Genomic signatures of 60 years of bidirectional selection for 8-week body weight in chickens

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ABSTRACT Sixty years, constituting 60 generations, have passed since the founding of the Virginia body weight lines, an experimental population of White Plymouth Rock chickens. Using a stringent breeding scheme for divergent 8-week body weight, the lines, which originated from a common founder population, have responded to bidirectional selection with an approximate 15-fold difference in the selected trait. They provide a model system to study the genetics of complex traits in general and the influences of artificial selection on quantitative genetic architectures in particular. As we reflect on the 60th anniversary of the initiation of the Virginia body weight lines, there is opportunity to discuss the findings obtained using different analytical and experimental genetic and genomic strategies and integrate them with a recent pooled genome resequencing dataset. Hundreds of regions across the genome show differentiation between the 2 lines, reinforcing previous findings that response to selection relied on standing variation across many genes and giving insights into the haplotype complexity underlying regions associated with body weight.

Key words: body weight, quantitative trait, polygenic selection, chicken, haplotype complexity

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INTRODUCTION

In the study of complex traits, different approaches have been used across innumerable organisms to uncover the genetic basis of phenotypic variation. The genetic factors influencing long-term selection of a quantitative trait have been explored using various analytical methods in the Virginia body weight lines, an experimental population of White Plymouth Rock chickens, established in 1957. With a generation interval of one yr, 2017 marks the hatching of the 60th generation. The 2 lines, high weight selected (**HWS**) and low weight selected (**LWS**), were selectively bred for divergent body weight at 8 wk of age with a breeding regime structured so to reduce inbreeding and the subsequent stochastic fixation of alleles typical for small breeding populations (Siegel, 1962; Marquez et al., 2010). Selection, when expressed in standard deviation units, reflects a 2-fold difference for both lines, which is essentially a 15-fold difference between the means (Jambui et al., 2017a).

Response to bidirectional selection in the lines has been strong (Figure 1). Although the HWS continued to show some generational increase in BW until the last couple of generations, the LWS appears to have plateaued after generation 30. The plateau in LWS is the likely result of anorexia, resulting in higher mortality of the chicks and reduction in birds reaching sexual maturity (Zelenka et al., 1988). Differences in feed intake and feed efficiency between the lines became apparent in generation 5 (Siegel and Wisman, 1966), and beginning in generation 18, feed intake in the HWS was restricted after 8 wk to address obesity and reproductive issues (Dunnington and Siegel, 1996). The interface of age, body composition, and BW with sexual maturity in the selected and relaxed lines have been discussed by Liu et al. (1995), Dunnington and Siegel (1996), and Jambui et al. (2017b).

The Virginia body weight lines represent a unique resource to investigate the genetic architecture of this complex trait and the consequences of long-term selection. The genetics underlying response to selection within these lines are well documented in the earlier literature and demonstrate that many loci contribute to the observable differences in BW. Initial QTL mapping revealed 13 loci affecting growth in an F_2 population (crossed from HWS and LWS at generation 41; Jacobsson et al., 2004; Jacobsson et al., 2005). Sweep scans have revealed over 100 regions of differentiation (Wahlberg et al., 2009; Johansson et al., 2010;

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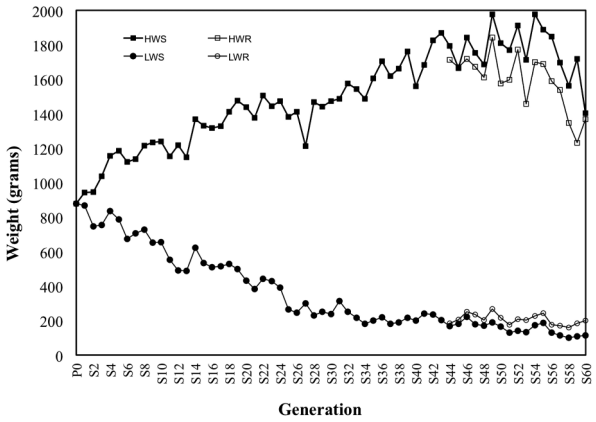

Figure 1. Mean 8-week BW (BW8) for high body weight selected (HWS) males (filled squares) and low body weight selected (LWS) males (filled circles), and high body weight relaxed (HWR) males (empty squares) and low body weight relaxed (LWR) males (empty circles). Selected generation (SX) indicated on the x-axis, starting from the mean body weight of the founders (P0). (Note: Relaxed lines, generated from selected generation 44, are plotted relative to selected generations, e.g., relaxed generation 0 is concurrent to selected generation 44).

