EFFICIENCY OF SINGLE-STEP GBLUP IN GENOMIC EVALUATION AND GWAS IN BROILER CHICKENS

by

XINYUE ZHANG

(Under the Direction of Ignacy Misztal)

ABSTRACT

Genomic selection has been a hot topic in the poultry industry during the last couple of years. Many tools have been built to conduct genomic evaluation and to inspect changes in genetic prediction before and after selection. Here, we evaluate selected features of the single-step genomic best linear unbiased prediction (ssGBLUP) statistical method. This method can predict genomic estimated breeding values (GEBVs) by blending traditional pedigree relationships with realized relationships derived from genetic markers. Subsequently, GEBVs can be utilized in a genome-wide association study (GWAS) by conversion of GEBVs to marker effects and their weights.

The dissertation utilized ssGBLUP in 4 studies. In the first study, the signatures of selection in male and female broiler breeds selected for the same goals were analyzed. Results indicated that the male breed had undergone stronger selection compared with the female breed in terms of allele frequency change. Furthermore, female breed had a greater heterozygosity change compared with the male breed. No overlapping selection region was found in the two breeds.

In the second study, five options for weighted ssGBLUP (WssGBLUP) were tested. Simulated data sets included 5, 100, and 500 quantitative trait loci (QTLs). Weights were calculated based on formulas for single or segment single-nucleotide polymorphism (SNP) variance. Prediction accuracy for WssGBLUP improved at 2nd to 4th iterations by updating the mean, max or summation of u_i^2 among every 20 (SNP), where u_i is the effect of SNP i. Accuracy reached a plateau after iteration 3 or 5 by using

weights proportional to u_i^2 plus a constant. Except in the 5-QTL scenario, realized accuracies with all WssGBLUP procedures were higher compared with those with BayesB and C. Noise in Manhattan plots was small with 5 and 100 QTLs but large with 500 QTLs.

In the third and fourth studies, (co)variance components and prediction accuracy in linear and threshold, univariate, bivariate, and multivariate models were compared using ssGBLUP and BLUP methods for disease traits of binary or categorical nature. Uni- and multivariate threshold models surpassed linear models in obtaining higher heritabilities. A univariate threshold model surpassed a linear model in predicting (G)EBVs. Bivariate models and the ssGBLUP method did not have an advantage over univariate models and the BLUP method, respectively.

INDEX WORDS: signatures of selection; WssGBLUP; mortality; threshold model

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by

XINYUE ZHANG

B.S., China Agricultural University, China, 2009

M. Sc., Ohio State University, 2012

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by

XINYUE ZHANG

Major Professor:	Ignacy Misztal
Committee:	Romdhane Rekaya
	J. Keith Bertrand
	Samuel Aggrey
	Robyn Sapp

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
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CHAPTER 1

INTRODUCTION

Genomic selection has been a hot topic in the poultry industry during the last couple of years. Many tools have been built to conduct genomic evaluation and to inspect changes in genetic prediction before and after selection. Here, we evaluate selected features of the single-step genomic best linear unbiased prediction (ssGBLUP) statistical method. This method can predict genomic estimated breeding values (GEBVs) by blending traditional pedigree relationships with realized relationships derived from genetic markers. Subsequently, GEBVs can be utilized in a genome-wide association study (GWAS) by conversion of GEBVs to marker effects and their weights.

The objectives of the current dissertation were 1) to inspect change in the genetic architecture for two breeds of broiler chickens under selection, 2) to modify weighted ssGBLUP (WssGBLUP) and compare GEBV and GWAS accuracies with those from other methods using simulated data, 3) to determine and compare (co)variance components of mortality and diseases traits for broiler chickens using different models, and 4) to predict and compare the (G)EBVs of mortality and body weight for broiler chickens using different models.

CHAPTER 2

LITERATURE REVIEW

Signatures of selection

Selection changes the allelic frequency of the underlying causative genes. At a population level, the variability within and between species is modified by selection (Nielsen, 2005). Different selections modify the variability in different ways. By combining the patterns of the ratio of inter- to intraspecific variability and frequency spectrum, types and effects of selections are able to be classified. Selection of livestock belongs to positive directional selection that favored new advantageous mutations, and the inter- to intraspecific variability between populations or between generations increases (Lewontin & Krakauer, 1973).

Molecular signature of selection, or selective sweep, is a type of new, strong positive directional selection. It is a region in the genome that has been preferentially increased in frequency and fixed in a population recently because of its functional importance in specific processes (O'Brien et al., 2014). Therefore, by detecting signatures of selection, it is able to provide heuristic genomic information for artificial selection by the livestock industry. Signatures of selection form because nucleotides adjacent to the favorable mutation also tend to increase in frequency in a sort of "hitchhiking" process (Smith & Haigh, 1974). This leads to distributions of nucleotides around favorable mutations that differ statistically from that expected purely by chance (Kim & Stephan, 2002). These regions can be detected because of their lower genetic variability and specific regional linkage disequilibrium (LD) patterns (O'Brien et al., 2014).

Popular statistical methods for signatures of selection

Lots of statistical methods have been developed to test the frequency spectrum over selection. Some test the intraspecific population variance, some test the ratio of inter- to intraspecific variance, and some test the allele frequency. Here I present some popular methods, including the ones that were used in my study.

Tajima's D test. This is the most famous neutral mutation test. In this test, the average number of nucleotide differences between pairs of sequences is compared with the total number of segregating sites (SNP, sequence, etc.). If the difference between these two measures of variability is larger than what is expected from the standard neutral model, this model is rejected (Tajima, 1989). The test statistic is

$$D = \frac{d}{\sqrt{\hat{V}(d)}} = \frac{\hat{k} - \frac{S}{a_1}}{\sqrt{e_1 S + e_1 S(S-1)}},$$

where d is the difference between observed and expected of average number of (pairwise) nucleotide differences between the DNA sequencesis $\hat{V}$ the variance of d, $\hat{k}$ is the observed difference as a part of d, S is the number of segregating sites, a_1 is the summation of the reciprocal of sample size, and e_1 is a function of sample size of $\hat{k}$ and S. In the null hypothesis, d is equal to 0. This test captures the information regarding the frequency spectrum.

Spatial pattern of selective sweep. This is an improvement of the neutrality test developed by Kim & Stephan (2002). The method estimates the location and the strength of the selective sweep by modeling the selective phase, which is the time needed for a substitution of a beneficial mutation that causes a hitchhiking effect to take place. The formula is

$$x(t) = \frac{\xi}{\xi + (1-)e^{\alpha(t-t_s)}},$$

where $x(t)$ is the allele frequency x at the time t, ξ and 1-ξ are the beginning and end frequency, respectively, α is 2 times the effective population size, and t_s is the length of the selective phase.

F_{ST} test. This test created by Akey et al. (2002) utilizes the differentiation among populations as an indicator of selective sweep. When a selective sweep occurs in one but not other populations of a species, the F_{ST} test can show the significant level against the null hypothesis of neutrality. The formula is

$$F_{ST} = \frac{MSP - MSG}{MSP + (n_c - 1)MSG},$$

where F_{ST} is the genetic differentiation, MSP is the observed mean square errors for loci across populations, MSG is the observed mean square errors for loci within populations, and n_c is the number of populations. If F_{ST} is significantly different from 0, then neutral selection is rejected and selective sweep is accepted. The F_{ST} test requires multiple loci and thus is good for large-scale genomic data.

Heterozygosity test.

Heterozygosity is the frequency of heterozygotes over all genotypes at a bi-allelic locus. High heterozygosity denotes high fitness as a pattern of balanced or natural selection. Rubin et al. (2012) used window-based heterozygosity to analyze large-scale genomic data. The equation is

$$H_P = \frac{2\sum n_{MAJ} \sum n_{MIN}}{(\sum n_{MAJ} + \sum n_{MIN})^2},$$

where H_P is the heterozygosity, $\sum n_{MAJ}$ is the sum of major allele frequencies, and $\sum n_{MIN}$ is the sum of the minor allele frequency (MAF) in a window.

Wright's fixation index. McEachern et al. (2009) combined F_{ST} with heterozygosity and formed a new F_{ST} as

$$F_{ST} = \frac{H_T - H_S}{H_T},$$

where F_{ST} is Wright's F_{ST} index, H_T is the heterozygosity of the total population, and H_S is the heterozygosity in subpopulations. This index with a high value denotes strong positive selection, but this formula does not account for sampling error (Weir & Cockerham, 1984).

Application of statistical methods in livestock

For domestic animals, there already have been several studies on the allele frequency spectrum of signature of selection. Elferink et al. (2012) investigated heterozygosity of commercial and noncommercial chicken breeds and identified 26 chromosomal regions with evidence of strong selection; 13 of the regions contained new candidate genes related to performance. Moradi et al. (2012) used Wright's fixation index and found novel regions of increased homozygosity that associated with fat deposition in thin and fat tail sheep breeds. Ribin et al. (2012) used pooled heterozygosity H_P and ZH_P and found 3 loci on a domestic pig chromosome associated with elongation of the back and increased number of vertebrae. However, those studies used cross-generational data; therefore, their results were affected by both recent and historical selection. Furthermore, most previous studies only have allele frequency data after completion of selection, and initial allele frequencies and their changes are unknown.

Cons of statistical methods

Most methods applicable to population genetic data rely on strong assumptions regarding the demography of the population (e.g., no subpopulation structure), which often results in confounding with selection. Comparative methods are free of such assumptions. However, they need sequence and reference information that can provide synonymous and nonsynonymous mutations (Nielsen, 2005).

Use of SNP data

To detect incomplete selective sweeps, it should be possible to utilize genome-wide changes in the allele frequency spectrum over time in populations under selection. One important consideration is that the process by which SNPs have been selected affects LD levels (Nielsen & Signorovitch, 2003), the frequency spectrum (Nielsen & Signorovitch, 2003), the level of population subdivision (Wakeley et al., 2001), and outlier definition (Nielsen, 2005). Therefore, the SNP ascertainment process needs to be taken into account.

GWAS and ssGBLUP

GWAS in livestock has been a hot topic since 2001. In mainstream prediction modeling, the marker effects are derived from GEBVs of genotyped animals. In livestock, the data structure often includes a large population with phenotypes, with a small proportion of it genotyped. In many situations, the genotyped animals are young and their phenotypes are not yet collected. To obtain the GEBVs of these animals in multistep methods, the pseudo phenotype [e.g., daughter yield deviation (DYD)] and deregressed progeny-test EBV (EBV_{DP}) as GEBV equivalents are created before GWAS analysis (Garrick et al., 2009; VanRaden & Wiggans, 1991). The pseudo phenotypes as well as GEBVs have some problems: 1) animals with few progeny have low reliability, 2) in multiple traits, different amounts of information may cause heterogeneity, 3) selection bias may exist for genotyped young animals, 4) systematic effect cannot be accounted for, 5) advantage cannot be taken of phenotypes and genotypes if they are mutually exclusive, 6) EBV_{DP} does not include parent average, whereas DYD has some degree of double counting, and 7) extension to complicated models is difficult (Garrick et al., 2009; VanRaden et al., 2009; Vitezica et al., 2011).

The above problems can be solved by the ssGBLUP approach proposed by Misztal et al. (2009) and Christensen and Lund (2010) that integrates phenotypes, genotypes, and pedigree information simultaneously. The integrated relationship matrix is

$$\mathbf{H}^{-1} = \begin{bmatrix} \mathbf{A}^{11} & \mathbf{A}^{12} \\ \mathbf{A}^{21} & \mathbf{A}^{22} \end{bmatrix} + \begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{G}^{-1} \end{bmatrix} - \begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{A}_{22} \end{bmatrix},$$

where **H** is the integrated relationship matrix, **A** is the traditional pedigree relationship matrix, **G** is the realized genomic relationship matrix, and $\mathbf{A}^{22}$ is the inverse of the pedigree relationship matrix of genotyped animals. Therefore the Henderson mixed-model equation becomes

$$\begin{bmatrix} \mathbf{X'X} & \mathbf{X'Z} \\ \mathbf{Z'X} & \mathbf{Z'Z} + \lambda\mathbf{H}^{-1} \end{bmatrix} \begin{bmatrix} \mathbf{b} \\ \mathbf{u} \end{bmatrix} = \begin{bmatrix} \mathbf{X'y} \\ \mathbf{Z'y} \end{bmatrix}$$ (Aguilar et al., 2010),

where **X** is the incidence matrix for fixed effects, **Z** is the incidence matrix for random effects, λ is the ratio of residual to additive variance, **b** is the best linear estimator, **u** is the best linear unbiased estimator, and **y** is the phenotype vector.

Weighted GBLUP (WGBLUP)

GBLUP usually assumes equal weights for all markers (Goddard & Hayes, 2009; Meuwissen et al., 2001; VanRaden, 2008). This assumption is biologically incorrect but makes the statistics robust by eliminating the number of unknowns (Meuwissen et al., 2001). Nonlinear methods such as BayesA and BayesB assume heterogeneous variances of SNP effects, with emphasis on the SNPs with major effects (Meuwissen et al., 2001; Meuwissen & Mike, 2004). The performance of these methods has been proved to be better than BLUP approaches in simulation studies assuming a few QTLs with large effects and many QTLs with small effects (Guo et al., 2010; Lund et al., 2009; Meuwissen et al., 2001; Meuwissen & Mike, 2004). However, experiences with real dairy cattle data indicate that these methods have resulted in reduced accuracy because of ignoring SNPs with small effects (Cole et al., 2009; Su et al., 2010) and that BLUP approaches performed well for most traits (Aguilar et al., 2010; Chen et al., 2011; Forni et al., 2011; Hayes et al., 2009; VanRaden et al., 2009; Wang et al., 2014)

One way to correct the equal-variance assumption of GBLUP without increasing the number of unknowns is to weight the SNPs. If those weights are known, WGBLUP provides GEBVs similar to those of a Bayesian procedure using the same weights (Legarra et al., 2009). WGBLUP and WssGBLUP were developed to allow for the estimation of weights within GBLUP or ssGBLUP, respectively.

Studies of WGBLUP

Sun et al. (2011) developed two procedures for calculating weights in WGBLUP. In the first one, the weights are calculated as $w^{(i)} = \hat{a}_j^{(i)2}$, where $w^{(i)}$ is the weight of SNP *j* at iteration *i* and $\hat{a}_j^{(i)}$ is the effect of SNP *j* at iteration *i*. This procedure is effective for identifying top QTLs but excessively shrinks small SNP effects; thus, the accuracy of GEBV is reduced. The highest accuracy of GEBV was achieved by modifying the formula for weights to $w^{(i)} = \hat{a}_j^{(i)2} + t$, where $t = \frac{\sigma_g^2}{2\sum_{j=1}^{m} p_j q_j}$, σ_g^2 is the genetic variance; *p* and *q* are the minor and major allele frequencies at locus *j*, respectively, and m is the number of SNPs. This procedure introduced a constant to avoid SNPs with no effect and brought the accuracy of GEBV close to that by BayesC but yielded "noisy" Manhattan plots.

Wang et al. (2012) evaluated WssGBLUP with simulation data using $d_{i(t)} = u_{i(t)}^2 [2p_i(1-p_i)]$, where $d_{i(t)}$ is the weight of SNP *i* at iteration *t*, $u_{i(t)}^2$ is the effect of SNP *i* at iteration *t*, and p_i is the MAF. They iterated either on SNP alone or on GEBV and SNP. The first option gave a good identification of top QTLs, and the second option provided a higher accuracy of GEBVs compared to BayesB, but only at the second iteration.

Su et al. (2014) used group-marker variance from BayesR as a weighting factor on GBLUP in the study of dairy cattle. They achieved up to 1% higher reliability and reduced bias

by 11% on average for 4 production traits and mastitis when using the mean variance of a 30-SNP window compared with single SNPs. However, with or without grouping, BayesR was still 1.7 to 2% more accurate compared with GBLUP. Xu (2013) demonstrated improved predictability in diploid plant QTL mapping using an artificial bin of LD-linked neighboring markers.

Procedures in WssGBLUP

Wang et al. (2012) built a routine procedure for WssGBLUP. The weighted matrix **D** is updated via iteration *t* for every SNP *i*:

1. $t = 0,\ \mathbf{D}_{(t)} = \mathbf{I};\ \mathbf{G}_{(t)} = \mathbf{Z}\mathbf{D}_{(t)}\mathbf{Z}'\lambda$.
2. Compute $\hat{a}_g$ by ssGBLUP.
3. Calculate $\hat{u}_t = \mathbf{D}_{(t)}\mathbf{Z}\mathbf{G}^{*-1}_{(t)}\hat{a}_g$.
4. Calculate $d^*_{i(t+1)} = \hat{u}^2_{i(t)} 2p_i(1-p_i)$ for all *i*.
5. Normalize $\mathbf{D}_{(t+1)} = \dfrac{tr(\mathbf{D}_{(0)})}{tr(\mathbf{D}^*_{(t+1)})}\mathbf{D}^*_{(t+1)}$.
6. Calculate $\mathbf{G}^*_{(t+1)} = \mathbf{Z}\mathbf{D}_{(t+1)}\mathbf{Z}'\lambda$.
7. $t = t + 1$.
8. Exit, or loop to step 2 or 3.

$\mathbf{G}^*_{(t+1)}$ is the new realized relationship matrix at iteration *t*, **Z** is the animal-by-genotype matrix, λ is the ratio of genetic to marker variance, $\hat{a}_g$ is the EBV, $\hat{u}_t$ is the estimated SNP effect, and p_i is the MAF of SNP *i*. The loop to step 2 is to update both GEBV and weight, whereas the loop to 3 is to only to update weight. The weight of SNP *i* is defined as square of the SNP effect times the variance of binomial distribution.

Modeling of discrete traits

Traits such as mortality and disease are recorded in discrete categories. These traits were treated as secondary traits in livestock and inferior to production traits for quite a long time (Thornton, 2010). Because genomic selection for production has been thoroughly studied and become routine in many industries worldwide, these secondary traits recently have become of interest. Linear models for continuous traits were not appropriate for discrete traits, because the latter are not normally distributed (Gianola, 1982; Thompson, 1979). When a case-controlled experiment is conducted to get phenotypes, extension of linear models to use this information is also tricky. Common models such as logit and probit can analyze traits with binomial distribution. However, those two models cannot be used for individual records.

Threshold models have been developed to provide genetic evaluation of categorical traits (Gianola & Foulley, 1983; Gilmour et al., 1985; Harville & Mee, 1984). Such models include an extra latent variable, also called an underlying variable or liability, which is normally distributed. In the case of a binary observational phenotype, the threshold model assumes that the phenotype becomes 1 when the liability reaches a certain threshold; otherwise, it remains 0. For example, in an additive mixed model,

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e} ,$$

where **y** is the discrete observations, **X** is the incidence matrix for fixed effects, **β** is the fixed effects, **Z** is the incidence matrix for random effects, **a** is the random additive genetic effects, and **e** is the residual. This model assumed an underlying distribution L of the discrete traits y, the response of which was modeled with the following distribution:

$$\begin{aligned} & f(y \mid L) = \prod_{i=1}^{n} f(y_i \mid L_i) \\ & = \prod_{i=1}^{n} I(L_i < t_1) I(y_i = 1) + I(t_1 < L_i < t_2\ ) I(y_i = 2) + I(t_2 < L_i < t_3\ ) I(y_i = 3) \end{aligned},$$

where n is the number of records; t_1, t_2, and t_3 are thresholds that define the three categories of response; and I is an indicator function that has a value of 1 if the condition specified is true or a value of 0 otherwise. The procedure is a nonlinear transformation of best linear unbiased estimation (BLUE) and BLUP.

To obtain the solution of the liability, it is treated as an unknown and sampled in Bayesian methodology. Fouley et al. (1983) and Janss and Foulley (1993) extended the threshold methodology to multitrait analysis with one continuous correlated trait or more and unequal design. Albert & Chib (1993) and Moreno et al. (1997) generalized the procedure to Markov-chain Monte Carlo. Albert & Chib (1993) and Sorenson et al. (1995) generated algorithms that allow empty categories in fully conditional distributions. Van Tassell et al. (1998) built a multiple-trait Gibbs sampler for animal models (MTGSAM) program that allows several continuous and categorical variables in a threshold-linear model with Gibbs sampling.

Performance in simulation and field data

The advantages of threshold over linear models have been shown in several studies. For discrete traits, the predictability of breeding values from a threshold animal model is higher than those from an equivalent linear animal model (Casellas et al., 2007; Ramirez-Valverde et al., 2001; Varona et al., 1999). Furthermore, the correlations of breeding values between linear and threshold models are above 0.99, and animal rankings are very similar (Weller et al., 1988; Weller & Ron, 1992). However, advantages of linear over threshold models have also been reported (Hagger & Hofer, 1989; Ramirez-Valverde et al., 2001). Jamrozik et al. (1991) reported that when categorical traits are nearly normal (e.g., 18 categories), the threshold model does not have an advantage over a linear model. Ramirez-Valverde et al. (2001) reported lower predictability of maternal breeding values in threshold models compared with linear models in sire-maternal grandsire models. The mechanism behind the inconsistency is not clear. Matos et al. (1997)

indicated no difference between goodness-of-fit and predictability of reproductive traits in linear and threshold models.

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CHAPTER 3

PRIOR GENETIC ARCHITECTURE IMPACTING GENOMIC REGIONS UNDER SELECTION: AN EXAMPLE USING GENOMIC SELECTION IN TWO POULTRY BREEDS[1]

[1] Zhang. X, I. Misztal, M. Heidaritabar, J. W. M. Bastiaansen, R. Borg, R. Okimoto, R. L. Sapp, T. Wing, R. R. Hawken, D. A. L. Lourenco, Z. G. Vitezica, H. H. Cheng, and W. M. Muir. 2015. *Livestock Science,* 171:1-11.

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Abstract

The objective of this study is to investigate if selection on similar traits in different populations progress from selection on similar genes. With the aid of high-density genome wide single-nucleotide polymorphism (SNP) genotyping, it is possible to directly assess changes in allelic frequencies and regions under selection and address the question. We compared the allele frequencies before and after two generations of selection on an index containing body weight at 6 wk, ultrasound measurement of breast meat, and leg score in two commercial chicken breeds with different selection histories: M breed was primarily selected for rapid growth and commonly used as a broiler breeder sire line; F breed was primarily used as dual-purposed dam line selected for both egg production and growth. After quality control, 52,742 and 52,639 SNPs in M breed and F breed were kept in 4,922 and 4,904 animals, respectively. The average allele frequency change for both breeds on the autosomes was 0.049. Threshold value for detecting selected regions, where allele frequency changes exceeded expectations under drift were 0.140 and 0.136 for breeds M and F, respectively. According to the criterion used in this study, there were 25 and 17 selection regions detected on breeds M and F, respectively, without any overlap of regions between the breeds. Average heterozygosity change in F breed was greater compared to M breed (0.008 vs. 0.002, $P<0.01$). Also, there was no overlapping of selected regions with high heterozygosity change between breeds M and F. The results indicate that in newly selected populations, even using the same criteria and selection methods, the historical selection goals and breed development determine the loci that most impact selection progress. These results are consistent with quantitative genetic theory that contribution of loci to selection progress depends on initial allele frequency. Therefore it should not be assumed that the same loci would be under selection in different populations even if similar selection goals and methods were used.

Keywords: SNP; allele frequency change; genomic evaluation

Introduction

An interesting academic question with practical implications is, “does selection on similar traits in different populations progress from selection on similar genes?”. In practice the question is “will genes found to be important in one breeder of a given trait also be important for the same trait in another?”. Because selection on traits changes the allelic frequency of the underlying causative genes (Nielsen, 2005), the interspecific to intraspecific variability between populations or between generations increases (Lewontin and Krakauer, 1973). Directional selection is different from other evolutionary factors that either reduce the ratio of within and between population genetic variability, or have no effect on the genetic variability. Selective sweeps, which are genomic region that have recently become fixed due to the selection of advantageous alleles, reduces the variability in the causative genes and flanking sites. To detect incomplete selective sweeps, it should be possible to utilize genome-wide changes in the allele frequency spectrum over time in populations under selection.

For domestic animals, there were already several studies on the allele frequency spectrum of signature of selection by investigating heterozygosity (Elferink et al., 2012), Wright’s fixation index (*Fst* test) (Moradi et al., 2012), and relative extended haplotype homozygosity (*REHH* test) (Sabeti et al. 2002). However, those studies used cross-generation data during selection, therefore, their results were impacted by both recent and historical selections. Furthermore, most previous studies only have allele frequency data after completion of selection, leaving the initial and change in allele frequencies unknown.

In order to separate the results caused by historical and new selection, our study used two methods: the straightforward allele frequency change from initial to last generation was used to detected genomic change in a recent selection experiment in broiler (meat-type) chickens; and heterozygosity change in above time cession was used to detect selective sweep. Two selection breeds from different origins, a sire breed (M) historically selected for rapid growth, and a dam

breed (F) historically selected for both egg production and growth, were used. These breeds were selected for body weight at 6 wk (BW), ultrasound measurement of breast meat (BM), and leg score (LS) using the same index in both. Genotypes on animals in these breeds were collected for genomic selection. From this data, we attempted to identify the changes in allele frequency spectrum across chromosomes for each generation that should provide insights into how the genome responds to selection.

METHODS

Data structure

Data was provided by Cobb-Vantress Inc. (Siloam Springs, AR). Animals from two pure breeds of commercial broilers were used. M breed was characteristic of a line primarily selected for rapid growth and commonly used as a primary broiler breeder sire line, and F breed was characteristic of a dual-purpose line selected for egg production and growth and commonly used as primary broiler breeder dam breed. In the experiment, both breeds were selected at 6 wk of age for body weight (BW, g), ultrasound measurement of breast meat (BM, cm^2), and leg score (LS, 'acceptable' or 'not acceptable') (Chen et al., 2011). The initial training dataset contained 2,000 animals from 2 generations (G-1 and G0), which was used to estimate SNP effects. From G0, selection was performed for 3 generations with about 800 animals genotyped as selection candidates in each generation of each breed. Then about 20 males and 200 females were selected for breeding. ssGBLUP method was used for estimation of genomic breeding values (GEBV) (Aguilar et al., 2011), except for G-1 of F breed where selection for LS was done with GEBV from a BayesA (Meuwissen et al., 2001). The initial data set for the prediction of GEBV of animals in generation G0 contained 183,784 and 164,246 broilers in M breed and F breed, respectively.

Pedigree information included sires and dams without records in 2 historical generations and 3 selection generations. The total number of records at the end of the experiment was 297,017 for M breed, and 277,051 for F breed.

Genotype data

Genotypes for 57,636 SNPs were obtained using the chicken IIIumina Infinium iSelect Beadchip (Groenen 2009). Total number of genotyped animals was 4,922 in M breed and 4,904 in F breed (Table 3.1). In M breed, 4,994 SNPs were removed because the call rate was less than 0.90, the MAF was 0, or the location was on unassigned chromosomes or incomplete chromosomes (16 and W); 51 animals were removed because of low call rate (<0.90) or parent-progeny conflicts. In F breed, 4,997 SNPs and 130 animals were similarly removed.

