Literature review:

The role of genomics for the improvement of dairy and beef cattle

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Introduction

The past few decades were characterized by a number of major discoveries and technological developments in the field of molecular genetics. Some highlights include Tautz (1989) discovering the hypervariable region in the human genome that led to the mapping of different DNA based markers. This was followed by the development of PCR technology by Mullis (Fore *et al.,* 2006) and automated sequencing becoming available during late nineties. The human genome was the first genome to be sequenced and this project was completed in 2001 (Adams, 2008). Genome sequencing projects followed for most farm animal species (including cattle, small stock, pigs and poultry), thus creating new opportunities for genetic improvement in livestock that was previously beyond researchers' reach.

In using DNA technology to perform genome mapping and sequencing a number of useful single genes were mapped in small stock (Van der Werf, 2007) and in beef and dairy cattle (Dekkers & Hospital, 2002) and some of these were successfully commercialized for application in the livestock industry. The focus of many research groups moved to searching for quantitative trait loci (QTL) for use in Marker Assisted Selection (MAS) or gene introgression (Anderson, 2001). The identification of these specific regions of interest that affect economically important traits in farm animals held great interest for the livestock industry. It was thought that DNA-markers and genomic information had the most potential for increasing genetic progress in quantitative traits with low heritability, which are difficult and / or costly to measure and often sex-limited (Dekkers, 2004). In the search for QTL and major genes different approaches were investigated including genome wide scans using high density panels of microsatellite or SNP markers together with genome wide association studies or candidate gene approaches (Hayes & Goddard, 2010). Currently the possibility exists to genotype an animal in a single assay using 50 000 SNP markers providing sufficient genomic information to be incorporated in breeding value estimations. "Genomics" as is now referred to by animal breeders was by no means an easy process. The aim of this review is to provide an overview of the developments that took place over the past two decades to lay the foundations of genomics for application in farm animals with specific reference to dairy and beef cattle.

The Bovine genome & the search for QTL

In order to appreciate the current molecular information available for cattle it is necessary to have a brief look at the bovine genome and the first efforts to compile DNA marker maps. Cattle have 58 autosomes and two sex chromosomes. The first genetic maps for cattle were constructed with 746 and 1250 microsatellite markers by Barendse *et al.* (1997) and Kappes *al.* (1997) respectively. The map compiled by Kappes *et al.* (1997) had genome coverage of 2990 cM with an average interval of 3.0 cM. This map was improved by adding more than a thousand markers with improved coverage and decreased marker intervals (on average 1.4cM) that resulted in a high density map with 3802 microsatellite markers (Ihara *et al.,* 2006). It is quite

important to note that 880 000 genotypes were generated from the USDA MARC cattle reference families in order to compile this map. The creation of these high density linkage maps were however essential for studying and fine mapping QTL and regions of interest.

QTL refer to regions in the genome that can be associated with traits of economic importance. As previously mentioned these are the traits that prove to be more difficult and slow to improve by means of quantitative methodology. Identification of relevant loci linked to the genes governing the traits or the genes themselves would therefore be ideal. The strategies for QTL mapping include either a candidate gene or a genome scan approach (Hu *et al.,* 2009). In both cases a resource population would have to be sampled and phenotypic data recorded for the traits of interest. For the candidate gene approach, candidate genes are selected and animals are only genotyped for these, followed by analyses of both the genotypic and phenotypic information (Kadarmideen *et al.,* 2006). In the genome scan approach genetic markers covering the whole genome are selected and typed in the reference population, followed by the construction of a linkage map and statistical analyses of the genetic and phenotypic data.

In these QTL-identification strategies three available marker types can be used. Three genetic marker types exist, namely direct markers where the loci are associated with the functional mutation, the LD markers that are in population wide linkage disequilibrium and the LE markers that are in population wide equilibrium with the mutation (Dekkers, 2004). Unfortunately direct markers are limited in number and difficult to find, except for some single gene traits (Anderson, 2001). LD markers result in a consistent association between the genotypes and traits, which will allow for selection based on genotypes. The use of LE markers for selection is very limited, and these markers find application in other molecular studies i.e. parentage verification, phylogenetics etc. Various strategies and marker types have potential for QTL detection and should be evaluated in context with the research question in mind. Although LE markers have the advantage of being random anonymous markers, estimates are within family and accuracy is lower. LD markers may provide higher accuracy for potential QTL across the population, but high density maps and candidate genes are required (Dekkers, 2004).

