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Breed-adjusted genomic relationship matrices as a method to account for population stratification in multibreed populations of tropically adapted beef heifers

Christie L. Warburton[A,F], *Roy Costilla*[A], *Bailey N. Engle*[A], *Nicholas J. Corbet*[B], *Jack M. Allen*[C], *Geoffry Fordyce*[A], *Michael R. McGowan*[D], *Brian M. Burns*[E] *and Ben J. Hayes*[A]

[A]Centre for Animal Science, Queensland Alliance for Agriculture and Food Innovation, University of Queensland, St Lucia, Qld 4067, Australia.
[B]Formerly Central Queensland University, School of Health, Medical and Applied Sciences, Rockhampton, Qld 4700, Australia.
[C]Agricultural Business Research Institute, University of New England, Armidale, NSW 2350, Australia.
[D]The University of Queensland, School of Veterinary Science, St Lucia, Qld 4067, Australia.
[E]Formerly Department of Agriculture and Fisheries, Rockhampton, Qld 4700, Australia.
[F]Corresponding author. Email: c.warburton@uq.edu.au

Abstract

Context. Beef cattle breeds in Australia can broadly be broken up into two subspecies, namely, *Bos indicus* and *Bos taurus*. Due to the time since divergence between the subspecies, it is likely that mutations affecting quantitative traits have developed independently in each.

Aims. We hypothesise that this will affect the prediction accuracy of genomic selection of admixed and composite populations that include both ancestral subspecies. Our study investigates methods to quantify population stratification in a multibreed population of tropically adapted heifers, with the aim of improving prediction accuracy of genomic selection for reproductive maturity score.

Methods. We used genotypes and reproductive maturity phenotypes from 3695 tropically adapted heifers from three purebred populations, namely, Brahman, Santa Gertrudis and Droughtmaster. Two of these breeds, Santa Gertrudis and Droughtmaster, are stabilised composites of varying *B. indicus* × *B. taurus* ancestry, and the third breed, Brahman, has predominately *B. indicus* ancestry. Genotypes were imputed to three marker-panel densities and population stratification was accounted for in genomic relationship matrices by using breed-specific allele frequencies when calculating the genomic relationships among animals. Prediction accuracy and bias were determined using a five-fold cross validation of randomly selected multibreed cohorts.

Key Results. Our results showed that the use of breed-adjusted genomic relationship matrices did not improve either prediction accuracy or bias for a lowly heritable trait such as reproductive maturity score. However, using breed-adjusted genomic relationship matrices allowed the capture of a higher proportion of additive genetic effects when estimating variance components.

Conclusions. These findings suggest that, despite seeing no improvement in prediction accuracy, it may still be beneficial to use breed-adjusted genomic relationship matrices in multibreed populations to improve the estimation of variance components.

Implications. As such, genomic evaluations using breed-adjusted genomic relationship matrices may be beneficial in multibreed populations.

Keywords: adjusted genomic relationship matrix, allele frequency, genomic selection, admixed cattle population.

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Introduction

Beef cattle breeds in Australia can broadly be broken up into two subspecies, namely, *Bos indicus* and *Bos taurus* (Davis 1993; Bolormaa *et al*. 2011, 2013). It is believed that these two subspecies diverged from a common ancestor between ~332 000 years ago (Achilli *et al*. 2008) and 2.0 million years

ago (Hiendleder *et al*. 2008). Due to the time since divergence, it is likely that mutations affecting quantitative traits have developed independently in the two subpopulations (Bolormaa *et al*. 2013; Kemper *et al*. 2015). The northern Australian beef industry is dominated by *B. indicus* and *B. indicus* × *B. taurus* composite cattle breeds (Bolormaa *et al*. 2011). So as to develop genomic evaluations for this industry, prediction models will need to accurately predict performance across purebreeds and composites of both subspecies.

It is estimated that between 2% (Bolormaa *et al*. 2013) and 10% (Bolormaa *et al*. 2011; Koufariotis *et al*. 2018) of the Australian Brahman genome is *B. taurus* in origin. Studies have shown that quantitative trait loci (QTL) affecting quantitative traits do segregate differently in *B. indicus* and *B. taurus* (Bolormaa *et al*. 2013; Porto-Neto *et al*. 2013). This will affect the prediction accuracy of multibreed genomic selection and the genomic evaluation of composite animals when animals of both ancestral subspecies exist within populations (Bolormaa *et al*. 2013; Kemper *et al*. 2015). This is because single nucleotide polymorphisms (SNP) in linkage disequilibrium (LD) with a QTL in chromosome segments from one subspecies may not be in LD with a QTL in the other subspecies, or in the same phase. For accurate across-breed genomic evaluations to be developed, it may be essential to account for the genomic architecture of traits by considering breed-specific ancestral alleles (Bolormaa *et al*. 2013; Porto-Neto *et al*. 2013; Kemper *et al*. 2015).