Pettersson et al., 2013), which demonstrate widespread selection on standing genetic variants. From these differentiated and fixed regions in the lines, 16 showed significant association to BW in the F_{15} of an advanced intercross line (Sheng et al., 2015). Zan et al. (2017) extended this analysis also to include QTL regions, revealing 20 independent and 2 epistatic loci associated with the selected trait these cross. Allelic complexity across these loci suggests that multiple alleles, linked loci, and epistasis contribute to the polygenic architecture of body weight in the intercross (Zan et al., 2017). Though it may appear that only a small proportion of the differentiated loci were associated with the selected trait, the power is limited in an intercross analysis of <1,000 individuals. This means that it is expected that the many small effect loci of this highly polygenic trait that together contribute the 40% unexplained additive genetic variance in this population will remain undetected.

To explore the wider genomic impact of these processes, a pooled genome approach was employed to investigate the genomic signatures in regions associated with BW in combination with previous SNP-based studies, candidate selective sweeps, and regions of extreme differentiation in these lines. Thus, we aim to further understand the consequences of long-term divergent selection for this complex trait with a moderate heritability by integrating and discussing findings obtained from different analytical and experimental strategies.

MATERIALS AND METHODS

All animal procedures were carried out by experienced handlers and in accordance with the Virginia Tech Animal Care Committee animal use protocols (IACUC-15–136).

Table 1. Pooled population samples and genome information.

Sample[1]	Number of individuals	Coverage	Heterozygosity[2]	Nucleotide diversity (π) (%)[3]
HWS40	29	28.6	0.195	0.205
50	30	21.0	0.168	0.180
55	30	27.7	0.169	0.178
HWR09	30	29.8	0.158	0.165
11	19	35.1	0.152	0.156
LWS40	30	27.4	0.210	0.223
50	9	30.7	0.182	0.193
55	30	26.5	0.176	0.186
LWR09	30	32.2	0.184	0.187
11	30	28.1	0.169	0.178

[1]HWS: high body weight selected line; HWR: high body weight relaxed line; LWS: low body weight selected line; LWR: low body weight relaxed line; numbers indicate generation number within respective line.
[2]Mean genome-wide expected heterozygosity.
[3]Nucleotide diversity calculated over 5,000 bp windows, averaged across genome (%).

Selection Lines

The founder population for the Virginia body weight lines was comprised of the progeny of crosses of 7 partially inbred lines of White Plymouth Rock chickens (Siegel, 1962). Selection was initiated for high and low BW at 8 wk of age, and the resulting closed lines were designated HWS and LWS. The initial breeding regime used 8 sires and 48 dams from generations 1 to 4, then 12 and 48 from generations 5 to 25, and 14 and 56 from generation 26 onwards (Dunnington and Siegel, 1996). Each generation was hatched on the first Tuesday in March. A second "insurance" hatch 2 wk later was used if there were inadequate numbers (approximately 150 to 250 individuals per line) in the first hatch (Dunnington et al., 2013). All generations were hatched in the same incubators and reared in the same pens, on the same diet. The relaxed sublines (**HWR** and **LWR**, respectively) for both HWS and LWS were generated from selected generation 44. The advanced intercross line (**AIL**) was initiated by reciprocal F_1 matings produced from HWS and LWS lines at generation 41 (Jacobsson et al., 2005; Park et al., 2006)

Samples

Chickens from selected generations 40 (**S40**), 50 (**S50**), 55 (**S55**), and from generations 9 and 11 from the relaxed line (**R09** and **R11**, respectively) were sampled for genomic analysis. DNA for the genomic analyses was prepared from blood samples collected from 9 to 30 individuals from each line and pooled in equimolar ratios prior to library construction (Table 1).

