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OPTIMIZING SHEEP BREEDING PROGRAMS WITH GENOMIC SELECTION

J.H.J. van der Werf[1,2], B.J. Horton[1,3] and R.G. Banks[1,4]

[1]Cooperative Research Centre for Sheep Industry Innovation, Armidale, NSW 2351, Australia
[2]School of Environmental and Rural Science, University of New England, Armidale, NSW, 2351
[3] Tasmanian Institute of Agricultural Research, University of Tasmania,
Kings Meadows, TAS 7249
[4] Meat and Livestock Australia, c/o. Animal Science, UNE Armidale, NSW 2351, Australia

SUMMARY

We discuss genomic selection as a way to provide information on breeding values for traits that are difficult to select for. A brief review of genomic prediction methods shows that currently in sheep, genomic prediction requires selection candidates to be genetically related to a reference population although it allows information of more distantly related individuals to contribute to selection accuracy. Subsequently we discuss genomic selection in a sheep breeding program context and discuss possible ways to optimize genotyping strategies in a breeding nucleus. Genotyping a proportion of pre-selected young males saves costs without compromising genetic gain, making genotyping cost effective even at a high testing cost. When only counting expressions of genetic gain in two tiers, the optimal proportion of males genotyped becomes lower and genotyping becomes prohibitive if testing costs are above $100 per head, unless breeding males can be used in the first year.

INTRODUCTION

Breeding programs are mainly driven by the choice of traits in the breeding objective, and their relative importance, the investment in trait measurement, and decisions about selection and mating based on estimated breeding value. Currently, the main tools available to breeders are estimated breeding values (EBVs) and indices. EBVs are best predictions of an animal's breeding value given all data available on phenotypic measurement and pedigree, and this can be enhanced by genomic information. This is particularly useful for traits that have a low EBV accuracy at the time of selection. One of the key questions for individual breeders is what information should be collected to drive breeding programs. With the advent of genomic selection, a typical question that arises is 'should I DNA test and if so, which animals should I genotype'?

To predict breeding value based on genomic information requires a reference population that needs to be large (thousands of animals measured) and to some extent represents the lineages and breeds found in the commercial breeding population. The question about the genetic constitution of a reference population for genomic selection is challenging for sheep breeding in Australia where the population consists of a diversity of breeds and lines within breeds. It is relevant to know whether breeding animals can be predicted based on a DNA test if they have no strong genetic relationship to the reference population.

The aim of this paper is to discuss breeding program options for sheep that allow for genomic selection and for selection on traits not normally measured by stud breeders. We first discuss genomic selection with specific emphasis on the value of genomic information to selection accuracy, and the accuracy of genomic prediction depending on an individual's relationship to a reference population. Subsequently we look at the breeding program context and optimize the proportion of rams to be genotyped in a breeding nucleus.

GENOMIC PREDICTION

Principle and Methods. Genomic selection involves collection of DNA samples on young breeding animals. These samples are sent for genotyping and based on information from thousands of DNA markers (single nucleotide polymorphisms - SNPs) an estimate can be made of breeding value by comparing the DNA information on the breeding animal with that of a reference population of animals that have information on DNA as well as phenotypes. Genomic selection was first proposed by Meuwissen *et al*. (2001) and is based on the proposition that if the marker density is high enough, each quantitative trait locus (QTL) is bound to be in linkage disequilibrium with a marker. This allows estimation of SNP effects across the whole genome in a set of animals with phenotypes and genotypes measured, then based on such estimates the breeding value of animals that have no phenotypes can be predicted. The term 'prediction equation' is often used, indicating that the genomic breeding value is calculated from a multiple regression equation of SNP genotype: GBV = $\sum b_i x_i$ where b_i is the effect of SNP genotype x_i. Various statistical methods have been proposed to estimate **b**. With tens of thousands of markers, it is not possible to estimate a regression effect for each marker as the number of data points is generally much smaller. Therefore, markers are usually treated as random effects. Depending on the prior assumption of SNP effects, such models can assume equal variance at each locus, a different variance at each locus, or a different variance at a small subset of loci with the remaining loci assumed to have no effect. In the original paper of Meuwissen *et al.* (2001) these methods were termed "BLUP", "BayesA" and "BayesB", respectively. These and slight variations of the methods have been used ever since data on SNP chips has become available, and in most cases, there appears to be little difference in the predictive ability of SNP effects that were obtained with any of these methods. This is an indication that the model underlying genetic variation is probably based on many small effects at many different loci, also known as the infinitesimal model. Clark *et al.* (2010) found through simulation that the BayesB method should be superior if much of the genetic variation of a trait is affected by few loci with large effects, but methods converge to a similar prediction accuracy under the infinitesimal model.