Studies have shown that the use of multibreed reference populations can improve the prediction accuracy of across-breed genomic evaluations (Bolormaa *et al*. 2013; Hayes *et al*. 2019). However, the magnitude of this improvement has generally been modest (Bolormaa *et al*. 2013; Hayes *et al*. 2019). Kemper *et al*. (2015) have shown that genome-wide association studies using multibreed reference populations can sometimes incorrectly identify SNP as segregating in multiple breeds when they are segregating only in a single breed. This limited power to detect SNP that segregate across, and have an effect upon QTL in multiple breeds of cattle, may explain why the use of multiple-breed reference populations in genomic evaluation has resulted only in small improvements in genomic prediction accuracy (Kemper *et al*. 2015).

Inclusion of genetically divergent breeds within genomic evaluations can reduce prediction accuracy due to differences in the allele frequencies and LD between marker and QTL and markers among breeds (Calus *et al*. 2014; Moghaddar *et al*. 2014). The genomic relationship matrix (**GRM**) in multi-breed genomic evaluations is typically weighted by the average allele frequencies across breeds (in fact, just the average allele frequencies in the reference population), and if there is disparity in the allele frequencies among breeds, this may have a negative effect on the prediction accuracy of multibreed genomic evaluations (Moghaddar *et al*. 2014). One proposed method to alleviate this issue is to model breed-specific allele effects to account for disparity in allele effects among breeds in multibreed genomic predictions (Ibáñez-Escriche *et al*. 2009; Makgahlela *et al*. 2013; Calus *et al*. 2014; Moghaddar *et al*. 2014; Lourenco *et al*. 2016; Lopes *et al*. 2017; Gurman *et al*. 2019; Sevillano *et al*. 2019; Duenk *et al*. 2019). This method has shown promising results in sheep (Gurman *et al*. 2019).

The objective of the present study is to account for breed-specific allele frequencies in construction of the **GRM** in a multibreed population of tropically adapted heifers, with the aim of improving prediction accuracy of genomic selection for reproductive maturity score, an economically important fertility trait. We hypothesise that the use of a breed-adjusted genomic relationship matrix will improve the prediction accuracy of genomic selection of admixed and composite populations that include both ancestral subspecies of beef breeds, namely, *B. indicus* and *B. taurus*.

Materials and methods

Animals

Full details of the datasets and genomic best linear unbiased prediction (GBLUP) models used in the present paper have been described previously (Warburton *et al*. 2020). Briefly, reproductive maturity-score data were obtained from the Queensland Smart Futures population, collected in the Next Generation Beef Breeding Strategies project (Burns *et al*. 2016; Engle *et al*. 2019). Full information on data recording and herd management of this population has been described in Burns *et al*. (2016) and Engle *et al*. (2019). In total, 3695 reproductive maturity scores (RMS) were measured in this population as a proxy trait for age at puberty (Engle *et al*. 2019). Reproductive maturity score is a single ultrasound measurement recorded when a heifer reaches ~600 days of age (Burns *et al*. 2016; Engle *et al*. 2019). It is measured on a 0–5 scale, where 0 = infantile reproductive tract, 1 = small ovarian follicles, 2 = ovarian follicles with a diameter larger than 10 mm, 3 = presence of corpus luteum, 4 = pregnancy to 10 weeks, and 5 = pregnancy longer than 10 weeks (Burns *et al*. 2016; Engle *et al*. 2019). Heifers in this dataset represented three breeds, Brahman (n = 979), Santa Gertrudis (n = 1802) and Droughtmaster (n = 914). Two of these breeds are stabilised composites of *B. indicus* × *B. taurus* origins, namely, Santa Gertrudis and Droughtmaster, whereas the Brahman is considered a purebred *B. indicus* breed. We have recently shown that the heritability for single ultrasound puberty traits, using SNP chip and sequence data, is low-to-moderate, 17–35% (Engle *et al*. 2019; Hayes *et al*. 2019; Warburton *et al*. 2020).

Genotypes

Heifers were genotyped using the Geneseek GGP-LD array, consisting of 24 121 SNP (Hayes *et al*. 2019). These Geneseek GGP-LD genotypes were initially imputed up to the BovineHD array with 728 785 SNP (800K) using the FImpute software (Sargolzaei *et al*. 2014) and a panel of 1500 BovineHD array genotyped animals from representative breeds (Hayes *et al*. 2019). After imputation, two other commercially available marker panels were constructed, the BovineLD array (6K) and the BovineSNP50 BeadChip (50K), by selecting SNP from the BovineHD array that were present on each of these lower-density panels.