Sequencing and Genome Alignments

Genome sequencing library construction and sequencing were carried out by SciLifeLab (Uppsala, Sweden) using 2 lanes on an Illumina Hiseq 2500. Reads were aligned to the *Gallus gallus* genome (Galgal5; INSDC Assembly GCA_0,00002315.3, Dec 2015) using

BWA (Li and Durbin, 2009). Duplicates were marked with Picard (v1.92; http://picard.sourceforge.net). GATK (v3.3.0; McKenna et al., 2010) was used for realignment around indels. Genome alignments were visualized in IGV (v2.3.52; Robinson et al., 2011; Thorvaldsdottir et al., 2013). GATK UnifiedGenotyper was used to generate allele calls at all sites (option: emit all sites) and with ploidy = 30 (18 for LWS50) to account for the pooled genome sample. Sites were filtered to include only those with >10 and <100 reads, wherefrom allele frequency, heterozygosity, and pairwise F_{ST} between all populations were calculated. Samtools (Li et al., 2009; v1.1; Li, 2011) was used to generate mpileup files for PoPoolation2 (v1.201; Kofler et al., 2011b), which was used to calculate F_{ST} over 1,000 bp sliding windows with 50% overlap between the selected population samples using the Karlsson et al. (2007) method, with minimum count 3, minimum coverage 10, maximum coverage 100, and minimum coverage fraction 1. Nucleotide diversity (π) was calculated across 5,000 bp windows for each population pool using Popoolation (v1.2.2; Kofler et al., 2011a). Genomic/haplotypic signatures within regions of interest were visualized by adjusting allele frequencies as used in Lillie et al. (2017) (similar to the allele polarization step in the haplotype-block reconstruction approach used by Franssen et al., 2017) to the generation of lowest complexity, then plotted using custom R scripts.

Differentiated Regions

Windows with F_{ST} greater than the 95% percentile of F_{ST} values in generation 55 (F_{ST} cutoff = 0.953) were used to limit the number of candidate regions due to drift. Windows with F_{ST} values above this cutoff were clustered into differentiated regions when less than 100 kb from each another. Clusters with less than 2 SNP or less than 100 kb were removed from the dataset to retain only the strongest candidate regions. Mean and median heterozygosity were calculated for each line within each differentiated region. Previously reported chicken body weight QTL (downloaded from the chicken QTL database; Hu et al., 2016) were compared to differentiated regions to determine overlaps using bed file comparisons in BEDOPS (Neph et al., 2012).

RESULTS AND DISCUSSION

General Genome Diversity

The pooled sequencing approach yielded between 21 × to 35 × genome coverage across the generation samples (Table 1). After filtering, a set of 7,656,897 SNP sites that showed polymorphism across the whole pooled genome dataset was retained, providing allele frequency estimates for all sequenced generations at each site.

The general decline in heterozygosity and nucleotide diversity observed in later generations (Table 1) was similar to that reported previously from 60k SNP data (Pettersson et al., 2013). There was a greater decline in diversity in the relaxed than in the selected lines. This may reflect a greater degree of inbreeding in the relaxed lines, which experienced an altered breeding scheme since the relaxation of selection. The selected lines are pedigree mated with restricted truncated selection to reduce inbreeding, whereas the mating populations for the relaxed lines are smaller and used pooled semen (Dunnington and Siegel, 1996; Marquez et al., 2010). The use of pooled semen introduces the possibility of sperm competition with some males contributing more progeny to the next generation than others.

Diversity was also higher in the LWS than in the HWS line. This could indicate that the HWS line experienced more fixation events, or it could also reflect the limits of selection for low BW. A selection plateau has been observed in the LWS line from approximately generation 30, and a proportion of each generation could never reproduce because they did not reach sexual maturity. Taken together, this may indicate a situation in which fixation for low weight haplotypes cannot be realized in this population, leading to the maintenance of relatively more diversity.

Genomic Footprint of Long-Term Divergent Selection

The first DNA-based measures of the genetic divergence between the BW lines were obtained after 31 generations using DNA fingerprinting (Dunnington et al., 1990; Haberfeld et al., 1992) and after 40 generations using 4777 SNP markers (Wahlberg et al., 2009). A comprehensive, genome-wide selective sweep analysis was later conducted employing the 60k SNP chip (Groenen et al., 2011) across generations 40 and 50. This chip reports 54,293 genotypable SNP markers across autosomes GGA1 to GGA28, the sex chromosomes, and 2 linkage groups, from which 32,846 were polymorphic in generation 40 (Johansson et al., 2010). Potential selective sweeps were characterized by clustering SNP fixed for alternative alleles together when there was a maximum of 1Mb (Pettersson et al., 2013) between subsequent fixed SNP and at least 2 SNP per cluster (or a more stringent 5 SNP per cluster) (Johansson et al., 2010). This methodology and the stringent criterion identified 65 clusters in generation 40, covering 8.6% of the chicken genome, which increased to 102 clusters by generation 50 (Johansson et al., 2010).