GBLUP. An interesting analogy was reported by Habier *et al*. (2007) who showed that the BLUP method for genomic selection is equivalent to the usual animal model where the numerator relationship matrix that is based on pedigree (the A-matrix) is replaced by a genomic relationship based on similarity of genotypes across the genome (G-matrix). This is because in a BLUP model for genomic selection the variance of the observations can be written as $XX' + \lambda I$, where X links animal phenotypes to all marker effects, i.e. it contains the animals' genotypes. XX' gives the cross-products of animals' genotypes, or 'correlations between genomes' and these elements have the same expectation as additive genetic relationships in the A-matrix. This has led to an interesting discussion regarding the information actually used in predicting genomic breeding values. Habier *et al.* (2007) argued that even if linkage disequilibrium (LD) did not exist, genomic prediction would still have a non-zero accuracy as genomic prediction could simply be based on relationships. However, simulation results showed that predictions based on relationships wear out quickly across generations whereas prediction based on LD persist for longer. A BayesB method would be more based on LD-type predictions and was therefore proposed as the preferred method. This was also concluded by Clark *et al.* (2010) who showed that that the BayesB method is generally more robust as it also captures relationships.

Another consequence of Habier's result is that both conceptually and computationally the genomic prediction is now simplified. One can easily predict genomic breeding values using

software such as ASReml (Gilmour *et al.*, 2009), where data on 'n' animals is combined with a genomic relationship matrix of 'n + q' animals, with n being the number of animals in the reference population with both phenotypes and genotypes, and q the number of animals without phenotypes but with genotypic data such that their breeding value can be predicted from genomic information. ASReml allows fitting an animal model where the inverse of the G-matrix that is computed from the genotypic data can be used to fit the covariance structure among the animal effects. The mixed model equations look like

$$\begin{bmatrix} X'X & X'X & 0 \\ Z'X & Z'Z + G^{11} & G^{12} \\ 0 & G^{21} & G^{22} \end{bmatrix} \begin{bmatrix} b \\ g_1 \\ g_2 \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \\ 0 \end{bmatrix}$$

where G^{11} pertains to the inverse of the genomic relationship among the animals in the reference set and G^{22} pertains to the set of animals to be predicted, and G^{12} pertain to genomic relationships between these two sets. Hence, the genomic breeding values of the animals without phenotypes is estimated as

$$\hat{g}_2 = -(G^{22})^{-1}G^{21}\,\hat{g}_1 \qquad [1]$$

which can be interpreted as a genomic regression of breeding values of animals without data on breeding values of animals with data. This approach is usually referred to as the GBLUP method. However, note that the genomic relationship matrix (G) can be constructed in many ways, differing in how they weight similarity at each locus. When all loci are weighted equally, the method is equivalent to Meuwissen *et al.* (2001) BLUP approach for genomic selection. When loci are weighted according to the mount of variation explained by it, these mixed model equations can give the same solutions as BayesA or BayesB approach, depending on how their variances were estimated, which depends on the prior distribution assumed for QTL effects.