Genomic relationship matrix (***GRM***) construction

Due to computational efficiency, **GRM** were constructed in Julia (Bezanson *et al*. 2017), a free open-source programming

language. Julia allowed us to efficiently manipulate big matrices with a modest use of RAM memory. For ease of inversion and numerical stability, any animals that had exactly the same genotypes on any of the marker panels were removed from the analysis. Two methods were used to construct **GRM**, Yang's ($\mathbf{GRM_Y}$) (Yang *et al*. 2010) and VanRaden's ($\mathbf{GRM_{VR}}$) (VanRaden 2008).

$$W_{ij} = \frac{M_{ij} - 2p_j}{\sqrt{2p_j\left(1 - p_j\right)}} \quad (1)$$

$$\mathbf{GRM_Y} = \frac{\mathbf{WW'}}{m} \quad (2)$$

Yang's $\mathbf{GRM_Y}$ was constructed using Eqns 1–2, where M_{ij} is the genotype of animal i at locus j, expressed as the number of copies of the reference allele (0, 1 or 2) and p_j is the allele frequency of the reference allele at locus j. Furthermore, **W** is a $n \times m$ matrix, where n is the number of animals and m is the number of markers on the genotype panel being used to construct the $\mathbf{GRM_Y}$.

$$W_{ij} = M_{ij} - P_j \quad (3)$$

$$P_j = 2 * \left(p_j - 0.5\right) \quad (4)$$

$$\mathbf{GRM_{VR}} = \frac{\mathbf{WW'}}{d} \quad (5)$$

$$d = 2\sum_j p_j * \left(1 - p_j\right) \quad (6)$$

In comparison, VanRaden's $\mathbf{GRM_{VR}}$ was calculated using Eqns 3–6, where M_{ij} is defined as in Yang's method and P_j is the allele frequency of the reference allele for locus j expressed as a deviation from 0.5, calculated using Eqn 4, where p_j is the allele frequency of the reference allele at locus j. The $\mathbf{GRM_{VR}}$ is then calculated using Eqn 5, where **W** is a $n \times m$ matrix calculated using Eqn 3 and d is twice the sum of the frequency of the reference allele, multiplied by the frequency of the non-reference allele across all loci (6). The resulting $\mathbf{GRM_{VR}}$ from each method is a $n \times n$ matrix of estimated genomic relationships between animals.

Two **GRM** were calculated for each method, a control **GRM** and a breed-adjusted **GRM**. Control **GRM** were calculated using both Yang's (Yang) and VanRaden's (VanRaden) methods described above, with population allele frequencies calculated using all heifers. Breed-adjusted **GRM** were also calculated using Yang's (YangBA) and VanRaden's (VanRadenBA) methods. Rather than using population allele frequencies for each locus; however, the dataset was split by breed (Brahman, Santa Gertrudis and Droughtmaster) and breed-specific allele frequencies were used to calculate each **GRM**. Using Yang's methods, all p_j estimates used in Eqn 1 were calculated for the reference allele in each breed at each locus. To avoid dividing by 0 or 1 in our breed-adjusted matrices, any alleles that had an allele frequency greater than 0.99 or less than 0.01 were set to 0.99 and 0.01 respectively. After calculating a **W** matrix for each breed using Eqn 1, all three **W** matrices were combined. After combining the three breed-adjusted **W** matrices to form a single matrix, a breed-adjusted $\mathbf{GRM_Y}$ was calculated using Eqn 2. By combining the **W** matrices before calculating the $\mathbf{GRM_Y}$, we were able to account for the genomic relationships among heifers of different breeds in the $\mathbf{GRM_Y}$, while adjusting for breed-specific allele frequencies.

When using VanRaden's methods, it was also possible to use breed-specific allele frequencies to calculate **W** in Eqn 3. Although, after all of the breed-specific **W** matrices were combined to calculate **W'W** in Eqn 5, it was problematic to use a breed-specific d (6) estimate to calculate the $\mathbf{GRM_{VR}}$ in Eqn 5. So as to circumvent this, we used a breed-specific allele frequency (P_j) to calculate **W** and then a population allele frequency (d) to calculate the $\mathbf{GRM_{VR}}$, thus, making the VanRadenBA $\mathbf{GRM_{VR}}$ only partially breed-adjusted.