The general dynamics of these sweep regions also have been characterized, including allele frequency changes, linkage disequilibrium (**LD**) block structure, and divergence between time points, by integrating 60k SNP chip genotyping of generation 53 of the selected lines and generation 9 of the relaxed lines (Pettersson et al., 2013). There was a general trend

of small allele frequency changes across a large number of loci, with the exception of a region from 167 Mb to 168 Mb on chromosome 1, which displayed a rapid allele frequency change and represents the strongest candidate of recent selection in HWS (Pettersson et al., 2013). The LD blocks identified were long and unique to the selected line, reflecting that distinctive selective histories have influenced the genomes within each line over the course of the selection experiment.

Regions with high differentiation between lines suggest strong sweep candidates containing polymorphisms contributing to the selection response. They also could result from unavoidable inbreeding and/or random genetic drift given the population sizes of the lines. To identify those with associations with 8-week BW, 252 SNP markers in 99 genomic regions [corresponding to 106 divergent regions defined by Johansson et al. (2010)], Sheng et al. (2015) genotyped in 825 individuals from the F_{15} generation of the AIL. In total, 16 regions showed an association with 8-week BW (with 20% FDR), explaining up to 51% of the total additive genetic variance in this intercross (Sheng et al., 2015).

With the higher SNP density afforded by pooled genome sequencing, as well as covering more of the genome, here we identify additional regions of differentiation between HWS and LWS lines (Figure 2). Between 704 and 1,012 differentiation regions were revealed in the genome across the generations studied. After filtering for regions greater than 100 kb in length, 244 regions were retained in selected generation 40, covering 99.6 Mb or 8.1% of the genome (Supplementary Table 1). This increased to 370 differentiated regions in selected generation 50 (Supplementary Table 2), and 395 regions in generation 55, which covered 174.5 Mb, or 14.2% of the genome (Supplementary Table 3). Due to the sample size in LWS50 (9 individuals), emphasis will be on comparisons between selected generations 40 and 55.

The candidate sweep regions identified by Johansson et al. (2010) were mainly congruent with differentiated regions defined by the current pooled genome approach. With the greater SNP density and genome-wide coverage, there is better ability to define additional regions of differentiation and investigate the haplotypic diversity therein. The higher resolution made it possible to use a smaller distance threshold to define differentiated regions, useful at indicating the underlying haplotypic complexity (Figure 3). Figure 3 shows the differences in haplotype complexity underlying 2 candidate sweep regions of similar length. For the region on GGA5 between 40 to 44 Mb, the region of differentiation extends from approximately 40.7 to 43.3 Mb. Within both lines, there is very low heterozygosity, and through adjusted allele frequencies, we can ascertain that the lines are fixed for alternative haplotypes within this differentiated region. By comparing LWS40 with LWS55, it is evident that there was another haplotype present in generation 40 that shared this central region, which was lost by generation 55. Figure 3B shows a cluster of differentiated regions extending from approximately 9.5 to 12 Mb on GGA6. The major haplotype in this region was fixed in the LWS lines by generation 40. In contrast, several haplotypes continue to segregate in HWS, contributing to the disrupted differentiation signature. When comparing generations 40 and 55, the haplotype frequency change resulted in fixation of the region 9.4 to 10.1 Mb, which is highly differentiated from that in LWS. These examples demonstrate the variation in haplotype structures that underlie differentiated regions observed in the pooled genome resequencing of the lines. In the case of GGA5, an approximately 3 Mb region was fixed for alternative haplotypes by generation 55, whereas on GGA6, an approximately 0.7 Mb region fixed for alternative haplotypes was fixed by generation 55, with multiple haplotypes continuing to segregate in HWS.