Breeding structure

The populations spanned several generations. G0 was the base population randomly selected from a historical set G-1, generation G1 were offspring of randomly selected parents from G0, G2 were offspring of parents selected from G1 on the index, and finally G3 were offspring of parents selected from G2 on the index. Allele frequency differences were obtained between all animals in G0 and all animals in G2, which are separated by 1 generation of random and 2 generations of directional selection. In other words, G3 data represented selected animals in G2, whereas G0 data represented the same generation.

Allele frequency changes

Allele frequencies (f) were computed in G0 and G2 by counting. The absolute values of changes in allele frequencies ($d_{02} = | f_2 - f_0|$) between two generations within each breed were calculated. Large allele frequency differences in allele frequencies between G0 and G2 generations were considered as putative selected regions. The running averages of 11 adjacent d_{02} values were plotted against the location of the middle SNP along chromosomes to emphasize the

systematic changes of frequencies in a window. Window size of 11 was chosen on a criterion if the frequency distribution is clear enough but not too disintegrated (results not shown). Threshold values for significant changes in allele frequency were obtained by simulating the flow of alleles through the real pedigree, and gene dropping performed using HaploSim (Coster and Bastiaansen, 2010). Haplotypes were simulated with 20 loci along one chromosome with 0 mutation rate and 0.5 initial allele frequency. A 0.5 starting frequency gives the largest possible drift variance and leads to a conservative threshold. The haplotypes were simulated for the founder animals (1,165 animals in M breed, and 1,154 in F breed) in the pedigree (Table 3.2). Genotypes were subsequently assigned to offspring according to Mendelian transmission rules. The changes in allele frequency from G0 to G2, d_{02}, were computed for 1,000 replicates. Then a distribution of the d_{02} was obtained from the 1,000 replications of 20 SNPs. A threshold for evidence of selection was determined as the 95% upper bound of the distribution obtained under drift ($P<0.05$).

Genetic variability between the breeds

Genetic variability was assessed by looking at absolute heterozygosity change between G0 and G2 (H_{02}) calculated in an overlapping sliding window approach with window size of 5 by modifying the equation by Rubin et al. (2010). The equation for heterozygosity within generation is $H_P = \frac{2\sum n_{MAJ}\sum n_{MIN}}{(\sum n_{MAJ} + \sum n_{MIN})^2}$, where $\sum n_{MAJ}$ is the sum of major allele frequencies, and $\sum n_{MIN}$ is the sum of the MAF in a window. Then the genetic variability was calculated as the absolute difference between H_P of G0 and G2. The threshold for extreme high or low heterozygosity change was defined as 4 times the standard deviation of genetic variability across chromosomes.

Genomic selection response from ssGBLUP

GEBV of putative selection regions and genomic selection response was estimated by using the mixed model below:

$$\begin{bmatrix} \mathbf{X'X} & \mathbf{X'Z} \\ \mathbf{Z'X} & \mathbf{Z'Z}+\lambda\mathbf{H}^{-1} \end{bmatrix}\begin{bmatrix} \mathbf{b} \\ \mathbf{u} \end{bmatrix}=\begin{bmatrix} \mathbf{X'y} \\ \mathbf{Z'y} \end{bmatrix},$$

where **X** and **Z** are the incidence matrices corresponding to fixed effects and additive genetic effects, respectively; **b** is a vector of fixed effects including an overall mean, hatch number and breed; **u** is the vector of random additive direct genetic effects; λ is the ratio of residual to additive genetic variances, where the residual effect is assumed independent and followed a normal distribution; $\mathbf{H}^{-1}$ is the inverse of a matrix that combines pedigree and genomic relationships (Aguilar et al., 2010); and **y** is the vector of phenotypic records, in a multi-trait scenario.

GEBV of SNP regions were calculated as the summation of SNP content times SNP effects for that region for all genotyped animals within a generation. Thereafter, genomic response was accounted for by the change of average GEBV from G0 to G2. GEBV and genomic selection response for each of the three traits were analyzed separately for each of the two breeds. The sex chromosomes were excluded from the model.

Results

Effect of selection traits on the change of genetic variation

Changes in allele frequency between G0 and G2 (d_{02}) in M breed and F breed were calculated to compare the response to selection. Whole-genome patterns of allele frequency change in M breed and F breed were different with respect to the positions, the ranges of putative select regions, and values of the most extreme d_{02} (Figures 3.1 and 3.2). Thresholds for significant d_{02} determined by gene dropping method were 0.140 for M breed and 0.136 for F breed (Figure S3.1). None of the selected regions were overlapping between the two breeds. The average changes in allele frequency ($\bar{d}_{02}$) on autosomes were the same, 0.049, in both breeds (Table 3.3). As expected for the sex chromosome, and aggravated by the smaller number of male versus

female parents, chromosome Z had a larger average allele frequency change compared to the autosomes. This change was greater in M breed than that found for F breed (0.070 vs. 0.061, respectively, P<0.01). The $\bar{d}_{02}$ of all chromosomes for M breed and F breed are 0.051 and 0.049. Also, the average minor allele frequency (MAF) of G2 is higher than the MAF of G0 in both breeds, again in both the Z chromosome and in autosomes (average MAF difference, autosomes: 0.002 for both breeds; chromosome Z: 0.016 and 0.008 for M breed and F, respectively, P<0.01). In selected regions, the average allele frequency changes were 0.177 for M breed, and slightly smaller, 0.176 for F breed, but not significantly different between the breeds (P=0.7). The distribution of d_{02} values showed a longer tail in M breed than F breed, indicating that SNPs in M breed have more extreme allele frequency changes after two generations of selection (Figure 3.3).

Selected regions

With both GBLUP selected breeds, less than half of the chromosomes contained extreme regions where the running average of d_{02} exceeded the threshold (Figures 3.1 and 3.2, Tables S3.2-S3.6). The threshold was exceeded on 12 and 9 chromosomes, and in 25 and 17 regions, in M breed and F breed, respectively. The total length of selected regions was 11,531 kb and 8,396 kb; and the average length was 494 kb and 461 kb for M breed and F breed, respectively. No overlapping regions were found between breeds under resolution of 23kbp/SNP (Tables S3.3 and S3.4). The greatest changes in the running averages of d_{02} values were found on chromosome 2, 9, 10 and Z on M breed; and on chromosomes 4, 12 and Z for F breed (Tables S3.5 and S3.6). Total numbers of 322 out of 44,770 and 296 out of 44,895 SNPs surpassed the threshold in breeds M and F, respectively.

Divergence and genetic variability among the breeds

Heterozygosity was expected to decrease in regions of selection (Allendorf 1986; Barton, 1998; Kim and Stephan, 2002). The results shown in Table 3.4 indicates that there is a positive

average change in heterozygosity ($\bar{H}_{02}$) across all autosomes between G0 and G2 in both breeds (G0-G2 = 0.004 for M breed and 0.008 for F breed, P<0.01), and the change in F breed is much larger compare to M breed (P<0.01). However, the Z chromosome has a bigger but increased $\bar{H}_{02}$ (G0-G2 = -0.086 and -0.088 for M breed and F, respectively, P<0.01).

The threshold values for significant heterozygosity changes (H_{02}) are 0.136 and 0.125 for breeds M and F, respectively. The running average of H_{02} showed multiple regions above the thresholds (Figure 3.4 and 3.5) that overlapped with significantly selected regions based on d_{02} (Figures 3.1 and 3.2) in both breeds. In M breed, chromosomes 2, 3, 9, 10, 15 and 18 each have one region that was identified by both methods. In F breed, one region each on chromosomes 3, 4, 7, 11 and 12, and two regions on chromosome 6 also overlapped between the two methods.

Discussion

Our results indicate that both breeds M and F have many genome regions where allele frequency changes are observed after 2 generations of selection. For both breeds, the average MAF was higher in G2 than in G0, implying a certain level of selection for minor alleles. The average absolute allele frequency changes on autosomes were the same for both breeds, which was expected since they had similar effective size, leading to similar impact of drift, and both were selected for two generations. However, the patterns of d_{02} were vastly different for breeds M and F: non-overlap of selection regions; more and larger selected regions in M breed compared to F breed; and also larger d_{02} values in M breed than in F breed. The larger number of selected regions implies that more genes or functional elements were selected and on top of that the larger peaks in d_{02} indicates a stronger selection on those regions.

The breeds experienced the same recent selection goal and intensity, density of genomic data, and had similar effective population sizes, which means that the distinct genetic backgrounds of M breed and F were responsible for the diversity in their allele frequency changes

(Falconer 1960). QTLs associated with the traits in breeds M and F started at different initial allele frequencies, given their differences in genetic architecture due to different historical selection goals, numbers and sizes of QTL affecting the traits, and LD (Lewontin, 1988). And the initial allele frequency of biallelic loci determines how and how strong the frequency changes along selection (Kimura, 1957), assuming no selective advantage to consider, as it was not natural selection. The larger selected regions and d_{02} in M breed compared to F breed indicates association with historical selection, where M breed was selected historically on growth traits with higher heritability than reproductive traits in which selection of F breed was based on. Nevertheless, for F breed there might be more genes but less selection intensity involved in the historical genetic architecture due to dual-purpose selection, resulting in the selection response being more distributed across the genome and fewer regions that pass the threshold. An interesting finding was that regions of d_{02} peaks appeared in breeds M and F were totally different. This attests that the same selection goal does not necessarily mean selection of the same genes, even in the same species. For example, alleles already fixed in M breed would not change in frequency, but still could be selected in F breed.

Unlike allele frequency change and heterozygosity that could be affected by recent and historical events, genomic selection response based on GEBV changes of SNP in a region, however, measured the change of genetic effect responding to the current selection. Results demonstrate that most putative selection regions based on allele frequency change did not show peak values in genomic selection response (Table S3.7 and S3.8). For autosomes, out of 19 regions in M breed, only 1 region exceeded 3 standard deviations from the mean (region 17, chromosome 23); and no region in F breed exceeded 3 standard deviations from the mean. Also, no region in either breed had top GEBV at G2 that ranked outside 3 standard deviations from the mean. The low heritability (0.24, 0.27 and 0.12 for BW, BM, and LS, respectively) may explain part of the inconformity. More importantly, based on the above assumption of historical selection,

the putative selection regions were more affected by historical selection hence might not necessarily overlap with current selection regions.

Ascertainment bias is not supposed to influence our results, although it was created at the same time the SNP chip was created because only common SNPs were placed on the chip. The impact of ascertainment bias is that rare alleles were neither present nor tested for. However this bias has little or no impact on our results for 2 reasons: 1) if rare alleles were selected for or against, the contribution of those SNPs to the total genetic variation will be small since that contribution is $2p(1 - p)u^2$, where p is MAF and u is marker effect, meaning that even if the effect of the allele is large, the weight will be small. Such loci will eventually contribute to total genetic variation as the allele frequency approaches 0.5. 2) Because we assumed the most conservative setting, i.e. P=0.5, if such alleles were present, and could have been tested for, we most likely would not have detect them due to the small effect such rare alleles have on genetic variance.

In other studies, QTL have been discovered across the whole genome, located on all macro-, intermediate-, micro-chromosomes and on chromosome Z. Using the same populations analyzed in this study, Wang (2013) identified the top 10 genome regions that explained genetic variance of the 3 traits that breeds M and F were selected on. These associated genome regions were detected using classical GWAS with WOMBAT (Meyer, 2007), ssGBLUP, and Bayes B methods. Only one of the selected regions identified in our study overlaps with the associated regions found by Wang (2013). The overlap was found in F breed, where the selected region is located on chromosome 6, from 19,539,027 bp to 20,308,725 bp. The corresponding region was associated with body weight at 6 wk was located from 19,470,652 bp to 19,901,892 bp and explained 5.97% of genetic variance according to the WOMBAT analysis (Wang 2013). The ssGBLUP and BayesB methods also identified an association in this region between 19,916,663 bp and 20,267,429 bp, accounting for 2.2% and 4.24% of the genetic variance, respectively. The lack of consistency between association and selection results could due to genetic drift, mutation

rate, as well as the fact that the model only accounted for additive genetic effect. On the other hand, genetic analysis only seeks for effective SNPs, not their favorable haplotypes. Lastly, the results of association analysis were shown to be method-sensitive (Wang, 2013). Current experience with GWAS indicates that although many associations are detected in several regions, only a few of them are found in similar studies.

Heterozygosity changes on autosomes from G0 to G2 indicated that selection reduces heterozygosity ($P<0.05$). The pattern is different from d_{02}, but both breeds have overlap between d_{02} peaks and H_{02} peaks. The overlapped regions confirmed that certain haplotypes have been selected within those areas. The peak regions that only appear in d_{02} but not in H_{02} can occur when a haplotype that was favored contains the minor allele for some SNPs and the major allele for others. The peak regions that only appear in H_{02} but not in d_{02} may indicate no unique haplotype was favored. Heterozygosity pattern of past selection gives the position of selective sweep (Barton, 1998; Kim and Stephan, 2002), which is a wide range of adjacent alleles that became fixed under strong directional selection. In our case, a change of heterozygosity, instead of fixation, was used to identify selective sweeps due to recent selection.

Chromosome Z is different from autosomes in a number of ways, e.g., higher major allele frequencies, larger $\bar{d}_{02}$ lower average heterozygosity but larger $\bar{H}_{02}$ from G0 to G2, which increased rather than decreased as generation of selection increases. It is important to note that heterozygosity analysis was done with genotypes of males only, as females are hemizygous (ZW) in chicken. Sundstrom et al. (2012) observed that when male effective population sizes are smaller, as is the case in many livestock selection programs, a selective sweep will reduce levels of genetic variability on the Z chromosome more drastically than on autosomes. Moreover, the recombination rate on Z chromosome is about 1.3cM/MB, ~2.5 times less than the average autosomal recombination rate (Levin et al., 1993), thus the effects of selection on linked neutral sites on chromosome Z would stretch much farther on average than autosomes. As expected from

these observations, we found that heterozygosity on chromosome Z changed more drastically than on autosomes in both breeds. Interestingly though, the genetic variability raised rather than reduced. Storchová and Divina (2006) found enrichment of male-biased genes (genes expressed preferentially or exclusively in male, e.g. genes coding sperm) but underrepresentation of female-biased genes on chicken Z chromosomes. Bellott et al. (2010) found that chicken Z chromosomes are more uniquely responding to selection for traits that benefit male sex traits more than female. Therefore, the heterozygosity increases on chromosome Z under selection was probably linked to male sex traits indirectly affected by selection breed.

Average heterozygosity of pooled autosomes and sex chromosomes in M breed ranged from 0.346 to 0.352. Elferink et al. (2012) used the same SNP array on commercial and non-commercial chickens where heterozygosities ranged from 0.39 to 0.43 for broiler sire breeds, and 0.35 to 0.42 for broiler dam breeds. These values are larger than in layers, given larger N_e and possibly less historic selection intensity in broilers compared with layers.

Previous studies investigating selective sweeps on domestication of chicken also showed effects of selection on genetic variability (Elferink et al., 2012; Wang, 2013). These studies analyzed the genetic variation across current generations to discover the impact of past selection. Of our 41 putative selected regions, 6 of them overlapped with reference studies (Table S3.6.), however, most selective sweeps from previous studies do not show overlap with our results, presumably because the fixation in their results was generated by historical selection, and our study of recent selection cannot change allele frequencies in a large scale if those regions were already under fixation process. Previous studies confirmed that selection is the major cause of the frequency spectrum pattern change on chromosomes.

Characterizing biological functions in putative selected regions

We were also interested to see if QTLs within the selected regions overlap with known QTLs from chicken. QTL were identified from the animal QTL database

(http://www.animalgenome.org/cgi-bin/QTLdb/GG/index) and compared with selected regions found in our study. The QTL in regions with large d_{02} changes were found to be related to either production or health. Selected regions in M breed overlapped with 4 QTL for body weight, 2 for growth, 1 for residual feed intake, 1 for muscle weight, 1 for muscle size, 1 for number of eggs, and 1 for age at first egg. Selected regions in F breed overlap with 1 QTL for egg shell thickness, 3 for carcass weight, 2 QTL for carcass components, 1 QTL for feather pecking, and 6 QTL associated with health traits.

Of all the genes located within the selected regions, the interesting candidate genes are listed in Table S9. Of interest in M breed, carboxypeptidase B1 (CPB1) is located in the highest peak d_{02} region on chromosome 9. Carboxypeptidase B1 is a necessary enzyme especially in the processing of recombinant insulin, and insulin is a vital hormone regulating the carbohydrate and fat metabolism in the body (Ladisch and Kohlmann 1992). Epidermal growth factor receptor (EGFR-CHICK) located on chromosome 2, which is also the 3rd highest peak of d_{02}, EGF stimulates the growth of various epidermal and epithelial tissues in vivo and in vitro and of some fibroblasts in cell culture (Groenestege et al., 2007**)**. MYOCD (myocardin) is located in the 4th region on chromosome 18 and plays a crucial role in cardiogenesis and differentiation of the smooth muscle cell lineage (myogenesis) (Du et al., 2002). Adenylated cyclase 10 (ADCY10) on chromosome Z has a critical role in mammalian spermatogenesis. In human, it produces the cAMP which mediates in part the cAMP-responsive nuclear factors indispensable for maturation of sperm in the epididymis. It induces sperm capacitation and is involved in ciliary beat regulation. (Geng et al., 2005; Schmid et al., 2007).

In F breed, on chromosome Z, the highest d_{02} region contains lipoprotein lipase (LPL), which catalyze the hydrolysis of triglycerides of circulating chylomicrons and very-low-density lipoproteins (VLDL) (Nilsson-Ehle et al., 1980). In the 4th region on chromosome 4, the gene zygotes arrest 1 (ZAR-1) is found, which in human is essential for female fertility and may play a

role in the oocyte-to-embryo transition (Wu et al., 2002). The second highest peak contains lipid phosphate phosphatase-related protein type 1-like motif (LOC427306), but its function is uncharacterized so far.

These genes associated in breeds M and F validate our assumption that different genes respond to the same selection direction in different breeds. Further studies on molecular pathways may need to illustrate the mechanism of different response.

Conclusions

The effect of selection goals and breeds on change of genomic variation was investigated across the entire genome of two breeds of broiler chicken. Twenty-five and seventeen regions with evidence of selection were detected after GBLUP selection in a male and a female broiler breeds, respectively. Our study shows that even using the same method (GBLUP) and the same selection index, changes in genomic variation are different between breeds. Given that both breeds have the same genes, this result implies that the historical goal during breed development changed the genetic architecture of each breed such that the regions currently selected were altered. These results are consistent with quantitative genetic theory that the contribution of loci to selection progress is dependent on initial allele frequency. Also, several QTLs overlap with the regions detected by allele frequency and heterozygosity changes indicating that these methods may have potential to identify genes that are functionally linked to the breeds.

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Table 3.1. Total number of genotyped animals and number of animals that were selected based on EBV.

Breed	Total genotyped animals	Selected animals			
		G0		G2	
		Female	Male	Female	Male
M	4,922	200[a]	20	200	200
F	4,904	200	20	20	20

Table 3.2. Number of genotyped animals retained after QC.

Breed	G0	G2	Total
M	1,165	1,009	4,871
F	1,154	1,028	4,774

Table 3.3. Average difference in allele frequencies ($\bar{d}_{02}$) and major allele frequencies (*f*) of autosomes and chromosome Z within generations between breeds.

Breed		G2-G0		G0		G2	
		1-28[a]	Z	1-28	Z	1-28	Z
M		$\bar{d}_{02}$	$\bar{d}_{02}$	f_0[d]	f_0	f_2[e]	f_2
	Average	0.049	0.07	0.734	0.809	0.731	0.793
	SD[b]	0.04	0.051	0.142	0.153	0.14	0.149
	N[c]	43,250	1,520	44,056	1,659	43,542	1,533
F		$\bar{d}_{02}$	$\bar{d}_{02}$	f_0	f_0	f_2	f_2
	Average	0.049	0.061	0.739	0.748	0.736	0.74
	SD	0.029	0.048	0.143	0.152	0.142	0.148
	N	43,533	1,362	42,629	1,414	42,180	1,375

[a]Chromosome 16 excluded, MAF=0 excluded

[b]SD: standard deviation

[c]N: total number of SNPs

[d]f_0: allele frequency at generation 0

[e]f_2: allele frequency at generation 2

Table 3.4. Average heterozygosity (H_P), mean difference of heterozygosity ($\bar{H}_{02}$) and standard deviation by breeds and generations.

Chromosome		M			F		
		G2-G0	**G0**	**G2**	**G2-G0**	**G0**	**G2**
1-28[a]		$\bar{H}_{02}$	H_{P0}[d]	H_{P2}[e]	$\bar{H}_{02}$	H_{P0}	H_{P2}
	Average	-0.004	0.362	0.36	-0.008	0.358	0.353
	SD[b]	0.062	0.142	0.145	0.058	0.143	0.148
	N[c]	45,063	44,056	43,542	42,161	42,629	42,180
Z		$\bar{H}_{02}$	H_{P0}	H_{P2}	$\bar{H}_{02}$	H_{P0}	H_{P2}
	Average	0.086	0.027	0.126	0.094	0.051	0.146
	SD	0.056	0.027	0.735	0.051	0.063	0.082
	N	1,502	1,659	1,533	1,372	1,414	1,375

[a]Chromosome 16 excluded, MAF=0 excluded

[b]SD: standard deviation

[c]N: total number of SNPs

[d] H_{P0}: heterozygosity at generation 0

[e] H_{P2}: heterozygosity at generation 2

Table S3.2. Chromosome regions with evidence of selection by GBLUP and their size in M breed.

Number	Chr	Start Region (b)	End Region (b)	Size (Kb)	#SNPs
1	1	185,202,760	185,413,126	210.366	9
2	2	50,574,392	54,731,865	4157.473	67
3	2	83,264,437	83,539,060	274.623	9
4	3	98,094,228	98,413,125	318.897	11
5	8	614,142	767,604	153.462	9
6	9	9,453,053	9,633,402	180.349	12
7	9	10,943,233	11,177,961	234.728	14
8	9	11,470,525	11,907,809	437.284	22
9	10	6,024,285	6,155,943	131.658	7
10	10	6,806,958	6,942,529	135.571	12
11	10	8,128,457	8,308,720	180.263	10
12	10	9,150,224	9,367,350	217.126	14
13	14	4,018,870	4,219,431	200.561	15
14	15	5,207,296	5,481,121	273.825	27
15	15	5,593,782	5,690,280	96.598	11
16	18	772,099	1,186,725	414.626	36
17	23	1,204,542	1,370,482	165.94	17
18	23	1,399,841	1,476,553	76.712	10
19	26	245,579	317,938	72.359	10
20	Z	1,694,169	2,132,656	438.487	19
21	Z	11,576,834	12,057,649	480.815	8
22	Z	17,156,388	17,411,947	255.559	11
23	Z	26,835,196	27,640,939	805.743	17
24	Z	62,933,907	63,349,183	415.276	13
25	Z	72,563,048	73,766,594	1202.546	17

Table S3.3. Chromosome regions with evidence of selection by GBLUP and their size in F breed.

Number	Chr	Start Region (b)	End Region (b)	Size (Kb)	#SNPs
1	3	33,983,511	34,455,760	472.249	21
2	4	16,923,316	17,188,954	265.638	10
3	4	39,593,070	39,691,336	98.266	6
4	4	65,831,538	66,207,979	376.441	17
5	5	1,191,392	1,468,963	277.571	12
6	6	13,057,220	13,306,619	249.399	13
7	6	16,365,513	16,695,335	329.822	18
8	6	19,539,037	20,208,725	769.688	36
9	7	31,502,486	32,511,463	1007.977	21
10	11	17,539,521	17,677,955	138.434	9
11	12	11,286,072	11,681,733	395.661	33
12	12	12,611,675	12,989,064	377.389	16
13	13	16,025,668	17,537,992	1512.324	84
14	Z	44,183,374	44,571,786	388.412	8
15	Z	45,488,677	45,700,011	211.334	7
16	Z	53,597,683	54,396,139	798.456	25
17	Z	64,478,414	65,205,624	727.21	16

Table S3.4. Number of total and direction of allele frequency changes after 2 generations of selection for GBLUP peaks in M breed.