A simple example can illustrate the GBLUP method. Let animals 1-4 have a phenotype and animal 1 is a parent of 2 and 3. Animal 5 is a third offspring of animal 1 but has no record. We ignore fixed effects and assume them known, and the observations y are deviations from their expectations (e.g. contemporary mean). If we use a pedigree based BLUP method, we can get estimates of the breeding values of those 5 animals as $\hat{u} = (Z'Z+\lambda A^{-1})^{-1}Z'y$ and when using a GBLUP method the prediction is $\hat{u} = (Z'Z+\lambda G^{-1})^{-1}Z'y$ with A and G being

A=

1	0.5	0.5	0	0.5
0.5	1	0.25	0	0.25
0.5	0.25	1	0	0.25
0	0	0	1	0
0.5	0.25	0.25	0	1

G=

1	0.5	0.5	0.02	0.5
0.5	1	0.20	0.015	0.20
0.5	0.20	1	0.025	0.30
0.02	0.015	0.025	1	0.025
0.5	0.20	0.30	0.025	1

The A-matrix is based on the path coefficients derived from the pedigree, whereas the G-matrix is an arbitrary example in which based on the genomic data, animals 3 and 5 are genomically more similar to each other and more distinct from animal 2 than based on expected degrees of relationship for half sibs. Also, animal 4 is now genomically somewhat related to the others, although not a direct relative and animal 4 shares more genomic information with animals 3 and 5 than with animal 2. When assuming a heritability of 0.25, the breeding value for the animal without phenotype (animal 5) would be estimated under regular BLUP similar to [1] as $\hat{u}_5 = 0.5\hat{u}_1$

(note that $\hat{u}_1$ contains also phenotypic information about animals 2 and 3) whereas under GBLUP this prediction according to [1] would be $\hat{g}_5 = 0.4999\hat{g}_1 - 0.026\hat{g}_2 + 0.0622\hat{g}_3 + 0.0144\hat{g}_4$.

The genomic regression coefficients themselves are not always insightful due to them being partial regression coefficients. For example, it may seem odd that to predict animal 5, the breeding value from animal 2 has a negative weight, whereas that of animal 4, which is much less related to animal 5, is positive. The reason is that information from animal 2 is also used to predict $\hat{g}_1$. Regression of genomic breeding value on phenotypes would avoid this confusion. These can be calculated as $\hat{u} = GZ'V^{-1}y$ and for animal 5 this gives

under regular BLUP $\hat{u}_5 = 0.1136.y_1 - 0.0455.y_2 + 0.0455.y_3$
whereas under GBLUP $\hat{g}_5 = 0.1135.y_1 + 0.0328.y_2 + 0.0591.y_3 + 0.0519.y_4$.

The accuracy would be computed from the diagonal of the inverse of the coefficient matrix (C^{ii}) for animal 5 as $r = \sqrt{(1-\lambda C^{55})} = 0.282$ under BLUP and 0.285 under GBLUP.

This example illustrates a number of points when using GBLUP; 1). There is a large degree of similarity between pedigree-based BLUP and genomically-based GBLUP predictions. A GBLUP prediction uses a more accurate covariance structure among relatives and therefore gives a more appropriate weighting to the information of relatives. For example, some sibs have genomically more in common than others, even though based on pedigree they may have the same expected numerator relationship. Visscher (2008) presented expected values for mean and variance of the proportion of the genome that individuals share identical by descent. For the human genome they found the standard deviation of relationship to be 0.039 for full sibs and 0.027 for half sibs, i.e. half sibs have a mean relationship of 0.25 but can vary between 0.20 and 0.30. Note that this variation in relationships is larger when fewer genes are involved, e.g. in the extreme case of single locus traits the relationship could be either 0 or 1, making the difference between BLUP and GBLUP larger. 2) Under both BLUP and GBLUP, most of the information to predict an animal's breeding values comes from relatives. 3) Information from distant relatives is often ignored in BLUP as it falls outside the known pedigree whereas in GBLUP such relationships may be detected and the information on distant relatives can be used.

Remaining Questions. The example above showed that to predict genomic breeding values, it is very useful to have relatives in a reference population. Information from distant 'relatives' could also contribute, but many more records on such distant relatives are usually needed to achieve a similar accuracy. Using simulation, Clark *et al.* (2011) found that GBLUP can give considerably higher accuracy of breeding value prediction than the pedigree-based BLUP method for animals that have no direct relatives in a reference population. This gives some confidence for the feasibility and utility of reference populations for genomic selection as selection candidates may not all need to have direct relatives in this resource.

