (EBV). The EBV is a representation of the additive genetic components of the individual's genotype, providing an indication of the genetic component that can be inherited in subsequent generations. The EBV is determined using random coefficients from a linear mixed model (BLUP; Henderson 1984). This method requires the recorded phenotypes and knowledge of an animal's pedigree. The approach to analysis has evolved from a single-trait sire model sometimes including dam or maternal grandsire terms, to a multi-trait animal model analysis system, which incorporates growth, reproduction and carcass trait information (Quaas and Pollak 1980; Graser *et al.* 2005). BREEDPLAN has been very successful, with genetic gains being realised in most farmed species. However, despite this success, there is

increasing interest to identify the genes and polymorphisms controlling traits as a new means of animal selection through simply inherited genetic markers (Goddard *et al.* 2010).

Appendix 1.2 Marker Assisted Selection (MAS)

Advances in molecular genetics have led to the identification of multiple genes or genetic markers that have an association with genes affecting important traits in livestock. The integration of these identifiable genes or genetic markers into breeding and/or selection decisions is referred to as marker assisted selection (Dekkers and Hospital 2002). Application of MAS for genetic gain relies on the ability to successfully genotype animals for marker loci of interest. Three types of marker loci are discernible; causal polymorphisms that code for functional mutations, loci in weak linkage disequilibrium (LD) with the functional mutation within families, also termed linkage equilibrium (LE) loci, and loci in strong LD across the population (Dekkers 2004);

Causal polymorphisms affecting the traits of interest can be incorporated into animal selection criteria for breeding programs. However, in practice, only a few mutations that cause genetic abnormalities and a small number of polymorphisms which have large effects on quantitative traits have been identified (Dekkers 2004). The Myostatin gene in cattle (Charlier *et al.* 1995), Callipyge gene in sheep (Cockett *et al.* 1994) and DGAT1 in Dairy cattle (Grisart *et al.* 2002) are examples of such causal polymorphisms. The majority of traits of economic importance are quantitative or complex traits and are controlled by a large number of segregating genes/QTL, each contributing a small effect sensitive to the environment (Mackay 2001). Therefore each contributing QTL explains only a small proportion of genetic variance in the breeding objective or trait (Dekkers 2004). This limits the usefulness of using a small number of causal polymorphisms.

Microsatellites were the first class of genetic markers to span across the genome. Typically, 100-200 microsatellites were used to provide wide but imprecise coverage of genome. QTL were detected based on linkage within full-sib or half-sib families (Georges *et al.* 1995; Zhang *et al.* 1998). A big limitation to these early MAS studies was that the QTL was mapped very imprecisely and the marker and QTL were in weak linkage disequilibrium so that the linkage phase varied between families. While LE markers are readily detectable (Andersson 2001) this meant that the linkage phase had to be determined within a family before the microsatellites could be used for selection (Goddard *et al.* 2010). Fernando and Grossman (1989) presented a generalised method for estimating animal breeding values using MAS where the first step was to detect and map genes underlying traits of interest (i.e. QTL) and the second step was to include this information into the BLUP-EBV. However this method of MAS resulted in small gains. Other logistical limitations of implementing LE in selection have been discussed in the literature (Dekkers 2004). In Genome wide association studies (GWAS) the number of tests is equal to the number of genotyped markers, i.e. single nucleotide polymorphisms (SNPs), with each SNP effect being tested independent of all other SNPs. Because the number of tests would often be many thousands, the multiple testing problems become so great that very stringent significance tests are required. This resulted in only the largest QTL being found , for example DGAT1 affecting fat content in milk (Grisart *et al.* 2002). Utilisation of a marker type that does not require linkage phase to be determined for each family and that utilizes all QTL is optimal for genetic improvement of livestock.

Meuwissen *et al.* (2001) attempted to estimate the effects of approximately 50,000 markers simultaneously from a limited source of phenotypic records in a simulation study. The markers were evenly spaced 1 cM apart and were in linkage disequilibrium with the QTL associated with the traits of interest. Their results showed that using this methodology could lead to large increases in response to selection, which has become known as 'genomic selection' (Goddard

et al. 2010). Genomic selection has become feasible due to the identification of many of thousands of SNP markers and with the declining cost of SNP-Chip genotyping technology. It is perhaps the most promising method of marker-assisted selection, being distinctly unique in that it utilises SNP markers that are in LD with QTL of interest, spanning across families and populations.