Chr	Position	f_0	f_2	d_{02}	Name
2	50623780	0.382	0.187	0.196	Gga_rs15990155
2	50670751	0.618	0.821	-0.202	Gga_rs15990227
2	50849141	0.575	0.720	-0.145	Gga_rs14181868
2	50869811	0.351	0.152	0.199	Gga_rs14181874
2	50893951	0.649	0.847	-0.198	Gga_rs15990496
2	50917682	0.649	0.847	-0.198	Gga_rs15990523
2	50929779	0.351	0.153	0.199	GGaluGA147120
2	50939795	0.351	0.152	0.199	Gga_rs14181886
2	50979433	0.428	0.281	0.147	Gga_rs15990558
2	51051310	0.572	0.719	-0.147	Gga_rs15990661
2	51075233	0.428	0.280	0.148	Gga_rs14181956
2	51075438	0.572	0.719	-0.147	Gga_rs14181958
2	51130285	0.590	0.749	-0.159	Gga_rs14182084
2	51159111	0.590	0.750	-0.159	GGaluGA147158
2	51176916	0.572	0.720	-0.148	Gga_rs15990836
2	51210778	0.590	0.749	-0.159	Gga_rs14182120
2	51280108	0.590	0.749	-0.159	Gga_rs10724628
2	51357095	0.428	0.281	0.147	Gga_rs15991100
2	51375819	0.410	0.252	0.158	Gga_rs14182263
2	51425256	0.572	0.719	-0.147	Gga_rs14182280
2	51444234	0.572	0.719	-0.146	Gga_rs14182344
2	51463919	0.590	0.758	-0.167	Gga_rs14182365
2	51483614	0.590	0.752	-0.162	Gga_rs14182386
2	51498096	0.572	0.719	-0.146	Gga_rs14182398
2	51525854	0.410	0.250	0.160	Gga_rs14182405
2	51647563	0.590	0.749	-0.159	Gga_rs15991322
2	51649545	0.590	0.749	-0.159	Gga_rs14182485
2	51708066	0.410	0.252	0.158	GGaluGA147234
2	51733546	0.410	0.250	0.160	Gga_rs15991494
2	51795747	0.590	0.749	-0.159	Gga_rs14182566
2	51826860	0.428	0.281	0.146	Gga_rs14182574
2	51851106	0.410	0.251	0.159	GGaluGA147253
2	51937287	0.590	0.750	-0.159	Gga_rs14182702
2	51965459	0.572	0.719	-0.146	GGaluGA147267
2	51995708	0.590	0.754	-0.164	Gga_rs14182727
2	52007022	0.590	0.749	-0.158	Gga_rs14182733
2	52064693	0.428	0.282	0.146	Gga_rs14182760
2	52109734	0.542	0.692	-0.150	GGaluGA147286
2	52123508	0.572	0.718	-0.146	Gga_rs15991956
2	52147801	0.590	0.749	-0.159	Gga_rs14182785
2	52210164	0.410	0.251	0.158	Gga_rs14726948

2	52216023	0.590	0.749	-0.159	GGaluGA147298
2	52253171	0.590	0.750	-0.159	GGaluGA147301
2	53807784	0.590	0.749	-0.159	Gga_rs14182788
2	53826736	0.410	0.249	0.161	Gga_rs15992055
2	53876105	0.410	0.251	0.159	Gga_rs14182822
2	53883624	0.590	0.756	-0.165	Gga_rs14182832
2	53902578	0.410	0.238	0.172	Gga_rs14182844
2	53997807	0.590	0.750	-0.159	Gga_rs14182879
2	54022986	0.590	0.750	-0.159	Gga_rs14182890
2	54122331	0.410	0.252	0.158	Gga_rs14183051
2	54208753	0.590	0.749	-0.159	Gga_rs14183091
2	54251297	0.590	0.749	-0.159	Gga_rs13616286
2	54334101	0.410	0.250	0.159	Gga_rs13616358
2	54383830	0.590	0.749	-0.159	Gga_rs14183353
2	54446447	0.590	0.749	-0.159	GGaluGA147366
2	54487968	0.410	0.251	0.158	Gga_rs14183201
2	54556413	0.410	0.251	0.159	Gga_rs14183274
2	54622092	0.410	0.251	0.159	Gga_rs15993166
2	54632827	0.590	0.750	-0.159	Gga_rs14183401
2	54731865	0.590	0.749	-0.159	Gga_rs13616387
9	11470525	0.718	0.874	-0.156	Gga_rs16655354
9	11471460	0.282	0.124	0.158	GGaluGA338557
9	11591855	0.332	0.186	0.146	Gga_rs15949367
9	11638384	0.683	0.839	-0.156	GGaluGA338623
9	11648946	0.664	0.434	0.230	Gga_rs14661884
9	11675204	0.664	0.434	0.230	GGaluGA338635
9	11675228	0.664	0.433	0.231	Gga_rs15949232
9	11697657	0.616	0.404	0.212	Gga_rs14661820
9	11744404	0.335	0.558	-0.223	Gga_rs10727978
9	11764236	0.357	0.580	-0.224	GGaluGA338661
9	11809684	0.662	0.436	0.226	Gga_rs13763666
9	11826808	0.404	0.612	-0.207	Gga_rs14661681
9	11848962	0.644	0.419	0.225	Gga_rs14661674
9	11873116	0.333	0.167	0.165	GGaluGA338715
9	11896431	0.315	0.151	0.163	Gga_rs13763624
9	11907809	0.644	0.418	0.226	Gga_rs15948757
10	9186849	0.413	0.247	0.166	Gga_rs14004211
10	9249855	0.482	0.282	0.200	Gga_rs15573306
10	9262744	0.482	0.283	0.199	Gga_rs14004240
10	9270971	0.483	0.280	0.202	GGaluGA068302
10	9284540	0.483	0.283	0.200	Gga_rs14004251
10	9314643	0.483	0.281	0.202	Gga_rs14004260
10	9320467	0.517	0.717	-0.200	GGaluGA068309
10	9347264	0.483	0.282	0.201	Gga_rs14004281
10	9367350	0.591	0.764	-0.174	GGaluGA068321
Z	1694169	0.613	0.450	0.163	Gga_rs15714460

Z	1733849	0.309	0.106	0.202	Gga_rs14067770
Z	1770223	0.648	0.884	-0.235	GGaluGA346277
Z	1820638	0.687	0.487	0.200	GGaluGA346299
Z	1835845	0.642	0.884	-0.241	GGaluGA346304
Z	1890405	0.152	0.343	-0.191	GGaluGA346332
Z	1923265	0.466	0.204	0.262	Gga_rs14067631
Z	1934775	0.510	0.207	0.302	GGaluGA346347
Z	1986078	0.530	0.812	-0.283	GGaluGA346376
Z	2002023	0.529	0.800	-0.271	GGaluGA346383
Z	2052364	0.255	0.433	-0.178	Gga_rs15714064
Z	2132656	0.112	0.342	-0.230	Gga_rs14067402

Table S3.5. Number of total and direction of allele frequency changes after 2 generations of selection for GBLUP peaks in F breed.

Chr	Position	f_0	f_2	d_{02}	Name
4	39608740	0.655	0.513	0.142	GGaluGA254530
4	39645198	0.654	0.512	0.142	Gga_rs14453946
4	39685604	0.552	0.367	0.185	GGaluGA254549
4	39691336	0.448	0.634	-0.186	Gga_rs14454004
4	65920557	0.622	0.427	0.195	Gga_rs16425301
4	65926490	0.622	0.429	0.193	Gga_rs14482124
4	65970742	0.305	0.478	-0.173	Gga_rs14482147
4	66009865	0.378	0.572	-0.194	GGaluGA262868
4	66022973	0.378	0.574	-0.196	GGaluGA262871
4	66055249	0.378	0.545	-0.167	Gga_rs14482197
4	66078516	0.622	0.455	0.167	Gga_rs14482208
4	66089362	0.550	0.361	0.189	Gga_rs15602304
4	66120967	0.451	0.640	-0.189	Gga_rs15602345
4	66167705	0.378	0.546	-0.167	Gga_rs14482252
4	66207421	0.378	0.548	-0.170	Gga_rs13548944
4	66207979	0.622	0.454	0.168	Gga_rs13548947
12	11318812	0.605	0.754	-0.149	Gga_rs14979711
12	11319599	0.395	0.247	0.148	Gga_rs14041150
12	11340723	0.409	0.254	0.154	GGaluGA085694
12	11346391	0.415	0.257	0.158	Gga_rs14041187
12	11357247	0.599	0.753	-0.154	GGaluGA085708
12	11379238	0.596	0.751	-0.155	Gga_rs13612028
12	11395657	0.581	0.748	-0.167	GGaluGA085732
12	11408668	0.588	0.759	-0.172	GGaluGA085745
12	11418973	0.591	0.760	-0.170	Gga_rs14041266
12	11428043	0.591	0.759	-0.169	Gga_rs14041284
12	11438067	0.408	0.238	0.170	Gga_rs13612067
12	11467656	0.591	0.758	-0.168	Gga_rs15654369
12	11470248	0.409	0.242	0.167	Gga_rs14041325
12	11487871	0.426	0.252	0.174	Gga_rs14041332
12	11500578	0.574	0.748	-0.174	Gga_rs14041333
12	11505029	0.574	0.750	-0.175	GGaluGA085780
12	11521935	0.574	0.748	-0.174	Gga_rs14979861
12	11535596	0.591	0.758	-0.168	Gga_rs14041369
12	11546970	0.591	0.761	-0.170	GGaluGA085805
12	11557768	0.426	0.251	0.174	Gga_rs14979909
12	11562607	0.574	0.748	-0.174	Gga_rs13612115
12	11578458	0.408	0.239	0.169	GGaluGA085823
12	11589524	0.426	0.251	0.175	Gga_rs14041454
12	11611160	0.389	0.237	0.152	GGaluGA085851
12	11626941	0.416	0.259	0.157	GGaluGA085867

12	11634177	0.416	0.259	0.157	GGaluGA085873
12	11664560	0.588	0.740	-0.151	GGaluGA085883
12	11681733	0.594	0.750	-0.156	Gga_rs14041513
33	53676177	0.276	0.446	-0.170	Gga_rs14770696
33	53708376	0.449	0.671	-0.222	Gga_rs14770716
33	53764200	0.259	0.447	-0.188	Gga_rs14770738
33	53770190	0.259	0.450	-0.191	GGaluGA353629
33	53858804	0.741	0.554	0.186	Gga_rs14770809
33	53869481	0.259	0.446	-0.186	Gga_rs16772057
33	53956105	0.554	0.348	0.206	Gga_rs14770869
33	53975701	0.448	0.650	-0.202	GGaluGA353674
33	54008397	0.552	0.354	0.198	Gga_rs13769150
33	54029926	0.553	0.356	0.197	GGaluGA353682
33	54089802	0.553	0.358	0.195	GGaluGA353690
33	54151572	0.445	0.627	-0.182	Gga_rs14771051
33	54168493	0.554	0.373	0.181	Gga_rs14771061
33	54205228	0.554	0.372	0.183	Gga_rs14771088
33	54209320	0.554	0.372	0.183	Gga_rs14771094
33	54302973	0.446	0.627	-0.181	Gga_rs14771149
33	54323063	0.557	0.401	0.156	Gga_rs14771155
33	54328818	0.557	0.402	0.154	Gga_rs14771157
33	54376509	0.557	0.401	0.156	GGaluGA353725
33	54396139	0.443	0.600	-0.156	GGaluGA353728
33	64718176	0.575	0.818	-0.243	Gga_rs14775506
33	64740695	0.574	0.818	-0.244	Gga_rs14775520
33	64777119	0.695	0.871	-0.177	Gga_rs14775580
33	64935210	0.425	0.183	0.242	Gga_rs16774878
33	64973798	0.693	0.871	-0.177	Gga_rs14775780
33	65029649	0.425	0.196	0.229	Gga_rs16121221
33	65062597	0.307	0.137	0.170	Gga_rs16121384
33	65125500	0.247	0.086	0.161	Gga_rs14775906
33	65178185	0.423	0.264	0.160	Gga_rs14775949
33	65205624	0.578	0.728	-0.150	Gga_rs14775967

Table S3.6. Selected regions overlapping with selection signals detected in other studies.

Chr	Breed	Selected region in this study		Selected region in other studies		Line type used in other study[reference]
		Start region (bp)	End region (bp)	Start region (bp)	End region (bp)	
2	M	50574392	54731865	50670751	54230105	commercial, broiler sire line, brown layer [a]
				50740000	50780000	commercial broiler [b]
				51880000	51920000	domestic line [b]
				51800000	51860000	domestic line [b]
				51940000	51980000	commercial broiler [b]
				52040000	52080000	commercial broiler [b]
9	M	9453053	9633402	9440000	9620000	domestic line [b]
10	M	9150224	9367350	9249855	9314643	dutch, dutch new breeds[a]
18	M	772099	1186725	465378	615438	broiler, broiler sire line[a]
6	F	19539027	20208725	19400000	19460000	commercial broiler [b]
11	F	17539521	17677955	17522895	17594419	broiler dam line[a]

[a]Elferink et al. 2012

[b]Rubin et al. 2010

Table S3.7. Percentage of window SNP variance for the alleles at peak of allele frequency changes on autosomes in M breed.

Region number	#SNPs	Selection response[a]	GEBV in G2[b]
1	9	-0.017	-0.008
2	67	0.024	0.065
3	9	0.016	0.015
4	11	0.006	-0.011
5	9	0.001	0.002
6	12	0.03	0.044
7	14	0.051	0.111
8	22	0.025	0.048
9	7	0.023	0.036
10	12	0.021	0.046
11	10	-0.033	-0.057
12	14	-0.027	-0.088
13	15	0.059	0.115
14	27	0.06	0.034
15	11	0.029	-0.009
16	36	0.015	-0.021
17	17	0.085	0.121
18	10	0.017	0.002
19	10	0.003	-0.005

[a] average single SNP GEBV change was $9\times10^{-5}\pm1\times10^{-3}$

[b] average single SNP GEBV in G2 was $1\times10^{-4}\pm6\times10^{-3}$

Table S3.8. Percentage of window SNP variance for the alleles at peak of allele frequency changes on autosomes in F breed.

Region number	#SNPs	Selection response[a]	GEBV in G2[b]
1	21	0.0285	0.0107
2	10	0.0187	0.0078
3	6	-0.0121	0.0078
4	17	-0.0324	-0.0300
5	12	-0.0179	-0.0098
6	13	0.0049	-0.0034
7	18	0.0083	0.0104
8	36	-0.028	-0.0481
9	21	0.0935	0.044
10	9	0.0007	0.0195
11	33	-0.0575	-0.0821
12	16	0.0487	0.0966
13	84	-0.0503	0.096

[a] average single SNP GEBV was $7\times10^{-5}\pm1\times10^{-4}$

[b] average single SNP GEBV in G2 was $1\times10^{-4}\pm2\times10^{-3}$

Table S3.9. Interesting genes located in regions of selection in breeds M and F.

Chr	Breed	Selected regions		Candidate gene acronym[a]	Candidate gene name
		Start region (bp)	End region (bp)		
1	M	185202760	185413126	CCDC67; SLC36A4	coiled-coil domain containing 67; solute carrier family 36 (proton/amino acid symporter), member 4;
2	M	50574392	54731865	CNTNAP2; TPK1; EGFR_CHICK	contactin associated protein-like 2; thiamin pyrophosphokinase 1; Epidermal growth factor receptor
9	M	9453053	9633402	TRIP12	thyroid hormone receptor interactor 12
9	M	10943233	11177961	PLSCR1; PLSCR5	phospholipidscramblase 1; phospholipid scramblase family member 5
9	M	11470525	11907809	CPB1 NCBP2	carboxypeptidase B1; nuclear cap binding protein subunit 2, 20kDa
10	M	8128457	8308720	MYO5A	myosin VA (heavy chain 12, myosin)
18	M	772099	1186725	MYOCD	myocardin
23	M	1399841	1476553	EYA3	Eyes Msent homolog 3
Z	M	11576834	12057649	EGFLAM; LIFR	EGF-like, fibronectin type III and laminin G domains; leukemia inhibitory factor receptor alpha
Z	M	72563048	73766594	ADCY10; LOC100858602	adenylatecyclase 10 (soluble); adenylatecyclase type 10-like
4	F	65831538	66207979	ZAR-1	Zygotes arrest 1
5	F	1191392	1468963	LOC428827; COR6; OR5AS1; LOC770492;	olfactory receptor 1052-like chick olfactory receptor 6; olfactory receptor, family 5, subfamily AS, member 1;

				IGHMBP2	ras-related and estrogen-regulated growth inhibitor-like immunoglobulin mu binding protein 2
6	F	19539027	20208725	IDE; MYOF	insulin-degrading enzyme; myoferlin
7	F	31502486	32511463	LRP1B	
13	F	16025668	17537992	FGF1	Fibroblast growth factor 1
Z	F	53597683	54396139	LPL	Lipoprotein lipase
Z	F	64478414	65205624	LOC427306	lipid phosphate phosphatase-related protein type 1-like

[a] resource: NCBI chicken genome overview, UniProt, GO

Figures

Figure 3.1. Pattern of genetic variation after two generations of selection for M breed. Running average of allele frequency distribution of 44,770 SNPs along the whole genome is plotted against the sequence. The deviations above the threshold show signals of selection.

Chromosome 33 is chromosome Z.

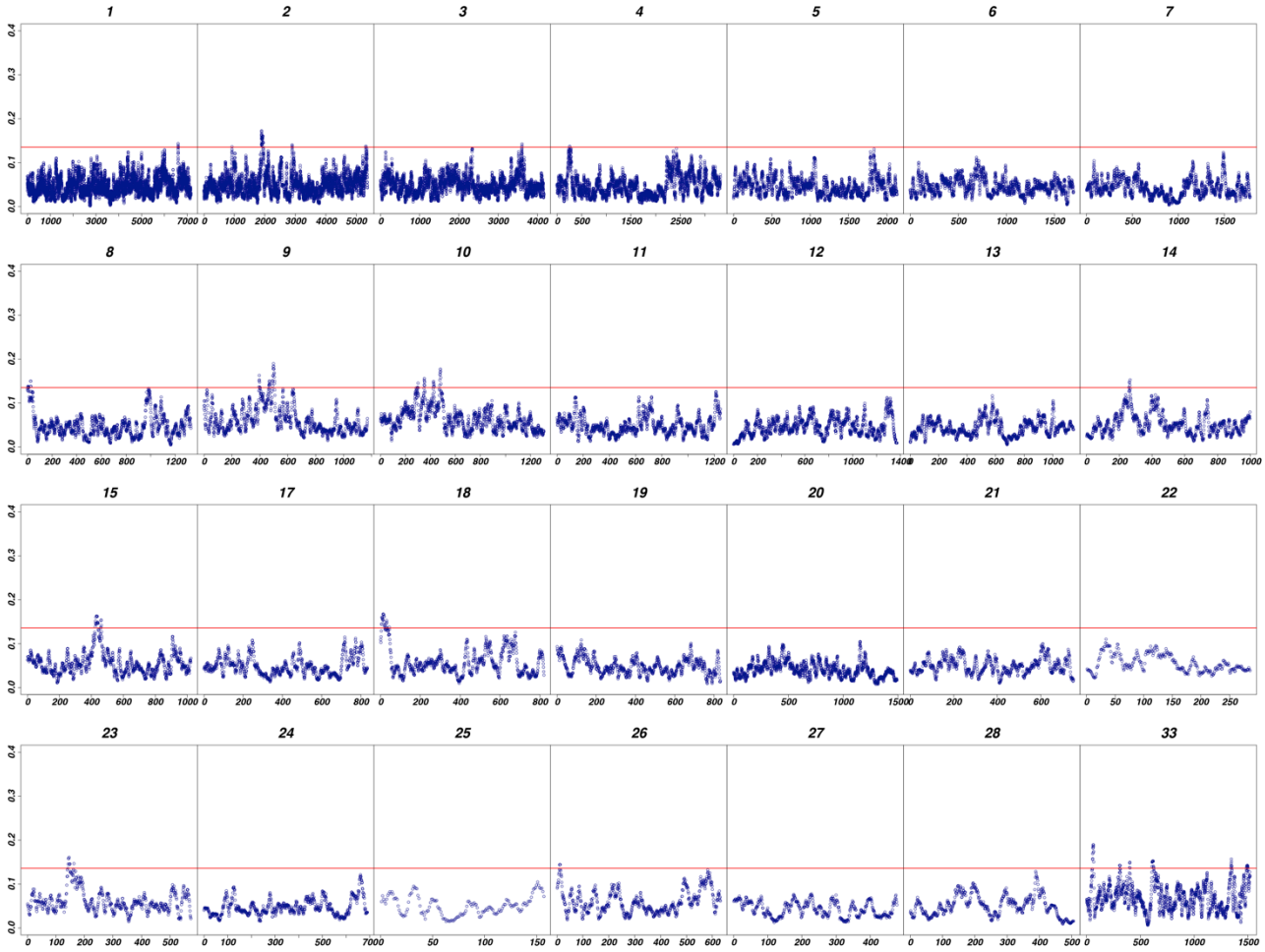


Figure 3.2. Pattern of genetic variation after two generations of selection for F breed. Running average of allele frequency distribution of 44,895 SNPs along the whole genome is plotted against the sequence. The deviations above the threshold show signals of selection.

Chromosome 33 is chromosome Z.

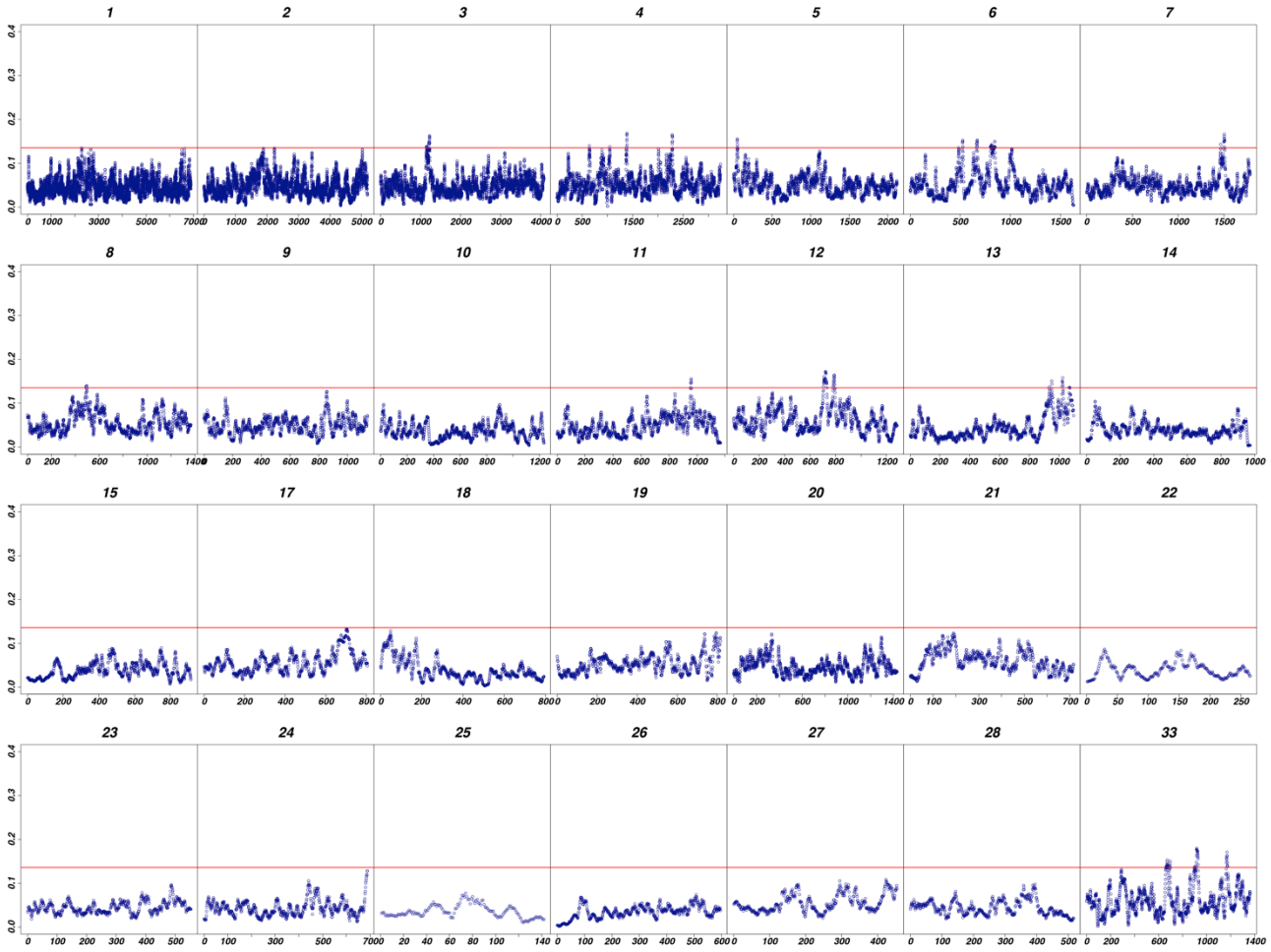


Figure 3.3. The distribution of d_{02} after two generations of selection on GBLUP breeding values. X-axis is d_{02} value, and y-axis is the number of bins.

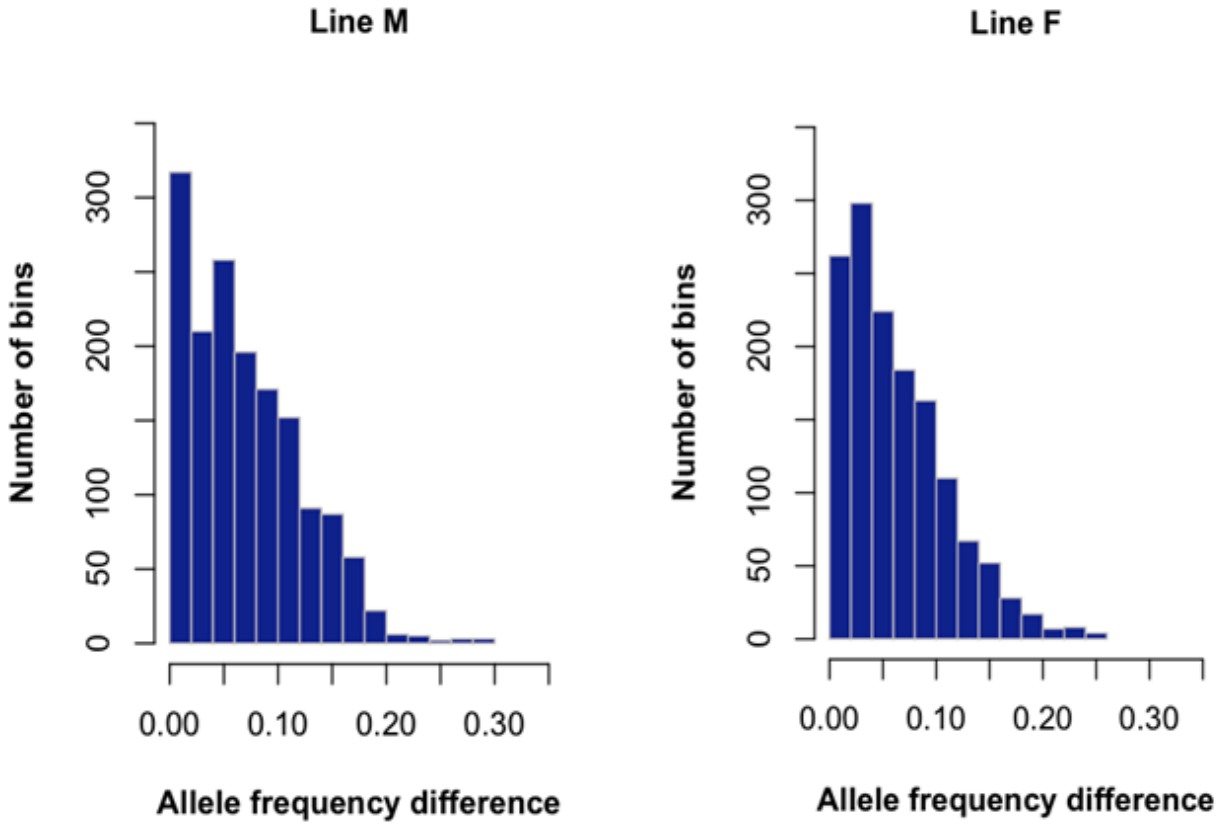


Figure 3.4. Pattern of heterozygosity after two generations of selection for M breed. Running average of allele frequency distribution of 46,293 SNPs along the whole genome is plotted against the sequence. The deviations above the threshold show signals of selection.

Chromosome 33 is chromosome Z.

