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ORIGINAL ARTICLE

Systematic differences in the response of genetic variation to pedigree and genome-based selection methods

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Genomic selection (GS) is a DNA-based method of selecting for quantitative traits in animal and plant breeding, and offers a potentially superior alternative to traditional breeding methods that rely on pedigree and phenotype information. Using a 60 K SNP chip with markers spaced throughout the entire chicken genome, we compared the impact of GS and traditional BLUP (best linear unbiased prediction) selection methods applied side-by-side in three different lines of egg-laying chickens. Differences were demonstrated between methods, both at the level and genomic distribution of allele frequency changes. In all three lines, the average allele frequency changes were larger with GS, 0.056 0.064 and 0.066, compared with BLUP, 0.044, 0.045 and 0.036 for lines B1, B2 and W1, respectively. With BLUP, 35 selected regions (empirical $P<0.05$) were identified across the three lines. With GS, 70 selected regions were identified. Empirical thresholds for local allele frequency changes were determined from gene dropping, and differed considerably between GS (0.167–0.198) and BLUP (0.105–0.126). Between lines, the genomic regions with large changes in allele frequencies showed limited overlap. Our results show that GS applies selection pressure much more locally than BLUP, resulting in larger allele frequency changes. With these results, novel insights into the nature of selection on quantitative traits have been gained and important questions regarding the long-term impact of GS are raised. The rapid changes to a part of the genetic architecture, while another part may not be selected, at least in the short term, require careful consideration, especially when selection occurs before phenotypes are observed.
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INTRODUCTION

Traditional selection of livestock applies a method called best linear unbiased prediction (BLUP), which uses phenotypes and pedigree information to predict breeding values, and has been successfully employed for many traits. Through the use of molecular genetic tools, the genetics of quantitative traits has become better understood and, consequently, genetic markers have the potential to predict genetic values more accurately (Dekkers, 2004) and increase genetic gain through marker-assisted selection. Despite the potential benefits of marker-assisted selection in breeding programs, its implementation has faced problems, especially in animal breeding, because discovery of markers with useful effects has been limited. Meuwissen *et al.* (2001) proposed a solution that does not require discovery of marker effects but uses all markers simultaneously in a method called genomic selection (GS). In GS, the genomic breeding value (GEBV) is estimated based on the estimates of marker effects covering the whole genome. This approach has become possible because of rapid developments in molecular genetics, in particular the identification of large numbers of single nucleotide polymorphisms (SNPs) and the development of low cost high throughput genotyping methodologies (Wang *et al.*, 2009). GS can increase rates of genetic gain per unit of time, because GEBVs typically have higher reliabilities than BLUP EBVs, particularly for young animals without phenotypic performance. Having reliable GEBVs before phenotypes can be recorded have clear advantages in terms of costs and reduction of generation intervals (Schaeffer, 2006).

Directional selection has an impact on allelic diversity. When genome-wide marker panels are used for selection, it is possible to use these markers to investigate the dynamics of allelic diversity across the genome. Most methods developed for assessing the allelic diversity through genomic analysis are based on calculating population genetics statistics such as allele frequencies (either directly or indirectly) (Elferink *et al.*, 2012) and linkage disequilibrium (LD) (Ennis, 2007). Previous studies have shown that frequencies of the favorable alleles, as well as alleles in neighboring regions, increase over time when a favorable mutation occurs in a population under selection (Smith and Haigh, 1974; Barton, 2000). This process can lead to a signature of selection. When signatures of selection are discovered, they are taken as indications that genetic variants are, or were, present with some measurable effect on the phenotype. Studies into signatures of selection measure the reduction in variation after selection and information such as allele frequencies before selection are typically unknown.

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Table 1 Number of selection candidates selected based on their GEBV, number of selected parents in the base and first generations of GBLUP selection and N_e for lines B1, B2 and W1

	GEBV												
Line	G0-GBLUP[a]						G1-GBLUP[b]						
	Selection candidates		Selected parents		p(i)	p(i)	Selection candidates		Selected parents		p(i)	p(i)	N_e[c]
	F	M	F	M	F	M	F	M	F	M	F	M	
B1	389	130	59	15	0.152 (1.554)	0.115 (1.688)	507	138	58	15	0.114 (1.688)	0.109 (1.709)	48
B2	476	133	57	15	0.120 (1.667)	0.113 (1.709)	516	143	58	15	0.112 (1.709)	0.105 (1.732)	40
W1	617	166	48	15	0.078 (1.872)	0.090 (1.804)	630	166	44	15	0.070 (1.918)	0.090 (1.804)	34

Abbreviations: F, female animal; GBLUP, genomic best linear unbiased prediction; GEBV, genomic estimated breeding value; *i*, selection intensity (*i* was derived from *p* (Supplementary notes)); M, male animal; *p*, proportion of candidates selected.
[a]G0-GBLUP is the base generation of GBLUP.
[b]G1-GBLUP is the first generation of GBLUP.
[c]The method used to calculate N_e is given in Supplementary notes.

Table 2 Size of training data for all generations in lines B1, B2 and W1

Line	G0-GBLUP[a]	G1-GBLUP[b]	G2-GBLUP[c]
B1	715	1096	1355
B2	611	990	1232
W1	734	972	1220

Abbreviation: GBLUP, genomic best linear unbiased prediction.
[a]G0-GBLUP is the base generation of GBLUP.
[b]G1-GBLUP is the first generation of GBLUP.
[c]G2-GBLUP is the second generation of GBLUP.

Most studies into the impact of GS have been done using simulations (Meuwissen *et al.*, 2001; Muir, 2007; Bastiaansen *et al.*, 2012). A number of questions are still unanswered regarding the use of GS, for instance, what impact GS has on genetic variation.

We aimed to broadly assess the response of the allele frequencies across the whole genome in populations that underwent selection for two generations based on two different estimated breeding values (EBV). In this study, pedigree BLUP EBV and genomic EBV (GEBV) were used to separately select the top animals within each of three layer chicken lines. Data from the GS experiment has been used to assess the potential and impact of this new method over two generations of selection in a commercial breeding program. It was expected that GS applies selection pressure directed to specific regions of the genome and leads to faster increase in the frequency of favorable allele, as was already shown in some simulations (Sonesson and Meuwissen, 2009; Jannink, 2010; Kinghorn *et al.*, 2011). Genetic variation was evaluated by measuring changes in allele frequencies across the whole genome that allowed the identification of genomic regions under selection. Besides the general insight into how the genome responds to selection, it was important to compare how the response to selection changed when breeding values were estimated with genetic markers instead of pedigree.

MATERIALS AND METHODS

Data structure

Three lines of commercial layers; two Brown lines (B1 and B2) and one White line (W1) were used. Having three lines allowed a comparison of the changes in genomic variation for related populations. A selection experiment was carried out to compare responses with genomic- and pedigree-based BLUP selection. For each line, a group of males and females were taken to be the base for the GS experiment in February 2009 (Table 1). All males born from 2005–2008 were genotyped and used as training data, except that for the base generation of GS (GBLUP), males hatched in January and February 2008 were not included in the training data, because they did not have progeny with phenotypes until June 2009. The size of the training set increased for each generation of selection by the addition of more phenotyped and genotyped animals; that is, for each generation, the newly genotyped animals with own or offspring phenotypes were added to the training set (Table 2).