Appendix 1.3 Advantages of Genomic Selection

Genomic selection has many advantages in cattle breeding; in particular it is beneficial for traits that are difficult to improve by traditional pedigree based selection. This includes traits where measurement of phenotype is difficult, expensive, sex-limited, only possible later in life or is not possible on selection candidates e.g. carcass traits (Dekkers 2004). For example, traits such as milk yield, which cannot phenotypically be measured on a bull, have been improved by progeny testing. This leads to an accurate estimate of the bulls breeding value but at the expense of a long generation interval. Genomic selection is beneficial in this scenario as bulls, and heifers, can be selected earlier in life, reducing the generation interval, and need for progeny testing, which can approximately double genetic gain per year (Pryce *et al.* 2010). Additionally, in multi-sire joining programs it has been hard to implement genetic improvement programs based off of pedigree information because they are logistically complex. Genomic selection might be more practical as the prediction equation would not require pedigree recording, although performance recording would still be necessary, and implementation would require only a DNA sample from each animal and laboratory facilities (Goddard *et al.* 2010). Genomic selection also has the potential to utilise information coming from a different environment as long as GxE effects are minimal.

Goddard (2012) described an example of this whereby milk production in the USA is a different, but correlated, trait to milk production in Australia. Phenotypic information from

the USA predicts breeding value in Australia less accurately than the same phenotypic information from Australia, due to the genotype by environment interaction. This inaccuracy can be overcome by using genomic selection where genotype information from the USA bull can be used in the equation to predict breeding value in Australia. Assuming the linkage disequilibrium (LD) between SNPs and causal QTL are the same in the Australian and American populations genomic selection should increase the internationalisation of breeding programs (Goddard 2012). Additionally Kinghorn (2012) reviewed the use of genomics in livestock management rather than for breeding, suggesting that genomic selection could be useful in grouping like animals together to direct towards a market where they will be most likely to meet specifications. This involved ideas such as identifying animals that will produce different meat qualities as well as animals that would perform better in different feeding schemes, i.e. long vs short grain feeding regime, with continued applications being presented along the entire supply chain.

Appendix 1.4 Methodology for Genomic Selection

Appendix 1.4.1 Cleaning of Genotypes

In between genotyping and constructing genomic predictions, there is an important step involving the cleaning of marker genotypes. Markers may be kept in the analysis or omitted based on whether they are polymorphic and/or if they have a minor allele frequency (MAF) greater than the lowest permitted cut off. The minor allele frequency is the amount at which the second most common allele appears in the population for a given marker. SNPs are omitted from the analyses that are monomorphic because they do not contribute to explaining the relationships between animals, meaning only polymorphic SNPs are retained. Of those polymorphic SNPs that are retained a subset are removed that have minor allele frequencies below a specified cut off point, below which variation may be considered genotyping technical errors. The aim here is to remove genotyping errors that could imply the

relationship between two animals is greater or lesser than it actually is. By doing this though, there is the potential to remove rare variants in the population; however they contribute such a minor effect that excluding them results in little difference.

Another important consideration is duplication in the data. Correlations between genotypes can be used to identify duplicate genotypes. Duplicate genotypes pose an issue when it comes to inverting the genomic relationship matrix (see below). In some instances, duplicate genotypes can appear as a result of monozygotic twins which will have identical genotypes but different phenotypes. Excluding these animals from the analysis does mean removing phenotypic information but in reality these animals may be 2 of thousands and so contribute a small amount to the analysis.

Appendix 1.4.2 Genomic estimated breeding values (GBLUP)

In GBLUP, genomic estimated breeding values (GEBVs) for animals are estimated using phenotypes and genomic relationships estimated from genome-wide dense SNP data. The genomic relationship between two animals is calculated as the correlation between their SNP genotypes and thus the GBLUP method is very similar to traditional BLUP with the exception that pedigree relationships (pedigree relationship matrix: **A**) have been replaced with genomic ones (genomic relationship matrix: **G**).