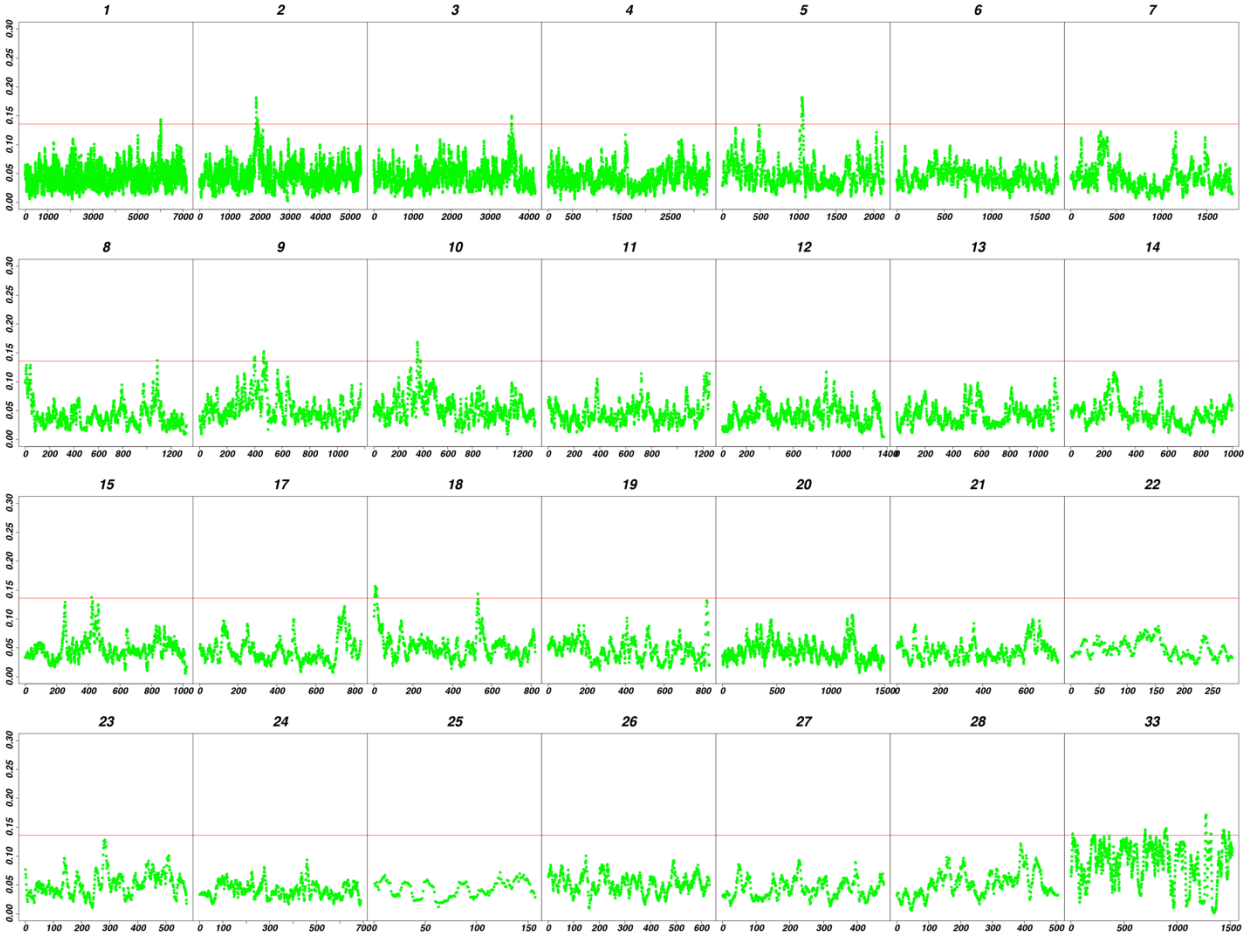


Figure 3.5. Pattern of heterozygosity after two generations of selection for F breed. Running average of allele frequency distribution of 43,253 SNPs along the whole genome is plotted against the sequence. The deviations above the threshold show signals of selection.

Chromosome 33 is chromosome Z.

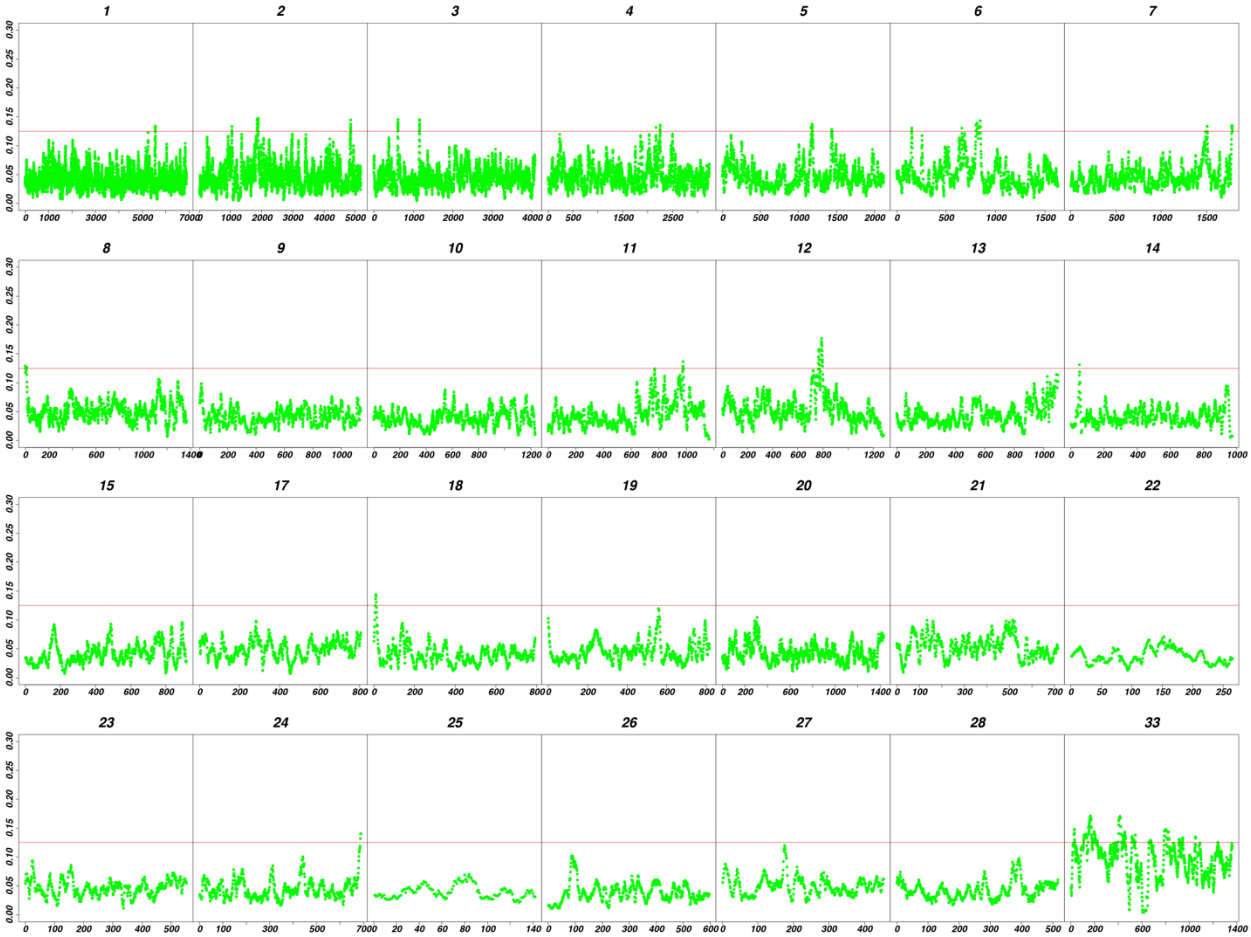
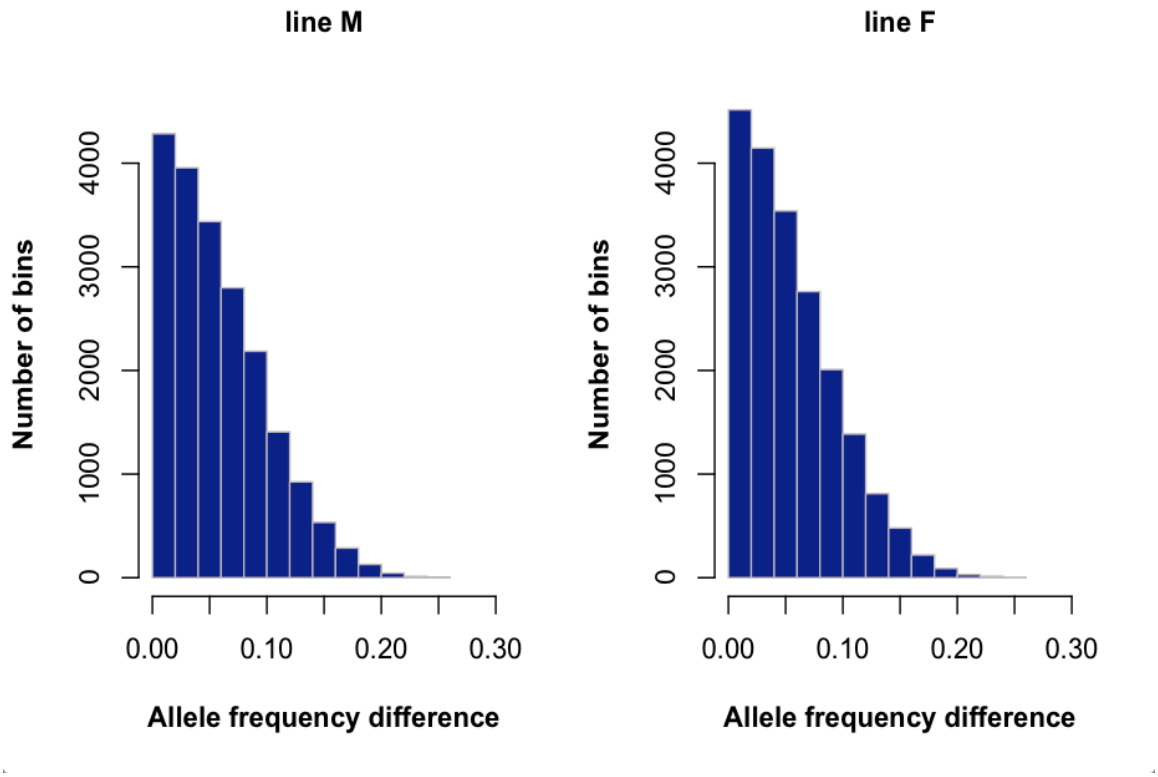


Figure S3.1. The distribution of allele frequency difference value obtained from gene dropping method.



CHAPTER 4

WEIGHTED SINGLE-STEP GENOMIC BLUP: AN ITERATIVE APPROACH FOR ACCURATE CALCULATION OF GEBV AND GWAS[1]

[1] Zhang, X., D. A. L. Lourenco, Legarra A., and I. Misztal. To be submitted to *Frontier Genomics*.

Abastract

Weighted single-step GBLUP (WssGBLUP) can improve both the accuracy of GEBV prediction and the estimate of marker effects. However, the improvements are limited and even weakened with a greater number of iterations. In the current study, five different procedures were implemented to calculate weights for a genomic relationship matrix to restrict the shrinkage along iterations of WssGBLUP. The procedures as well as BayesB and BayesC were tested with three simulated data sets with 5, 100, and 500 true QTLs. Prediction accuracy of WssGBLUP improved at iterations 2 through 4 by updating the mean, maximum, or summation of u_i^2 among every 20 SNPs, where u_i is the effect of SNP i.. Accuracy reached a plateau after iteration 3 or 5 by using weights proportional to u_i^2 plus a constant. Except in the 5-QTL scenario, accuracies with all WssGBLUP procedures were higher compared with those from BayesB and BayesC. Noise in the Manhattan plots was small with 5 and 100 QTLs but large with 500 QTLs. The presented procedures enhanced the accuracy of both GEBVs and marker effects.

Keywords: GWAS, WssGBLUP, BayesB, BayesC

Introduction

GBLUP usually assumes equal weights for all SNPs, whereas Bayesian methods give different weights to SNPs. If those weights are known, WGBLUP provides GEBVs similar to those of a Bayesian procedure using the same weights (Legarra et al., 2009). Different methods (WGBLUP and WssGBLUP) were developed to allow for the estimation of weights within GBLUP (Sun et al., 2011; Sun et al., 2012) or ssGBLUP (Aguilar et al., 2010; Misztal et al., 2009; Wang et al., 2012), respectively. Sun et al. (2011) developed two procedures for calculating weights in WGBLUP. In the first one, the weights are calculated as $w^{(i)} = \hat{a}_j^{(i)2}$, where $w^{(i)}$ is the weight of SNP *j* at iteration *i* and $\hat{a}_j^{(i)}$ is the effect of SNP *j* at iteration *i*. This procedure is effective for identifying top QTLs but excessively shrinks small SNPs; thus, the accuracy of GEBVs is reduced. The highest accuracy of GEBVs was achieved by modifying the weight formula to $w^{(i)} = \hat{a}_j^{(i)2} + t$, where $t = \frac{\sigma_g^2}{2\sum_{j=1}^{m} p_j q_j}$, σ_g^2 is the genetic variance; *p* and *q* are the minor and major allele frequencies at locus *j*, respectively, and *m* is the number of SNPs. This procedure introduced a constant to avoid SNPs with no effect and brought the accuracy of GEBVs close to that by BayesC but yielded noisy Manhattan plots. Wang et al. (2012) evaluated WssGBLUP with simulation data using $d_{i(t)} = u_{i(t)}^2 [2p_i(1-p_i)]$, where $d_{i(t)}$ is the weight of SNP *i* at iteration *t*, $u_{i(t)}^2$ is the variance of SNP *i* at iteration *t*, and p_i is the MAF. They iterated either on SNPs alone or on GEBVs and SNPs. The first option gave a good identification of top QTLs, and the second option provided a higher accuracy of GEBVs compared with BayesB, but only at the second iteration.

Recently, it was found that assigning a common weight to markers on a chromosomal region yielded more accurate estimates. Su et al. (2014) used group-marker variance from BayesR as a weighting factor for GBLUP in the study of dairy cattle. They achieved up to 1% higher reliability and reduced bias by 11% on average for 4 production traits and mastitis when using the mean variance of 30-SNP window compared with single SNPs. However, with or without grouping, BayesR was still 1.7 to 2% more

accurate compare with GBLUP. Xu (2013) demonstrated improved predictability in diploid plant QTL mapping using an artificial bin of LD-linked neighboring markers.

Because ssGBLUP is easy to apply and is usually the most accurate among tested methods (Wang et al; 2014; Wang et al., 2012), the objectives of this study were to present new procedures to calculate weights for SNPs in WssGBLUP and to compare the accuracy and SNP effects with those computed by BayesB (Meuwissen et al., 2001) and BayesC (Kizilkaya et al., 2010) using simulated data.

Materials and Methods

Data simulation

One additive trait with a mean of 1.0, phenotypic variance of 2.0, and heritability of 0.5 was simulated using QMSim (Sargolzaei & Schenkel, 2009). A total of 20 chromosomes with an average length of 82 cM and containing 45,000 evenly distributed SNPs were created. Three scenarios were considered involving different numbers of randomly placed QTLs (5, 100, and 500) were considered to simulate simple traits defined by major effects and complex traits or indices affected by numerous minor effects. All QTLs were selected among SNPs. For the first scenario, QTL effects were sampled from the normal distribution with a minimum absolute value of 0.2. For the latter two scenarios, QTL sampling was determined by the gamma distribution with a shape factor of 0.4. Both SNPs and QTLs were biallelic with no overlap between their positions. The simulated population was randomly selected from 205 generations, which was preceded by a historical population with 1,000 generations of random mating. Overall, 200 males and 2,600 females were selected to mate in each generation with a litter size of 1, forming an effective population size of 743. Generations 200 to 204 were treated as a training population and generation 205 as a validation population, with 1,240 and 300 genotyped animals, respectively. The complete datasets contained 18,400 individuals in the pedigree, of which 13,000 were phenotyped and 1,540 were genotyped.

Quality control was conducted as described in Wiggans et al. (2010) using the methodology by Aguilar et al. (2011). SNP and animal call rates were 0.90, MAF was 0.05, and Hardy-Weinberg equilibrium difference was 0.15. Monomorphic SNPs were deleted.

Models and computation.

The model for the simulation analysis included a population mean, a random SNP effect, and a random residual error term. For WssGBLUP, GEBV and SNP effects were obtained by BLUPF90 (Misztal et al., 2002) modified for genomic analyses (Aguilar et al., 2010). For BayesB and BayesC, EBV_{DP} with $c = 0.05$ (where c is the fraction of genetic variation not explained by markers) as well as EBVs were calculated from BLUP estimates as in Garrick et al. (2009). Subsequently, the SNP effects were obtained by GenSel (Fernando and Garrick, 2009) using a chain length of 41,000 to 50,000 with first 1,000 to 10,000 chains as burn-in. Degrees of freedom for genetic variances and residual were set to 4 and 10, respectively; 10 Metropolis-Hasting iterations per chain were set for BayesB.

Statistical analysis.

The weighted genomic relationship matrix was constructed, as suggested by Vanraden (2008):

$$\mathbf{G} = \frac{\mathbf{ZDZ'}}{2\sum p_i(1-p_i)},$$

where p_i is the MAF of SNP i and **D** is the matrix of weights, where d_{ii} is the weight for SNP i. The weights were derived from SNP solutions. Improvements in the SNP weights can be obtained iteratively either by recomputing only the SNP effects or by recomputing the GEBVs (Wang et al., 2012). The latter was chosen for this study. Six options were used to calculate the SNP weights in ssGBLUP: 1) default: weight is proportional to u_i^2, where u_i is effect of SNP i; 2) constant: weight is proportional to u_i^2 plus a constant, where the constant was chosen as the weight of the top SNP in the first iteration; 3) nonlinear A: weights were $v^{|s^{-2}|}$, where v is a scale standing for the departure from normality and s is the number of standard deviations from the mean for each $2\sum p_i(1-p_i)u_i^2$; 4) large window: the largest effect (u_i^2)

among every 20 SNPs and default weighting was used; 5) mean window: the mean effect of every 20 SNPs; and 6) summed window: the summation of every 20 SNPs.

Accuracy was defined as the correlation between true breeding value (TBV) and GEBV in the validation population. Correlation between TBV and direct genomic values (DGV) (Aguilar et al., 2010; Wang et al., 2012) was also computed. Comparisons were made among the six options and also with BayesC using EBV_{DP} from BLUP computed by WssGBLUP with default weighting using only phenotypes and π, the proportion of markers with no effect, of 0.5, 0.9, and 0.99.

Results and Discussion

Simulation

QTL effects ranged from 0.2 to 1.2 in the 5-QTL scenario, from 0.0 to 0.8 with 100 QTLs, and from 0 to 0.6 with 500 QTLs. An average of 36,000 SNPs was collected after quality control. Average LD r^2 (correlation between loci pair) at last generation was about 0.29. Average allele frequency for last three generations was 0.49.

Genetic estimates

Figure 4.1 and Table S4.1 show accuracies of GEBVs for six different methods under three scenarios. The average accuracies of six methods were 0.873, 0.803, and 0.769 under 5-, 100-, and 500-QTL scenarios. Standard deviations among 10 iterations ranged from 0.020 to 0.067. With default weighting, the accuracy increased initially but declined later. As the number of QTLs increased, the inflection point came earlier (0.909, 0.826, and 0.810 on iterations 4, 3, and 2 for 5-, 100-, and 500-QTL scenarios, respectively). The declined accuracy with iteration was the result of continuously adding weight to SNPs with large effects while shrinking SNPs with small effects. Consequently, GEBVs gradually decreased with iteration because the number of SNPs with no effect increased.

For early iterations ($\leq$5), large, mean, and summed windows were most accurate at iteration 4 (5-QTL scenario), iteration 3 (100-QTL scenario), and iteration 2 (500-QTL scenario). A mean window had

the highest accuracies and improved peak accuracy of default weighting by 0.9% (0.917 vs. 0.909), 1.6% (0.839 vs. 0.826), and 0.2% (0.812 vs. 0.810) under 5-, 100-, and 500-QTL scenarios, respectively. Window options performed better than options with single SNP weighted because the uncertainty was smaller (Su et al., 2014). A window size of 20 SNPs was chosen over 5, 10, 50, and 100 based on accuracy. Many factors, including size of reference population and population structures, influence the optimum window size (Su et al., 2014). Window options maintained high accuracy with 5 QTLs but lost the superior performance in late iterations with more QTLs. A summed window decreased in accuracy fastest among all window options, especially under the 500-QTL scenario, because it gave the greatest weight to the windows with large SNP effects and least weight to those with small SNP effects. This over- and under-weighting introduced bias into the solutions. In regard to real genetic evaluation of massive data, the performance of iterations 4 and later may not matter because one iteration usually takes from several hours up to several weeks.

Weights that included a constant were introduced to retain all SNP effects with the same base value, which was chosen to be the top effect at first iteration. The best average constants for 5-, 100-, and 500-QTL scenarios were 8, 40, and 13, respectively. These relatively small values avoid SNPs with no effects while not deviating large effects significantly. The results indicated that although the option with a constant did not have as high accuracy at early iteration as the window options, accuracy remained stable after the peak was reached (0.880, 0.834, and 0.811 at iterations 5, 5, and 3 for 5, 100, and 500 QTLs, respectively). These exceeded accuracy of the default option by 1.0 and 0.6% under the 100- and 500-QTL scenarios, respectively, but not for the 5-QTL scenario, where most SNPs did not have effects. Adding a constant to avoid under-weighting was redundant and counterproductive. The plateau accuracies exceeded GBLUP by 14.6% (0.880 vs. 0.768), 8.8% (0.834 vs. 0.767), and 2.8% (0.811 vs. 0.789) under the 5-, 100-, and 500-QTL scenarios (Figure 4.1 and Table 4.1). The 8.8% increase in accuracy is higher than the 7.4% (0.87 vs. 0.81) in Sun et al.'s (2011) study that used WGBLUP in a similar simulation of 10,000 SNPs and 33 QTLs. For the option with a constant, any constant that is not too large to reduce the

original scale (e.g., <3 × peak SNP effect) improved the accuracy of GEBVs. However, the mechanism behind picking the right constant is unclear; e.g., the average genetic variance *t* in Sun et al. (2011) derived from GBLUP was too small for ssGBLUP. Theoretically, a threshold between zero and peak SNP effects increases the bottom line of the absolute value for SNPs with no effects. This threshold should both guarantee high accuracy of EBVs and differentiate SNP effects. Number of QTLs, LD, and distribution of QTL effects are related to this threshold, but in reality these are unknown.

Muir (2007) found that the optimum accuracy for GBLUP was reached at a ratio of 10:1 marker:QTL loci when the markers and QTLs were evenly distributed on the genome. The markers cannot capture all the genetic variance when the QTL loci outnumber the marker loci. This explained the average accuracy decline when the number of QTLs increased in the simulation with randomly placed QTLs. WssGBLUP raised the accuracy under all scenarios by up to 1.6%. These results indicated an increasingly greater ability for weight manipulation to improve accuracy when a relatively small number of markers loses the ability to capture the QTLs.

VanRaden (2008) developed a nonlinear prediction A to define weight of SNP i as $1.25^{|s-2|}$, where *s* is the number of standard deviations from the mean, and 1.25 represents the departure from normality. For this study, the *s* with the best accuracy was smaller and ranged from 1.06 to 1.12. This option gave more weight to SNPs with smaller effects, thus preventing the drastic decrease in accuracy. Its inferior performance compared with other options occurred for two reasons. First, for oligogenic traits with few large QTLs where mean effect is close to zero, it assigns more weight to SNPs with effects but not to those with no effects; thus, it introduces bias into GEBVs. Results in the 5-QTL scenario indicated that the nonlinear-A option did not greatly increase the accuracy through iteration compared with the default option (0.880 vs. 0.909). Secondly, the narrow weight range from 1 to about 2 is not very flexible. This study showed that nonlinear A performed as well as other options (0.809) only under the 500-QTL scenario.

The accuracies of DGVs from Bayesian methods were compared with accuracy of GEBVs from WssGBLUP (Figure 4.1 and Table 4.2.1). Except for the 5-QTL scenario, all WssGBLUP options under all scenarios surpassed BayesC and BayesB in accuracy before iteration 6. BayesC with π = 0.99 was 5.3% lower compared with the peak accuracy of the default option under the 100-QTL scenario (0.782 vs. 0.826) and 13.3% lower under the 500-QTL scenario (0.702 vs. 0.810). Decreases for BayesB were 13.3% (0.716 vs. 0.826) with π = 0.99 under the 100-QTL scenario and 40.9% with π = 0.5 under the 500-QTL scenario (0.479 vs. 0.810). This is consistent with previous studies (Daetwyler et al., 2010; Zhong et al., 2015), which indicated that Bayesian methods perform well when the number of QTLs is small, whereas WssGBLUP performs better when the number of QTLs is large (>50, results not shown) because it uses an infinitesimal model that includes a polygenic effect. Moreover, pedigree relationships contribute to the accuracy of ssGBLUP (Legarra et al., 2009; Aguilar et al., 2010; Christensen & Lund, 2010), whereas DGVs of Bayesian methods exclude parent average (Garrick, 2009). With both pedigree and parent average removed (GBLUP line shown in Figure 1), the difference was smaller. BayesC with π = 0.99 was 14. 4 and 2.0% higher compared with GBLUP under the 5- and 100-QTL scenarios, respectively (0.879 vs. 0.768 and 0.782 vs. 0.767) but 10.4% lower under the 500-QTL scenario (0.702 vs. 0.789). BayesB with π = 0.99 was 17.6% higher (0.903 vs. 0.768) under the 5-QTL scenario but 6.6% lower under the 100-QTL scenario (0.716 vs. 0.767) and 39.3% lower with π = 0.5 under the 500-QTL scenario (0.479 vs. 0.789). Sun et al. (2011) also found 9.9% (0.87 vs. 0.81) higher accuracy for BayesC but 2.5% (0.83 vs. 0.81) higher accuracy for BayesB with π = 0.99 compared with GBLUP. Posterior variances from BayesB and BayesC were used to weight **G** in ssGBLUP, but the result did not (Results not shown).

QTL identification

Figure 4.2 shows the Manhattan plots of SNP effects (graph A) for all methods (graphs B–G) and scenarios (Figures 4.2.1a, 4.2.2a, and 4.2.3a) for the iteration with the best accuracy (iteration 2 for all scenarios). Figures 4.2.1b, 4.2.2b, and 4.2.3b show the Manhattan plots of BayesC and BayesB with different π. Under all scenarios, window options reduced the noise. Although up to 20% of the QTLs did

not create large peaks, most QTLs with large effects were identified and few peaks were false. The option with a constant reduced the difference between large and small SNP effects; hence, the plot looks noisy. A similar pattern was found in the nonlinear-A option because the weighting factors had a limited range for all SNPs. Bayesian methods, especially BayesB, estimated SNP effect best under the 5-QTL scenario; however, under the 500-QTL scenario, it captured <1% of the SNPs and assigned extremely high weight to them. This is the result of neglecting polygenic effects among SNPs with small effects, which caused bias in estimating SNP variances (Calus & Veerkamp, 2007). BayesB and BayesC patterns depend on the choice of π.

Computation time

Less than 10 min was required to compute the breeding values and SNP effects in ssGBLUP.