Software has been developed to incorporate large and complex pedigrees in the statistical analyses for identification of QTL (Seaton *et al*., 2002, 2006). In a few instances QTL identification studies have resulted in the identification of causative mutations, i.e. DGAT for milk fat content in dairy cattle (Grisart *et al.,* 2002) and MSTN for double muscling in beef cattle (Charlier *et al.,* 1995). Several putative QTL have been identified and in some cases even applied in MAS, specifically in dairy cattle. Genome scans were conducted for QTL associated with milk, health and conformation traits in dairy cattle using granddaughter designs and outbred families (Heyen *et al.,* 1999, Zhang *et al.,* 1998 ; Schrooten *et al.,* 2000). In all of these studies several QTL were identified for these traits, and these effects were included in breeding programs. Potential advantages were clearly shown by using QTL information in dairy programs (Abdel-Azim & Freeman, 2002; Boichard *et al*., 2006). The French dairy industry genotyped more than 70 000 bulls for 14 chromosomal regions over a 7 year period and results were sufficient to reduce the number of bulls for progeny testing by 15% (Boichard *et al.,* 2006). Despite the results they obtained, only a part of the genetic variance for the traits under study could be explained by QTL.

Genome–wide association studies followed where many more SNP markers were tested for association with the trait of economic importance still based on the assumption that a significant association was seen due to linkage disequilibrium between SNP with the causative mutation. Using this approach Charlier *et al.* (1995) identified five recessive disorders using a discovery

population with 25 000 - 50 000 SNP markers. Genome-wide association studies (GWAS) were performed in dairy cattle using approximately 43 000 SNP and associations were confirmed with feeding level and response of milk production to heat stress (Hayes & Goddard, 2010). Unfortunately these QTL only explained 1.5% and 2% of the genetic variance for the two traits.

Despite the efforts of performing GWAS the effects of the individual QTL identified for the desired traits were small and MAS was much less effective that what was originally expected (Hayes & Goddard, 2010). As quantitative traits are affected by many genes, it was found that any one gene explained a very small amount of the phenotypic variance. Many of the genes have a very small contribution, and the estimation of these effects is difficult. Even research done on human height by Visscher *et al.* (2010) could only explain 1-2% of the variation using a large number of QTL. It was clear that for complex traits GWAS would not be a feasible option and an alternative approach was needed to use all the available marker information simultaneously.

Whole genomic sequencing and SNP discovery

After the completion of the human genome sequencing in 2001, sequencing of the genomes of most farm animal species followed. The bovine genome sequence, completed in 2004, was the first genome with high SNP coverage of the Cetartiodactyla mammals (Family Bovidae). The DNA of a Hereford cow (L1 Dominette) and individuals representing six other breeds were used in this project (Tellam *et al.,* 2009). In this process SNP or single nucleotide polymorphisms were identified. A SNP is the genetic variation in a DNA sequence that occurs when a single nucleotide in a genome is altered. SNP markers are abundant single-locus markers that are usually bi-allelic and are located approximately every 700 bp in the *Bos Taurus* genome with an estimated total of nearly 4 million SNP (Seidel, 2010).

The frequency of more than 37 000 SNP markers were analyzed in 497 cattle by the Bovine HapMap Consortium that set the scene for further expansion of SNP discoveries (Eck *et al.,* 2009; Seidel, 2010). Van Tassel and co-workers added another 23 000 SNP to the Bovine collection studying 66 cattle that included breeds such as the Holstein, Angus, Red Angus, Gelbvieh, Hereford, Limousin and Simmental. This resulted in the compilation of a 50 000 SNP array that is currently applied in animal breeding as a technology referred to as “genomic selection” (Eck *et al.,* 2009; Seidel, 2010). Identification of SNP variation in a population is currently done by applying commercially available SNP chips. Depending on the genetic make-up of the animal the SNP may be a zero (not present for the specific individual), one copy or two copies (inherited from the dam, sire or both). Different SNP chips have been commercialized and are available as 50K SNP, 10K SNP and other smaller varieties. Ilumina (a USA based company) was one of the first to produce a 50K SNP chip for cattle. This is currently being used in the dairy industry in several first-world countries (Seidel, 2010).