In order to calculate the genomic relationship G the definition of two matrices M and P is required. As in VanRaden (2008), let **M** denote the matrix of marker genotypes, with dimensions as the number of individuals (*n*) by the number of loci (*m*). Elements of **M** are set to 0, 1 or 2 for the homozygote, heterozygote and other homozygote respectively. Equations can include marker information using *n* x *n* matrix $\mathbf{MM}^{\mathbf{T}}$ where diagonals count the number of homozygous individuals for each loci and off-diagonals count the number of times alleles at different loci were inherited by one individual.

The matrix **P** contains allele frequencies for each loci expressed as a difference from 0.5 and multiplied by 2 such that column j_i of **P** is $2(p_j - 0.5)$; where p_j is the frequency of the second allele at locus *j*. Subtraction of matrix **P** from **M** gives **Z** which results in setting the mean values of the allele effects to 0. The genomic relationship matrix, **G**, can then be calculated as follows;

(1) $$\boldsymbol{G} = \frac{\boldsymbol{ZZ'}}{2\sum p_i(1-p_i)}.$$

Division by $2\sum p_i(1-p_i)$ ensures the scale of **G** is similar to the scale of **A**. With this method, matrix **G** is generally positive semidefinite, but can be singular if the number of loci (*m*) are limited or if the number of individuals (*n*) is greater than the number of loci (*m).* Additionally identical twins, clones or duplicate samples can cause singularity to occur. The form of **G** in equation (1) was proposed by VanRaden (2008) for purebred populations however can be modified to account for differing allele frequencies in multi-breed (composite and crossbred) populations as demonstrated by Harris and Johnston (2010) and Erbe *et al.* (2012). The genomic relationship matrix can be further improved by taking a weighted average of the relationship estimated from each marker, i.e. the weighted average across SNPs, as described by Yang *et al.* (2010). Goddard *et al.* (2011) argued that a regression of elements of **G** towards **A** was necessary to account for sampling error in estimating coefficients of **G**. It was proposed that an unbiased genomic relationship matrix, **G***, is calculated as in (1) with the adjustments described by Yang *et al.* (2010) and then regressed towards **A** such that;

(2) $\mathbf{G^*} = \mathbf{A} + b(\mathbf{G} - \mathbf{A})$,

Where *b* is equal to Var(**G**)**/** [Var(**G**) + 1/*m*] and Var(**G**) is the variance of the non-diagonal elements of **G** (Goddard *et al.* 2011). While this is perhaps the most unbiased method it would not be suitable in commercial circumstances where **A** is not known. In this instance the version of **G** proposed by VanRaden (2008) or Yang *et al.* (2010) would be suitable.

There are many ways to describe genomic relationships among individuals other than the equation for **G** presented above, although that is a common method. While other methods may appear to be different (i.e. don't include estimation of allele frequencies) and may be based on different assumptions, providing varying estimates of additive genetic variance, the estimation of genetic merit of the population is similar (Tier *et al.* 2015) i.e. GEBVs are similar.

Once the genomic relationships have been determined, the breeding value (BV) of an individual i can be determined as;

(3) $\mathrm{BV}_i = \sum_{j=1}^{N_q} x_{ij} a_j,$

Where a_j is the additive effect of the jth QTL and x_{ij} is the genotype of the *i*th individual at the *j*th QTL coded as 0, 1 or 2 (described above). However, in practice the QTL position and effects are not known rather we detect the QTL based on linkage-disequilibrium (LD) with SNPs. Therefore the BV is estimated rather than truly determined and is coined a genomic estimated breeding value (GEBV). The GEBV for individual *j* becomes;

(4) $\widehat{GEBV}_i = \sum_{j=1}^{n} m_{ij} \hat{\boldsymbol{g}}_j$

Where the number of SNPs is n, m_{ij} is the genotype of individual *i* at the *j*th SNP and $\hat{g}_j$ is the apparent effect of the *j*th SNP, in LD with one or more QTL, on the quantitative trait estimated from data (Goddard *et al.* 2010).