For BLUP, parents were chosen from two groups of males (88 and 110 weeks old) and two groups of females (44 and 66 weeks old). Animals were selected from multiple hatch dates in each generation. On average, parents for BLUP selection were selected from nearly 6000 females and 600 males (Table 3).

Within each line, the top animals were selected based on either their EBV from BLUP or their GEBV from GBLUP analysis. The number of selection candidates and selected parents are in Table 1 for GBLUP selection and Table 3 for BLUP selection. Average selection pressure was approximately the same for GBLUP and BLUP. In addition, average selection pressure was nearly the same for males and females (Tables 1 and 3) (Selection intensities were calculated based on the records in the pedigree. The pedigree does not include all hatched animals, as there was a pre-selection during rearing based on parents' performance. It means only the animals housed in the laying house or being genotyped are included in the pedigree file). Selection had been performed on a commercial index that contained 15–18 traits. Selected animals were mated at random, except that full and half-sib matings were avoided. Restrictions were applied to ensure selection from a large number of families to limit inbreeding. The population for GBLUP was smaller (Table 1). The rationale for the smaller population was that selection could be performed within full sib families, whereas for BLUP, all full sibs had the same breeding values based on sib performance. The number of phenotypes required was also smaller for GBLUP.

Pedigree data were available for up to 14 generations before the current experiment. The total number of pedigree records ranged between 205 000–227 000 animals for each of the three lines. The number of pedigree records within the 14 generations was about 18 000 for each line and included information on animal identification number, sex, father and mother identification number and hatch date of each animal.

Collection of DNA samples and genotyping

DNA samples were extracted from individual blood samples. In total, 57 636 SNPs were included on the chicken IIlumina Infinium iSelect Beadchip (Illumina Inc., San Diego, CA, USA) (60 K chip). Genotyping and quality control were done using the standard protocol for Infinium iSelect Beadchips and raw data were analyzed with Genome Studio v2009.2 (Illumina Inc.) as previously described (Groenen *et al.*, 2011).

Genotyped data

The genotypes were derived from four generations of the training set (Table 2), all selection candidates in two generations of GBLUP selection, and the base

Table 3 Number of selection candidates selected based on their EBV, number of selected parents in the base and first generations of BLUP selection and N_e for lines B1, B2 and W1

	EBV												
Line	G0-BLUP[a]						G1-BLUP[b]						
	Selection candidates		Selected parents		p(i)	p(i)	Selection candidates		Selected parents		p(i)	p(i)	N_e[c]
	F	M	F	M	F	M	F	M	F	M	F	M	
B1	7424	1229	812	162	0.109 (1.709)	0.132 (1.627)	2603	443	297	50	0.114 (1.688)	0.113 (1.709)	99
B2	7682	1214	781	164	0.102 (1.755)	0.135 (1.608)	2594	414	254	59	0.098 (1.767)	0.143 (1.590)	83
W1	9026	1565	788	199	0.087 (1.817)	0.127 (1.627)	2450	645	153	78	0.062 (1.968)	0.121 (1.667)	121

Abbreviations: GBLUP, genomic best linear unbiased prediction; EBV, estimated breeding value; F, female animal; *i*, selection intensity (*i* was derived from *p* (Supplementary notes)); M, male animal; *p*, proportion of candidates selected.
[a]G0-BLUP is the base generation of BLUP.
[b]G1-BLUP is the first generation of BLUP.
[c]The method to calculate N_e is given in Supplementary notes.

Table 4 Number of genotyped selection candidates used to calculate d_{02} for BLUP and GBLUP selection in lines B1, B2 and W1

Line	G0-BLUP[a]		G2-BLUP[b]		G0-GBLUP[c]		G2-GBLUP[d]	
	F	M	F	M	F	M	F	M
B1	248	1058	0	110	248	126	296	130
B2	0	953	0	110	238	128	297	130
W1	230	1205	0	150	230	141	0	150

Abbreviations: BLUP, best linear unbiased prediction; GBLUP, genomic best linear unbiased prediction; F, female animal; M, male animal.
[a]G0-BLUP is the base generation of BLUP. G0-BLUP included genotyped grandparents of G2-BLUP plus their genotyped hatch mates.
[b]G2-BLUP is the second generation of BLUP.
[c]G0-GBLUP is the base generation of GBLUP.
[d]G2-GBLUP is the second generation of GBLUP.

(G0) and second generation (G2) of BLUP selection (Table 4). The genotypes of all individuals in the training generations and three generations of selection were obtained with the 60 K chip, except the female genotypes from the last generation that were imputed from 3 K based on reference haplotypes from the population. The accuracy of imputation was 0.95–0.97.

Breeding values from BLUP

The following mixed model was used to estimate the EBV:

$$\begin{pmatrix} X'X & X'Z \\ Z'X & Z'Z+\lambda * A^{-1} \end{pmatrix}\begin{pmatrix} b \\ a \end{pmatrix} = \begin{pmatrix} X'Y \\ Z'Y \end{pmatrix},$$

where Y was the phenotypic record of animal i, b was a vector of fixed effects, including an overall mean, hatch date, line and cage tier (the row and level of the cage in the henhouse). a was the vector of random animal effects, X was the design matrix corresponding to fixed effects, Z was the design matrix that corresponds the records to the animal effects. λ was σ_e^2/σ_a^2 in which σ_e^2 was the residual variance and σ_a^2 was the additive genetic variance. Residuals were assumed independent and following a normal distribution; $e \sim (0, I\sigma_e^2)$. For BLUP, only the pedigree information was used for building the relationship matrix (A).

Breeding values from GBLUP

The statistical model for GBLUP was the same as for BLUP, except that an H matrix (single-step GBLUP) (Misztal *et al.*, 2009) was used as the relationship matrix instead of the A matrix. The H matrix combines the numerator relationship matrix (A) based on pedigree information with the genomic relationship matrix (G) based on SNP information. Single step GBLUP has been used to distinguish between BLUP with the H matrix from BLUP with the G matrix. In this study, only BLUP with H has been applied. Therefore, we simply compare GBLUP (which included genomic information) with BLUP which excludes genomic information. The GBLUP model assumed that the SNP effects (g) were normally distributed; $g \sim (0, I\sigma_g^2)$, and that the variance of SNP effects was equal for all SNPs.

Generations

For GBLUP, the generations were discrete. The last generation of GBLUP-selected animals (G2-GBLUP) had their grandparents in the base generation (G0-GBLUP). However, for BLUP, the generations were overlapping (see data structure section) and therefore, not all grandparents of animals in the last generation of BLUP (G2-BLUP) were from G0-GBLUP. Allele frequencies of G0-BLUP were calculated on all the genotyped grandparents of G2-BLUP animals and their hatch mates, including grandparents that were not in G0-GBLUP (Table 4).

Allele frequency changes

Allele frequencies (f) were computed in G0-GBLUP, G2-GBLUP, G0-BLUP and G2-BLUP by counting. The absolute value of changes in allele frequencies ($d_{02} = |f_2 - f_0|$) within each line was calculated for all SNPs with minor allele frequency (MAF) > 0. The running averages of 11 adjacent d_{02} values were plotted against the location of the middle SNP to emphasize the systematic changes of frequencies in a region over the erratic pattern of individual SNPs.