Genomic Explorations of Regions Associated with the Selected Trait, 8-Week Body Weight

Genomic signatures of differentiation and haplotypic complexities have been useful in gauging the overall impact of bidirectional selection on the genomes of the Virginia body weight lines. Key insights are now attainable by investigating regions with supported associations to the selected trait, 8-week BW. Below is a summary of associated regions previously identified in the Virginia body weight lines and a description of the signature of selection revealed within these by pooled genome sequencing.

The first studies to map the genetic basis of the quantitative trait variation in the lines were based on DNA fingerprinting in crosses between HWS and LWS individuals after 31 generations of selection (Dunnington et al., 1992; Dunnington et al., 1993). These were followed by genome-wide QTL mapping studies in an F_2 cross of HWS and LWS individuals from generation 40 using 145 microsatellite markers. Loci were mapped for BW and growth-rate measurements from hatch to 70 d of age. Five significant and 8 suggestive QTL for growth were identified and designated *Growth1* to *Growth13* (Jacobsson et al., 2005). Implementing an improved high-density linkage map and additional SNP markers with wider genome coverage (434 genetic markers polymorphic in the lines, covering 31 chromosomes), Wahlberg et al. (2009) confirmed *Growth1*, *4*, *6*, *7*, *9,* and *12* to have significant or suggestive contributions to these growth traits. They provided circumstantial evidence supporting the existence of *Growth2, 3*, and *8* as well, but concluded that the remaining 4 may reflect false positives in earlier work that were vetted in subsequent studies by using denser markers.

To facilitate fine-mapping of QTL identified from the F_2 cross, the AIL was generated from HWS and LWS individuals from generation 40 to introduce more