G-BLUP utilises a linear mixed model for the calculation of breeding values as follows;

(5) y= Xb + Wu + e

where **y** is the response vector, **b** is the fixed parameters in the model, **u** is the vector of genomic breeding values (BLUPS), which assumes SNP effects are normally distributed with a mean of 0 and have constant variance and ***e*** is the residual or environmental error. Since tens

of thousands of SNPs are potentially utilised, the normality assumption implies that each SNP contributes a small effect on the trait of interest which is akin to the traditional infinitesimal model for quantitative traits (Goddard *et al.* 2010).

If the number of individuals is less than the number of SNPs then there is a relationship between **g** and **u**, the vector of genomic breeding values, such that **u**=**Mg** where the variance of **u** is **~** $N(0, \mathbf{MM}^{T}\sigma^{2}_{u})$. This is akin to the conventional animal model used in BLUP estimates, however here it has been adapted for GBLUP.

GBLUP is more accurate than BLUP because genomic relationships are able to better partition the similarity in genotype between animals compared to pedigree relationships. Pedigree relationships assume that, for example between full-sibs, that they have 50% of their alleles in common to one another. This assumption would be correct given an infinite number of unlinked genes. However genes on the same chromosome are linked which means they are not inherited independently of one another, resulting in variation around this 50% assumption. Using genomic relationships it has been demonstrated that, between full-sibs, only 40% of their alleles might be shared (Hayes *et al.* 2009b). This can be detected due to dense marker genotyping.

Appendix 1.4.3 Back-solving from GBLUPs to estimate marker effects

GBLUP analysis does not use marker effects to estimate animal breeding values. Rather the markers are used to define genomic relationships through the **G** matrix which is then utilised in the calculation of breeding values, equivalent to the traditional pedigree relationship matrix. However the effects of individual markers can be estimated by back-solving from predicted animal GBLUPS such that,

(6) $\mathbf{g} = \mathbf{Z}\,(\mathbf{Z}^{T}\,\mathbf{Z})^{-1}\mathbf{u}$

With these marker effects an animal within the population can then be genotyped and the marker effects (i.e. its genotype) can be multiplied by the G matrix to ascertain a breeding value, analogous to (7) below. .

Appendix 1.4.4 Bayesian approaches

The best approach to genomic selection will depend on the genetic architecture of the trait. If there are a large number of QTL, each contributing a small effect, then the GBLUP method (described above) is more appropriate. Conversely, if only a few markers have a large effect then Bayesian methods provide better estimates than GBLUP. Examples of Bayesian methods include the 'Bayesian alphabet', i.e. Bayes A and Bayes B (Meuwissen *et al.* 2001), Bayes C (Habier *et al.* 2011) and Bayes R (Erbe *et al.* 2012). The prior distribution of SNP effects described by Bayesian methods may make more sense biologically than the assumption that all SNPs have an effect, although small.

Bayes A assumes that SNP effects follow a scaled *t* distribution with a small number of degrees of freedom. In this scenario, SNPs of large effect are more probable due to the *t* distribution having greater kurtosis (thicker tails) than a normal distribution (Meuwissen *et al.* 2001). Some polymorphisms with large effects on quantitative traits are known and so the Bayes A prior assumption may more closely reflect the true situation although it still assumes that all marker effects are non-zero.

Bayes B is an alteration on Bayes A in that a proportion of the marker effects follow a scaled *t* distribution and the remaining markers have no effect. Again this distribution may allow for SNPs with large effects but differs in that all SNPs are no longer considered to be having an effect (Meuwissen *et al.* 2001). Bayes C is different again as this method assumes that SNPs with effects are normally distributed having a constant variance (Habier *et al.* 2011). Bayes C

doesn't allow for SNPs with large effects but **Bayes R** does, assuming a mixture of normal distributions for SNPs with effect (Erbe *et al.* 2012).