Conclusion

The procedures for calculating SNP weights in WssGBLUP can be effective in improving both the accuracy of GEBVs and GWAS. WssGBLUP GEBVs were more accurate than those from BayesB and BayesC, although different priors and π in the latter could change the ranking of the methods. Window options may be the best choices given that the true number of QTLs may not be known in real data. The WssGBLUP method is especially useful for GWAS when the population contains many ungenotyped animals and when complex models preclude accurate deregression.

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Table 4.1. Accuracy of BayesB and BayesC using different response variables with different π under three simulations.

			Number of simulated QTLs					
			5		100		500	
Response variable	**Method**	π	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
EBV_{DP}[1]	**BayesC**	0.50	0.66	0.05	0.61	0.04	0.63	0.10
		0.90	0.75	0.06	0.67	0.03	0.65	0.09
		0.99	0.87	0.04	0.76	0.07	0.68	0.07
	BayesB	0.50	0.85	0.07	0.56	0.06	0.48	0.17
		0.90	0.85	0.07	0.60	0.07	0.44	0.16
		0.99	0.87	0.07	0.63	0.07	0.41	0.15
EBV	**BayesC**	0.50	0.70	0.05	0.64	0.03	0.66	0.09
		0.90	0.78	0.06	0.69	0.03	0.68	0.08
		0.99	0.88	0.05	0.78	0.02	0.70	0.07
	BayesB	0.50	0.88	0.08	0.65	0.07	0.42	0.12
		0.90	0.89	0.07	0.68	0.06	0.41	0.09
		0.99	0.90	0.06	0.72	0.05	0.47	0.09

[1]Deregressed-proved EBV with $c = 0.05$.

Figure 4.1.1. Accuracies of different WssGBLUP under 5-QTL simulation.

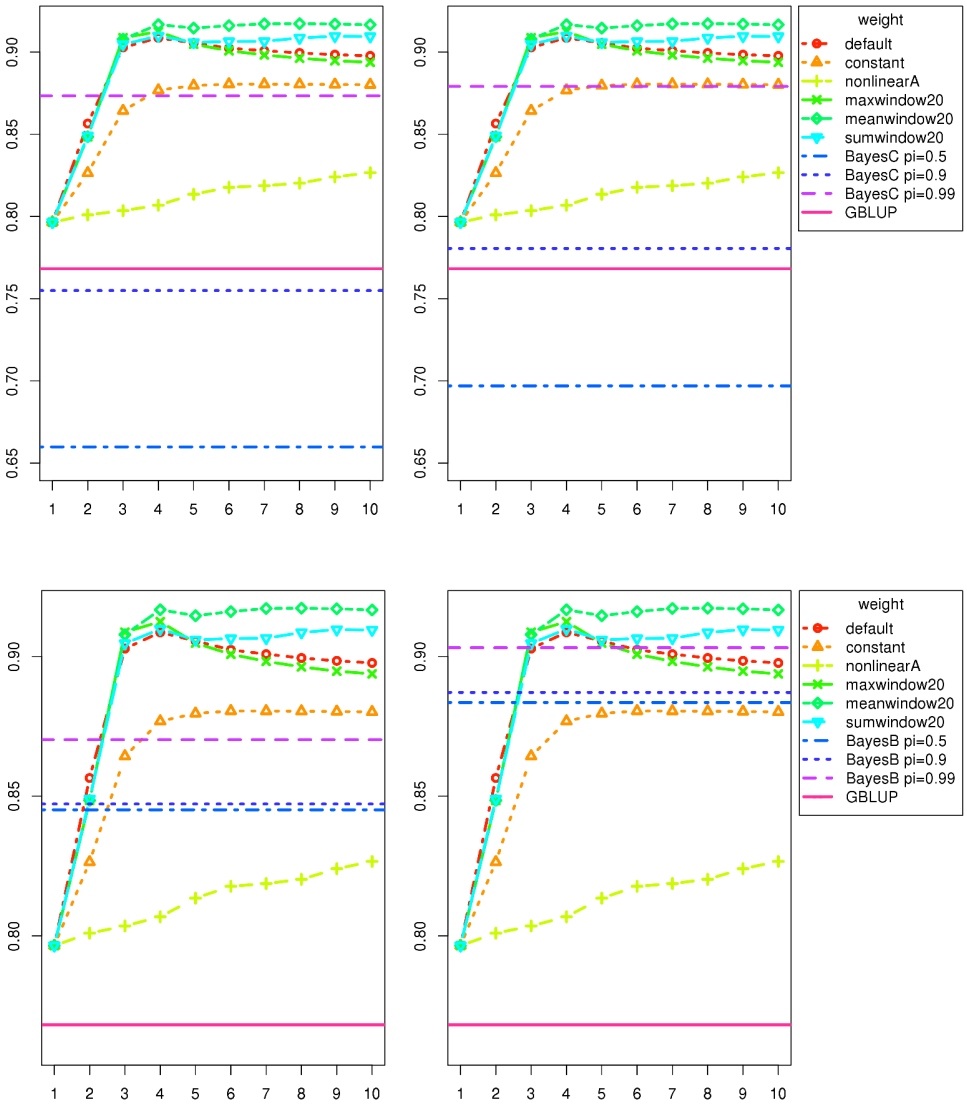


Figure 4.1.2. Accuracies of different WssGBLUP under 100-QTL simulation.

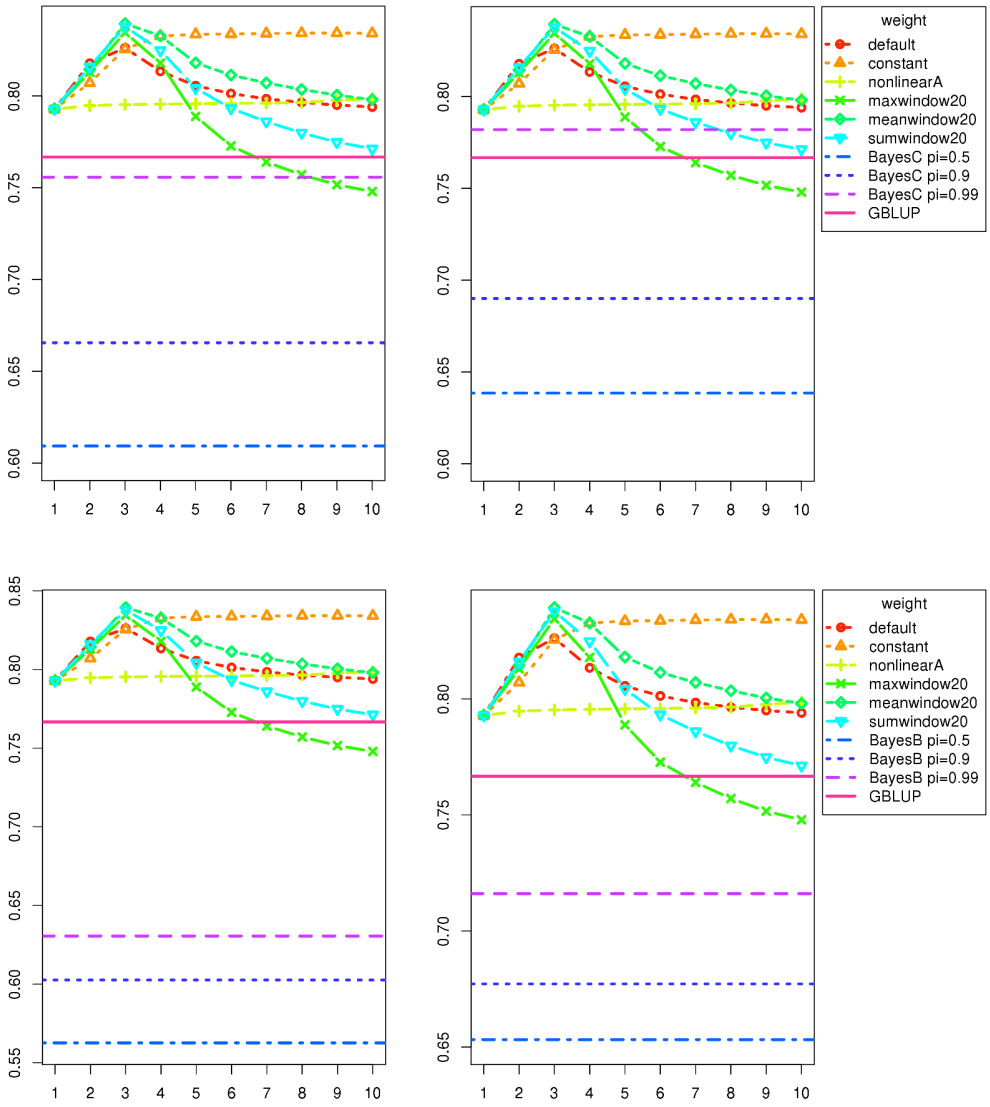


Figure 4.1.3. Accuracies of different WssGBLUP under 500-QTL simulation.

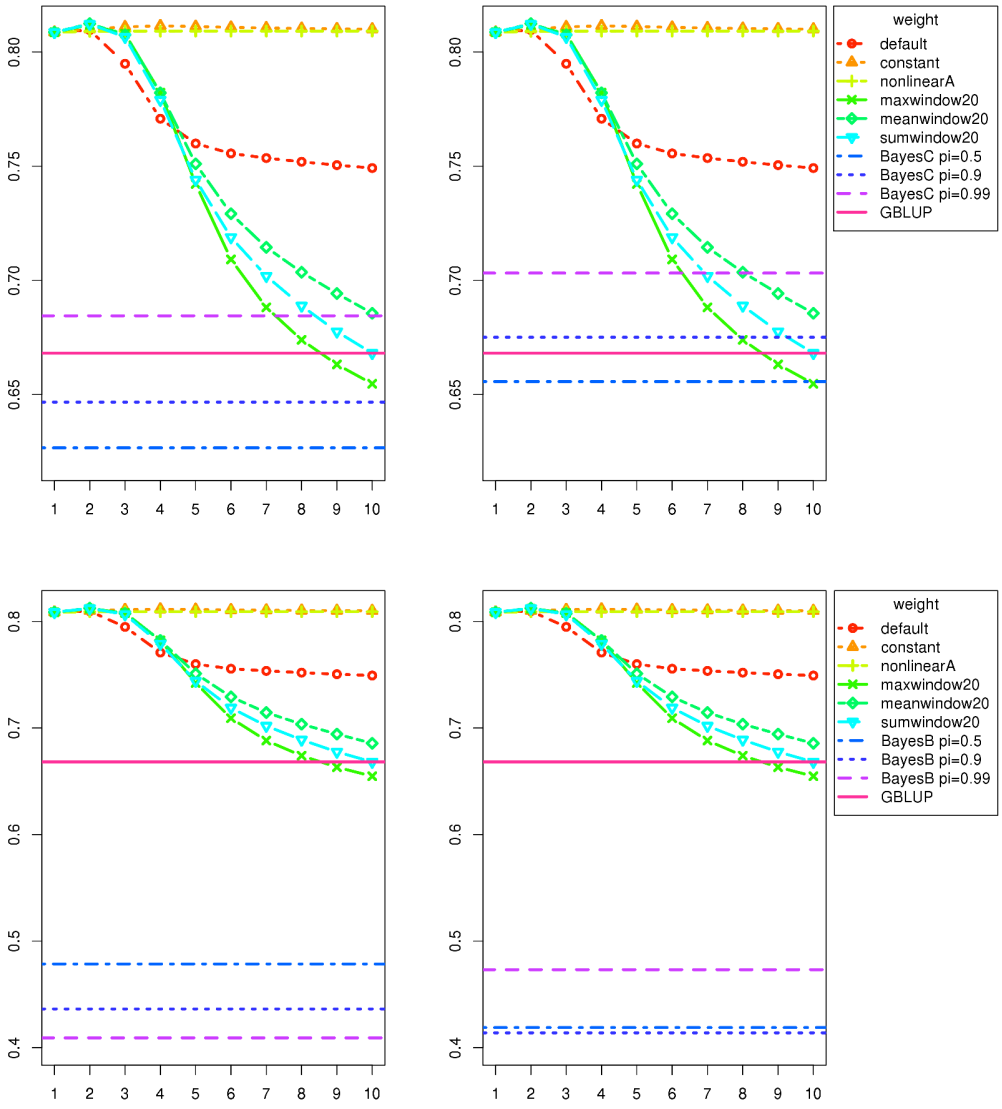


Figure 4.2.1. Proportion of variance explained by QTL effects and absolute SNP effects for different methods under 5-QTL simulation. a: A: true QTL; B: default; C: constant; D: nonlinear A: weights as $v^{|s^{-2}|}$, where v is a scale standing for the departure from normality, and s is number of standard deviation from mean for each u_i^2; E: large window; F: mean window; G: sum window. **b:** A: true QTL; B: BayesC π = 0.5; C: BayesC π = 0.9; D: BayesC π = 0.99; E: BayesB π = 0.5; F: BayesB π = 0.9; G: BayesB π = 0.99.

a.

b.

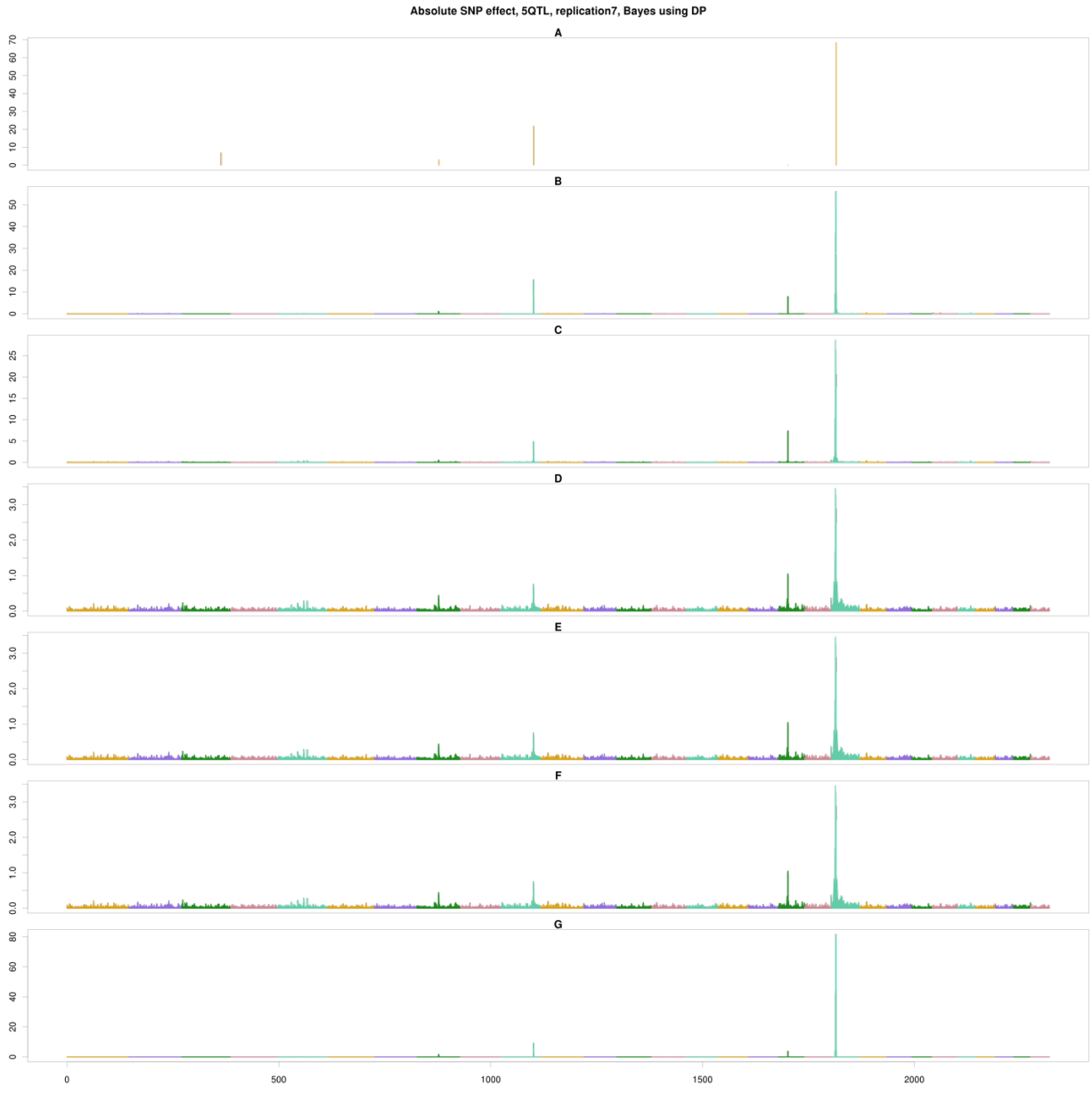
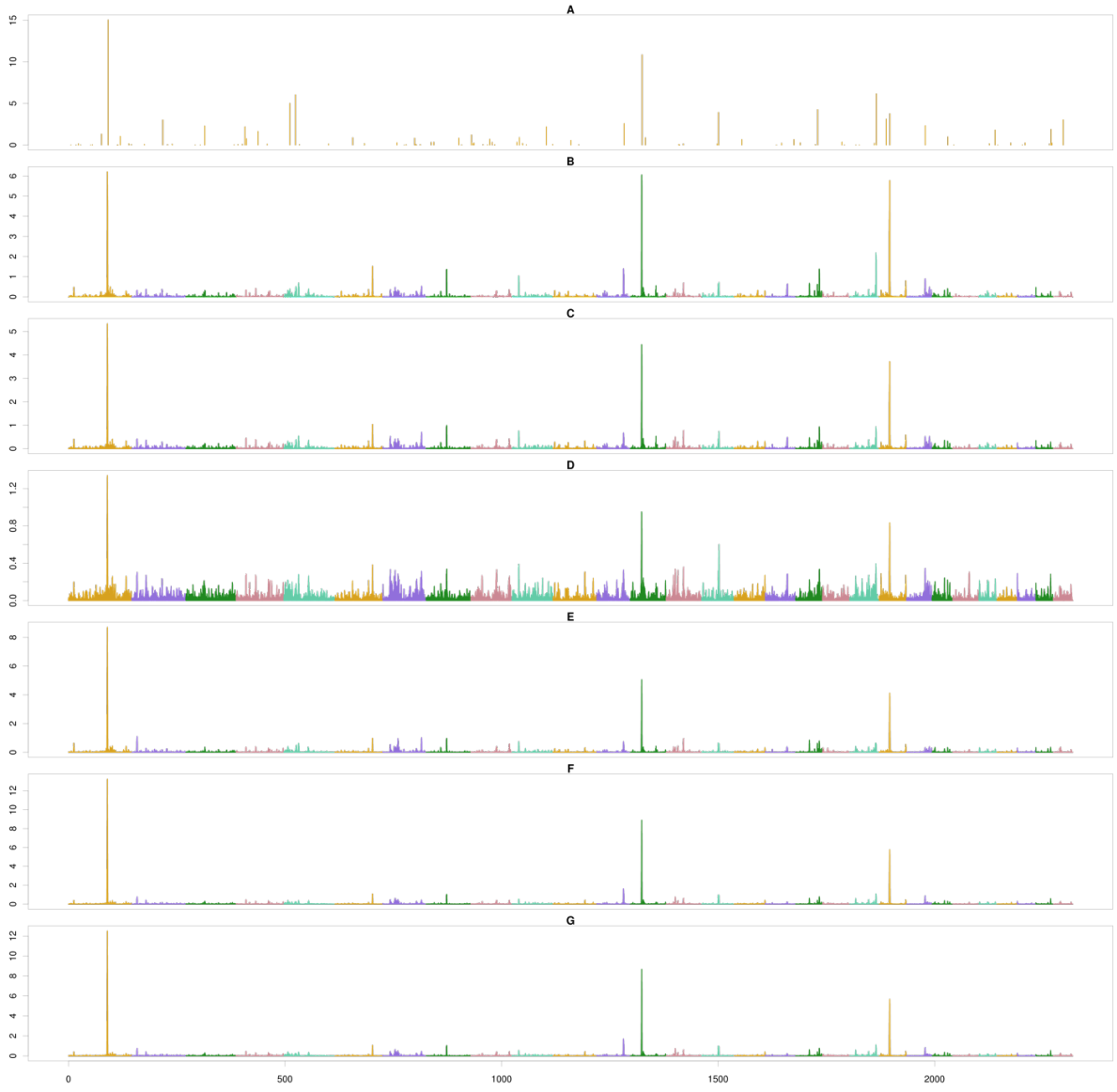

Absolute SNP effect, 5QTL, replication7, Bayes using DP
A
B
C
D
E
F
G
0
500
1000
1500
2000

Figure 4.2.2. Proportion of variance explained by QTL effects and absolute SNP effects for different methods under 100-QTL simulation. a: A: true QTL; B: default; C: constant; D: nonlinear A: weights as $v^{|s^{-2}|}$, where v is a scale standing for the departure from normality, and s is number of standard deviation from mean for each u_i^2; E: large window; F: mean window; G: sum window. **b:** A: true QTL; B: BayesC π = 0.5; C: BayesC π = 0.9; D: BayesC π = 0.99; E: BayesB π = 0.5; F: BayesB π = 0.9; G: BayesB π = 0.99.

a.



b.

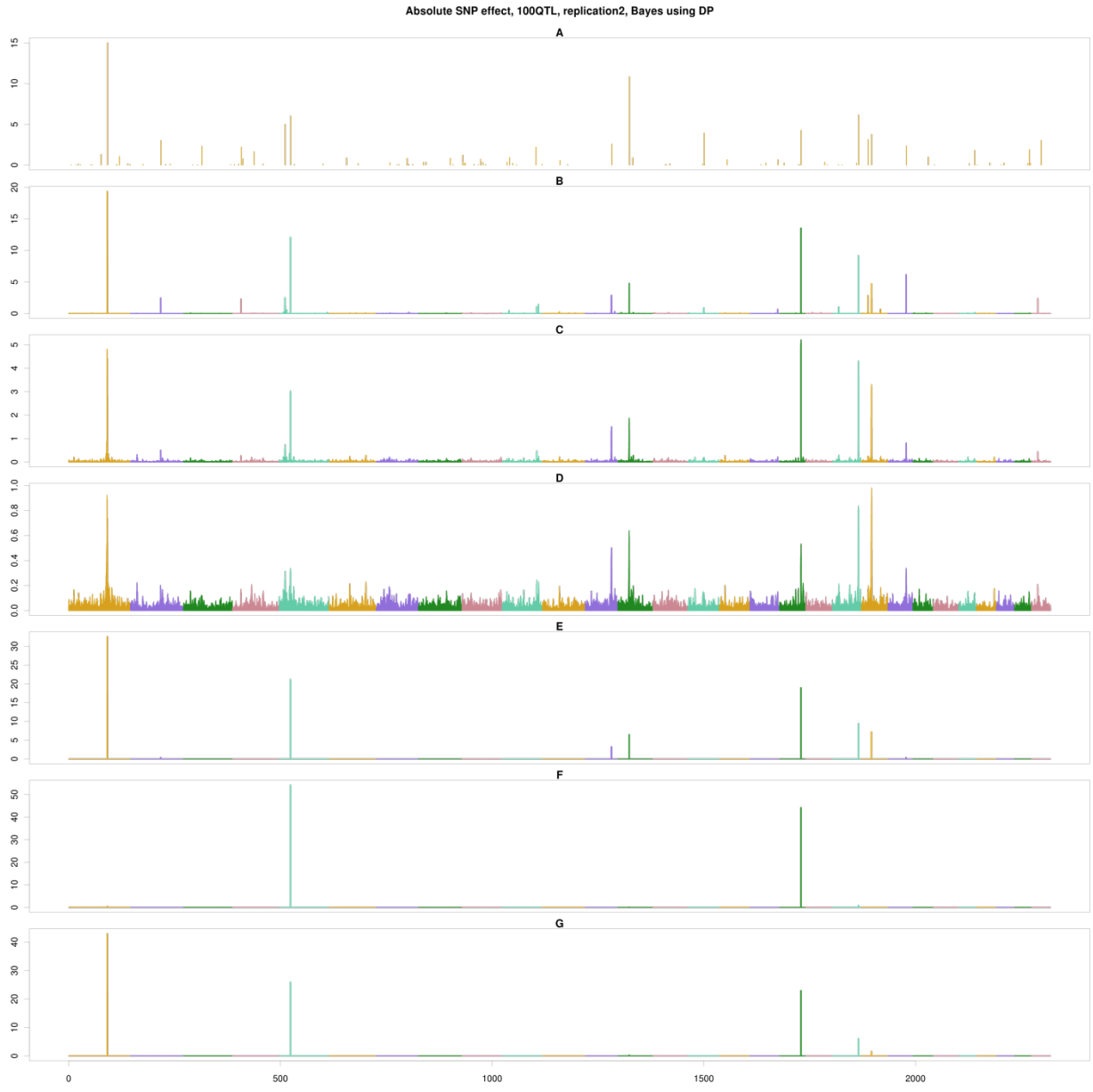
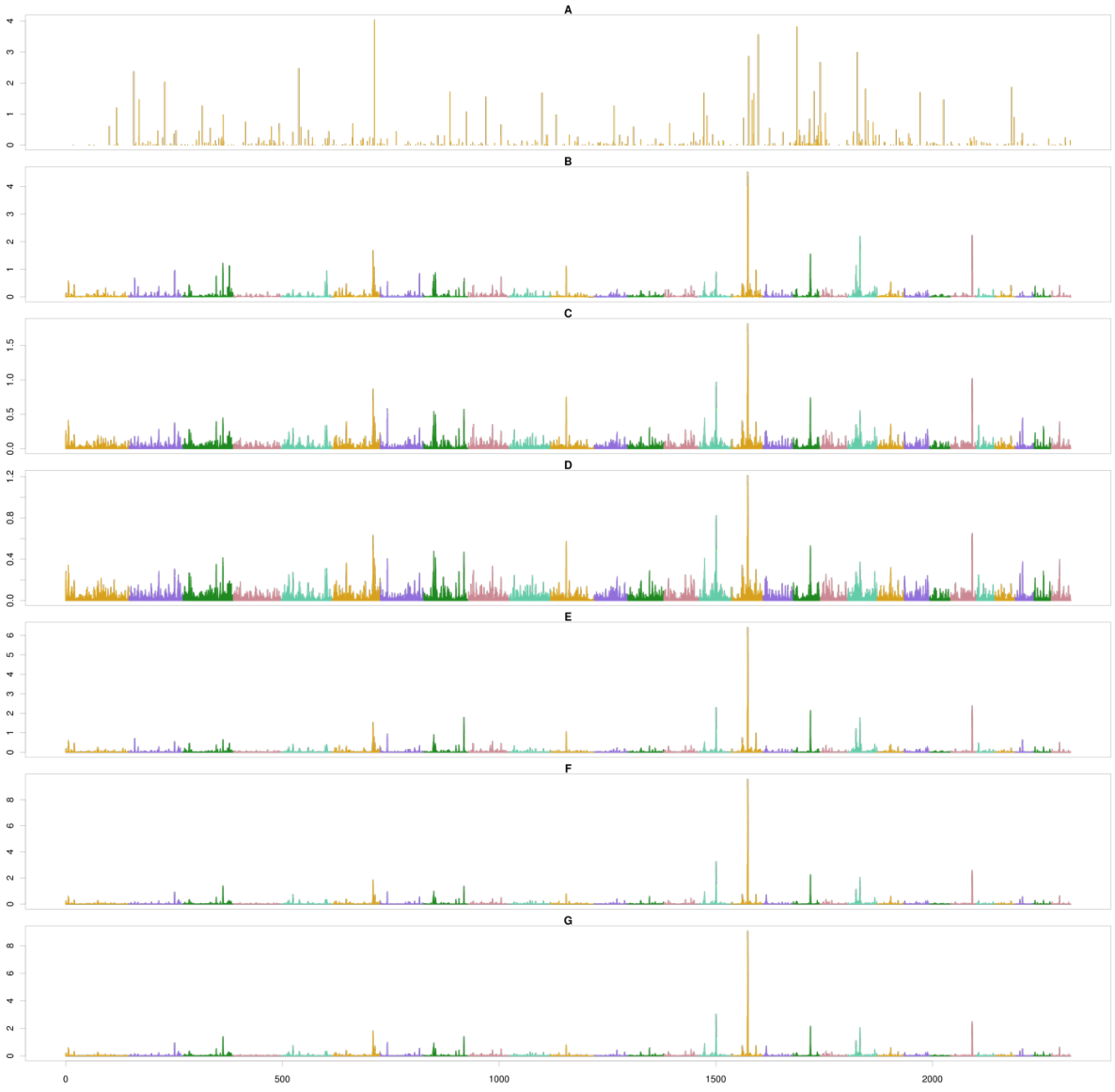

Absolute SNP effect, 100QTL, replication2, Bayes using DP
A
B
C
D
E
F
G
0
500
1000
1500
2000

Figure 4.2.3. Proportion of variance explained by QTL effects and absolute SNP effects for different methods under 500-QTL simulation. a: A: true QTL; B: default; C: constant; D: nonlinear A: weights as $v^{|s^{-2}|}$, where v is a scale standing for the departure from normality, and s is number of standard deviation from mean for each u_i^2; E: large window; F: mean window; G: sum window. **b:** A: true QTL; B: BayesC π = 0.5; C: BayesC π = 0.9; D: BayesC π = 0.99; E: BayesB π = 0.5; F: BayesB π = 0.9; G: BayesB π = 0.99.

a.



b.