Estimation of threshold values for putative selected regions

An empirical threshold was determined using the gene dropping method (MacCluer *et al.*, 1986). Gene dropping was done by dropping alleles along the existing pedigree. The process was done by simulating one chromosome that contained 20 loci with zero mutation rate and 0.5 starting allele frequency. The haplotypes were simulated for the founder animals in the pedigree. Genotypes were assigned to offspring in each generation based on the Mendelian transmission rules (random sampling). Changes in allele frequency were computed between the same generations, including the same animals as in the real data. The distribution of allele frequency changes was obtained from 1000 replicates. Values of d_{02} beyond the 95% threshold ($P < 0.05$) of the empirical distribution (Supplementary Material 1) were taken to be indicative of selection.

Distribution of d_{02} under drift and selection

To compare the observed changes in allele frequencies with their expectation, we divided the observed d_{02} of each SNP by SD_t, which is the s.d. of the allele frequency after t generations of pure drift.

$$SD_t \approx \sqrt{pq(1 - e^{-\left(\frac{t}{2N_e}\right)})}, \quad (1)$$

where p and q were the initial allele frequencies of the SNP, and N_e was the effective population size. As the rate of genetic drift is proportional to N_e, the realized N_e from the gene dropping analysis was used. Values obtained for N_e

were 48, 40 and 34 for GBLUP and 99, 83 and 121, for BLUP in lines B1, B2 and W1, respectively (Tables 1 and 3). t was equal to 2. A histogram of the standardized allele frequency changes, d_{02}/SD_t, across all SNPs was compared to the expected distribution of $SD_t = 1$.

RESULTS

Data quality control

Genotypes from 57 636 SNPs were obtained from the chicken IIIumina Infinium iSelect Beadchip (60 K) (Groenen *et al.*, 2011). Of these SNPs, 1144 were unmapped on the genome build WASHUC2 (Groenen *et al.*, 2011) and were removed from the data. Furthermore, two linkage groups and chromosomes 16, 31 and 32 were excluded from the analysis because of insufficient SNP coverage resulting in low information content on these chromosomes. After exclusions, ~37 K SNPs for the brown layer line, B1, 36 K SNPs for the brown layer line, B2, and 26 K SNPs for the white layer line, W1, were found segregating and retained for analyses (Table 5).

Response to selection

Change in mean of index values from G0-BLUP to G2-BLUP and from G0-GBLUP to G2-GBLUP were taken as response to selection (Table 6). For all lines, there was a higher response with GBLUP than BLUP, with the largest difference of 62% (0.33 s.d. units extra response) in line B1. Across the three lines, the response to selection was 39% higher in GBLUP than BLUP based on the index values, hence GS was effective (Table 6).

Effect of selection method on allele frequencies

To compare the impact of selection methods on the allele frequencies and to identify the genomic regions that have been under selection, allele frequency differences, d_{02}, were calculated between generation zero (G0) and generation two (G2), for both BLUP- and GBLUP-selected lines. Patterns of d_{02} across the whole genome were very different between BLUP and GBLUP-selected lines (Figures 1–3). Changes in allele frequencies were on average larger with GBLUP than with traditional BLUP. The absolute changes in allele frequency, d_{02}, were on average, 0.056, 0.064 and 0.066 for GBLUP compared with 0.044, 0.045 and 0.036, for BLUP in lines B1, B2 and W1, respectively. The distribution of d_{02} values showed a longer tail of high d_{02} values for GBLUP than for BLUP (Figure 4).

The standardized changes in allele frequencies, d_{02}/SD_t, were on average 1, 1.08 and 1 for GBLUP compared with 1.12, 1.05 and 1.01 for BLUP in lines B1, B2 and W1, respectively. From the histogram of standardized allele frequency changes, we observed that both BLUP and GBLUP-selected lines had fewer d_{02} values near zero than expected, and more d_{02} values in the tails of the distribution (Figure 5) indicating that selection does have an impact on changes in allele frequencies. Selection changes allele frequency in addition to changes that are expected from drift that are indicated by the solid line in Figure 5. The comparison of d_{02} from BLUP and from GBLUP shows that GBLUP has a higher density close to zero and in the tail (Figure 6), but a lower density in the range from 1.0 or 1.5 standardized d_{02} to 2.5 or 3.5 standardized d_{02}.

Table 5 Number of SNPs retained after exclusions in the genome of BLUP and GBLUP-selected animals

Line	*GBLUP*	*BLUP*
B1	37 197	37 254
B2	36 582	36 731
W1	26 302	26 337

Abbreviations: BLUP, best linear unbiased prediction; GBLUP, genomic best linear unbiased prediction.

Threshold values for putative selected regions

Significant thresholds to declare significant selected regions ($P < 0.05$) were obtained from gene dropping (MacCluer *et al.*, 1986) and were 0.167 for line B1, 0.184 for line B2 and 0.198 for line W1 in GBLUP. The thresholds for BLUP were lower; 0.115, 0.126 and 0.105 for lines B1, B2 and W1, respectively. These values confirm the expectation that random fluctuations in allele frequencies would be bigger in GBLUP than BLUP, because of the pedigree structure and smaller N_e for GBLUP (Tables 1 and 3).

Selected regions

With GBLUP selection, the majority of chromosomes contained regions in which the running average of d_{02} values exceeded the threshold (Figures 1–3, Supplementary Materials 2–4). Chromosomes without significant evidence of selection were mostly the micro and intermediate-size chromosomes, whereas others had multiple locations of selection. Most chromosomes that contained more than one region with evidence of selection were macrochromosomes, but there was no evidence of clustering of significant peaks in specific regions of the genome. With BLUP, fewer regions showed evidence of selection (Figures 1–3, Supplementary Materials 5–7). No overlap was observed between selected regions responding to BLUP selection and regions responding to GBLUP selection. In selected regions, the average d_{02} were 0.241, 0.220 and 0.204 for GBLUP compared with 0.121, 0.156 and 0.135, for BLUP in lines B1, B2 and W1, respectively. Although the number of selected regions, number of SNPs in selected regions, and the average d_{02} were higher for GBLUP, the average length of selected regions was nearly similar for GBLUP and BLUP (Table 7).

Overlap of selected regions between lines

Of the 70 GBLUP-selected regions in all lines, few were found to overlap between lines, and therefore most of the selected regions were line specific. The only region that overlapped between two brown layer lines was near position 15 Mb on chromosome 8. This region represents the highest peak in line B2 and was among the five highest peaks in line B1. In line W1 and B1, the highest peaks were at regions 41–44 Mb on chromosome 4 and near position 4 Mb on chromosome 21, respectively. There was no overlap for these regions with significant regions in other lines.

When lines are very different, it may be expected to see limited overlap between the genomic regions that contribute to genetic variance and hence, would respond to selection. The divergence between the lines was assessed by measuring the diversity (Fst) between lines within the base generation, as well as the second generation. The method for calculation of Fst is given in Supplementary notes. These comparisons revealed, as expected, that lines B1 and B2 (brown layers) are the least divergent lines (Table 8).