The model used for Bayesian methods can be simply written as;

(7) $y_i = \mu + I_1\ X_{i1}\ b_1 + I_2\ X_{i2}\ b_2 + \ldots + I_j\ X_{ij}\ b_j \ldots + I_m\ X_{im}\ b_{m,}$

where y_i is the phenotype of animal i, μ is the overall mean, I_j is an indicator variable with a value of 0 or 1 indicating whether SNP_j is having an effect or not (in this case I_1 would be the indicator variable of SNP 1), X_{ij} is the genotype of animal i for SNP j and b_i is the estimate of effect of SNP_i i.e. a vector of marker effects, m is the total number of SNPS (Meuwissen *et al.* 2016).

For linear predictions the estimation of **g** would involve directly utilising the genomic relationship matrix as described above (See equations [2] and [3] in VanRaden 2008). However this is not the case in Bayesian predictions. As described by Goddard *et al.* (2010) in their review, the vector of marker effects, **g**, should be estimated as $\hat{\boldsymbol{g}} = E(\boldsymbol{b}|data)$ where the estimate of **g** is conditional upon the data. The data utilised in genomic selection consists of a phenotyped and genotyped reference population, and assuming the data have been corrected for all other effects, i.e. environmental and management effects, then $\hat{\boldsymbol{g}}$ can be calculated using Bayes theorem such that;

(8) $$\hat{g} = E(\boldsymbol{g}|data) = \frac{\int \boldsymbol{g} p(\boldsymbol{y}|\boldsymbol{g}) P(\boldsymbol{g}) \mathrm{d}b}{\int p(\boldsymbol{y}|\boldsymbol{g}) p(\boldsymbol{g}) \mathrm{d}b},$$

Where $p(\boldsymbol{g})$ is the prior assumption of distribution of SNP effects (**g**), and $p(\boldsymbol{y}|\boldsymbol{g})$ is the likelihood of the data given **g** (Goddard 2009). Where the prior assumption of **g** follows a normal distribution with the same variance of all markers then (7) reduces to a BLUP estimate of **g**. However, where non-linear assumptions of **g** are considered, closed form solutions are not available for equation (7) so SNP effects are calculated utilising Markov Chain Monte Carlo

methods, such as using a Metropolis- Hasting algorithm, in combination with Gibbs sampling methodology (Meuwissen *et al.* 2001; Habier *et al.* 2011; Erbe *et al.* 2012).

Appendix 1.4.5 Comparing GBLUP and Bayesian results

Bayesian methods assume a prior distribution of SNP effects that may make more sense biologically than the linear assumption where SNP effects follow a normal distribution, and since the Bayesian distribution may more closely resemble the true distribution, a higher accuracy can be achieved. The reliability of genetic merit using Bayesian genomic selection methods, in simulated Holstein and Jersey bulls, was shown to be higher (approximately 3%) than GBLUP, while both methods has considerably higher reliabilities than the traditional relationship matrix (VanRaden 2008). Meuwissen and Goddard (2010) showed using simulated whole-genome sequence data that non-linear methods clearly outperform GBLUP as they take maximum advantage of the genome sequence data. Similar results have been reported in real data investigating milk traits in Holstein and Jersey populations with Bayes R increasing the average accuracy of the traits across both breeds by 0.05 when 30,000 SNPs are used (Erbe *et al.* 2012). Bolormaa *et al.* (2013) showed Bayes R accuracies to be, on average, greater by 0.03 compared to GBLUP for residual feed intake, carcass and meat quality traits in *Bos taurus, Bos indicus* and composite cattle. Brøndum *et al.* (2015) reported similar results in Nordic dairy populations with analysis under a Bayesian model yielding generally higher accuracies with 54,000 SNP data compared to GBLUP.

While the argument above suggests that Bayesian methods are higher performing than GBLUP, this does not mean that GBLUP is poor performing in the field. The fact that there are many genes affecting economically important traits in livestock and that linkage disequilibrium can extend over large genomic distances explains the good performance of GBLUP within breeds (Meuwissen *et al.* 2016) . The biggest advantage of GBLUP over Bayesian

methods is its capability to be integrated with current genetic evaluation programs, Australia wide, that currently utilise pedigree relationships (**A** matrix) as the genomic relationship matrix can replace it. Genomic breeding values have been successfully incorporated using a 'blending approach' with traditional EBVs (Harris and Johnson 2010). However there are further challenges ahead regarding genomic selection in beef and sheep populations which need to be rectified before genomic selection is widely adopted by these industries.