Genomic best linear unbiased predictions (GBLUP)

Genomic GBLUP were performed in Julia (Bezanson *et al*. 2017) using Eqn 7, where **y** is a vector of phenotypes, **X** is design matrix allocating records to animals, **b** is a vector of fixed effects and **Z** is an incidence matrix relating phenotypes to animals. Furthermore, **a** is a vector of random animal effects where the distribution is assumed to be $N(0,\ \mathbf{GRM}\sigma^2_g)$, where **GRM** is one of the four $n \times n$ matrices calculated above and σ^2_g is SNP genetic variance. Random error terms were modelled in vector **e** with an assumed distribution $N(0,\ \mathbf{I}\sigma^2_e)$. The unexplained proportion of variation in the model is σ^2_e and **I** is an $n \times n$ identity matrix. The fixed effects modelled in this analysis were age at measurement, which was fitted as a covariate and contemporary group, defined as herd, year and season. Breed was not fitted in this model as contemporary groups in this dataset were single breed and, as such, the effect of breed was accounted for in the contemporary group fixed effect.

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e} \quad (7)$$

Genomic predictions were performed using a five-fold cross-validation technique. Multibreed validation groups were populated by randomly allocating 20% of the data into one of five groups. This strategy of using randomly allocated mixed-breed validation groups was used as it reflects the admixed populations in the northern Australian beef industry. Each animal occurred only in a single validation group and these groups were maintained for each of the analyses to enable fair comparisons between methods.

$$LRT = -2 \times \left(Log\_Likelihood_{Full} - Log\_Likelihood_{Reduced}\right) \quad (8)$$

Variance components were estimated for all **GRM** by importing our custom **GRM** into genomic-wide complex trait analysis and performing restricted maximum-likelihood analysis (Yang *et al*. 2011). Log-likelihood estimates for the full and reduced models for each **GRM** were used to calculate a log-likelihood ratio test statistic (*LRT*) using Eqn 8. Significance tests between the *LRT* statistic of breed-adjusted and control **GRM** for each method (Yang and VanRaden) and each marker panel were conducted to determine whether **GRM** were significantly different. These significance tests assumed a Chi-square distribution on 1 degree of freedom and a significance threshold of

$p = 0.05$, resulting in a Chi-squared statistic of 3.841. A breed-adjusted **GRM** was considered to be significantly different from a control **GRM** if breed-adjusted *LRT* was greater than the control *LRT* plus the Chi-squared statistic of 3.841. Variance components specific for each **GRM** were used in Julia BLUP estimations to calculate genomic estimated breeding values (GEBVs) for animals in each validation group.

Prediction accuracy was calculated as the correlation between GEBV and the phenotype adjusted for fixed effects. Correlations were averaged across each validation group and the average correlation was divided by the square root of the heritability of the trait estimated on both Yang's and VanRaden's 800K control **GRM** ($h^2 = 0.20$) to obtain prediction accuracy. Prediction accuracy standard errors were calculated as the standard error of the mean for the five validation groups (s.e.). Furthermore, prediction bias was calculated as the regression coefficient between the adjusted phenotype and GEBV for each validation group and analysis. Standard errors for bias estimates were calculated as the standard error of the mean for the five validation group bias estimates (s.e.). Prediction accuracies and estimated regression bias were plotted in R (R Core Team 2020) using the ggplot2 package (Wickham 2016).

Results

To understand the genomic relationships among the three breeds of heifers in these data, we used principal-component analyses of the 50K control **GRM** to illustrate the genomic relationships among breeds, shown in Fig. 1. In our analyses, breed is breeder defined; however, observation of Fig. 1 shows that this may not be the most accurate definition of breed. Generally, the three breeds in this dataset exist in discrete clusters within the principal-component analysis plot, suggesting that user-defined breed is reasonably accurate. There are some animals, most notably Brahmans, that may genomically be more related to the Droughtmaster breed (Fig. 1). When using breed-adjusted **GRM**, it will be vitally important to accurately identify an animal's breed or breed of origin, so as to obtain the best estimate of breed-specific allele frequencies. Very few animals in this dataset appear to have

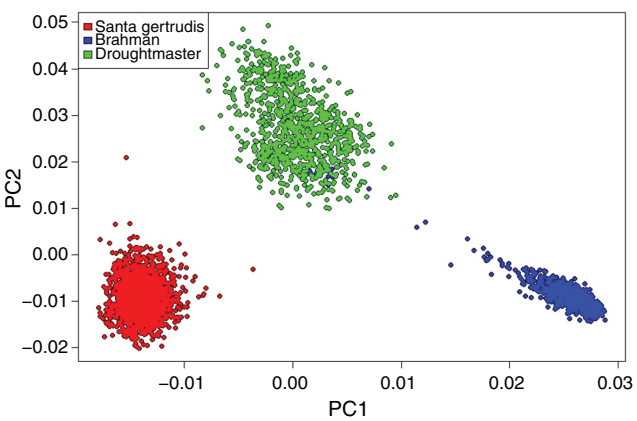

Fig. 1. Principal component analysis of the Queensland Smart Futures research herd heifers, estimated using the 50K Yang's control genomic relationship matrix.

incorrect breed of origin assignment; as such, this will likely have little impact on the accuracy of our results. In future analyses, it will be essential to develop methods to improve the accuracy of breed determination when accounting for population structure in multibreed populations.