Daetwyler *et al.* (2011) investigated the accuracy of predictions across breed and found these to be low when sheep breeds are distant. Sheep breeding programs have a multiplicity of different breeds, which makes it difficult to set up reference populations if a large number of animals from each breed needs to be represented. A solution might be to use denser markers (Goddard *et al.,* 2006) as with shorter distances between marker and QTL it is more likely that there is LD across populations such that the marker becomes predictive across populations. Prediction across breeds would also require locus effects to be at least similar across breeds. Such a hypothesis has not been widely tested in whole genome prediction. The LD paradigm that underlies the original Meuwissen *et al.* (2001) paper would require dense markers for accurate genomic predictions, and denser markers are needed to predict more distantly related animals. The genomic relationship approach may suggest that much sparser markers are sufficient to predict genomic relationships.

Whether denser markers would allow prediction of more distantly related individuals more accurately needs to be investigated.

GENOMIC SELECTION

Prediction accuracy. Genomic information can increase the accuracy of EBVs in young breeding animals, particularly for traits that are difficult to measure on-farm and early in life. Modeling of sheep breeding programs has shown that the predicted additional rates of genetic gain could be 30% for wool sheep and 20% for meat sheep (van der Werf, 2009). The advantage in wool sheep is mainly an increased accuracy of predicting merit for life time production (wool and lambs) when selecting at an early stage. The advantage in meat sheep is mainly the prediction of carcass and meat quality traits that cannot be measured on breeding animals. The CRC for sheep industry innovation in Australia has used more than 7000 records from the Information Nucleus Flock as well as from the Sheep Genomics Project to predict genomic breeding values which were compared with Australian sheep breeding values (ASBVs) from progeny tested industry rams. The prediction accuracy was based on a 50k SNP chip and was shown to be highest for merino sires, with accuracies of ~0.6 for wool and ~0.5 for meat traits, because the reference population was mainly based on a merino genetic background (Daetwyler et al, 2010). Prediction accuracies were between 0.2 and 0.5 in maternal and terminal sire breeds. Further work is being undertaken to add additional data about phenotypes and genotypes.

Commercialization. The commercial delivery of genomic information to breeders in Australia can be via the existing genetic evaluation system (OVIS) where various methods have been explored to combine genomic and phenotypic information into predicted breeding values. This has recently been tested in a pilot project and breeders have received estimated breeding values for young rams for existing traits but with improved accuracy, as well as for new traits that are not routinely measured. To the breeder, genotype information will appear as improved accuracies of EBVs for existing traits or EBVs for traits that were not measured on-farm before, e.g. meat quality. This seems an easy model for introducing genomic selection into the industry. However, there are two important hurdles that need to be taken. First, investing in genotyping needs to be cost effective for a breeder; hence the cost of genotyping should not exceed the returns from improved accuracy of breeding values. These returns may be hard to capture, especially when achieved in traits that are valued further down the supply chain. Sheep production systems are predominantly pastoral based and extensive in nature and the number of commercial expressions resulting from most stud rams is low. This makes it difficult for individual breeders to invest much in trait recording or DNA testing even though the cost-benefit of investments in breeding from a national perspective is usually favourable due to the multiplication of benefit across multiple tiers. Cost-benefit from the individual breeder's perspective could be evaluated by only counting cumulative benefits of selection superiority as expressed in direct offspring of sires (rams) sold, e.g. see *Dominik et al.,* (2011).

A second hurdle is that to predict breeding value based on a DNA test, a large reference population needs to exist and to some extent represent all lineages and breeds found in the commercial breeding population. For traits that cannot be measured on-farm, such as carcass and meat quality traits, this requires investment in phenotypic measurement such as is currently achieved in the information nucleus model. As not all industry benefits of genetic improvement flow back to breeders, this investment is unlikely to come solely from breeders. Other traits such as adult wool measures, adult weight and reproduction could be measured on farm. Without genomic selection this information is hard to utilise in selection decisions as it becomes available after animals are selected for the stud breeding program. Genomic selection could use information

on previous generations efficiently and for such traits the reference populations might well consist of the ancestors of the current selection candidates across all trait recording flocks.