Appendix 1.5 Implementation in Breeding Programs

Appendix 1.5.1 Multi-Step genomic selection versus Single Step

The inclusion of genomic information in Australian genetic evaluations programs, such as BREEDPLAN, used to be very *ad hoc* and involved a selection index approach (Hayes *et al.* 2009a; Harris and Johnson 2010) where de-regressing EBVs and GEBVs by their accuracies 'blends' or combines the two values together post-analysis. This approach was used to determine BREEDPLAN EBVs for animals that had both SNP and pedigree information and was conducted for each trait separately. For traits without genotypes, GEBVs could be blended with those traits that do when a high correlation between the two traits exists (Swan *et al.* 2012). For example, carcass rump fat depth and carcass rib fat depth are highly correlated traits and therefore the GEBV of one can be blended with the EBV of the other.

To more broadly apply genomic selection in beef, it is likely that most of the animals are not going to genotyped, but those ungenotyped animals have pedigree and phenotypic records that want inclusion in the estimation of breeding values and similarly the genotyped animal may not be phenotyped e.g. for a sex-limited trait the bull may be genotyped but is not phenotyped. Because of this it is necessary to use pseudo-phenotypes which is a projection of the phenotypes of individuals close to the genotyped individual. First a regular genetic evaluation based on pedigree is run which is then used to create pseudo-phenotypes. After

this, then a genomic evaluation model is used. This process is clumsy and is referred to as Multi-Step GBLUP with potential losses of information as well as inaccuracies and biases (Legarra *et al.* 2014).

All sources of information such as pedigree, performance and genomic should be combined together in as single analysis and not blended after the fact to more easily accommodate animals with different levels of information (Swan *et al.* 2012). This is what is referred to as the single step method or ssGBLUP and the basic theory was developed in parallel by Legarra *et al.* (2009) and Christensen and Lund (2010). Single step has now been adopted by BREEDPLAN. Accuracy is usually as equal to, if not greater than, any other genomic selection method i.e. multi-step (Legarra *et al.* 2014). One shortcoming of the single-step methodology is that so far it does not work when utilising Bayesian distributions of SNP effects although some solutions have been proposed in the literature (Legarra and Ducrocq 2012).

Single-Step GBLUP, or ssGBLUP, integrates genomic relationships (G) with pedigree based relationships (A) intro a combined relationship matrix (H) also known as the extended relationship matrix. The logic of BLUP still holds and the only other change is to use **H** instead of the relationship matrix where for **y**= **Xb** + **Wu** + **e**,

$Var(\boldsymbol{u}) = \boldsymbol{H}\sigma_u^2$;

$Var(\boldsymbol{e}) = \boldsymbol{I}\sigma_e^2$, and the solutions to the mixed model equations are (Legarra *et al.* 2014);

$$\begin{pmatrix} \boldsymbol{X'X} & \boldsymbol{X'W} \\ \boldsymbol{W'X} & \boldsymbol{W'W} + \boldsymbol{H^{-1}}\lambda \end{pmatrix} \begin{pmatrix} \widehat{\boldsymbol{g}} \\ \widehat{\boldsymbol{u}} \end{pmatrix} = \begin{pmatrix} \boldsymbol{X'y} \\ \boldsymbol{W'y} \end{pmatrix}$$

Appendix 1.5.2 Reference Populations

In genomic selection, SNP effects are estimated using a reference population (sometimes called a discovery or training population) dataset which is made up of individuals that have both genotypic and phenotypic records. Dairy populations are favoured for genomic selection as they achieve high accuracy GEBVs. For example, Holsteins have a relatively low effective population size (approximately 100). This means that the LD between SNPs and QTL is high, so that approximately 40,000 SNPs explain most of the population's genetic variance. This low effective population size means that the effective number of chromosome segments is low, indicating reduced genetic diversity and therefore higher accuracies can be achieved when estimating their effects. In addition, progeny testing is widely practiced in the dairy industry which means relatively accurate estimates of genetic variance, and hence breeding values, can be obtained which reduces the residual error in the data (Goddard *et al.* 2010). The extensive amount of information in dairy breeds means that these datasets are said to be high quality.