After performing genomic evaluations for each reference population, we estimated the prediction accuracy of each validation group as a measure of the efficacy of our genomic evaluation models. These prediction accuracies were averaged across the five validation groups in each analysis and the results for each **GRM** and marker panel are presented in Fig. 2. These results showed that the higher-density marker panels, 50K and 800K, have higher prediction accuracy than does the lower-density marker panel, 6K. Furthermore, the prediction accuracy for RMS is similar between the 50K and 800K analysis, meaning that there is little benefit to using the higher-density marker panel in this population of heifers. The VanRaden breed-adjusted $\mathbf{GRM_{VR}}$ showed small improvements in prediction accuracy for RMS on all panels, but this difference is not statistically significant (s.e. = 0.05). In contrast, on all panels, the Yang breed-adjusted $\mathbf{GRM_{Y}}$ had a slightly lower prediction accuracy than did all other **GRM**, but again this was not statistically significant (s.e. = 0.05). These results suggest that there was no significant improvement in prediction accuracy for RMS in this population of heifers from using breed-adjusted **GRM**.

Estimation bias was calculated in this analysis as the regression coefficient between estimated breeding values and phenotype adjusted for fixed effects (Fig. 3). It should ideally be close to one. Similar to the results presented in Fig. 2, bias estimates for each of the five validation groups were averaged for each panel and **GRM** and are presented in Fig. 3. These results showed that the 6K regression coefficients were less than 1 and lower than in the 50K and 800K analysis, meaning that there is a general over-dispersion of GEBVs in this analysis. Bias estimates between the 50K and 800K analyses were similar and close to one, suggesting that both the 50K and 800K analyses were unbiased. There were no statistically significant differences among the estimated biases for each **GRM** on each marker panel. In all analyses, the Yang breed-adjusted $\mathbf{GRM_{Y}}$ generally had a slightly lower bias estimate than did the control $\mathbf{GRM_{Y}}$ and the VanRaden breed-adjusted $\mathbf{GRM_{VR}}$ had slightly higher bias estimates in the 6K and 50K analyses. These differences between breed-adjusted and control **GRM** were not significantly different (s.e. = 0.13).

Variance components were estimated for each **GRM** in each analysis to determine the effect of using breed-adjusted **GRM** (Table 1). Using the VanRaden method, there was no change in the estimates with or without breed adjustment across all SNP panels. In contrast, when using Yang's method, the Yang breed-adjusted $\mathbf{GRM_{Y}}$ showed a consistent decrease in residual variance across all marker panels in comparison with the other **GRM**. Furthermore, the estimated additive genetic variance in the Yang breed-adjusted $\mathbf{GRM_{Y}}$ was slightly higher than in other **GRM**. In combination, this reduced residual and increased additive genetic variance resulted in higher estimated heritabilities for the Yang breed-adjusted $\mathbf{GRM_{Y}}$ than for the other three