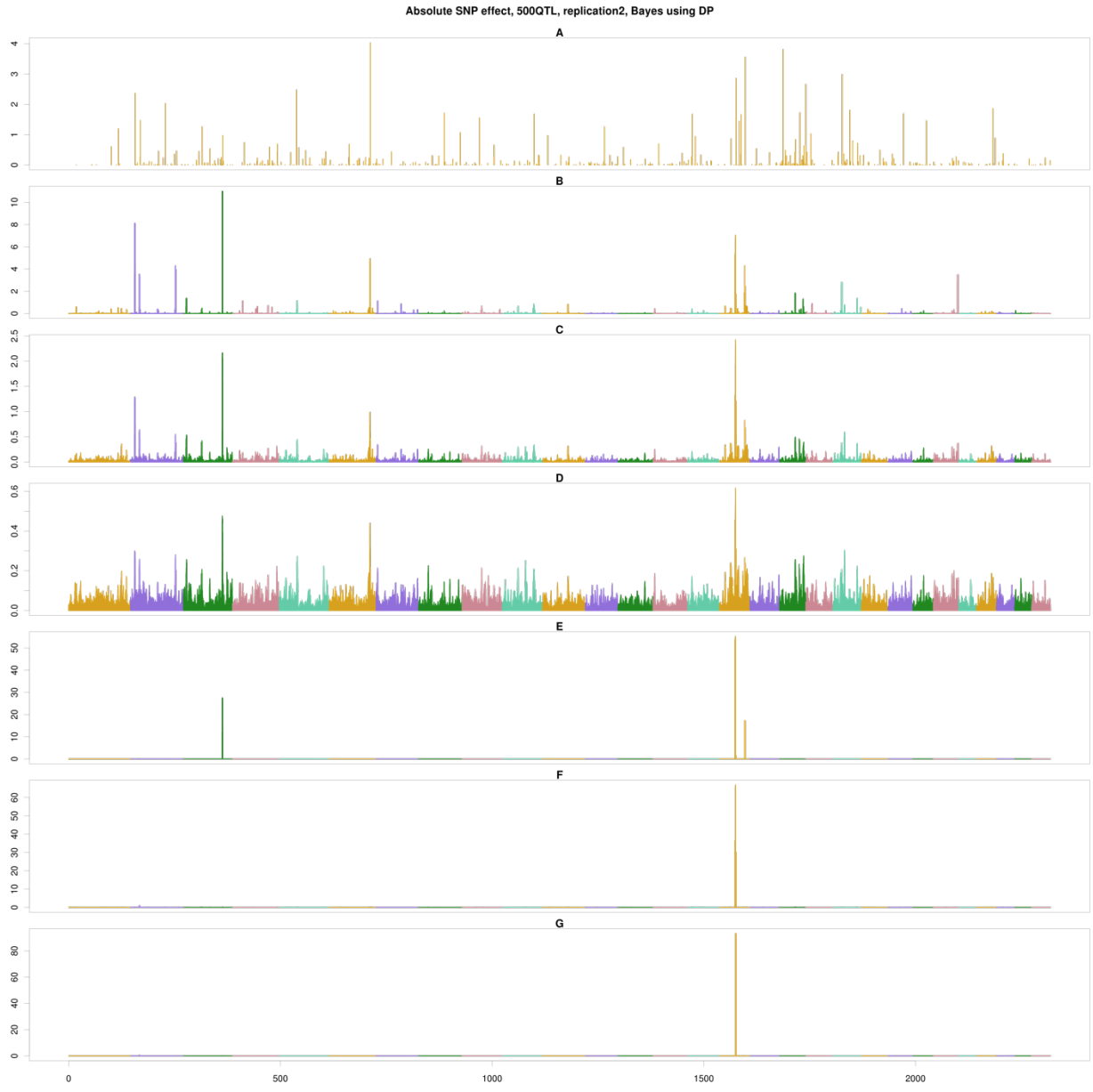

Absolute SNP effect, 500QTL, replication2, Bayes using DP
A
B
C
D
E
F
G
0
500
1000
1500
2000

Table S4.1. Accuracy for first 10 iterations of ssGBLUP with iterations with different methods under 5-, 100-, and 500- QTL simulation.

		5 QTLs		100 QTLs		500 QTLs	
Option	**Iteration**	**Accuracy**	**SD**	**Accuracy**	**SD**	**Accuracy**	**SD**
Default	1	0.80	0.04	0.79	0.02	0.81	0.04
	2	0.86	0.02	0.82	0.02	0.81	0.04
	3	0.90	0.03	0.83	0.02	0.79	0.04
	4	0.91	0.04	0.81	0.03	0.77	0.05
	5	0.91	0.04	0.81	0.03	0.76	0.05
	6	0.90	0.05	0.80	0.03	0.76	0.05
	7	0.90	0.05	0.80	0.03	0.75	0.06
	8	0.90	0.05	0.80	0.03	0.75	0.06
	9	0.90	0.05	0.80	0.03	0.75	0.06
	10	0.90	0.05	0.79	0.03	0.75	0.06
Constant	1	0.80	0.04	0.79	0.02	0.81	0.04
	2	0.83	0.03	0.81	0.02	0.81	0.04
	3	0.86	0.03	0.83	0.02	0.81	0.04
	4	0.88	0.03	0.83	0.02	0.81	0.04
	5	0.88	0.03	0.83	0.02	0.81	0.04
	6	0.88	0.03	0.83	0.02	0.81	0.04
	7	0.88	0.03	0.83	0.02	0.81	0.04
	8	0.88	0.03	0.83	0.02	0.81	0.04
	9	0.88	0.03	0.83	0.02	0.81	0.04
	10	0.88	0.03	0.83	0.02	0.81	0.04
Nonlinear A	1	0.80	0.04	0.79	0.02	0.81	0.04
	2	0.80	0.04	0.79	0.02	0.81	0.04
	3	0.80	0.03	0.80	0.02	0.81	0.04
	4	0.81	0.03	0.80	0.02	0.81	0.04
	5	0.81	0.02	0.80	0.02	0.81	0.04
	6	0.82	0.02	0.80	0.02	0.81	0.04
	7	0.82	0.03	0.80	0.02	0.81	0.04
	8	0.82	0.03	0.80	0.02	0.81	0.04
	9	0.82	0.03	0.80	0.02	0.81	0.04
	10	0.83	0.03	0.80	0.02	0.81	0.04
Large window	1	0.80	0.04	0.79	0.02	0.81	0.04
	2	0.85	0.02	0.81	0.02	0.81	0.04
	3	0.91	0.03	0.83	0.02	0.81	0.04
	4	0.91	0.04	0.82	0.02	0.78	0.04
	5	0.90	0.05	0.79	0.03	0.74	0.05
	6	0.90	0.05	0.77	0.03	0.71	0.05
	7	0.90	0.05	0.76	0.03	0.69	0.06
	8	0.90	0.05	0.76	0.03	0.67	0.06
	9	0.89	0.05	0.75	0.03	0.66	0.06
	10	0.89	0.05	0.75	0.03	0.65	0.06
Mean window	1	0.80	0.04	0.79	0.02	0.81	0.04
	2	0.85	0.02	0.81	0.02	0.81	0.04
	3	0.91	0.03	0.84	0.02	0.81	0.04
	4	0.92	0.04	0.83	0.02	0.78	0.05

	5	0.91	0.05	0.82	0.02	0.75	0.05
	6	0.92	0.04	0.81	0.03	0.73	0.06
	7	0.92	0.04	0.81	0.03	0.71	0.06
	8	0.92	0.04	0.80	0.03	0.70	0.06
	9	0.92	0.04	0.80	0.03	0.69	0.06
	10	0.92	0.04	0.80	0.03	0.69	0.07
Summed window	1	0.80	0.04	0.79	0.02	0.81	0.04
	2	0.85	0.02	0.82	0.02	0.81	0.04
	3	0.90	0.03	0.84	0.02	0.81	0.04
	4	0.91	0.05	0.82	0.02	0.78	0.05
	5	0.91	0.05	0.80	0.03	0.74	0.05
	6	0.91	0.05	0.79	0.03	0.72	0.05
	7	0.91	0.05	0.79	0.04	0.70	0.05
	8	0.91	0.04	0.78	0.04	0.69	0.05
	9	0.91	0.04	0.77	0.04	0.68	0.05
	10	0.91	0.04	0.77	0.04	0.67	0.06

CHAPTER 5

RELATIONSHIP BETWEEN MORTALITY AND SELECTION ON RESIDUAL FEED INTAKE AND RELATED TRAITS IN BROILER CHICKENS[1]

[1] Zhang, X., S. Tsuruta, D. A. L. Lourenco, I. Misztal, R. L. Sapp, and R. J. Hawken. To be submitted to *Poultry Science*.

Abstract

Three binary traits [mortality (MORT), ascites (ASC), and tibial dyschondroplasia (TD)] and one categorical trait [femur head necrosis (FHN)] were analyzed with Bayesian methodology and Gibbs sampler using linear and threshold models and a multivariate threshold-linear model with four other continuous traits [body weight (BW), residual feed intake (RFI), breast muscle percentage (BMP), and weight gain (WG)]. Field data included 186,596 records of commercial broilers from Cobb-Vantress Inc. THRGIBBS1F90 software was used to obtain estimates of the marginal posterior mean and standard deviation of the (co)variance components, heritabilities, and correlations from threshold and multivariate linear-threshold models. AIREMLF90 software was used to obtain the mean and standard error of the corresponding statistics from the linear models. The posterior means of direct heritability for binary and categorical traits from the threshold models were higher compared with those means from the linear models. The means and posterior means of direct and maternal heritability of all traits from the multivariate linear-threshold model were higher compared with the means from the linear models and posterior means from the threshold models except for FHN, for which the heritability was higher from the threshold model than from the multivariate linear-threshold model (0.29 vs. 0.19). The results confirmed that the posterior mean of the marginal distribution was suitable as a point estimate for univariate threshold and multivariate threshold-linear models.

Keywords: mortality; binary trait; threshold model

Introduction

Commercial broiler mortality has dropped from 18 to 3.9% since 1925 (The National Chicken Council, 2015); however, compared with commercial layers, the mortality of broilers younger than 1 year old is 7 times higher (European Commission, 2000). The peak mortality rate often occurs within the first week after the birth of the chicks, and the second peak gradually comes after the week 7 (McNaughton et al., 1978; Tabler et al., 2004). Recently, the early lay mortality has been associated with heavy, overconditioned hens in contrast to the early and mid-1990s when it was often associated with small and underfleshed hens. Studies showed that chickens bred for a higher yield of breast meat have a higher incidence of heart and circulatory disorders and are more susceptible to infectious diseases (Animal Welfare Working Group, June 1995; Julian, 1993).

ASC is the seeping of liver plasma into the body cavity as a result of pulmonary hypertension (Julian, 1993). It is the number one cause of broiler mortality in commercial and pastured poultry production, with an incidence rate as high as 25% in commercial broilers. ASC is responsible for 8% of all broiler deaths each year and 20 to 30% of all male broiler deaths (Mattocks, 2002). It is attributed primarily to superior growth characteristics combined with underdeveloped internal organs, primarily the lungs and heart (Julian, 1993; Pavlidis et al., 2007).

TD is the swelling of immature cartilage that causes bowing in the region of the hock joint. This leaves the growth plate prone to fracture, infection, and deformed bone development (Bradshaw et al., 2002). It is the potential cause of lameness, mortality, and carcass condemnations in young poultry (Julian, 2005; Okimoto, 2015, personal communication; Velleman, 2000). In broiler chickens, TD develops within 2 to 5 weeks of age (Lynch et al., 1992). Although the natural etiology of TD is not known (Rath et al., 2004), super growth and unbalanced nutrition are thougth to be major contributions in addition to genetics (European Commission, 2000; Julian, 1998; Leach & Monsonego-Ornan, 2007; Thorp, 1994). Wong-Valle et al. (1993) demonstrated that the incidence rate was higher in male broilers than in females.

FHN is one kind of proximal femoral degeneration most frequently is the results of bacterial chondronecrosis and osteomyelitis (Thorp et al., 1993) and accounts for 17.3% of lameness in broilers (McNamee & Smyth, 2000). It is triggered by poor calcification of the long bones in super-growth chickens, probably as early as the second week of age when osteoblast activity and bone formative processes decline and mineralization is insufficient (Prisby et al., 2014). Diagnose of early FHN is difficult (McNamee & Smyth, 2000; Prisby et al., 2014). Birds in which bacterial chondronecrosis with osteomyelitis has already developed are unlikely to respond to the treatment. Because of the multiple types of bacteria, a vaccine has not been fully developed yet (McNamee & Smyth, 2000).

Mortality and diseases are recorded in discrete categories. Threshold models have been developed to provide genetic analyses of categorical traits (Gianola & Foulley, 1983; Gilmour et al., 1985; Harville & Mee, 1984). Fouley et al. (1983) and Janss and Foulley (1993) extended the threshold methodology to multitrait analyses that consider one continuous correlated trait or more and unequal design. Albert & Chib (1993) and Moreno et al. (1997) generalized the procedure to Markov-chain Monte Carlo. Albert & Chib (1993) and Sorenson et al. (1995) constructed algorithms to allow empty categories in fully conditional distributions. Van Tassell et al. (1998) developed the MTGSAM program that allows several continuous and categorical variables in a threshold-linear model, and combined with Gibbs sampling.

The advantages of threshold over linear models have been shown in several studies. The predictability of breeding values from a threshold animal model is higher than from equivalent linear animal model for discrete traits (Casellas et al., 2007; Ramirez-Valverde et al., 2001; Varona et al., 1999). Furthermore, the correlations of breeding values from linear and threshold models are >0.99, and animal rankings are very similar (Weller et al., 1988; Weller & Ron, 1992). However, advantages of linear over threshold models also have been reported (Hagger & Hofer, 1989; Ramirez-Valverde et al., 2001).

The objectives of this study were 1) to build and analyze linear and threshold models for mortality and 2) to estimate variance components.

Materials and Methods

Data

Cobb-Vantress Inc. provided data for 188,936 fully pedigreed commercial broiler breeders from 20 overlapping mini-generations (MGs) from multiple breeder flocks. Eggs that were laid within several consecutive weeks constituted one MG, and each week was considered as one hatch. Approximately 14 MG were used in the study. Breeder source, MG, and hatches were used to define 420 contemporary groups (CGs). Four growth traits (BW, RFI, BMP, and WG), three binary traits (MORT, ASC, and TD), and one categorical trait (FHN) were recorded. Binary traits were classified as 0 (normal) and 1 (abnormal). FHN was scored from 0 to 6. Classification of MORT was based on observation. TD was scored using an X-ray machine as well as dissection of the legs. A random population of birds were sent each week to dissection, where breast meat FHN and TD were recorded. ASC was visually ascertained by fluid in the body cavity and cyanosis of the comb, wattles, and skin. Suspicious individuals were then dissected and confirmed according to the presence of fluid in the abdominal cavity and right ventricular hypertrophy (Enkvetchakul et al., 1993). TD incidence was determined by making a longitudinal cut across the right tibia, and the tibia was scored according to the white cartilage plug abnormality (Rekaya et al., 2013). FHN were scored as 0 (normal) to 3 (gross disintegration of the epiphysis, physis or metaphysis) for each leg, and scores for both legs were combined to obtain overall scores from 0 to 6 (Sapp, 2015, personal communication). MORT and ASC were recorded from hatch up to grading (recording of BW); BW, TD, FHN, and BMP were recorded around 5 weeks of age after grading. Subsequently, RFI was measured using a 1-week test, and WG was measured afterwards. BMP was measured after dissection and dead chickens were culled; therefore, no further traits were recorded for dissected and culled chickens.

Statistical models

The analyses were performed using animal models. Fixed effects included MG, sex, and CG. For linear models, fixed effects included MG, CG, and animal sex. For binary and categorical traits in threshold models, CG was treated as a random effect. Sex was not used for BMP because it was only recorded for roosters. In addition to direct breeding value, BW had maternal breeding value and maternal permanent effects.

Univariate Linear Model.

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z_1a} + \mathbf{Z_2m} + \mathbf{Z_3}\,\mathbf{pe}_m + \mathbf{e}\,,$$

where **b** were fixed effects; **a** were direct breeding values; **m** were maternal breeding values; $\mathbf{pe}_m$ were maternal permanent environmental effects; $\mathbf{X}$, $\mathbf{Z_1}$, and $\mathbf{Z_2}$ were incidence matrices that linked the data with fixed effects and direct and maternal breeding values, respectively; $\mathbf{Z_3}$ was a diagonal matrix; and **e** were residuals. This model was used for all 8 traits.

Univariate Threshold Model

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z_1a} + \mathbf{Z_2m} + \mathbf{Z_4cg} + \mathbf{e}\,,$$

where **cg** were random CG effects of based on farm source, MG and hatch and $\mathbf{Z_4}$ was an incidence matrix that linked the data with **cg**.

This model assumed an underlying distribution L of the binary (MORT, TD, and ASC) and categorical (FHN) traits y with the same effects as the univariate linear model, but the response of y was modeled with the following distribution:

$$\begin{aligned} &f(y \mid L) = \textstyle\prod_{i=1}^{n} f(y_i \mid L_i) \\ &= \textstyle\prod_{i=1}^{n} I(L_i < t_1) I(y_i = 1) + I(t_1 < L_i < t_2\ ) I(y_i = 2) + I(t_2 < L_i < t_3\ ) I(y_i = 3) \end{aligned}^{,}$$

where n is the number of records; t_1, t_2, and t_3 are thresholds that define the three categories of response and I is an indicator function that equals 1 if the condition specified is true or 0 otherwise. The procedure is a nonlinear transformation of BLUE and BLUP.

Transformation of heritability. The heritability from the linear model was transformed to a liability scale for binary traits using the formula from Dempster and Lerner (1950):

$$h_o^2 = \frac{\bar{z} h_l^2}{\bar{p}(1-\bar{p})},$$

where h_o^2 is the heritability on the observational scale, $\bar{z}$ is the height of the normal density function, h_l^2 is the heritability on the liability scale, and $\bar{p}$ is the incidence rate. The normal density function is a standard norm following Gianola and Foulley (1983).

For ordered categorical traits, the heritability from the linear model was transformed to a liability scale using the formula from Gianola (1979) with normal distributions for both breeding values and residuals:

$$h_o^2 = \frac{h_l^2 [\sum_{i=1}^{m-1} z_i (a_i + 1 - a_i)]^2}{\sum_{i=1}^{m-1} a_i^2 p_i (1-p_i) - 2\sum_{i=1}^{m} \sum_{j=1}^{m} a_i a_j p_i p_j},$$

where m is the number of categories, a_i is the threshold value of category i + 1, and p_i is the incidence rate of the category i.

Bivariate Linear-Linear Model

BW and MORT used the same model as for the univariate linear model. Genetic components of both traits were assumed to be correlated. Residual components were not assumed to be correlated. Genetic and residual effects were assumed to be independent of each other.

Bivariate Threshold-Linear Model.

BW used the same model as in LM, and MORT used the same model as in TM. Genetic and residual components shared the same assumptions as in LLM.

Multivariate Threshold-Linear Model.

Continuous traits used the same model as for the univariate linear model, and binary and continuous traits used the same model as for the univariate threshold model. Genetic components were assumed to be correlated. Random CG effects were not assumed to be correlated. Residual components were also assumed to be correlated, except for the binary traits with extreme category problems (ECPs); e.g., if chickens were dissected to confirm their disease status, then only healthy chicken in category 0 would have records that were measured afterwards.

Genomic BLUP Models.

ssGBLUP was used as a genomic model.

$$\begin{bmatrix} \mathbf{X'X} & \mathbf{X'Z} \\ \mathbf{Z'X} & \mathbf{Z'Z}+\lambda\mathbf{H}^{-1} \end{bmatrix}\begin{bmatrix} \mathbf{b} \\ \mathbf{u} \end{bmatrix}=\begin{bmatrix} \mathbf{X'y} \\ \mathbf{Z'y} \end{bmatrix},$$

where where **y** is a vector of phenotypic records in a multitrait scenario; **X** and **Z** are the incidence matrices corresponding to the fixed effects and additive genetic effects, respectively; **b** is a vector of fixed effects including an overall mean, hatch number, and breed; **u** is the vector of random additive direct genetic effects; λ is the ratio of residual to additive genetic variances, where the residual effect was assumed independently and followed a normal distribution; $\mathbf{H}^{-1}$ is the inverse of a matrix that combines pedigree and genomic relationships (Aguilar, 2010); and **e** is the vector of residual effects, which is assumed to be independent and follow a normal distribution [$e \sim (0, I\sigma_e^2)$].

Computation and software

AIREMLF90 (Misztal et al., 2002) was used to estimate variance components of linear models. The convergence criterion was 10^{-12}. THRGIBBS1F90 (Tsuruta & Misztal, 2006) was used to estimate variance components of threshold and threshold-linear models. The POSTGIBBSF90 program (Aguilar, 2010) was used to determine the burn-in and convergence and to calculate posterior means.

Results

Data summary

The statistics of binary, categorical, and continuous traits are listed in Tables 5.1.1 and 5.1.2. All 186,596 animals had at least one trait recorded. Incidence rates of MORT, ASC, and TD were 7.5, 1.2, and 3.5%. FHN had an incidence rate of 13.4% for category 1 and 8.6% for categories 2 through 6 combined; therefore, category 2 was redefined to include categories 2 through 6. Only roosters had BMP records. Average offspring per female was 54.

(Co)variance components

(Co)variance components and heritability from univariate linear, univariate threshold, bivariate linear-linear, and bivariate threshold-linear models are in Tables 5.2 and 5.3. Other models for BW (Table S5.1) and for BW and MORT (Table S5.2) were not used because of small or large heritability or difficulty with convergence. Correlations and heritabilities from the multivariate threshold-linear model are in Table 5.4, and (co)variance components are in Table S5.3. A constant of 1,000 was added to raw RFI records to avoid a value of 0, which would have been treated as a missing record in BLUP90IOD2 and CBLUP90IOD2. Raw BW, RFI, BMP, and WG records were divided by 10 to avoid overflow of THRGIBBS1F90. Number of burn-ins discarded in POSTGIBBSF90 ranged from 5,000 to 150,000.

By using a threshold model, heritabilities of binary and categorical traits were on a liability scale and, therefore, were different from those from linear models that were on a phenotypic scale. The heritabilities of binary and categorical traits from a threshold model were higher compared with those

from a linear model for TD (0.33 vs. 0.06), FHN (0.29 vs. 0.02), ASC (0.22 vs. 0.17), and MORT (0.12 vs. 0.05). By transforming heritability from the linear model to the liability scale, the new heritabilities were close to those from threshold models (TD, 0.34; FHN, 0.33; ASC, 0.24; and MORT, 0.17). With the multivariate threshold-linear model, heritabilities for BW (0.26 vs. 0.2), BMP (0.50 vs. 0.48), TD (0.34 vs. 0.33), MORT (0.13 vs. 0.12), and BW maternal genetic effect (0.08 vs. 0.04) were higher, but heritabilities for RFI (0.25 vs. 0.26), WG (0.21 vs. 0.22), FHN (0.19 vs. 0.22), and ASC (0.24 vs. 0.29) were lower compared with univariate linear and threshold models.

Genetic correlations between MORT and seven other traits were generally very low, except for maternal BW (−0.5) and ASC (0.77). BW had a positive correlation with TD (0.17), FHN (0.23), and ASC (0.27). Maternal BW also had a negative correlation with FHN (−0.13) and ASC (−0.37), but heritability of maternal BW was low (0.08). Residual correlations of ASC and MORT with other traits were significantly different from zero, although by definition they should be zero and likewise for TD and FHN with RFI. Other than those anomalies, medium to high correlations were found between MORT and ASC (0.73), BW and BMP (0.41), BW and WG (0.12), and RFI and BMP (0.16).

(Co)variance components from linear-linear and threshold-linear models for MORT and BW were estimated just for the predictability study that compared univariate and bivariate models. Heritability of BW was slightly higher compared with those from the univariate model (0.21 vs. 0.2), but heritability of MORT was not (0.05 for linear-linear and univariate linear models; 0.03 for threshold-linear model vs. 0.12 for the univariate threshold model). The correlation of genetic effect between BW and MORT was 0.12 for the linear-linear model, 0.28 for the threshold-linear model, and 0.13 for the multivariate threshold-linear model.