DISCUSSION

Directional selection acts on genetic variation (Przeworski *et al.*, 2005) and allele frequencies change as response to selection (Garnett and Falconer, 1975; Kimura, 1989). Currently, there is a great interest in using the patterns of variation to identify genomic regions under selection (Sabeti *et al.*, 2002). In our study, we compared the genome-wide response to selection obtained by traditional BLUP or GS (GBLUP). GBLUP was expected to apply selection pressure directed to specific regions of the genome resulting in a more rapid increase of

Table 6 Mean of index values in G0 and G2 of BLUP and GBLUP for lines B1, B2 and W1

Line	*GBLUP*				*BLUP*				*Difference in response between two methods (in standardized unit)*
	G0	*G2*	*G0–G2*	*G0–G2 (standardized unit)*	*G0*	*G2*	*G0–G2*	*G0–G2 (standardized unit)*	
B1	605.28	804.90	199.62	0.86	662.19	800.33	138.14	0.53	0.33
B2	440.15	705.03	264.88	0.90	479.23	707.31	228.07	0.74	0.16
W1	570.25	733.44	163.19	0.59	631.43	760.46	129.03	0.44	0.14

Abbreviations: BLUP, best linear unbiased prediction; GBLUP, genomic best linear unbiased prediction; G0, base generation; G2, second generation.

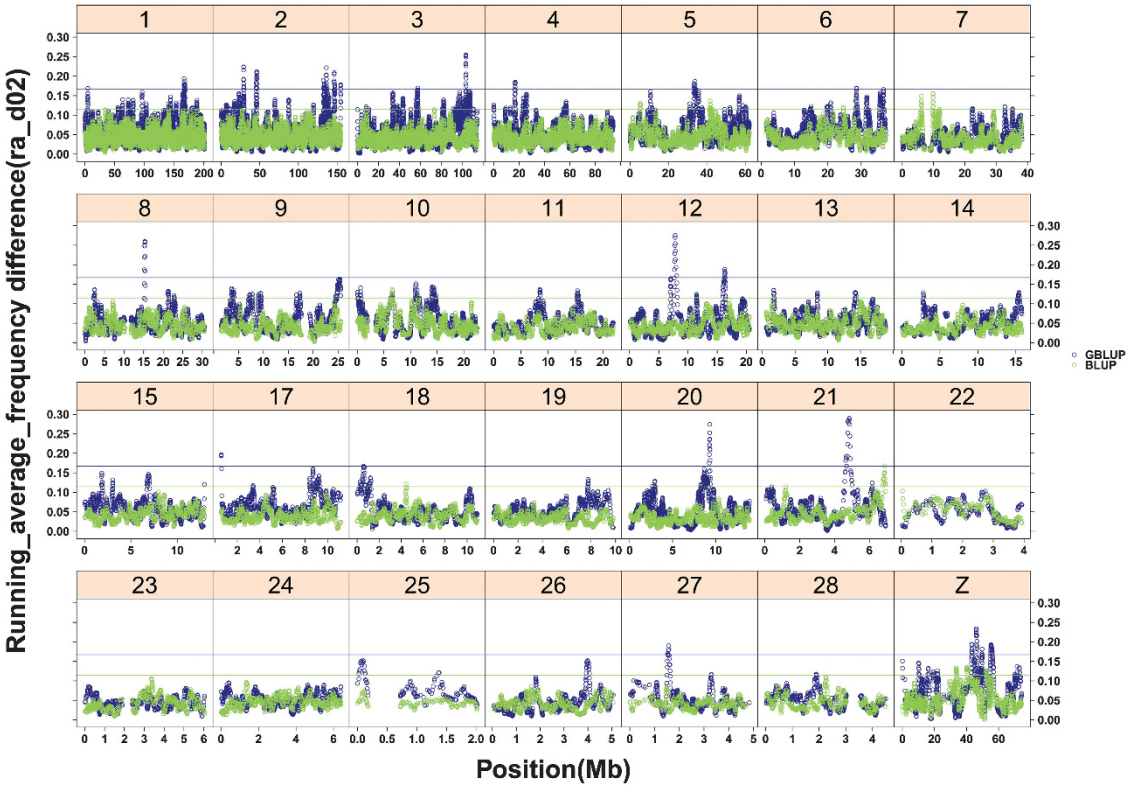

Figure 1 Pattern of genetic variation after two generations of selection for line B1. Running average of allele frequency distribution of 37197 SNPs (GBLUP) and 37254 SNPs (BLUP) along the whole genome is plotted against the physical position (Mb). The deviations above the threshold show signals of selection.

the frequency of favorable alleles, as was already shown in simulation studies (Sonesson and Meuwissen, 2009; Jannink, 2010; Kinghorn *et al.*, 2011).

Our results show that both GBLUP and BLUP selection cause genome-wide changes in allele frequencies after two generations of selection. Changes in allele frequencies were ~51% larger across the genome in GBLUP compared with BLUP selection and 64% larger in selected regions. With the larger changes in allele frequencies, GBLUP resulted in an ~39% larger average response to selection across all lines. The higher response to selection and the larger changes in allele frequencies can, at least partially, be explained by the smaller effective population size of GBLUP compared with BLUP. However, when using the drift thresholds from gene dropping, all these differences were taken into account, and yet a higher number of selected regions were detected for GBLUP in each of the three replicate populations. This difference in number of selected regions therefore seems to be systematic. The response to GS depends on the initial allele frequency at the markers that are used and their LD to the QTL, whereas the response to BLUP selection depends on the initial allele frequencies at the QTL (Goddard, 2009). BLUP will not distinguish between QTL based on different levels of LD between these QTL and the SNPs, whereas GBLUP can focus on a subset of QTL, when these are in LD with the SNP set. While GBLUP can focus on a subset of QTL, it can also select on many QTL when many SNPs have strong LD with the QTL, such that the QTL will be effectively tagged for GBLUP. In such a situation, and with a large training set, GBLUP can predict most (perhaps all) of the variance explained by QTL. Our current results indicate that GBLUP has focused on a more limited set of QTL to select, compared with BLUP.

SNPs at extreme allele frequencies or linked to QTL of small effect are unlikely to be used in GBLUP, because these markers are usually not discovered as having an effect on the target trait (Goddard, 2009) and subsequently not selected to higher frequencies. With BLUP selection, all QTL are responding to selection, including those with very small effects, which results in small changes of allele frequencies near, potentially many, QTL positions.