For beef breeds, it may not be possible to assemble such high quality datasets as that described for the Holstein breed except that it is expected that as the number of genotyped individual's increases in beef, the accuracy will increase, just as it did for dairy. The low accuracies reported in beef GEBVs (Swan *et al.* 2012) are likely due to the relatively low numbers of animals that have both genotypes and phenotypes included in the reference population to develop genomic prediction equations (Johnston *et al.* 2012). In this instance it would be desirable to combine data from multiple breeds within a species. Ibáñẽz-Escriche *et al.* (2009) and De Roos *et al.* (2009) reported that across population, i.e. across breed, genomic evaluations were preferable compared to evaluations within populations when: the populations included in the reference dataset were closely related, the SNP chip density used

was high, or the access to/or provision of phenotypic records is limited. However, De Roos *et al.* (2008) also showed when looking at diverged breeds like Holstein-Friesian, Jersey and Angus that including multiple breeds potentially has limitations in that the phase of LD between the SNPs and QTL might not be the same. They suggested that if denser SNPs are used i.e. approximately 300,000 markers or greater as demonstrated then results should be more consistent. When considering linkage disequilibrium purely between beef breeds with approximately 30,000 SNPs (purebred Angus, Charolais and crossbreds of varying Angus, Charolais, Simmental and Piedmontese content) it was found that correlations of LD phase were high for distances between marker pairs ≤ 70kb, but as distance increased the correlations reduced (Lu *et al.* 2012). When a high density (HD) SNP chip is used (e.g. 777,000) a similar pattern is observed. However, the HD SNP chip is advantageous in that associations can still be detected when physical distance between markers are larger (>50-70 kb) that otherwise would have been hidden if only 50,000 SNPs were used (Porto-Neto *et al.* 2014).

Pryce *et al.* (2011) reported that there was minimal advantage, under GBLUP or Bayes A analysis, of multi-breed genomic evaluations over single breed evaluations. However using two breeds in the reference population was generally better than only utilising one breed, when the goal was to predict GEBVs for a breed not included in the reference population. Moghaddar *et al.* (2014) reported that not including the target breed in the reference population, in sheep, gave prediction accuracies close to zero while including data from genetically distant sheep breeds was found to have a neutral to slightly negative effect on accuracy. This appears to be contradictory to the results presented by Pryce *et al.* (2011) however that investigation included three dairy breeds while the latter compared Border Leicester, Poll Dorset and White Suffolk (pure and first cross) with Merino. It then seems less surprising that using a purebred Merino reference population yielded accuracies close to zero when calculating GEBVs for the other three breeds as the breeds diverged to serve different

purposes (meat vs wool), compared to the dairy cattle breeds who likely diverged more recently.

Ventura *et al*. (2016) proposed a method to help improve genomic prediction accuracy within a multi-breed beef population when selecting for crossbred and purebred animals. Their method clusters animals based on their genotype and unlike the traditional approaches for genomic selection, that use a fixed reference population, genomic prediction using clusters chooses the best reference population that will result in the highest accuracy. They reported an overall gain in accuracy of 1.3% across all traits and scenarios investigated.

Appendix 1.5.3 Long-term response to genomic selection

There is evidence to suggest that long-term genomic selection, that is selection for several subsequent generations, may result in declining responses to selection when using the prediction equation estimated from the base population or generation (Muir 2007). This is because selection is on the SNP allele and not directly on the favourable QTL allele, and although the pair is linked, the SNP allele is driven towards fixation much more quickly. This diminishes the LD between the SNP and QTL which in turn reduces the effectiveness of genomic selection (Goddard 2009). Additionally, genomic selection is unlikely to effectively select for rare, favourable alleles due to poor correlations with common SNPs. Traditional selection methods i.e. selection on phenotype, don't result in diminishing responses to selection because increasing the occurrence of rare favourable alleles compensates for movement towards fixation of more common favourable alleles. However this balance is not likely to be achieved in genomic selection and decline in response to selection is expected. Although it is likely this decline will be slower if the trait of interest is controlled by numerous genes, each contributing a small effect as the rate at which the allele frequency changes will be slower (Goddard 2009).