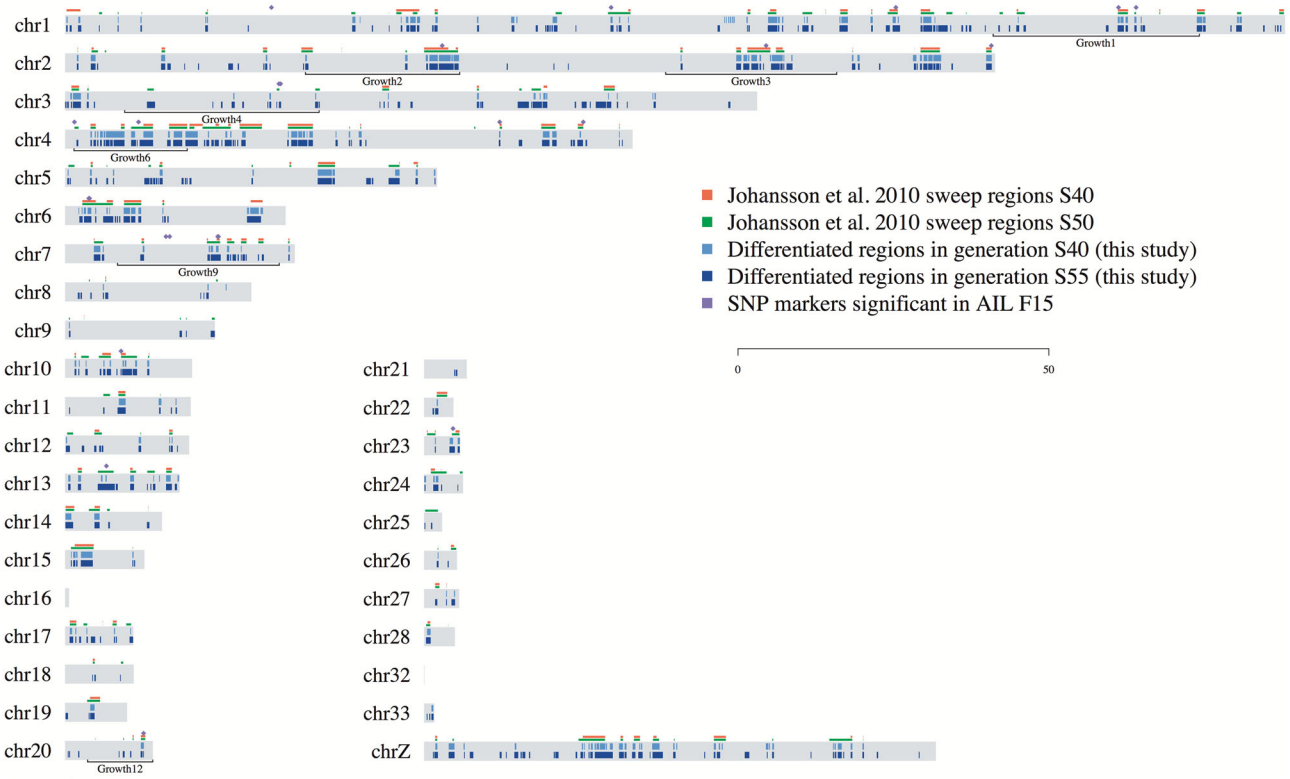

Figure 2. Distribution of differentiated regions across the chicken chromosomes (gray). Candidate sweep regions in generations 40 and 50 defined by Johansson et al. (2010) are represented by orange and red bars, respectively. Differentiated regions between HWS and LWS in generations 40 and 55, defined in this study, are represented by light blue and blue bars, respectively. Locations of SNP markers with significant associations to BW in intercross (Zan et al., 2017) are indicated with purple points above the gray chromosomes. GrowthX are names of QTL, as in Jacobsson et al. (2005), mapped in an intercross between the HWS and LWS at generation 40. Scale indicated is in megabases on the galgal5 genome assembly.

recombination events into the pedigree and decrease LD between markers and QTL, thus improving genomic resolution. Focusing on 9 genomic regions containing QTL (specifically *Growth1*, *2*, *3*, *4*, *6*, *7*, *8*, *9*, and *12*), 384 segregating SNP (average distance less than 1 cM between markers) were used to genotype 1,529 individuals from the F_2 to F_8 generations of the AIL (Besnier et al., 2011). By implementing a haplotype-based linkage mapping approach in a variance-component based model framework, 5 QTL (*Growth1*, *2*, *4*, *9*, and *12*) were confirmed with genome wide significance, and 4 were suggestive (Besnier et al., 2011). The size of *Growth1*, *2*, *4*, and *12* QTL were considerably decreased from the F_2 scan, and *Growth9* was fine-mapped into 2 distinct peaks (Besnier et al., 2011).

Association analysis in generations F_2 to F_8 of the AIL imputed to high-density genotypes provided strong implications that multiple alleles segregated within the HWS and LWS lines in several of the QTL, with several likely to contain multiple independent loci contributing to BW (Brandt et al., 2016). This was confirmed using data from generation F_{15}, where further fine mapping revealed that 5 of 9 explored QTL were either multiallelic, fine-mapped into multiple loci, or both (Zan et al., 2017). In addition, association analysis revealed 10 contributing loci associated with previously defined QTL and an additional 10 loci from putative sweep regions, which together explain approximately 30% of the phenotypic variance and more than 60% of the additive genetic variance (Zan et al., 2017).

To provide further support that differentiated regions are likely due to selection on body weight, previously mapped QTL for related traits in other populations can be compared to our differentiated regions. However, as the cumulative coverage of BW QTL reported in other populations spans the majority of the chicken genome, there is considerable overlap. Some QTL regions are, however, of greater interest because they have been either fine-mapped or identified in multiple populations. For instance, our differentiated region on chromosome 4 (76,706,500 to 77,903,000 bp) overlaps with recently fine-mapped BW QTL in an F_{10-12} intercross from inbred New Hampshire (NHI) and White Leghorn (WL77) lines (Lyu et al., 2017). This QTL has been replicated in other chicken populations, including a White Leghorn × Rhode Island Red cross (Sasaki et al., 2004), a Silky Fowl × White Plymouth Rock cross (Gu et al., 2011), and the Beijing-You line (Liu et al., 2013). This differentiated region also overlaps with an association to BW in a broiler x layer F_2 of Brazilian chicken populations (Pertille et al., 2017). We also saw overlap between differentiated regions on chromosome 1 (169,271,500 to 170,269,500 bp) and chromosome 27 (3,553,000 to 3,671,500 bp) and QTL mapped for BW,