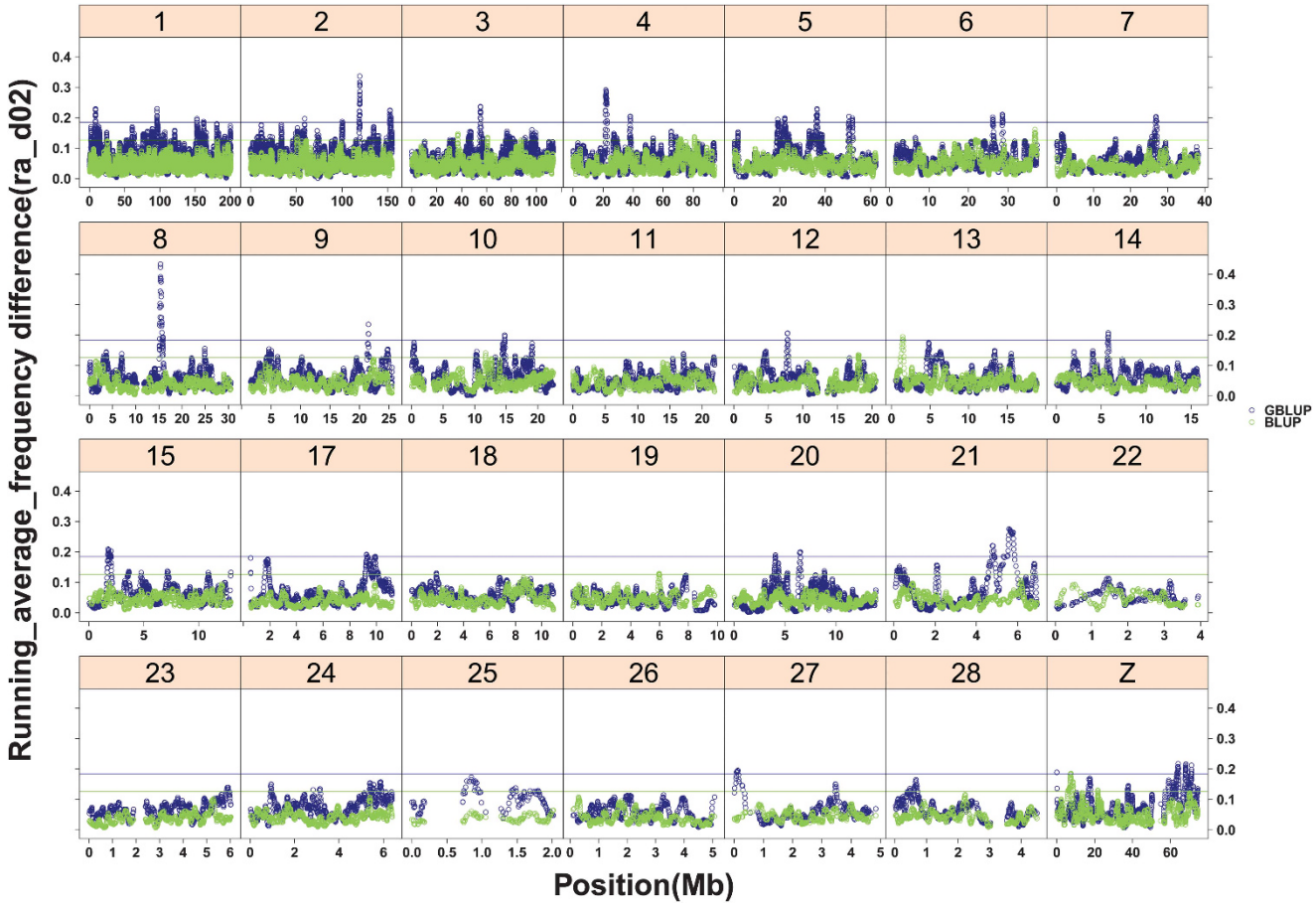

Figure 2 Pattern of genetic variation after two generations of selection for line B2. Running average of allele frequency distribution of 36582 SNPs (GBLUP) and 36731 SNPs (BLUP) along the whole genome is plotted against the physical position (Mb). The deviations above the threshold show signals of selection.

It appears that when GBLUP is progressing, it could lead to sequential waves of different regions being selected. In the long term, this may lead to suboptimal use of available genetic variation (Villanueva *et al.*, 2004). To sequentially select different regions, the effects of the SNPs need to change, which can happen when the model is retrained and effects are re-estimated. Continually re-estimating marker effects and including new markers in the breeding value prediction would be needed in the hope that new marker–QTL associations can be exploited (Goddard, 2009). In simulation studies (Muir, 2007; Sonesson and Meuwissen, 2009; Bastiaansen *et al.*, 2012), it was shown that if GS is practiced for many generations, without retraining, the rate of response will decline rapidly.

To distinguish a real selection signal from genetic drift, a suitable statistical method should be applied to distinguish whether observed changes in allele frequencies are the result of selection rather than random genetic drift. In this study, gene dropping through the real pedigree was used to set a threshold to differentiate regions under selection from fluctuations in allele frequencies that can be expected from genetic drift. Our simulation took into account the exact pedigree, to provide an empirical distribution of the changes in allele frequencies due to genetic drift for the pedigree under investigation. The threshold values were larger for GBLUP than BLUP, as expected from the smaller number of selected parents (smaller N_e). In addition, we found that selected parents for GBLUP were on average more related to each other than selected parents for BLUP (Table 9). This may seem counterintuitive, because GBLUP is expected to be better able to select across multiple families. However, selected parents of BLUP were from different generations and different hatch dates (overlapping generations), whereas for GBLUP, all selected parents were from one generation. Therefore, in this study, the relationship between selected parents for GBLUP was higher than for BLUP (Table 9). With fewer and more related parents selected for GBLUP, genetic drift had a much greater influence on allele frequency variation (Results section). However, the impact of drift was taken into account by applying the gene dropping method that accounted for the realized pedigree.

The observed d_{02} is a combination of effects from genetic drift and selection. If genetic drift and selection act in the same direction, we expect to see a large peak and if they act in the opposite direction, we may see a smaller peak. Separating the effects of drift and selection is not possible when only the sum of the two can be observed. However, using an estimate of the N_e, the SD_t of allele frequencies due to drift could be calculated, and with this SD_t, the observed d_{02} was standardized. The distribution of the observed d_{02} showed a larger variance than expected under drift, a clear indication that selection is affecting allele frequencies in both BLUP and GBLUP (Figure 5). The distribution of standardized d_{02} showed small but important differences between GBLUP and BLUP. GBLUP had a higher density than BLUP for both small values and large values of standardized d_{02}, whereas BLUP had a higher density at intermediate values of