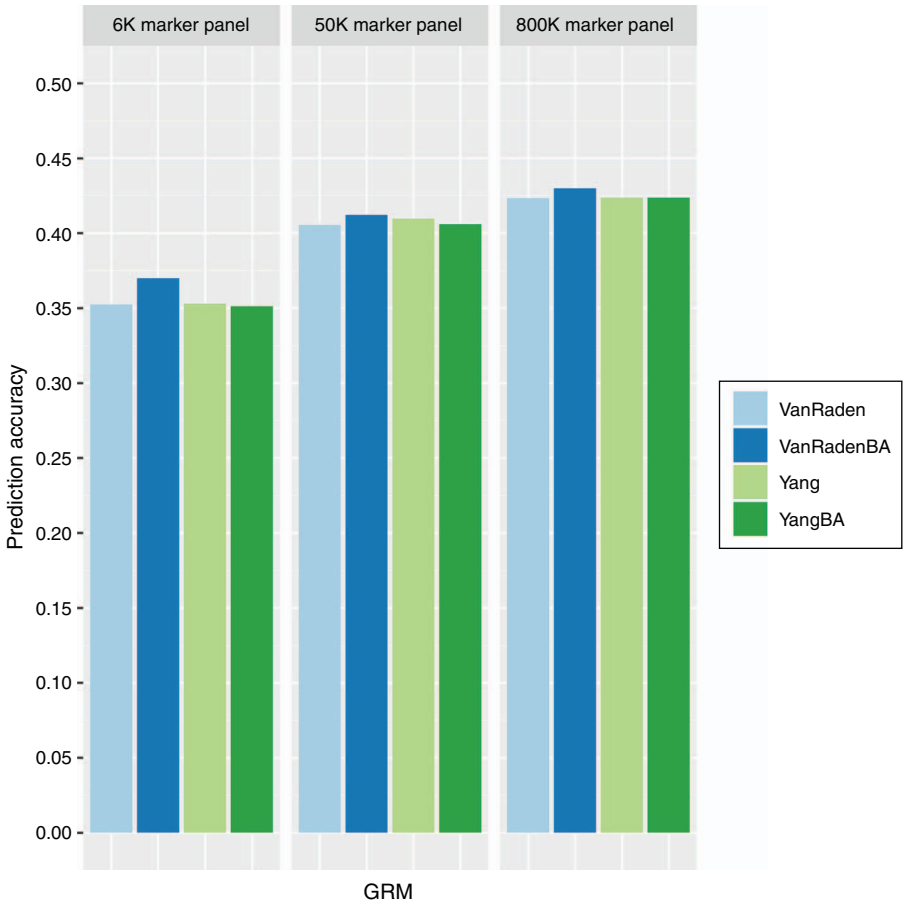

Fig. 2. Prediction accuracy for reproductive maturity score (RMS) measured on the 6K, 50K and 800K marker panels across four genomic relationship matrices, namely, VanRaden's (VanRaden), VanRaden's breed-adjusted (VanRadenBA), Yang's (Yang) and Yang's breed-adjusted (YangBA).

GRM. While this result was not statistically significant, this trend for a reduction in residual variance with this **GRM** suggests that the use of the Yang's breed-adjustment captures a higher proportion of the additive genetic effects.

Results from log-likelihood ratio testing showed that there was a consistent reduction in log-likelihood estimates for the Yang breed-adjusted $\mathbf{GRM_Y}$ across all models, suggesting that this **GRM** was more appropriate for these data. Furthermore, significance testing of the *LRT* results between control and breed-adjusted **GRM** for each method and marker panel showed that the *LRT* estimates for Yang breed-adjusted $\mathbf{GRM_Y}$ are significantly different from those for the Yang control $\mathbf{GRM_Y}$ across all marker panels.

Discussion

Ideally, genomic selection in the northern Australian beef industry will be performed across-breeds to allow beef cattle producers opportunities to select the best bulls for their production system, irrespective of breed constraints. Unfortunately, for many key traits such as fertility, the prediction accuracy of multibreed genomic evaluations remains quite low (Engle *et al*. 2019; Hayes *et al*. 2019). The objective of the present study was to use breed-specific **GRM** to account for population stratification in a multibreed population of tropically adapted heifers, with the aim of improving prediction accuracy for reproductive maturity score. We showed that the use of breed-specific allele frequencies to calculate breed-adjusted **GRM** did not result in either improved prediction accuracy or bias for reproductive maturity score evaluations in this population of heifers. However, our results showed that the Yang's breed-adjusted $\mathbf{GRM_Y}$ was the optimal model for genomic prediction in this population of heifers, on the basis of *LRT* significance tests. This was reflected in the variance component estimations using this $\mathbf{GRM_Y}$, showing a reduction in residual variance and an increase in the additive variance estimations for RMS in this population of heifers.

Variance component estimation is critical for the efficacy of any genetic evaluation (Hofer 1998). Our results showed that the use of Yang's breed-adjusted $\mathbf{GRM_Y}$ resulted in higher *LRT* estimates, which were significantly different from Yang's control *LRT* estimates. This suggests that Yang's breed-adjusted $\mathbf{GRM_Y}$ provided a better fit for the