Genomic selection in animal breeding

It should be emphasized here that the past few decades of genome mapping, marker discovery, GWAS, QTL detection and MAS was an essential process that established the basis for genomic selection (GS). GS in simple terms is based on the average effect of all the markers on a genome wide basis, while in MAS the effect was restricted to the one marker that showed significant linkage to the QTL (Hayed & Goddard, 2010). Due to more dense panels of markers being available, all QTL are now assumed to be in linkage disequilibrium with at least one SNP. To be able to use GS in practical breeding it had to be included in the Estimated Breeding Value

(EBV). Genomic data is now included as an additional source of information together with pedigree and performance records used in routine quantitative analyses.

From the vast amount of literature over the past five years it is clear that the implementation of GS is no simple matter and reference populations are required as well as molecular platforms for genotyping and refined statistical methodology to analyze the thousands of SNP generated. The first step for implementation is a reference population for the specific breed that will contain both phenotypic and genotypic information.

Reference population & SNP panels

The reference population (or often referred to as training population / data) (Meuwissen, 2007) need to consist of at least 1000 animals with phenotypic data and genotypes based on a large number of SNP. The phenotypic data may include individual performance records or EBVs from national evaluations. In the case of dairy cattle it also may consist of DYD records of average performance of offspring data (Calus, 2010).

In the construction of the reference population De Roos *et al.* (2007; 2008) and Van Raden *et al.* (2009) recommend that in dairy cattle proven bulls should be used as they already should have reliable phenotypic information and EBVs. The young candidate bulls will probably have close relationships with the proven bulls and therefore the estimation of their EBVs and the accuracy thereof will be improved. In theory the reference populations should include animals over the whole range of performance, including both inferior and superior animals, but this is not feasible in practice. The approach is therefore to have reference populations with the widest possible range of performance and with close relationships to the potential young selection candidates (Calus, 2010). It has also been shown that the reference populations should include more than one line that may relate to the young bulls, in order to ensure that there is LD across the specific family or lines for setting up the prediction equations.

It is important to note that the high density SNP marker panels were required to ensure that at least one SNP will be in LD with the trait across the families, lines or breeds. The number of SNP necessary depend on the extent of LD in the species or breed, the length of the genome (L) and the effective population size (N_e). It has been shown that with the current Holstein reference population, approximately 50 000 SNP will provide an accurate estimate within the Holstein, but at least 300 000 SNP are required for a prediction for Jersey cattle using the Holstein reference data (Calus, 2010).

It is clear from literature that for a breed to take part in an international evaluation based on SNP data there should be a sufficient number of bulls in the reference population to reflect the relevant line/family or with close genetic relationships to the population and with accurate phenotypic data (records or national EBV's). The USA is in a fortunate position as a DNA repository has been established storing semen from dairy bulls since 1999, consisting of major dairy bulls that have been progeny tested (Ashwell & Van Tassell, 1999).

Genome wide EBV estimation

Once the reference population has been established, the SNP genotypes that have been generated are used in the prediction equations for the GEBV (Hayes & Goddard, 2010). The prediction equations can also be used to estimate an EBV for young bulls that have been genotyped, but do not yet have a record for the trait. In Figure 1 the different sources of information necessary is shown for the prediction of genomic EBV's.

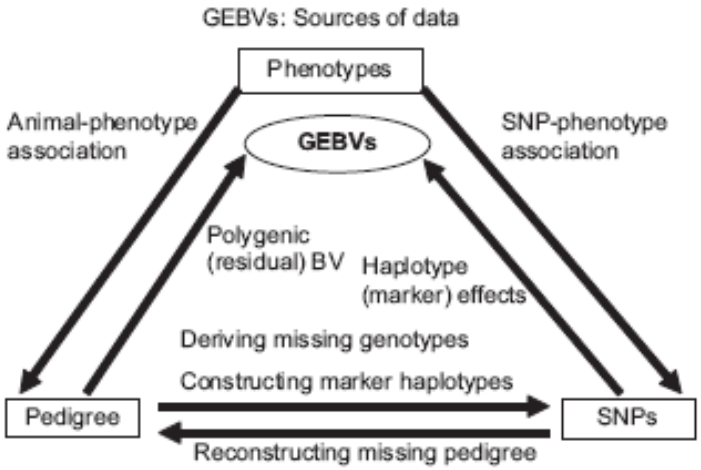

Figure 1 Sources of data for use in genome-wide prediction of breeding values (Calus, 2010)

The challenge in handling the data for the estimation of GEBV is based on having a large number of SNP genotypes (effects) and much less available phenotypes, approximately a thousand animals from the reference population. The statistical methodology had to be designed to solve this unequal distribution of equations and account for loci with very large or very small effects (Hayes & Goddard, 2001; Goddard, 2009). Different models based on certain assumptions have been described in literature that uses either a Baysian model (Meuwissen *et al.* 2001) or a mix model approach using Gibbs sampling. A summary of the assumptions for the different models were provided by Hayes & Goddard (2010) and is shown here in Table 1.