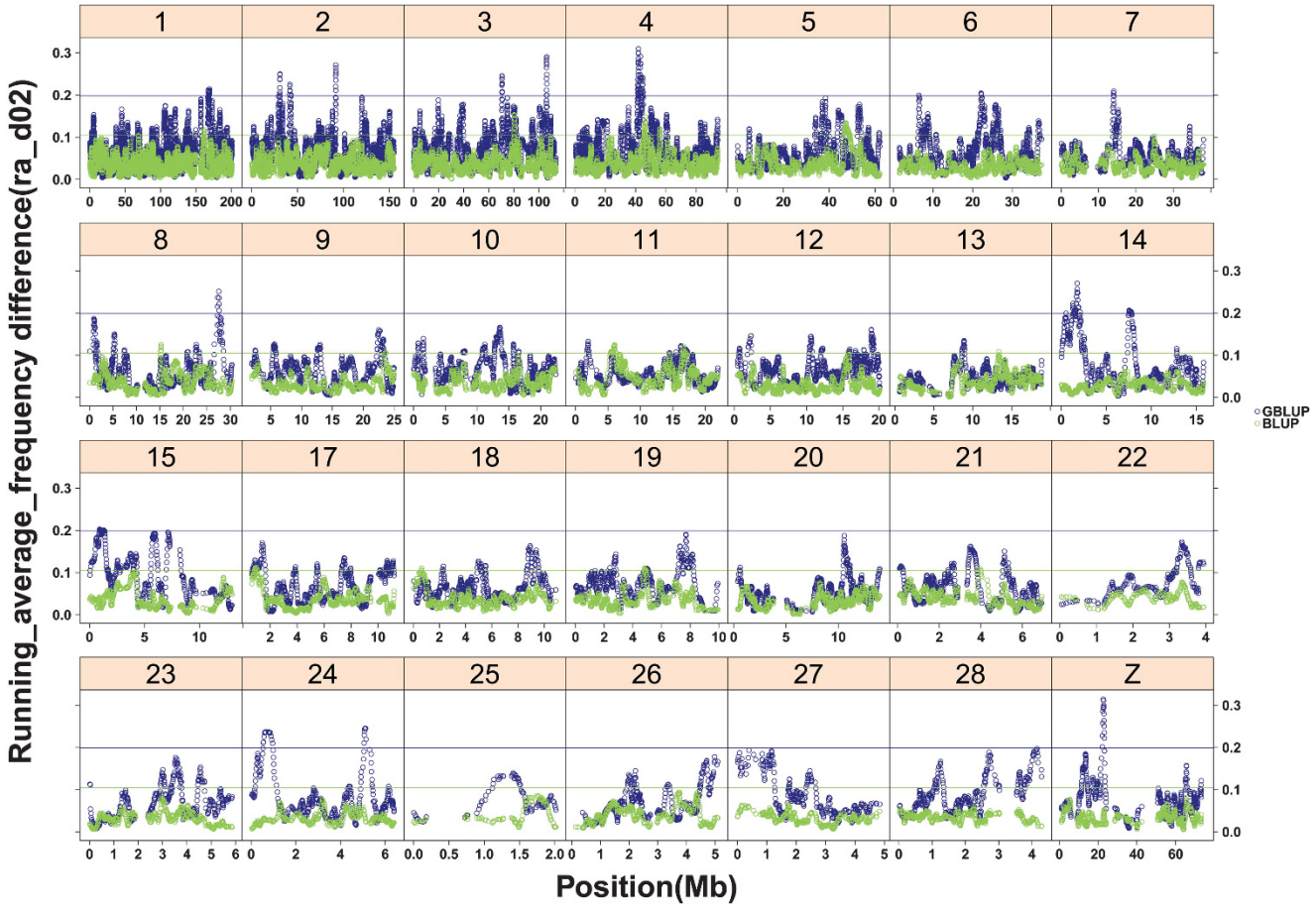

Figure 3 Pattern of genetic variation after two generations of selection for line W1. Running average of allele frequency distribution of 26 302 SNPs (GBLUP) and 26 337 SNPs (BLUP) along the whole genome is plotted against the physical position (Mb). The deviations above the threshold show signals of selection.

standardized d_{02}, roughly for values between 1.5 and 3.5. This result confirms the expectation that BLUP selects on all QTL that are affecting the index, whereas GBLUP appears to favor certain regions and ignores others. In the favoured regions, standardized d_{02} values were large, that is, more SNPs with standardized d_{02} above 4 for GBLUP compared with BLUP (Figure 6), and in the ignored regions, standardized d_{02} values were small, resulting in more SNPs with standardized d_{02} values near 0 for GBLUP compared with BLUP. Standardization was applied to correct for the differences in N_e between GBLUP and BLUP, so that remaining differences between the standardized d_{02} distributions were due to the method of selection. To confirm that standardization worked as expected, simulations were done with one of the training data sets, selecting a larger and smaller number of parents in two scenarios (resulting in different N_e). Observed d_{02} distributions showed the expected differences due to N_e, and we confirmed that after correction for N_e, the distributions of standardized d_{02} were comparable for the two scenarios with different N_e, both under selection on BLUP or GBLUP (results not shown). In addition, a simulation study by Liu *et al.* (2014) investigated the changes in allele frequency at QTL, SNPs and linked neutral loci with different selection methods; GBLUP and BLUP, in a population with equal N_e ($N_e = 200$) for both methods. They showed that after correction for drift, GBLUP moved the favorable alleles to fixation faster than BLUP and showed larger hitch-hiking effect than BLUP.

We asked whether the observed d_{02} peaks could be due primarily to selection and in an attempt to address this question, we tried to predict the additive effects responsible for the observed allele frequency peaks. This additive effect was estimated as:

$$a = \sigma_{\bar{i}} s/2i, \quad (2)$$

where $\sigma_{\bar{i}}$ was the s.d. of the index values for the candidates (males and females that could potentially be selected as fathers and mothers of next generation), s was the selection coefficient and i was selection intensity. s and i values for the allele frequency changes at peaks are given in Supplementary Material 8. Methods to calculate s and i are given in Supplementary notes. Note that as i was different for males and females, the average selection intensity for females and males was used. The predicted additive effects (standardized unit) that would cause the observed changes in allele frequencies were 0.28 on average (Supplementary Material 8). The variance explained by the five large peaks (5 loci) of each line was 2.3%, larger than typically reported variance explained by the associated SNPs. For example, for human height, the observed range of additive effects for 201 loci, as a percentage of genetic variance, was 0.04–1.13 (Park *et al.*, 2010). Hence, the genetic variance estimates for the peaks of d_{02} are likely to be over-estimated. Several possible explanations can be given for the overestimation of a from Equation (2). Selection coefficients can be overestimated due to several assumptions being made. Any