Computing time

For variance component estimation, THRGIBBS1F90 took 11 hours for 100,000 iterations with the univariate threshold model, 75 hours with the bivariate linear-linear model, 13 days with the bivariate

threshold-linear model, and 35 days for 200,000 iterations with the multivariate threshold-linear model with eight traits. AIREMLF90 took 5 minutes for the univariate linear model and 75 hours for the bivariate linear-linear model, depending on the initial value

Discussion

Heritabilities and correlations in different models

In a threshold model using maximum likelihood for binary or categorical response variables, heritability tends to be biased upward when the amount of information per fixed effect is small (Hoeschele & Tier, 1995; Moreno et al., 1997; Tempelman, 1998). Such ECPs (Misztal et al., 1989) have an observed value of only 0 or 1 at a certain level of a fixed effect. In the full dataset of this study, MGs had at least 2,651 samples, sex had at least 26,788 samples, and CGs had at least 39 samples at one level for a single binary or categorical trait. When splitting the data randomly in half, CGs would have more serious ECPs. The small sample size for CG levels was not a problem in this study because 1) the data were split by CG, which guaranteed that each level contained all samples in each subset, and 2) CG was treated as a random effect with a Gaussian distribution so that the bias in Monte Carlo error, autocorrelations and variance estimates would be decreased (Hoeschele & Tier, 1995; Luo et al., 2001; Moreno et al., 1997).

Gianola (1979) had illustrated that h^2 is higher on a liability scale compared with a linear scale, and this has been proved in many studies (Kadarmideen et al., 2004; Ramirez-Valverde et al., 2001; Varona et al., 1999), including this one. By transforming the linear scale to a liability scale, the univariate and multivariate h^2 become comparable with those from the threshold models.

Multivariate models were expected to have higher h^2 compared with univariate models, assuming strong correlations among different traits. This was the case for BW, BMP, TD, ASC, and MORT but not for FHN. RFI and WG h^2were very close in both models. FHN was the only categorical trait.

BW and MORT were also analyzed using bivariate models. BW was selected because its maternal genetic effect had the highest correlation with MORT and BW records were collected immediately after MORT records. However, the h^2 of MORT with the bivaruate threshold-linear model was smaller compared with those from the univariate and multivariate threshold models. The smaller h^2 was probably the result of the small heritability of the maternal genetic effect, which contributes very little to MORT, and the missing values of MORT, which were not handled properly by the bivariate model. In linear regression, the missing values were not considered; however, with the threshold model using THRGIBBS1F90, they were predicted from a Gaussian sampling distribution. With the multivariate model, the seven other traits probably provided information about the shape of the distribution (thus reducing the bias), but BW was the only other trait used for the bivariate model.

Heritabilities and genetic correlations of disease traits

González-Recio et al. (2008) reported a heritability of 0.02 for late mortality (14-42 dayes of age) in a commercial broiler population using Bayesian linear model with a incidence rate of 5%. in a commercial broiler population using a Bayesian linear model with an incidence rate of 5%. In a cold-challenge experiment with two commercial sire lines of chickens selected for production traits from birth to 35 days of age, De Greef et al. (2001) reported a heritability of 0.22 using a linear model with an incidence rate of 12% in the population. In a White Plymouth Rock broiler dam line up to 35 days of age, Pakdel et al. (2002) a heritability for mortality of 0.32 with a linear animal model and 0.16 for direct and 0.05 for maternal heritabilities with a linear maternal model. The correlation between direct and maternal effects was 0.21. The high heritability resulted mainly from the high incidence rate, as estimated heritability is a function of incidence with a linear model but not with a threshold model (Gianola, 1979). This discovery revealed a role of maternal effect in pullet mortality. Using a linear model with a maternal effect, this study found that the direct heritability of mortality was 0.02 and maternal heritability was 0.01 (Table S3.2.). The correlation between direct and maternal genetic effects was 0.02. Because of the very small portion for maternal heritability and smaller direct heritability compared with a simple model, the

maternal model was not used. The same was the case for the threshold model, where the correlation was 0.03. Differences in heritability could be the result of differences in definitions used by various researchers, animal age at measurement, scale used to describe the trait, sample size, and statistical and computational strategies used for estimation (Rekaya et al., 2013).

Pakdel et al. (2002) reported that the heritability of ASC-related continuous traits were 0.18 to 0.47. They also found a maternal heritability of 0.03 for ASC ventricular weights. Moghadam et al. (2001) reported heritabilities of 0.12 and 0.22 on the liability scale for ASC syndrome transformed from a linear scale in White Rock and Cornish broilers with an incidence rate of 1.5 and 1.1%, respectively. They also reported higher heritabilities for male broilers. De Greef et al. (2001) reported a heritability of 0.06 using a linear model for ASC-related mortality with an incidence rate of 4.2%. In an experiment for ASC susceptibility of male line chicks up 20 weeks of age, Pavlidis et al. (2007) reported that the heritability from a linear model was 0.30 ± 0.05 for the susceptible line and 0.55 ± 0.05 for the resistant line, with an average incidence rate of 75.3% for both lines.

Rekaya et al. (2013) that TD heritability was 0.12 ± 0.01 in a Cobb-Vantress commercial broiler line, which was slightly smaller than for this study. They used Bayesian implementation and a multivariate threshold-linear model. Akbas et al. (2009) reported that the heritability of TD heritability at 6 weeks of age in a commercial broiler population with an incidence rate of 7% was 0.06 using a linear animal model, which was the same as in this study, and 0.21 if transformed to a liability scale. Kapell et al. (2012) reported a TD heritability of 0.27 in commercial-broiler breeder lines recorded at 5 to 6 weeks of age for males only, with a prevalence of 7.8% using a linear animal model. Kuhlers and McDaniel (1996) reported that TD heritability in commercial male-line broiler breeders was 0.5 at 7 weeks of age using a linear animal model. The incidence rate was 11%.

No literature on the genetic component of FHN was found. One study on mineral density of human femoral neck bone reported a heritability of 0.43 (confidence interval of 0.16–0.67); no significant phenotypic relationship was found between mineral density and body weight at the same age.

Kadarmideen et al. (2004) reported a heritability of 0 for femur head score in Swiss Large White pigs using generalized mixed linear, logit, and probit animal models. They claimed that heritability was not estimable because of the extremely low incidence of 0.01%.

Genetic correlations between disease traits were generally negligible, expect for MORT and ASC. De Greef et al. (2001) reported a genetic correlation of 0.9 between mortality and ASC-related mortality, which is similar to the 0.77 of this study.

Heritabilities and genetic correlations of production traits

For production traits, the BW heritability estimate was smaller than the 0.33 of Rekaya et al. (2013) but larger than that reported by Chen et al. (2011). The heritability estimate of RFI in this study was close to the 0.26 of Rekaya et al. (2013) but lower than the 0.45 of Aggrey et al. (2010). The BMP heritability estimate was higher than the 0.39 reported by Liu et al. (2014). Studies on breast muscle weight reported heritabilities of 0.37 to 0.53 (Venturini et al., 2014). WG heritability ranged from 0.19 to 0.51 in previous studies (Aggrey et al., 2010; Gonzalez-Ceron et al., 2015). BW had no genetic correlation with RFI, which was expected because it was adjusted by BW. BMP was also a measurement related to BW, and BW was slightly negatively correlated with BMP, which differed from the correlation of 0.2 of De Greef et al. (2001). BW had a small genetic correlation with WG. RFI had an insignificant small positive relationship with BMP, which was also expected for the same reason as for BW. RFI had a small genetic correlation with WG, which was smaller than the 0.34 of Aggrey et al. (2010) and the 0.27 of González-Ceron et al. (2015). This implies that selection for higher RFI would result in greater WG. BMP had no genetic correlation with WG.

Genetic correlations between production and disease traits

In this study, BW had small positive genetic correlations with disease traits, which implies that t selection for BW slightly impairs health. Its genetic correlation with MORT, however, was not significantly different from zero. The insignificant genetic correlation between BW and MORT in this

study probably resulted from the bias introduced by data truncation. De Greef et al. (2001) reported a genetic correlation of −0.46 ± 0.11 between mortality and BW at 35 days of age. In swine, Roehe et al. (1999) reported that the genetic correlation between the number of pigs born alive and litter birth weight was -0.28 to -0.37, which implies a positive relationship between birth weight and mortality. Schneider et al. (2012), however, reported a positive genetic correlation (0.56) between number of pigs born alive and litter birth weight in crossbred swine. A similar result was reported by Dufrasne et al. (2013) in a crossbred pig population, which suggests that BW is negatively correlated with pre-weaning mortality with a genetic correlation of -0.52. Arango et al. (2005) reported a genetic correlation of -0.65 between number of piglets born dead and those reaching 113.3 kg in commercial Large Whites, which implies that selection for faster growth increased birth mortality.

Pavlidis et al. (2007) reported genetic correlations of 0.28 and 0.24 between body weight at 21 days of age and ASC-related mortality in susceptible and resistant lines selected for ASC, respectively, which implies that selection on ASC reduces BW. Other studies reported negative genetic correlations from −0.23 to −0.37 between BW and ASC indicator traits, which implies a positive relationship between BW and susceptibility to ASC (Pakdel et al., 2005; Zerehdaran et al., 2006). Closter et al. (2012) found a change in genetic correlation between BW and an ASC indicator trait from slightly positive to moderately negative from 2 to 7 weeks of age, and the change was more pronounced in males than in females, which suggests that males and females should be studied separately. The genetic correlation between BW before 7 weeks of age and TD ranged from −0.03 to 0.19 in previous studies (Kapell et al., 2012; Kuhlers & McDaniel, 1996; Rekaya et al., 2013)

RFI also had small positive genetic correlation with disease traits, and an insignificant genetic correlation with MORT. No literature on the relationship of chicken RFI and diseases were analyzed in this study was found, but a study of beef cattle found no relationship between RFI and perinatal mortality (Crowley et al., 2011). Rekaya et al. (2013) reported that the genetic correlation between RFI and TD was 0.01, the same as in this study.

BMP had insignificantly negative genetic correlations with disease traits, which implies no effect when selecting on BMP. The small correlation in this study is attributed mainly to the fact that BMP was only recorded for males and the sample size was very small. Although high BMP was widely considered as negatively affecting the health at a phenotype level, few studies examined the genetic relationship. De Greef et al. (2001) reported a genetic correlation of 0.02 ± 0.01 between mortality and BMP, which was close to the correlation of 0.04 ($P > 0.05$) in this study. Hoving-Bolink et al. (2000) indicated that chickens with high BMP were at higher risk of having ASC, mainly through lower capillary density that diminishes oxygen supply. Rekaya et al. (2013) reported genetic correlation of −0.08 between breast muscle yield and tibial dyschondroplasia.

Residual correlations

For the binary traits that were truncated early, their residual and phenotypic correlations with subsequent recorded traits were supposed to be zero (Table 5.4). The correlations of RFI with BMP, TD, and ASC were significantly different from zero but low. Correlation between BMP and ASC was high, in agreement with many studies, which suggests that males selected for breast muscle had high ASC prevalence (Mattocks, 2002; Moghadam et al., 2001; Pavlidis et al., 2007). Correlations of RFI with FHN, WG, and ASC and between WG and MORT were higher than 0.7, mostly because of the inaccurate covariance estimate from the Gibbs sampling procedure of THRGIBBS1F90. An unstructured covariance model may lead to unstable covariance parameter estimates with large posterior variances in an unbalanced design where the number of observations is small with respect to the number of traits, effects, and measurements. Model improvements, such as ante-dependence or a heteroscedastic residual (co)variance matrix, can account for unbalanced design and improve covariance estimates (Azevedo et al.).

Implication

In this study and many others, mortality was triggered by complicated causes specific to each genetic background, environment, management, etc. Therefore, the results of this study might not be extrapolatable. The background of this study indicated that ASC might explain the major cause of mortality. Some preventive measures, such as sufficient ventilation, can alleviate the incidence rate (Julian, 1993).

In a study including Cobb-500 male broilers, Goliomytis et al. (2003) found that leg problems were the principal cause of mortality of broilers beyond 70 days of age. Calcium insufficiency is a cause of immature bone that eventually leads to diseases that increase mortality (Zavala et al., 2011). BW and BMP are not direct causes of mortality, but are principal causes of leg problems (Animal Welfare Working Group, June 1995; Goliomytis et al., 2003; Julian, 1993; Sorensen et al., 1999). Breeding farm and hatchery environments are sources of bacterial infections in legs (McNamee & Smyth, 2000). Fewer times of meal feeding and early feed restriction were suggested as efficient means to reduce skeletal problems in meat-type birds (Sorensen et al., 1999; Su et al., 1999). However, another study showed that starvation could raise male broiler mortality (Zavala et al., 2011).

Other mortality factors may include sex, maternal effect from hens, and management. Female and male broilers had different causes and degrees of mortality. Males often had a higher mortality because of killing, feather pecking, and other aggressive attacks, which lead to bone impairments, such as fractures and infections (Zavala et al., 2011). Higher mortality in broiler chicks up to 8 weeks of age has been found to be correlated with younger age of hen at laying and lighter egg weight (McNaughton et al., 1978).

Zavala et al. (2011) suggested examining BW lost, atrophy, or obesity reflected by breast muscle score as well as and bone integrity, such as femur bone in necropsy, to determine the cause of mortality because the diseases that change physiological condition at the time of death are highly related to mortality.

Conclusion

Early mortality of broilers has a low to moderate heritability, which implies that it can be improved through selection. Selection of heavier maternal body weight may decrease offspring mortality. Selection on residual feed intake, on the other hand, does not influence mortality.

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Table 5.1.1. Number of observations (and incidence rates in parentheses) of disease traits and mortality.

Trait[a]	N	Trait score						
		0	1	2	3	4	5	6
TD	59,124	57,032 (96.5%)	2092 (3.5%)	—	—	—	—	—
FHN	16,870	11,112 65.86%	2,265 13.4%	1,372 8.1%	62 0.4%	22 0.1%	1 0%	6 0%
ASC	163,971	161,950 98.8%	2,021 1.2%	—	—	—	—	—
MORT	180,998	167,389 92.5%	13,609 7.5%	—	—	—	—	—
Total	186,596	—	—	—	—	—	—	—

[a]TD: tibia dischondroplasia; FHN: femur head necrosis; ASC: ascites; MORT: mortality.

Table 5.1.2. Summary statistics of production traits.

Trait[a]	N	Mean	SD	Median	Min	Max
BW (g)	161,984	1973.8	229.7	1956.1	1162.3	2863.3
RFI (g)	41,730	0.12	64.56	-1.52	-445.1	493.89
BMP (%)	7,087	25.5	1.9	15.3	15.3	33.3
WG (g)	41,730	693.0	139.7	686.0	300.0	1372.0
Total	186,596	—	—	—	—	—

[a]BW: body weight; RFI: residual feed intake; BMP: breast muscle percentage; WG: weight gain.

Table 5.2.1. Means (and standard errors in parentheses) for the animal (σ_a^2), maternal (σ_m^2), maternal permanent environment group ($\sigma_{pe_m}^2$), and residual (σ_e^2) variances, covariance between animal and maternal effect (σ_{am}^2) and direct (h^2) and maternal heritabilities (h_m^2) for production traits using linear models.

	Trait[a]			
Statistic	**BW**	**RFI**	**BMP**	**WG**
σ_a^2	49.25 (4.57)	10.87 (0.56)	0.01 (0.00)	27.79 (1.56)
σ_m^2	8.53 (2.46)	—	—	—
σ_{am}^2	-7.41 (2.67)	—	—	—
$\sigma_{pe_m}^2$	11.66 (1.43)	—	—	—
σ_e^2	181.23 (2.40)	31.50 (0.41)	0.01 (0.00)	96.99 (1.16)
h^2	0.20	0.26	0.48	0.22
h_m^2	0.04	—	—	—

[a]BW: body weight; RFI: residual feed intake; BMP: breast muscle percentage; WG: weight gain.

Table 5.2.2. Means (and standard errors in parerntheses) for animal (σ_a^2), contemporary group (σ_{cg}^2), and residual (σ_e^2) variances of linear models, posterior means and standard deviations in highest posterior-density regions for the variance components of threshold models, and heritabilities on linear (h^2) and liability (h_l^2) scales for disease and mortality traits.[a]

	Linear Model				Threshold Model			
	TD[a]	**ASC**	**FHN**	**MORT**	**TD**	**ASC**	**FHN**	**MORT**
σ_a^2	0.0011 (0.0001)	0.0001 (0.0000)	0.0650 (0.0060)	0.0031 (0.0002)	0.5323 (0.0465)	0.3087 (0.0179)	0.4473 (0.0567)	0.1418 (0.0115)
σ_{cg}^2	—	—	—	—	0.0604 (0.0116)	0.0918 (0.0152)	0.0431 (0.0082)	0.0261 (0.0028)
σ_e^2	0.0174 (0.0001)	0.0078 (0.0000)	0.3161 (0.0055)	0.0659 (0.0003)	1.0005 (0.0082)	1.0000 (0.0052)	1.0381 (0.0158)	1.0001 (0.0048)
h^2	0.06	0.02	0.17	0.05	—	—	—	—
h_l^2 [b]	0.34	0.24	0.33	0.17	0.33	0.22	0.29	0.12

[a]TD: tibia dischondroplasia; FHN: femur head necrosis; ASC: ascites; MORT: mortality.

[b] Transformation between observed and liability scales based on Dempster & Lerner (1950) and Gianola (1979).

Table 5.3. Means (and standard errors in parentheses) for animal (σ^2_a), maternal (σ^2_m), maternal permenental envrionment group ($\sigma^2_{pe_m}$), contemporary group (σ^2_{cg}), and residual (σ^2_e) variances of a linear-linear model, posterior means and standard deviations in highest posterior-density regions for variance components of a threshold-linear model, and heritabilities on linear (h^2) and liability (h^2_l) scales for mortality (MORT) and body weight (BW).

Statistics and traits	Linear-linear models				Threshold-linear models			
	$\sigma^2_{x1,x2}$	$r_{x1,x2}$	h^2	h^2_l	$\sigma^2_{x1,x2}$	$r_{x1,x2}$	h^2	h^2_l
σ^2_a **-MORT**[a]	0.003 (0.001)	—	0.05	0.16	0.028 (0.009)	—	—	0.03
σ^2_a **-BW**	51.240 (4.727)	—	0.21	—	51.010 (5.586)	—	0.21	—
σ^2_m **-BW**	6.722 (2.238)	—	0.03	—	11.729 (2.813)	—	0.05	—
$\sigma^2_{a,m}$ **-BW, BW**	-7.504 (2.571)	-0.403	—	—	-9.183 (2.675)	-0.380	—	—
$\sigma^2_{a,a}$ **-MORT, BW**	0.047 (0.024)	0.120	—	—	0.333 (0.173)	0.280	—	—
$\sigma^2_{a,m}$ **MORT, BW**	-0.071 (0.043)	-0.490	—	—	-0.513 (0.122)	-0.900	—	—
σ^2_{mpe} **-BW**	12.640 (1.414)	—	—	—	10.288 (1.589)	—	—	—
σ^2_{cg} **-MORT**	—	—	—	—	0.023 (0.003)	—	—	—
σ^2_e **-MORT**	0.066 (0.000)	—	—	—	1.007 (0.005)	—	—	—
σ^2_e **-BW**	180.200 (2.471)	—	—	—	180.360 (2.887)	—	—	—

[a]MORT: mortality, BW: body weight.

Table 5.4. Genetic correlations (upper right), residual correlations (lower left), and heritabilities from the multiple threshold-linear model by trait.

Trait	BW[a]	RFI	BMP	WG	TD	FHN	ASC	MORT	BW_{mat}
BW		0.00	-0.12[c]	0.28[c]	0.17[c]	0.23[c]	0.27[c]	0.13	-0.53[c]
RFI	0.04[c]		0.14[c]	0.22[c]	0.01	0.18[c]	0.08[c]	0.01	0.09
BMP	0.41[c]	0.16[cb]		0	-0.01	-0.15[c]	-0.06	0.04	0.11
WG	0.12[c]	-0.02[c]	-0.06		-0.03	-0.10	0.25[c]	0.14[c]	0.03
TD	-0.08[c]	-0.13[cb]	-0.01	0.06[b]		0.11	0.02	-0.02	0
FHN	0.03[c]	0.98[cb]	0.12	0.01[b]	-0.02		0.08	0.10	-0.13[c]
ASC	0.31[cb]	0.27[cb]	0.65[cb]	0.74[cb]	-0.11[cb]	0.23[cb]		0.77[c]	-0.37[c]
MORT	-0.13[cb]	0.20[cb]	0.15[cb]	0.82[cb]	-0.30[cb]	0.22[cb]	0.73[c]		-0.50[c]
BW_{mat}	—	—	—	—	—	—	—	—	—
h^2	0.26	0.25	0.50	0.21	0.34	0.19	0.24	0.13	0.08

[a]BW: body weight; RFI: residual feed intake; BMP: breast muscle percentage; WG: weight gain; TD: tibia dischondroplasia; FHN: femur head necrosis; ASC: ascites; MORT: mortality; BW_{mat}: BW maternal genetic.

[b]Should be 0 because of extreme category phenotype.

[c]Significantly different from 0 by 2 standard deviations.

Table S5.1. Means (and standard errors in parentheses) for the animal (σ_a^2), maternal (σ_m^2), maternal permenental group ($\sigma_{pe_m}^2$), phenotypic (σ_p^2), and residual (σ_e^2) variances, covariance between animal and maternal effect (σ_{am}^2), and direct (h^2) and maternal heritabilities (h_m^2) for body weight using full and reduced linear models.

Statistics	Full model	Reduce model 1	Reduce model 2
σ_a^2	49.25 (4.57)	46.36 (3.70)	108.29 (3.43)
σ_m^2	853.01 (2.46)	—	—
σ_{am}^2	-7.41 (2.67)	—	—
$\sigma_{pe_m}^2$	11.66 (1.43)	13.80 (1.00)	—
σ_e^2	181.23 (2.40)	181.34 (1.99)	151.03 (1.87)
σ_p^2	243.26	241.51	259.32
h^2	0.20	0.19	0.42
h_m^2	0.04	—	—

Table S5.2. Means and standard errors in parentheses) for animal (σ^2_a), maternal (σ^2_m), maternal permenental environment group (σ^2_{mpe}), contemporary group (σ^2_{cg}), and residual (σ^2_e) variances for an altertanive linear-linear model, posterior means and standard deviations in highest posterior-density regions for variance components of an alternative threshold-linear model, and heritabilities on linear (h^2) and liability (h^2_l) scales for mortality (MORT) and body weight (BW).

Statistics and traits	**Linear-linear model**				**Threshold-linear model**			
	$\sigma^2_{x1,x2}$	$r_{x1,x2}$	h^{2a}	h^2_l	$\sigma^2_{x1,x2}$	$r_{x1,x2}$	h^2	h^2_l
σ^2_a **-MORT**	0.002 (0.000)	—	0.03	0.09	0.065 (0.013)	—	—	0.06
σ^2_m **-MORT, MORT**	0.001 (0.000)	—	0.01	—	0.027 (0.007)	—	0.02	—
$\sigma^2_{a,m}$ **-MORT, MORT**	-0.001 (0.000)	-0.636	—	—	-0.015 (0.009)	-0.360	—	—
σ^2_a **-BW**	49.430 (4.626)	—	0.17	—	50.364 (4.895)	—	0.21	—
σ^2_m **-BW**	6.016 (2.161)	—	0.02	—	15.273 (2.168)	—	0.06	—
$\sigma^2_{a,m}$ **-BW, BW**	-6.759 (2.495)	-0.039	—	—	11.239 (2.810)	-0.140	—	—
$\sigma^2_{a,a}$ **-MORT, BW**	0.013 (0.026)	0.044	—	—	-0.200 (0.167)	-0.110	—	—
$\sigma^2_{m,m}$ **-MORT, BW**	0.012 (0.017)	0.165	—	—	-0.504 (0.107)	-0.780	—	—
$\sigma^2_{a,m}$ **-MORT, BW**	-0.039 (0.021)	-0.367	—	—	-0.129 (0.127)	-0.130	—	—
$\sigma^2_{a,m}$ **-BW, MORT**	-0.007 (0.026)	-0.032	—	—	0.550 (0.120)	0.470	—	—
σ^2_{mpe} **-MORT**	0.001 (0.000)	—	—	—	0.021 (0.004)	—	—	—
σ^2_{mpe} **-BW**	11.410 (1.365)	—	—	—	9.280 (1.421)	—	—	—
$\sigma^2_{mpe,mpe}$ **-MORT, BW**	-0.014 (0.009)	-0.014	—	—	—	—	—	—
σ^2_{cg} **-MORT**	-	—	—	—	0.026 (0.003)	—	—	—
σ^2_e **-MORT**	0.066 (0.000)	—	—	—	1.000 (0.005)	—	—	—

σ_e^2 **-BW**	235.400 (2.545)	—	—	—	180.660 (2.562)	—	—	—
$\boldsymbol{\sigma^2_{e,e}}$**-MORT, BW**	1.904 (0.019)	—	—	—	—	—	—	—

[a] Transformation between observed and liability scales based on Dempster & Lerner (1950) and Gianola (1979).