Genotyping Strategies. We used the sheep breeding model previously developed by Horton (1996) to examine the optimal proportion of males genotyped in a breeding nucleus. The model was adapted to consider the increase in information available for older breeding animals, both due to extra measurements and progeny information, and rams were selected optimally across age class. The model allows for the use of genomic information to improve selection accuracy. Since this requires expensive tests the model uses two stage selection of the nucleus rams. The young rams are tested using measured values (including information from relatives where available) at the age they could enter the breeding flock. Then a proportion of the best rams available are selected for genomic testing. The rams actually used in the nucleus are chosen using all the information available, including the genomic results. Rams not used in the nucleus are used in the multiplier or commercial levels as usual. After taking into account cost of measurement of phenotype and genotype, the breeding model was optimised using a differential evolution algorithm for a single objective or a multiple objective genetic algorithm, using the objectives $ value per ewe and efficiency ($ gain as % of $ invested) as the criteria of optimization. The proportion of the nucleus ram drop chosen for genomic testing is optimised by the genetic algorithm.

We initially considered a model for a three-tiered breeding system, with a nucleus, multiplier flocks and commercial flocks. The model was then modified to also be able to represent a two-tiered system, where the nucleus flock (possibly using genomic information) sold rams directly to commercial flocks rather than through multiplier flocks. With only two-tiers the nucleus must be able to provide returns from the selection methods by direct gains in the commercial flocks, rather than multiplying the genetic benefits through the multiplier tier. The two-tiered system was simulated by ensuring that the nucleus was large enough to produce sufficient rams for all the commercial flock and the 'multiplier flocks' did not use any selection for their rams. The value of the 'multiplier flock' cull rams was set to be the same as the value of wethers produced in the commercial flock, so these groups became equivalent for production purposes. The nucleus produced 10,000 lambs under the two tier system and it was 2,000 for the three tier system. The total number of ewes was 150,000 and 1 million, respectively. The model was used to test the potential value of genomics at a range of different costs, by determining the optimum proportion of nucleus rams to be tested at a given cost per test. The results of five runs of the model at each test cost are shown in Figure 1. Models were tested with rams first used at 19 months (i.e. lambs born when rams were 2 yo) and for rams used for mating at 7 months of age (lambs born when rams were 1 yo). Without genomic information, selection accuracies at 7 mo and 19 mo were 0.48 and 0.62 while with genomic selection these were 0.62 and 0.75, respectively. The coefficient of variation of the breeding objective was 10%.

For a three-tiered structure, if the cost of genomic testing was less than $500 per animal genotyped, the optimum strategy required the genotyping of about 75-80% of the ram drop. The initial selection was based on measured information including measurements on relatives, then using genomic tests to select the rams required in the nucleus. For the 2 yo ram system, at test costs below $100 the optimum proportion tested was unstable, either close to 80% or at 100% for different runs of the model. For a two-tiered structure, when rams were first used for mating at 19 months there was sufficient information to make the selection with reasonable accuracy based on data available at that age and with test costs greater than $110 the model did not use genomic selection. At $110 per test the solutions with the use of genomics were equal to those without genomics in terms of $gain per ewe in the system, while below $110 per test the use of genomic information improved the value of the breeding system. When rams were used for breeding at a

younger age there was less measured information available so the accuracy was much lower, unless genomic data was also used for selection. In this case the increase in accuracy was critical and with test costs of $300/animal about 43% of the rams were selected for genomic testing before use as breeders in the nucleus. Even with test costs at $500 per animal tested the optimal breeding system required the use of genomics when rams were used for breeding at 7 months.