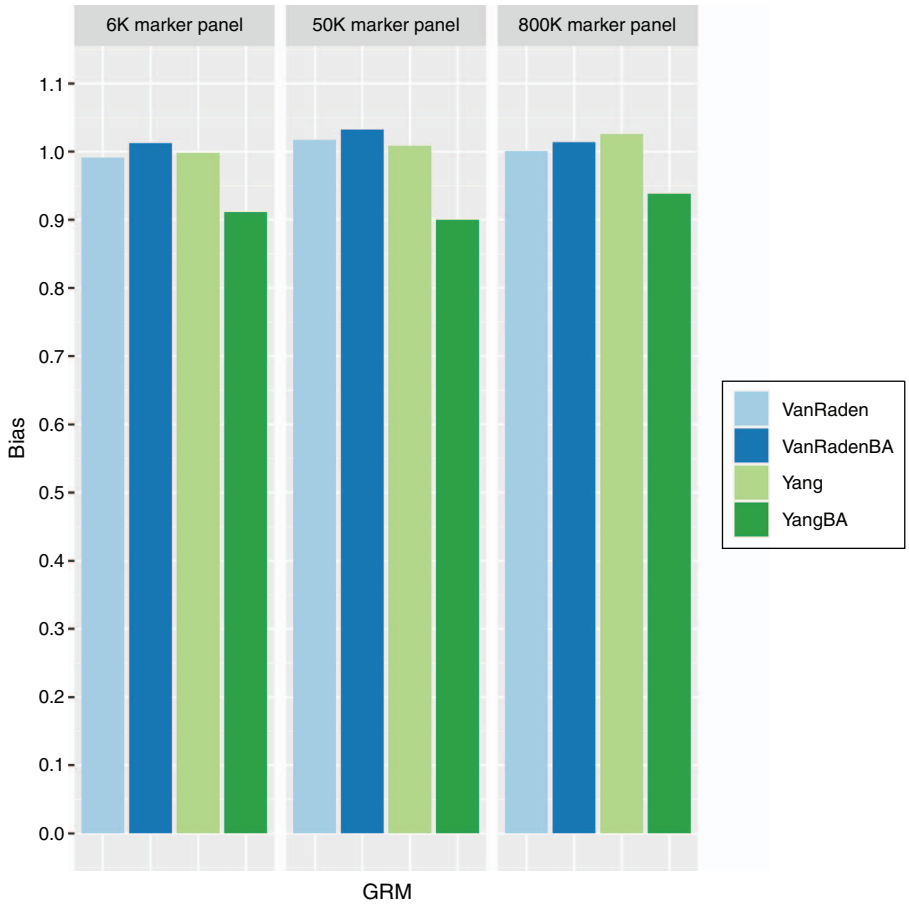

Fig. 3. Estimation bias for reproductive maturity score (RMS) measured on the 6K, 50K and 800K marker panels across four genomic relationship matrices, namely, VanRaden's (VanRaden), VanRaden's breed-adjusted (VanRadenBA), Yang's (Yang) and Yang's breed-adjusted (YangBA).

Table 1. Estimated variance components (s.e. in parentheses), phenotypic variance (V_p), additive genetic variance (V_a), residual variance (V_e), heritability (h^2), log-likelihood of the full model (*LogL*) and log-likelihood ratio test statistic (*LRT*) for reproductive maturity score in Queensland Smart Futures heifers

Estimated with the 6K, 50K and 800K marker panels using one of four genomic relationship matrices; Yang's (Yang), Yang's breed-adjusted (YangBA), VanRaden's (VanRaden) and VanRaden's breed-adjusted (VanRadenBA). *Denotes *LRT* statistic for a breed-adjusted GRM that is significantly different from the control. Tested using a Chi-squared test on 1 degree of freedom and a significance level of $P = 0.05$

GRM	V_p	V_a	V_e	h^2	*LogL*	*LRT*
			6K marker panel			
Yang	1.09 (0.03)	0.15 (0.03)	0.95 (0.03)	0.14 (0.02)	–2065.85	69.0
YangBA	1.09 (0.03)	0.16 (0.03)	0.93 (0.03)	0.15 (0.02)	–2050.39	81.9*
VanRaden	1.10 (0.03)	0.15 (0.03)	0.95 (0.03)	0.14 (0.02)	–2065.41	69.9
VanRadenBA	1.09 (0.03)	0.15 (0.03)	0.95 (0.03)	0.14 (0.02)	–2056.41	69.9
			50K marker panel			
Yang	1.10 (0.03)	0.20 (0.03)	0.91 (0.03)	0.18 (0.03)	–2050.71	91.7
YangBA	1.09 (0.03)	0.22 (0.03)	0.87 (0.03)	0.20 (0.03)	–2040.73	111.7*
VanRaden	1.10 (0.03)	0.19 (0.03)	0.91 (0.03)	0.18 (0.03)	–2050.88	91.4
VanRadenBA	1.10 (0.03)	0.19 (0.03)	0.91 (0.03)	0.18 (0.03)	–2050.88	91.4
			800K marker panel			
Yang	1.11 (0.03)	0.22 (0.03)	0.89 (0.03)	0.20 (0.03)	–2049.23	94.7
YangBA	1.10 (0.03)	0.24 (0.04)	0.86 (0.03)	0.22 (0.03)	–2039.25	114.4*
VanRaden	1.11 (0.03)	0.22 (0.03)	0.89 (0.03)	0.20 (0.03)	–2047.48	98.1
VanRadenBA	1.11 (0.03)	0.22 (0.03)	0.89 (0.03)	0.20 (0.03)	–2047.48	98.0