Table S5.3. Posterior means (**PM**), their standard deviation (**PSD**) and effective sample size (**ES**) in highest posterior-density regions for the animal (σ^2_a), maternal (σ^2_m), maternal permanent environment ($\sigma^2_{pe_m}$), contemporary group (σ^2_{cg}), and residual (σ^2_e) variances from a threshold-linear model and heritability on linear (h^2) and liability (h^2_l) scales for all traits.[a]

$\sigma^2_{x1,x2}$	PM	PSD	ES	$\sigma^2_{x1,x2}$	PM	PSD	ES
σ^2_{cg} **-TD**[a]	0.077	0.014	14.3	σ^2_a **-ASC**	0.343	0.033	9.0
σ^2_{cg} **-FHN**	0.032	0.004	23.3	$\sigma^2_{a,a}$ **-ASC, MORT**	0.172	0.016	18.2
σ^2_{cg} **-ASC**	0.070	0.008	11.3	σ^2_{am} **-ASC, BW**	-0.977	0.197	12.3
σ^2_{cg} **-MORT**	0.027	0.003	120.3	σ^2_a **-MORT**	0.146	0.011	21.5
σ^2_a **-BW**	63.786	5.311	13.0	σ^2_{am} **-MORT, BW**	-0.870	0.139	8.3
$\sigma^2_{a,a}$ **-BW, RFI**	-0.056	1.274	13.3	σ^2_m **-BW**	20.737	2.569	5.2
$\sigma^2_{a,a}$ **-BW, BMP**	-0.106	0.043	21.4	$\sigma^2_{pe_m}$ **-BW**	8.722	1.004	5.4
$\sigma^2_{a,a}$ **-BW, WG**	12.328	1.959	30.6	σ^2_e **-BW**	175.990	2.738	14.6
$\sigma^2_{a,a}$ **-BW, TD**	1.024	0.435	8.4	$\sigma^2_{e,e}$ **-BW, RFI**	2.599	0.849	16.8
$\sigma^2_{a,a}$ **-BW, FHN**	0.896	0.277	7.1	$\sigma^2_{e,e}$ **-BW, BMP**	0.635	0.029	19.2
$\sigma^2_{a,a}$ **-BW, ASC**	1.283	0.301	10.0	$\sigma^2_{e,e}$ **-BW, WG**	17.298	1.803	10.3
$\sigma^2_{a,a}$ **-BW, MORT**	0.383	0.259	5.3	$\sigma^2_{e,e}$ **-BW, TD**	-1.013	0.347	15.0
σ^2_{am} **-BW, BW**	-19.431	3.161	5.9	$\sigma^2_{e,e}$ **-BW, FHN**	0.457	0.177	8.4
σ^2_a **-RFI**	10.668	0.437	43.3	$\sigma^2_{e,e}$ **-BW, ASC**	4.147	0.570	2.8
$\sigma^2_{a,a}$ **-RFI, BMP**	0.052	0.025	3.6	$\sigma^2_{e,e}$ **-BW, MORT**	-1.745	0.211	11.7
$\sigma^2_{a,a}$ **-RFI, WG**	4.004	0.734	23.4	σ^2_e **-RFI**	32.496	0.352	37.4
$\sigma^2_{a,a}$ **-RFI-, TD**	0.025	0.148	8.3	$\sigma^2_{e,e}$ **-RFI, BMP**	0.107	0.033	3.8
$\sigma^2_{a,a}$ **-RFI-, FHN**	0.291	0.078	12.1	$\sigma^2_{e,e}$ **-RFI, WG**	-1.488	0.617	22.9
$\sigma^2_{a,a}$ **-RFI, ASC**	0.147	0.071	16.3	$\sigma^2_{e,e}$ **-RFI, TD**	-0.737	0.236	4.7
$\sigma^2_{a,a}$ **-RFI, MORT**	0.009	0.057	24.6	$\sigma^2_{e,e}$ **-RFI, FHN**	5.563	0.046	8.8

σ^2_{am} **-RFI, BW**	1.311	0.844	10.1	$\sigma^2_{e,e}$ **-RFI, ASC**	1.549	0.088	14.8
σ^2_{a} **-BMP**	0.013	0.001	9.7	$\sigma^2_{e,e}$ **-RFI, MORT**	1.144	0.126	7.3
$\sigma^2_{a,a}$ **-BMP, WG**	-0.003	0.027	7.7	σ^2_{e} **-BMP**	0.014	0.001	12.8
$\sigma^2_{a,a}$ **-BMP, TD**	-0.007	0.005	7.5	$\sigma^2_{e,e}$ **-BMP, WG**	0.080	0.049	10.2
$\sigma^2_{a,a}$ **-BMP, FHN**	-0.009	0.003	7.4	$\sigma^2_{e,e}$ **-BMP, TD**	-0.001	0.008	3.7
$\sigma^2_{a,a}$ **-BMP, ASC**	-0.004	0.004	3.4	$\sigma^2_{e,e}$ **-BMP, FHN**	0.014	0.004	7.8
$\sigma^2_{a,a}$ **-BMP, MORT**	0.002	0.002	13.1	$\sigma^2_{e,e}$ **-BMP, ASC**	0.077	0.004	11.4
σ^2_{am} **-BMP, BW**	0.059	0.032	12.3	$\sigma^2_{e,e}$ **-BMP, MORT**	0.017	0.005	12.5
σ^2_{a} **-WG**	30.462	1.832	9.9	σ^2_{e} **-WG**	114.790	1.640	11.5
$\sigma^2_{a,a}$ **-WG, TD**	-0.141	0.347	5.6	$\sigma^2_{e,e}$ **-WG, TD**	0.634	0.378	7.8
$\sigma^2_{a,a}$ **-WG, FHN**	-0.251	0.144	10.2	$\sigma^2_{e,e}$ **-WG, FHN**	0.155	0.202	9.6
$\sigma^2_{a,a}$ **-WG, ASC**	0.803	0.129	23.4	$\sigma^2_{e,e}$ **-WG, ASC**	7.941	0.284	3.3
$\sigma^2_{a,a}$ **-WG, MORT**	0.302	0.101	13.1	$\sigma^2_{e,e}$ **-WG, MORT**	8.833	0.187	6.6
σ^2_{am} **-WG, BW**	0.783	1.119	30.2	σ^2_{e} **-TD**	1.000	0.005	2,999.0
σ^2_{a} **-TD**	0.559	0.071	11.5	$\sigma^2_{e,e}$ **-TD, FHN**	-0.023	0.032	18.4
$\sigma^2_{a,a}$ **-TD, FHN**	0.040	0.035	7.2	$\sigma^2_{e,e}$ **-TD, ASC**	-0.112	0.042	5.8
$\sigma^2_{a,a}$ **-TD, ASC**	0.010	0.029	9.5	$\sigma^2_{e,e}$ **-TD, MORT**	-0.302	0.049	4.0
$\sigma^2_{a,a}$ **-TD, MORT**	-0.007	0.015	17.5	σ^2_{e} **-FHN**	1.000	0.005	2,767.4
σ^2_{am} **-TD, BW**	-0.014	0.202	6.8	$\sigma^2_{e,e}$ **-FHN, ASC**	0.234	0.032	3.6
σ^2_{a} **-FHN**	0.245	0.019	23.8	$\sigma^2_{e,e}$ **-FHN, MORT**	0.221	0.021	10.1
$\sigma^2_{a,a}$ **-FHN, ASC**	0.024	0.016	3.8	σ^2_{e} **-ASC**	1.000	0.005	2,999.0
$\sigma^2_{a,a}$ **-FHN, MORT**	0.020	0.010	5.8	$\sigma^2_{e,e}$ **-ASC, MORT**	0.728	0.020	6.5
σ^2_{am} **-FHN-, BW**	-0.300	0.137	10.5	σ^2_{e} **-MORT**	1.000	0.005	2,733.0

[a]TD: tibia dischondroplasia; FHN: femur head necrosis; ASC: ascites; MORT: mortality; BW: body weight; RFI: residual feed intake; BMP: breast muscle percentage; WG: weight gain.

CHAPTER 6

COMPARISON OF LINEAR VS. THRESHOLD AND SINGLE- VS. TWO-TRAIT ANALYSES IN MORTALITY OF COMMERICAL BROILER BREEDERS: PREDICABILITY[1]

[1] Zhang, X., S. Tsuruta, D. A. L. Lourenco, I. Misztal, R. L. Sapp, and R. J. Hawken. To be submitted to *Journal of Animal Science*.

Abstract

This study compared the predictability of several models and methods, for obtaining genetic evaluations of MORT as a binary variable in broiler chickens. Data were obtained from Cobb-Vantress Inc. and included 180,998 records of both MORT and BW. The incidence rate of MORT was 7.5%. Univariate threshold and linear animal models and bivariate linear-linear model and threshold-linear animal models were used for MORT and BW. The threshold animal models included fixed effects for MG and sex and random effects for CG (source, MG, and hatch), direct and maternal genetic effects, and maternal permanent environment. The linear animal models were similar to threshold animal models, except that CG was treated as fixed. Models were compared using a data splitting technique based on the correlation of EBVs from two samples, with half of the records discarded randomly by CG in the first sample and the remaining records discarded in the second sample. For each model, both BLUP and ssGBLUP methods were used. Reported predictabilities are average correlations of 10 replicates. The results obtained confirmed the slight advantage of threshold over linear for univariate models and threshold-linear over linear-linear for bivariate models with both BLUP (0.746 vs. 0.614 for univariate models and 0.614 vs 0.728 for bivariate models) and ssGBLUP (0.737 vs. 0.587 for univariate models and 0.720 vs. 0.597 for bivariate models). Bivarate models improved the predictability of breeding values for MORT compared with the univariate linear model with ssGBLUP but not for the threshold model with BLUP or ssGBLUP; ssGBLUP had no advantage over BLUP in the same models. For genetic evaluation of mortality in chickens, the threshold model appears more appropriate compared with the linear model. To add more traits to the model, data must be accurate and complete.

Keywords: mortality, binary trait, threshold model

Introduction

Threshold models have been developed to provide genetic evaluation of categorical traits (Gianola & Foulley, 1983; Gilmour et al., 1985; Harville & Mee, 1984). Fouley et al. (1983) and Janss and Foulley (1993) extended the threshold methodology to multitrait analyses that consider more than one continuous correlated trait and unequal design. Albert & Chib (1993) and Moreno et al. (1997) generalized the procedure to Markov-chain Monte Carlo. Albert & Chib (1993) and Sorenson et al. (1995) developed algorithms that allow empty categories in fully conditional distributions. Van Tassell et al. (1998) constructed a MTGSAM program that allows several continuous and categorical variables in a threshold-linear model with Gibbs sampling.

The advantages of threshold over linear models have been shown in several studies. The predictability of breeding values from a threshold animal model is higher than from equivalent linear animal model for discrete traits (Casellas et al., 2007; Ramirez-Valverde et al., 2001; Varona et al., 1999). Furthermore, the correlations of breeding values from linear and threshold models are >0.99, and animal rankings are very similar (Weller et al., 1988; Weller & Ron, 1992). However, advantages of linear over threshold models also have been reported (Hagger & Hofer, 1989; Ramirez-Valverde et al., 2001).

Chen et al. (2011) showed that the accuracy of breeding value for leg score could be improved significantly by including genomic information in the estimation, which suggests the possibility of using ssGBLUP in predicting breeding value.

Chapter 3 showed that the maternal effect of BW was the only factor that had a strong relationship with MORT. Therefore, BW was chosen for a bivariate model with MORT in this study. The objectives were 1) to compare linear and threshold models, 2) to compare univariate and bivariate models, and 3) to compare BLUP and ssGBLUP models in terms of MORT and BW.

Materials and methods

Phenotypic data

The phenotypic data were the same as described in Chapter 5.

Genotypic data

Overall, 52,232 SNPs on 28 autosomes and two commercial chromosomes from 18,947 selection candidates in MGs 184 to 198 were collected using an Illumina 60K BeadChip for chickens. Genotypes for some individuals from early MGs were imputed from an old panel to the new panel using the methodology of Browning and Browning (2009), which was also used for later MGs. The R^2 criterion in Beagle was 0.8. Quality control for imputation included a SNP error rate of <0.035, a correlation between raw and imputed SNPs of >0.85, single-SNP heritability of >0.8, a SNP error rate of <0.005, and Mendelian sampling errors of <0.5.

After imputation, all SNPs were processed using PREGSF90 (Aguilar et al., 2014). For quality control, SNPs with Mendelian conflicts, a call rate of <0.9, an MAF of <0.05, and Hardy-Weinberg equilibrium difference of <0.15 as well as individuals with a call rate of <0.9 were removed. Individuals with a parentage conflict were reassigned with correct parents or removed by comparing genomic and pedigree relationships.

Statistical models

The univariate linear and threshold models and bivariate linear-linear and threshold-linear models used were the same as those described in Chapter 5.

Comparison of models

The models were compared using two-fold crossvalidation, as described in Ramirez-Valverde et al. (2001). The data set was split into two samples. For the first sample, half of the 420 CGs were randomly chosen and their records discarded. For the second sample, records for the other half of CGs

were discarded. Thus, records in the two samples were mutually exclusive, but pedigree and genomic relationships were not split.

Ten replications were conducted using the above criteria, and the correlations of EBVs from the two samples were averaged across the 10 replicates. For each replicate, correlations were calculated for four subsets: all animals, young animals (the last generation), genotyped animals, and genotyped young animals. The correlation reflected the predictability of the model. A high correlation indicated a highly stable model prediction.

Computation and software.

BLUP90IOD2 (Misztal et al., 2002) was used to predict (G)EBVs for the linear model with a convergence criterion of 10^{-14}. CBLUP90IOD2 (Misztal et al., 2002) was used to predict (G)EBVs for threshold and threshold-linear models. The CBLUP90IOD2 convergence criteria were 10^{-14} for the threshold model and 10^{-12} for the threshold-linear model. The YAMS (Masuda et al., 2014a,b) option was used in CBLUP90IOD2 to solve the mixed-model equations iteratively over the dense genomic relationship matrix.

Results

Data summary

A total of 180,998 animals had BW and MORT data (Chapter 5, Tables 5.1.1 and 5.1.2). Animals with a MORT score of 2 did not have a BW record. The incidence of MORT was 7.5%.

Genotyped data

After quality control, 38,609 SNPs remained. Chromosomes 16, 25, and three commercial chromosomes were eliminated because of Mendelian conflicts. Sex chromosomes were eliminated because of asymmetry. Average allele frequency across the remaining SNPs was 0.49. Overall, 18,047 genotyped animals remained after quality control, which was 9.97% of the phenotyped animals.

Additionally, 12,969 of genotyped animals (71.86%) were selection candidates with complete records for both their sires and themselves, and only one dam did not have record. The numbers of records for each trait are listed in Table 6.1 for genotyped animals. Very few animals were genotyped in categories 1 and 2; for MORT, no dead animals were genotyped. Only 537 animals with BMP records were genotyped. Among all 420 CGs, 12.98% of animals were genotyped on average, with a maximum of 55.79% genotyped in CG 1871922, and a minimum of 0% genotyped in CG 1781854.

Model comparison

Mean correlations among EBVs for MORT and BW from univariate linear and threshold models and bivariate linear-linear and threshold-linear models that were used to analyze the split dataset are in Tables 6.2.1. and 6.2.2.

Univariate linear and threshold models

The predictability of MORT was higher with the threshold model than with the linear model for all four subsets of animals and both BLUP and ssGBLUP methods (Table 6.2.1). For genotyped young animals, the BLUP predictability of MORT was 0.746 ± 0.025 for the threshold model compared with 0.614 ± 0.035 for the linear model.

Bivariate linear-linear and threshold-linear models

The predictability of MORT was higher with the threshold-linear model than with the linear-lienar model for both BLUP (0.728 ± 0.021 vs. 0.614 ± 0.036) and ssGBLUP (0.720 ± 0.024 vs. 0.597 ± 0.025) for genotyped young animals (Table 6.2.1). For BW, predictability with the threshold-linear model was slightly lower than with the linear-linear model for both BLUP (0.697 ± 0.042 vs. 0.715 ± 0.040) and ssGBLUP (0.752 ± 0.013 vs. 0.760 ± 0.014) for genotyped young animals (Table 6.2.2). All replicates in all subsets of the two traits were consistent with the average.

Univariate threshold and bivariate threshold-linear models

The pattern of predictability of MORT for univariate threshold and bivariate threshold-linear models was complicated (Table 6.2.1). For genotyped young animals, the univariate threshold model was better than the bivariate threshold-linear model in prediction for both BLUP (0.746 ± 0.025 vs. 0.728 ± 0.021) and ssGBLUP (0.737 ± 0.027 vs. 0.720 ± 0.024). A similar pattern can be observed for the subset of young animals. However, for the datasets of all genotyped animals or all animals from all generations, the univariate threshold model was not as good a predictor as the bivariate threshold-linear model. For the subset of all genotyped animals, predictability was 0.719 ± 0.042 (univariate threshold model) vs. 0.752 ± 0.031 (bivariate threshold-linear model) with BLUP and correspondingly 0.721 ± 0.040 vs. 0.737 ± 0.025 with ssGBLUP.

Univariate linear and bivariate linear-linear models

For MORT, the bivariate linear-linear model outperformed the univariate linear model for all four subsets with both BLUP and ssGBLUP (Table 6.2.1). Predictability for genotyped young animals with BLUP was 0.614 ± 0.035 (univariate linear model) vs. 0.614 ± 0.036 (bivariate linear-linear model); corresponding predictability with ssGBLUP was 0.587 ± 0.024 vs. 0.597 ± 0.025. For BW, however, the pattern of predictability for univariate linear and bivariate linear-linear models was complicated (Table 6.2.2). With BLUP, the univariate linear model outperformed the bivariate linear-linear model for all subsets. Predictability for genotyped young animals was 0.722 ± 0.041 (univariate linear model) vs. 0.715 ± 0.040 (bivariate linear-linear model). However, with ssGBLUP, the univariate linear model was inferior to the bivariate linear-linear model for genotyped young animals (0.757 ± 0.018 vs. 0.760 ± 0.014) as well as for the genotyped animal and young animal subsets.

BLUP and ssGBLUP

For BW from the univariate linear model (Table 6.2.2), predictability was higher with ssGBLUP, which included genomic information, than with traditional BLUP (0.722 ± 0.041 vs. 0.757 ± 0.018) for

genotyped young animals. Only one replication out of 10 had a predictability that was 0.01 lower in ssGBLUP than in BLUP. Genotyped animals also had correspondingly higher predictability (0.727 ± 0.017 vs. 0.746 ± 0.024) as did the young animals of the last generation (0.771 ± 0.028 vs. 0.779 ± 0.024). For MORT from the univariate threshold model (Table 6.2.1), predictability for genotyped young animals was lower with ssGBLUP than with BLUP (0.746 ± 0.025 vs. 0.737 ± 0.027), but predictabilities for the other three subsets were higher with ssGBLUP. Predictability of genotyped young animals for MORT was also lower using a univariate linear model with ssGBLUP than with BLUP (0.614 ± 0.035 vs. 0.587 ± 0.024), which was also observed for the genotyped animal subset. For genotyped young animals, 6 out of 10 replicates were consistent with the average correlation using the univariate threshold model compared with 9 out of 10 for the univariate linear model. For both univariate models, predictability was higher with ssGBLUP than with BLUP for datasets of all animals and young animals.

For BW from bivariate models (Table 6.2.2), predictability was higher with ssGBLUP than with BLUP for genotyped young animals (0.715 ± 0.040 vs. 0.760 ± 0.014 for the linear-linear model and 0.697 ± 0.042 vs. 0.752 ± 0.013 for the threshold-linear model). All replications for all four subsets were consistent with the average. For MORT (Table 6.2.1), however, predictability was lower for genotyped young animals with ssGBLUP than with BLUP (0.614 ± 0.036 vs. 0.597 ± 0.025 for the linear-linear model and 0.728 ± 0.021 vs. 0.720 ± 0.024 for the threshold-linear model) as was also observed for the genotyped animal subset.

Standard errors for predictability with the univariate linear and bivariate linear-linear models generally were higher with BLUP than with ssGBLUP, but this was not always the case for univariate threshold and bivariate threshold-linear models.

Computing time

For prediction of breeding values, THRGIBBS1F90 took 1.5 hours for the univariate threshold model and 13 hours with genomic information, 4 hours for the bivariate threshold-linear model and 39 hours with genomic information. All results using THRGIBBS1F90 were solved at 11,000 iterations.

CBLUP90IOD2 took 2 minutes for the univariate threshold model and 1.5 hours with genomic information and 30 minutes for the bivariate threshold-linear model and 5 hours with genomic information. BLUP90IOD2 took 2 minutes for the linear univariate model and 30 minutes with genomic information and 20 minutes for the bivariate linear-linear model and 4 hours with genomic information.

Discussion

Predictability in this study ranged from 0.629 to 0.813, which was slightly higher than reported in previous studies using a bivariate threshold-linear model and THRGIBBSF90 (Brien et al., 2002; Ramirez-Valverde et al., 2001; Varona et al., 1999). When comparing within univariate or bivariate models, where the observations were the same across groups, threshold models were better compared with linear models in predicting MORT. For BW, however, a linear model was better than a threshold model, which is understandable given the properties of the data (Gianola, 1982; Thompson, 1979). In a bivariate model, predictability theoretically should be higher than in a univariate model, especially for traits with low heritability. However, in this study, the pattern varied among generations, traits, and presence or absence of genomic information. The heritability of MORT from the bivariate threshold-linear model was lower than from the univariate threshold model (0.03 vs. 0.05, Chapter 3, Table 3.2.2). One explanation could be that BW is only maternally highly correlated to MORT. However, in previous studies where bivariate models were better than univariate models, the continuous traits all had medium to high direct genetic correlations to the discrete traits (Negussie et al., 2008; Ramirez-Valverde et al., 2001; Varona et al., 1999).

Compared with BLUP, ssGBLUP was expected to have higher predictability in all scenarios. However, it appeared to be biased for MORT. Predictabilities for the genotyped and genotyped young animal subsets were not better for ssGBLUP compared with BLUP regardless of model. The mechanism behind the result is not clear. For MORT, category 1 had no genotyped animals (Table 6.1). Therefore, GEBVs of dead individuals were improved only through genotyped relatives with records. Nevertheless,

predictability for univariate threshold and bivariate threshold-linear models with ssGBLUP still was higher than for univariate linear and bivariate linear-linear models, respectively, in this study.

Binary and categorical traits in this study were almost all truncated and with known or unknown bias. For example, ASC, TD, and FHN were confirmed only by necropsy when a suspicious lesion was detected by X-ray or when the chicken expressed observational symptoms. Thus, the false negative rate is expected to be biased upwards by the error rate of the detection methods. MORT and ASC, on the other hand, were recorded only at early ages, and dead animals were not recorded for other traits, such as BW. This is a problem in bivariate but not univariate models. The breeding values of individuals with missing traits were predicted from relatives with phenotypes through pedigree and genomic relationships and, therefore, had low reliability.

Another reason for the variation in predictability may be that this dataset was far from balanced. The number of observations for continuous traits, number of observations for categories of discontinuous traits, and number of genotyped individuals in each CG varied from none to a very large number. In two-fold cross validation with split CG, the correlation between split data can vary depending on the relationships among animals with different CGs and the information in the related CGs.

Conclusion

A threshold model is more appropriate for predicting binary data. A bivariate or multivariate model that includes correlated traits can improve the predictability of binary traits. ssGBLUP using a mixed relationship matrix can improve predictability compared with BLUP. Bayesian methods with proper priors and proper sampling distribution may improve truncated and missing values.

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Table 6.1. Numbers of observations for genotyped animals by trait and generation.

Trait[a]	Genotyped animals				Genotyped young animals[b]			
	Category				Category			
	0	1	2	Missing	0	1	2	Missing
TD	16,678	40	—	1,320	2,134	7	-	241
FHN	417	81	41	17,508	60	5	5	2,312
ASC	17,998	19	—	30	2,371	1	—	10
MORT	18,045	0	—	2	2,381	0	—	1
BW	17,998			49	2,371			11
RFI	16,188			1,859	2,071			311
BMP	537			17,510	70			2,312
WG	16,188			1,859	2,071			311
Total	18,047			—	2,382			—

[a]TD: tibia dischondroplasia (binary trait); FHN: femur head necrosis (categorical trait); ASC: ascites (binary trait); MORT: mortality (binary trait); BW: body weight (continuous trait); RFI: residual feed intake(continuous trait); BMP: breast muscle percentage(continuous trait); WG: weight gain (continuous trait).

[b]Genotyped animals in generation 198.

Table 6.2.1. Correlations[a] (and standard errors in parentheses) of split datasets for breeding value solutions of mortality using univariate linear, univariate threshold bivariate linear, and bivariate threshold-linear animal models.

		Univariate		Bivariate	
Model	**Animal group**	**BLUP**	**ssGBLUP**	**BLUP**	**ssGBLUP**
Linear	All	0.519 (0.026)	0.538 (0.024)	0.537 (0.025)	0.553 (0.023)
	Young	0.663 (0.027)	0.671 (0.025)	0.665 (0.028)	0.670 (0.026)
	Genotyped	0.594 (0.037)	0.590 (0.031)	0.613 (0.036)	0.609 (0.030)
	Genotyped young	0.614 (0.035)	0.587 (0.024)	0.614 (0.036)	0.597 (0.025)
Threshold	All	0.641 (0.039)	0.671 (0.036)	0.681 (0.026)	0.696 (0.023)
	Young	0.780 (0.022)	0.792 (0.024)	0.764 (0.020)	0.756 (0.024)
	Genotyped	0.719 (0.042)	0.721 (0.040)	0.752 (0.031)	0.737 (0.025)
	Genotyped young	0.746 (0.025)	0.737 (0.027)	0.728 (0.021)	0.720 (0.024)

[a]Correlations are the average from 10 replicates.

Table 6.2.2. Correlation[a] (and standard errors in parentheses) for a split datasets for breeding value solutions of body weight using univariate linear, univariate threshold bivariate linear, and bivariate threshold-linear animal models.

		Univariate		Bivariate	
Model	**Animal group**	**BLUP**	**ssGBLUP**	**BLUP**	**ssGBLUP**
Linear	All	0.800 (0.0076)	0.799 (0.008)	0.792 (0.006)	0.793 (0.008)
	Young	0.771 (0.028)	0.779 (0.024)	0.759 (0.027)	0.781 (0.014)
	Genotyped	0.727 (0.017)	0.746 (0.024)	0.720 (0.015)	0.747 (0.014)
	Genotyped young	0.722 (0.041)	0.757 (0.018)	0.715 (0.040)	0.760 (0.014)
Threshold	All	—	—	0.781 (0.006)	0.785 (0.007)
	Young	—	—	0.737 (0.029)	0.768 (0.014)
	Genotyped	—	—	0.708 (0.015)	0.740 (0.013)
	Genotyped young	—	—	0.697 (0.042)	0.752 (0.013)

[a]Correlations are the average from 10 replicates.

CHAPTER 7

CONCLUSIONS

This dissertation inspected the changes in genetic architecture before and after selection on broiler chickens. In the study of signatures of selection (Chapter 3), the study of two breeds using the same selection goal and the same selection index showed quite different signatures of selection, which implies that the historical goal during breed development changed the genetic architecture of each breed such that the regions currently selected were altered. The overlap of signatures of selection and putative selection regions detected by GWAS via ssGBLUP indicates strong evidence for genomic selection. In the study of MORT (Chapter 6), the results from a threshold model indicate a low heritability of early MORT but a strong negative genetic correlation with maternal BW at early age. This implies that selection on heavier BW in hens decreases chicken MORT. Nevertheless, the low correlation between MORT and RFI implies that selection on the latter has no impact on MORT.

In Chapters 4 to 6, the studies in this dissertation confirmed that ssGBLUP is an effective statistical method for genomic evaluation and GWAS in broiler chickens. In the WssGBLUP study (Chapter 4), these methods improved the accuracy of breeding value prediction after weighting compared with traditional BLUP, GBLUP, BayesB, and BayesC, especially for complex traits with numerous underlying QTLs. The Manhattan plots indicated similar patterns for WssGBLUP and Bayesian methods, with more SNP captured by WssGBLUP under an infinitesimal model. The ssGBLUP is applicable to complex models, such as a multivariate model and a model with maternal genetic and maternal permanent effects. Furthermore, the blending relationship (**H** matrix) in ssGBLUP is able to be combined with Bayesian inference using Gibbs sampling. In the study of threshold models (Chapter 6), the accuracy of breeding value prediction was better with ssGBLUP compared with that of BLUP under both linear and

threshold models for both continuous and binary traits. In bivariate models, ssGBLUP had an advantage over BLUP for BW but no advantage for MORT as a binary trait.