To counteract this decline in response, Muir (2007) proposed re-estimating the prediction equation each generation which partially prevented the decline. Additionally, Goddard (2009) suggested decreasing selection pressure on common QTL with large effect; that is use a high density SNP chip in conjunction with Bayes B, so that only SNPs that are in close LD with the QTL have estimated effects greater than zero. This resulted in accuracies that persisted over time as the LD persisted over time, as demonstrated by Meuwissen and Goddard (2010). The level of inbreeding in the population should also be kept to a minimum as this reduces the long-term response to selection (Goddard *et al.* 2010).

Appendix 1.5.4 Number of SNPs and Imputation

When considering how many markers to use it is important to remember the markers are only used because the number and distribution of QTL of interest across the whole genome are unknown. Therefore markers, such as SNPs are utilised because they are located genome wide with a known location. The aim is to use enough markers so that all QTL are in complete linkage disequilibrium with at least one marker or a haplotype of markers, that is every potential QTL is 'tagged' by a marker (Goddard 2009) such as a SNP. This is the principle that genomic selection relies on.

In Japanese Black cattle approximately 90-97% of the genetic variance was estimated for carcass weight using 4,000, 6,000 and 10,000 SNPs respectively (Ogawa *et al.* 2014). For marbling score 10,000 SNPs were required to account for as much as 92% of the genetic variance. Ogawa *et al.* (2014) proposed a larger number of SNPs were required for marbling due to the trait being controlled by QTLs with relatively small effects in comparison to carcass weight where QTLs with large effects are known. Similar results have been reported in Holstein populations where accurate genomic evaluation can be achieved using 3,000-5,000 SNPs, evenly placed across the genome (Moser *et al.* 2010; Wiggans *et al.* 2012). However as

the number of SNPs utilised increases, so does the accuracy of prediction. Prediction of genetic value using simulated whole-genome sequence data has been shown to increase accuracies of prediction by greater than 40% relative to the use of 30,000 SNPs (Meuwissen and Goddard 2010).

The attractiveness of using a low density SNP panels is the comparatively low cost compared to higher density panels. For example, prior to the availability of the Illumina Bovine3K BeadChip (2010), female genotyping accounted for 38.7% of the dairy genotyped population in the US which rose to 59% by 2012 (Wiggans *et al.* 2012). Previously discussed literature, in this review, recommends utilising higher density SNP panels to ensure LD between SNPs and QTL across populations. This is where imputation becomes important.

Imputation is used to fill in missing genotypes after SNP-chip genotyping based on the genotypes and haplotypes identified in other animals. Imputation methods can also be used in conjunction with low density chips that are cheap. For this, key ancestors need to be genotyped with a high density SNP chip to identify the haplotypes in the population. Descendants can then be genotyped with the low density chip, which has enough SNPs to recognise which of the haplotypes the animal carries, allowing the missing information to be imputed (Meuwissen *et al.* 2016). The limited effective population sizes and population structures in livestock make imputation of high-density genotypes for sparse genotypes possible (Daetwyler *et al.* 2011).

Initially imputing 50K genotypes to 800K genotypes and then to sequence has been shown to result in a higher accuracy of imputation than imputing 50K directly to sequence; although SNPs with low minor allele frequencies are more difficult to impute correctly (Van Binsbergen *et al.* 2014).

Appendix 1.6 Summary of Genomic Selection

Genomic selection is possible due the availability of SNP markers throughout the genome. It is an attractive alternative to pedigree based evaluations due to the ability to predict phenotypes accurately from genotypes at a young age, rather than using mid-parent averages. It is advantageous for late in life, hard to measure traits such as fertility and carcass traits due to the utilisation of extensive reference populations. These populations are made accessible as SNP data replaces the need for extensive pedigree recording. Many methods of implementing genomic selection have been discussed and compared. Most promising, is the ability to combine data from different breeds in multi-breed evaluations under the correct conditions.

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