reproductive maturity score phenotype in this population of heifers. The use of this $\mathbf{GRM_Y}$ resulted in consistently lower residual variance and slightly higher additive genetic variance estimates across all three marker panels. This is significant as the reduction in residual variance suggests that this model is more accurately partitioning genetic variance and, therefore, model error variance is being reduced. This trend was not seen in the VanRaden breed-adjusted model. The VanRaden breed-adjusted equations are only partially using breed-specific alleles, when the **W** matrix is being calculated. Due to the way in which the $\mathbf{GRM_{VR}}$ is calculated in the VanRaden breed-adjusted model, the *d* statistic has to be calculated using population allele frequencies. Whereas, in the Yang breed-adjusted model, breed-specific allele frequencies were used in all calculations. This may explain why the VanRaden breed-adjusted model did not have the same affect on the estimated variance components as did the Yang breed-adjusted model.

There are limited estimates of published variance components for breed-specific **GRM** available in the literature, but one study has shown that the estimated variance components from a breed-adjusted **GRM** had the opposite effect in pigs (Sevillano *et al*. 2019). In their study, Sevillano *et al*. (2019) showed that variance components estimated with a breed-adjusted **GRM** had reduced additive genetic variances and, in some cases, reduced heritability estimates in comparison to using a non-adjusted **GRM**. In comparison, our results showed that the use of a breed-adjusted $\mathbf{GRM_Y}$ may improve the accuracy of variance component estimation in multibreed populations, and this warrants further investigation.

Industry-available genomic evaluations will be beneficial to end-users only if the accuracy of prediction is high. When performing genomic evaluations across genetically divergent populations, such as those found in the northern Australian beef industry, it will be essential to account for population stratification to improve the prediction accuracy of genomic evaluations (Bolormaa *et al*. 2013; Kemper *et al*. 2015). There are several reports in the literature from other species (pigs, dairy and sheep) that suggest that breed-specific allele frequencies do not result in improved prediction accuracies in other multibreed populations (Ibáñez-Escriche *et al*. 2009; Makgahlela *et al*. 2013; Moghaddar *et al*. 2014; Lopes *et al*. 2017). In contrast, a recent study showed that the use of breed-specific allele frequencies improved the prediction accuracy of forward cross-validation in single-step sheep genetic evaluations in comparison to using population allele frequencies to calculate the **GRM** (0.220 and 0.206 respectively); however, these results were not statistically significant (Gurman *et al*. 2019). Despite the recent results presented in the sheep industry, our results demonstrated that the use of breed-specific allele frequencies did not result in improved prediction accuracies for a lowly heritable trait such as reproductive maturity score. Population stratification will need to be accounted for in future evaluations, and it may be beneficial to investigate other methods to account for population differences, such as the use of haplotype analyses (Bolormaa *et al*. 2011; Koufariotis *et al*. 2018).

Estimation bias is another important parameter to review when considering the efficacy of genomic evaluations. In the present study, bias was reduced most by using a higher-density marker panel, that is, 50K or 800K marker panels. Similar to prediction accuracy, there was no significant improvement in bias estimations when using breed-adjusted **GRM** in this population of heifers. Bias estimates of breed-adjusted **GRM** evaluations are mixed in the literature. One study in dairy cattle showed no improvement to bias estimates by using breed-adjustment (Makgahlela *et al*. 2013). Another study in a crossbred population of chickens showed that the use of a breed-adjusted **GRM** reduced estimation bias (Duenk *et al*. 2019). Conversely, the study by Gurman *et al*. (2019) showed that bias increased slightly in sheep analyses when using breed-adjusted **GRM** in a single-step BLUP. While the effect of breed-adjusted **GRM** on estimation bias in GBLUP analyses remains inconclusive, no studies have shown large adverse estimates of bias using these methods. Therefore, from the results presented in the current study, it can be suggested that breed-adjusted **GRM** have little effect on the estimation bias in multibreed GBLUP in this population of heifers.

Conclusions

In conclusion, the use of breed-adjusted **GRM** did not improve the accuracy of prediction for reproductive maturity score in this multibreed population of tropically adapted heifers. Nevertheless, the use of Yang's breed-adjusted $\mathbf{GRM_Y}$ allowed the capture of a higher proportion of additive genetic effects (higher additive genetic variance) consistently across several SNP panels. This finding suggests that despite seeing no improvement in prediction accuracy, it may still be beneficial to use breed-adjusted $\mathbf{GRM_Y}$ in multibreed populations to improve the estimation of variance components.

Conflicts of interest

The authors declare no conflicts of interest.

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