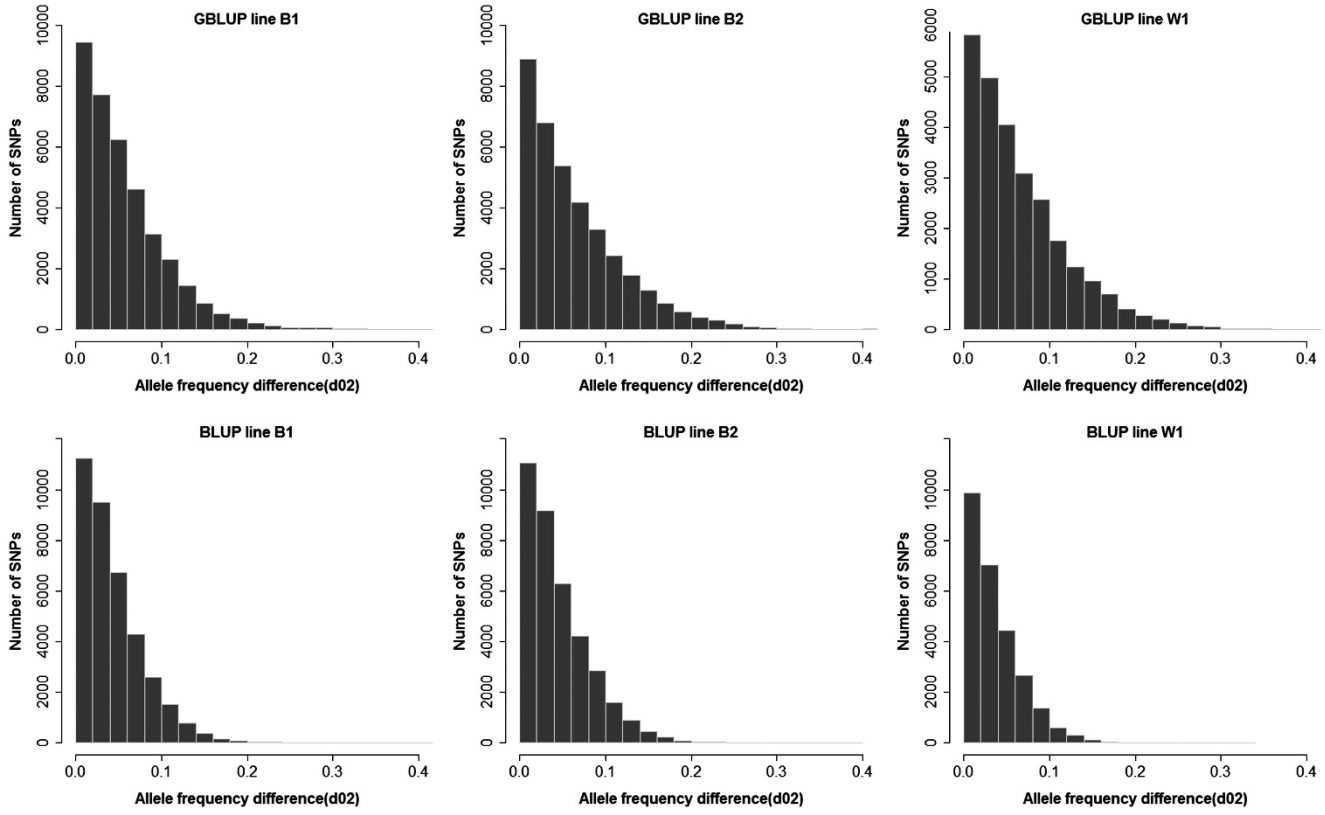

Figure 4 Distribution of d_{02} after two generations of selection on GBLUP or BLUP breeding values. On the *x* axis, d_{02} values are plotted and the number of SNPs is displayed on the *y* axis. The distribution of d_{02} values shows more extreme values for GBLUP than BLUP.

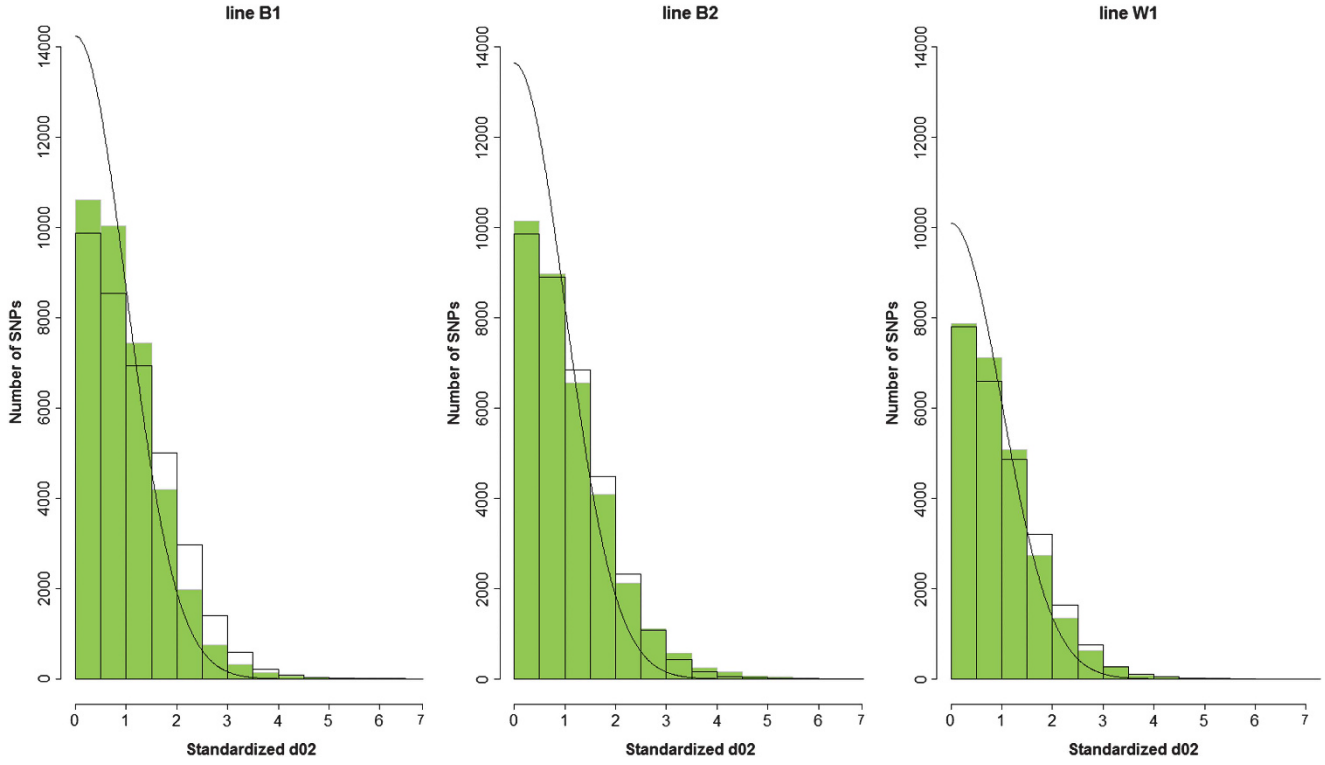

Figure 5 Distribution of standardized d_{02} (standardized based on drift s.d.) across all loci after two generations of selection of GBLUP (green bars) or BLUP (transparent bars). On the *x* axis, standardized d_{02} values are plotted and the number of SNPs is displayed on the *y* axis. The black solid line shows the expected variance of allele frequency changes under pure drift ($SD_t = 1$).