Table 1 Summary of assumptions for methods based on SNP markers for genomic EBV estimations (adapted from Hayes & Goddard, 2010).

Name	Assumed distribution of SNP effects	Implication
BLUP	Normal	A very large number of QTL of small effect
BayesA	t distribution	A large number of QTL of small effect and a small proportion with moderate to large effect
BayesB	Mixture distribution of zero effects and t distribution of effects	A large number of genome regions with zero effect, a small proportion of QTL with moderate effects
Bayesian LASSO	Double exponential distribution of effects	Very large proportion of SNP with effect of close to zero, small proportion of moderate to large effect
BayesSSVS	Mixture distribution of zero effects and t distribution of effects	A large number of genome regions with almost zero effect, a small proportion of QTL with moderate effects

Simulation studies were performed using the different models (Bayesian and mixture) based on reference populations with at least a 1000 animals and these models reported accuracies

ranging from 0.7 to 0.8 for animals without a phenotypic record. Marker density was assumed to be one SNP per cM (Meuwissen *et al.,* 2001; Habier *et al.,* 2007). In studies where real data was used from dairy cattle traits based on approximately 40 000 SNP, accuracies for GEBVs ranged from 0.36 to 0.77 (Schenkel *et al.,* 2009) to 0.52 to 0.82 (De Roos *et al.,* 2009). The size of the reference population affected the accuracies, with large populations (1200 bulls) having accuracy values of above 0.77 for GEBVs.

Application of GS

There is no doubt that genomic selection has significant advantages for improvement of farm animal genetics. Dairy cattle thus far have led the way and have experienced some of the advantages of having an added resource of information. GEBV can be obtained in a relative short period after birth compared to a 6-7 year period of progeny testing before a progeny-based EBV becomes available. GEBVs have distinct advantages for dairy cattle with regard to reducing costs on progeny testing and decreasing the generation interval (Boichard *et al*., 2006; Schaeffer, 2006).

Another advantage for the application of GS is that the platforms for large scale genotyping is now well established with several private companies offering 10K and 50K SNP panels. The Illumina Bovine SNP Bead chip has been developed in cooperation with USDA Bovine Functional Genomics Laboratory in Beltsville and the University of Missouri Research is underway to design a SNP chip suitable for use in Bos Taurus and Bos Indicus that will make commercial application possible for beef cattle ((Van Raden *et al*.,2009).

Genomic technology has been well received by dairy cattle breeders in the USA and Canada and indications are there that genomic evaluations will replace traditional evaluations in these countries (Van Raden *et al.,* 2009). The French dairy industry applied SNP methodology where 3200 bulls, including three breeds, were genotyped and evaluated for 25 traits. Due to the advantages of shown by using GS, Boichard *et al.* (2010), indicated that GS will be extended to all French dairy breeds by the end of 2011.

South African dairy and beef cattle breeders have shown interest in DNA technology by making use of DNA based parentage and other diagnostic tests available for genetic improvement of their stock (Van Marle Köster & Nel*,* 2003). It is envisaged that genomic selection will be important for South African beef and dairy cattle breeders to remain competitive and part of international genetic evaluation systems. It is however necessary to establish a reference population for South African dairy and beef breeds. This population will be used to obtain the relationship between the information from the SNP chips and the “true” breeding values for the different traits. A number of older and proven bulls with a large number of recorded progeny (with breeding value predictions with a very high reliability) should be genotyped and this information tested against “candidate sires” to establish the predictability associated with the models available.

Conclusion

DNA technology opened up a vast amount of new possibilities for genetic testing and improvement of farm animals. GS in selection programs holds potential for sex-limited traits, traits that are expressed late in life and functional traits with low heritabilities. Application of GS globally in both the dairy and beef industries has become inevitable and smaller countries with fewer resources will have to collaborate and carefully plan genetic programs to remain part of the international arena.

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