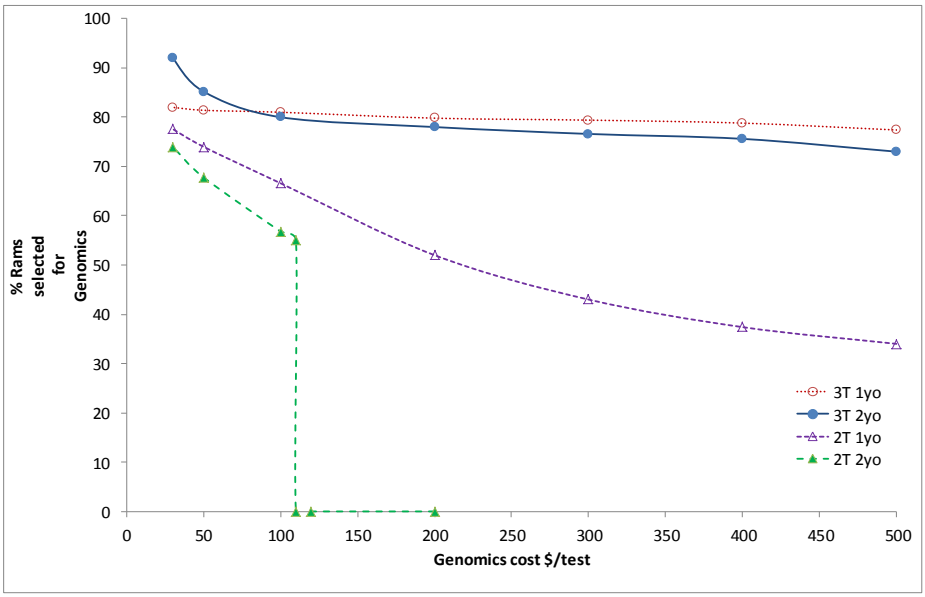

Figure 1. Cost of genomic tests and % of rams tested in the optimum model structure.

According to our modeling it is cost effective to genotype a substantial proportion of the breeding nucleus, even if genotyping costs are fairly high. This is because much of the genetic progress achieved is multiplied over many animals. For example in the three-tier system genetic improvement is expressed in 1 million commercial animals. In the two-tier model, the multiplication factor is lower and therefore the benefits per DNA tests are much lower and with genotyping costs above $100 it becomes uneconomical to genotype unless rams are used for breeding when little or no phenotypic information is available.

It is important the emphasize here that we did not simulate a specific breeding objective as used in the industry, but rather aimed at showing the principles by using a generic 'overall merit' objective with a genetic standard deviation of around $9. This is at the high end of the breeding objectives that underpin the indexes used by Sheep Genetics. Different objectives will have different benefit from genomic selection. The shape of the graphs displayed in Figure 1 will be largely unaffected by the particular breeding objective but the scale along the X-axis could vary.

The current model is a first attempt to optimize investment in genotyping and as such could be used for a broader scope of problems related to investment in information. For example, it can be extended to include measurement of individual traits and this could be achieved via multiple stage selection steps. The model would need to include multiple traits to reflect not only increased response for overall merit, but also a shift of response to traits for which more information is collected. Also the option of using reproductive technology would need to be considered as genomic selection would lead to increased benefits from such technologies. We have ignored the cost of the reference population when assessing genotyping strategies for individual breeders. Size,

genetic composition and measurement strategy of such a reference population could be determined with regard to the size and composition of the commercial breeding population that would benefit from it.

CONCLUSIONS

Genomic selection has potential in sheep breeding as accuracies have been reported that are of sufficient magnitude to cause a substantial improvement of selection response (e.g. see Daetwyler et al, 2010). Ongoing genomic selection requires a reference population with genotypes and phenotypic measurements on traits that cannot be easily selected for on-farm. The required size, as well as the genetic constitution of the reference population needs to be determined, and is dependent on the contribution from more distantly related individuals to a genomic prediction. Prediction accuracy is expected to improve with an increase in size of the reference population, and prediction across breeds may or may not improve with denser SNP panels, the latter depends on the assumption that consistent effects of loci or small regions on the genome can be estimated with sufficient accuracy across a wider range of genetic backgrounds. There is currently already a wealth of genotypic and phenotypic data in the sheep CRC and elsewhere that can contribute to resolving many of these questions. Experiences from cattle research can provide information about the added value of high density chips. Such information could be used to model expected outcomes from selection strategies and to optimize investment in trait measurement and genotyping. Business models have to be developed such that investment in breeding programs can be shared among those that benefit from genetic improvement. These are not only breeders, but also commercial producers, processors and ultimately consumers.

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