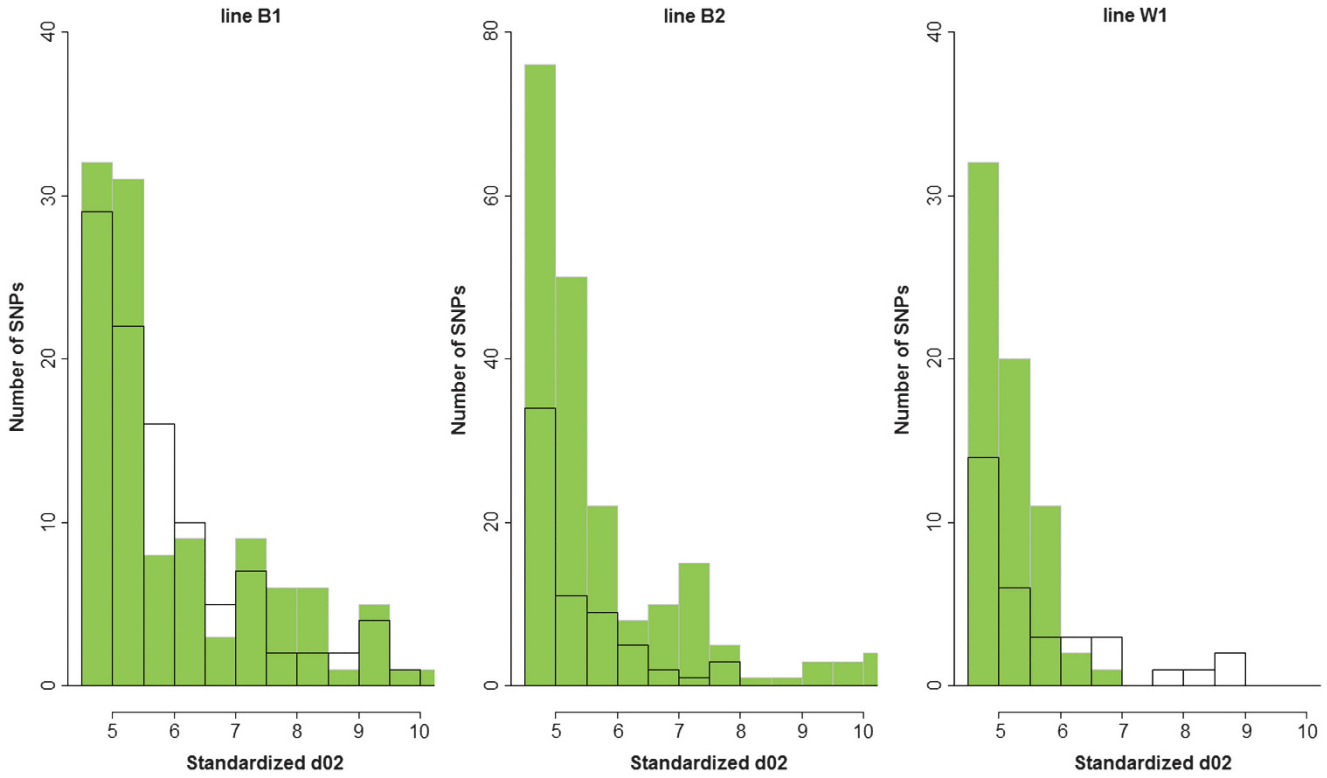

Figure 6 Distribution of standardized d_{02} (standardized based on drift s.d.) across loci with standardized $d_{02}>4$ (tail of distribution in Figure 5). Green bars shows the standardized d_{02} values of GBLUP and transparent bars shows the standardized d_{02} of BLUP. On the *x* axis, standardized d_{02} values are plotted and the number of SNPs is displayed on the *y* axis.

Table 7 Number of selected regions, number of SNPs in selected regions and the average length of selected regions for lines B1, B2 and W1

Line	*GBLUP*			*BLUP*		
	n	*Number of SNPs in selected regions*	*Average length (kb)*	n	*Number of SNPs in selected regions*	*Average length (kb)*
B1	24	240	518	10	88	643
B2	30	283	360	12	102	384
W1	16	204	645	13	162	527

Abbreviations: BLUP, best linear unbiased prediction; *n*, number of selected regions exceeding the drift threshold; GBLUP, genomic best linear unbiased prediction.

Table 8 Divergence between different lines using Fst values

Method	*G0*			*G2*		
	B2 and W1	*B1 and B2*	*B1 and W1*	*B2 and W1*	*B1 and B2*	*B1 and W1*
GBLUP	0.30	0.09	0.29	0.30	0.11	0.30
BLUP	0.29	0.08	0.28	0.29	0.10	0.30

Abbreviations: BLUP, best linear unbiased prediction; GBLUP, genomic best linear unbiased prediction; G0, base generation; G2, second generation.

effects of drift on the allele frequencies in the selected regions are attributed to the additive effect of a single gene, whereas the combined effect of several linked genes on d_{02} may have been observed. Other assumptions for the use of Equation (2) are that the allele frequency change was slow and that the selection coefficient was considered to be against an unfavorable homozygote. The large observed changes in allele frequencies should therefore be interpreted as the result of the combined action of drift and selection on a region that may contain multiple favorable alleles.

Table 9 Average genomic relationship between selected parents of G2-GBLUP and G2-BLUP

Line	*G2-GBLUP*[a]	*G2-BLUP*[b]
B1	0.066	0.040
B2	0.074	0.053
W1	0.092	0.037

Abbreviations: BLUP, best linear unbiased prediction; GBLUP, genomic best linear unbiased prediction.
[a]G2-GBLUP is the second generation of GBLUP.
[b]G2-BLUP is the second generation of BLUP.

QTL are discovered across the whole genome and therefore a random distribution of selection regions across the genome due to different contributions of regions to the variance was expected. Most significant selected regions were found in macrochromosomes (chromosomes 1, 2, 3, 4 and Z), which can be attributed to the fact that macrochromosomes form about 80% of the chicken genome. Moreover, there is less recombination in macrochromosomes compared with microchromosomes (Groenen *et al.*, 2009; Megens *et al.*, 2009) and regions under strong selection, which are located in genomic regions with low recombination rate (macrochromosomes) will be more readily detected, because they affect a wider window of SNPs.

All lines were under selection for the same traits and two of the lines (B1 and B2) were found to be more related to each other than to the other line based on Fst values (Table 8). However, only few selected regions overlapped, even between the two brown lines. This low level of concordance was surprising, but may be explained by the time since the B1 and B2 lines were split, ~15 generations ago. Both lines were selected during this period, which may have changed their genetic architecture, especially at loci that are important for the selection index. The historical separation of the lines leads to a number of possible reasons for lack of concordance. First, because selection is based on indexed phenotypes that include multiple traits, this leads to a large number of loci that are potentially selected. Chevin and Hospital (2008) showed that for quantitative traits, selection at specific quantitative trait loci may strongly vary in time and depend on the genetic background of the trait. Second, different lines can have differences in initial allele frequencies for potentially favorable alleles, resulting in differences in selection response. Starting allele frequencies are different between lines. Third, some lack of concordance might be due to the small effect of some alleles that could not be detected by GS. It is expected that the frequency of loci with the largest effects would rise more rapidly in the population and reach the detection threshold (Johansson *et al.*, 2010). Fourth, specific variants might have different effects in different lines. Fifth, epistatic interactions may change the allele substitution effect of the QTL, and therefore change the marginal effect of the marker.

In addition to the lack of concordance between different lines, overlap of selected regions was also limited between the two methods within each line. The correlation of d_{02} values from the two methods, within each line was small: 0.16 for line B1, 0.11 for line B2 and 0.15 for line W1. These correlations are positive but have low values, reflecting the differences in response to selection for the two methods (Figures 1–3).

Previous studies have shown the effects of selection on genetic variability (Rubin *et al.*, 2010; Elferink *et al.*, 2012). These studies analyzed the variation in the current populations to discover the impact of past selection. Congruence between these previous studies and the current study would provide confirmation that selection is the major cause for changes in allele frequencies at these overlapping selected regions. Of our 70 selected regions identified by GBLUP, 16 overlapped with regions that showed evidence of past selection (Amaral, 2010; Rubin *et al.*, 2010; Elferink *et al.*, 2012) (Supplementary Material 9). Four of the 16 overlapped regions had very high d_{02} in our results. Given the low concordance of selected regions even within the same line selected with different methods, the low concordance with other studies, applying different analyses in different populations, is not surprising. The most likely reason for the limited overlap with previous studies is that these previous studies aimed to identify regions where variation was presumably present in ancestral populations and was largely swept from the population. In our current experiment, the variation that was still available after historic selection and domestication was used to generate phenotypic change. When variation is already swept from the population, it will not contribute to current genetic progress.

Our experiment gives insight into how genomes respond to selection in general, and specifically how that response to selection is different if breeding values are estimated with or without genomic information. Not only will this allow a better use of knowledge on genomic variation in breeding programs, but it may also lead to identification of possible constraints related to the genome architecture (for example, recombination landscape), and to (local) inbreeding effects.

CONCLUSION

Seventy regions with evidence of selection were detected within the layer genome after selection by GBLUP compared with only 35 regions after selection by BLUP. With similar selection intensities, GBLUP directed selection pressure more locally than BLUP, favouring certain regions and ignoring others, whereas BLUP spreads the selection pressure more evenly along the genome. This localized selection pressure may lead to sequential waves of changing allele frequencies with unknown implications for the available genetic variation. The opportunity to select on GEBVs, before phenotypes of selection candidates are available, does require careful consideration of these issues, while at the same time includes promises for genetic improvement, as well as the understanding of genetic response to selection.

DATA ARCHIVING

The data will be available on request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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