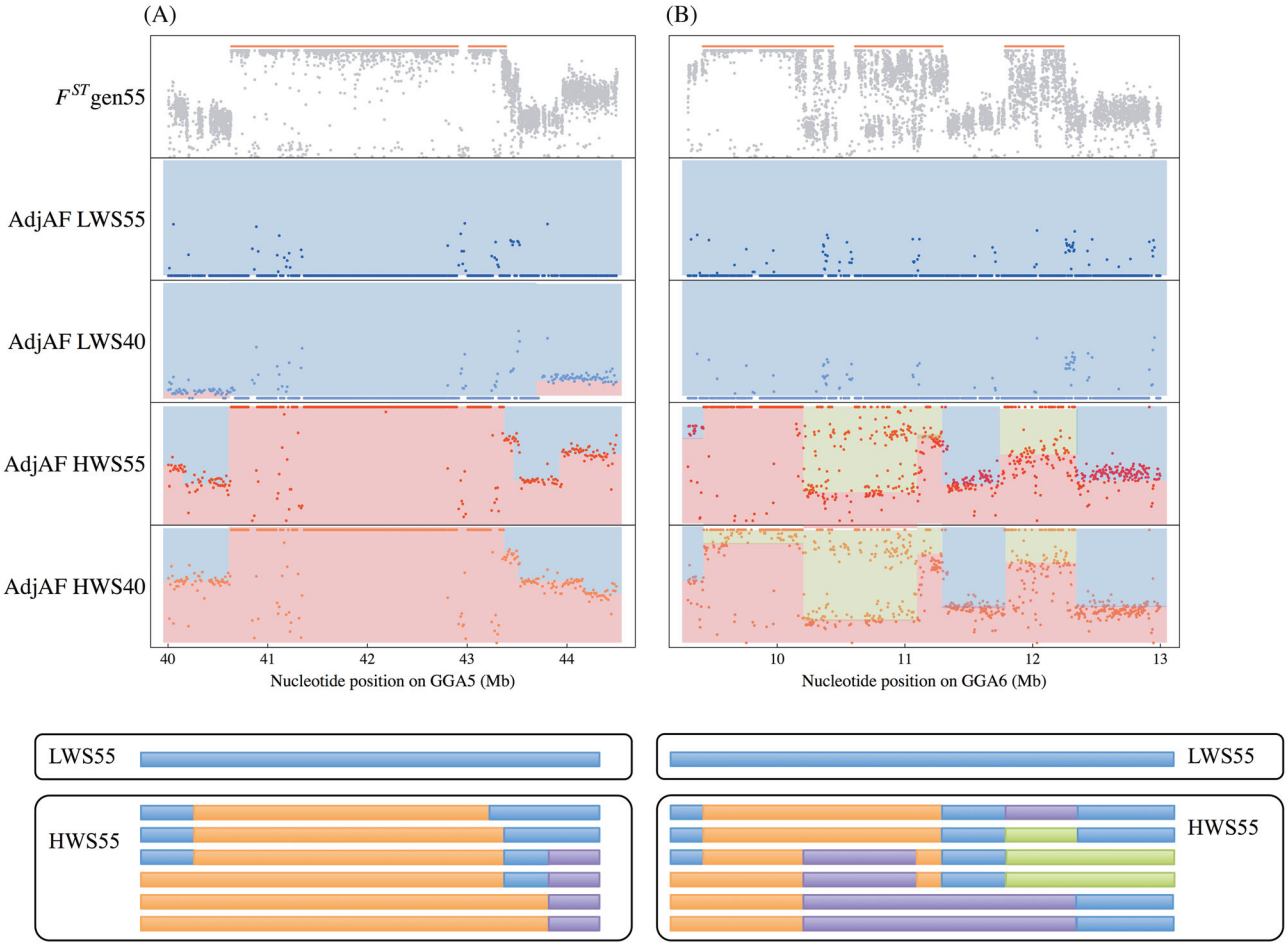

Figure 3. Comparison of population statistics across differentiated regions, revealing haplotype structure, including F_{ST} (0 to 1) between HWS55 and LWS55 and median adjusted allele frequency (0 to 1) in generations 40 and 55 for LWS and HWS, with conceptual schematic of haplotypes (lower panels) that could be contributing to these signatures. A. Regions 40 to 44.5 Mb on GGA5. The differentiated region results from fixation of different haplotypes between 40.5 and 43.2 Mb on this chromosome. B. Regions 9 to 13 Mb on GGA6. The differentiated regions between 9.5 and 12.5 Mb result from fixation of one haplotype in the LWS line, and the removal of this haplotype in the HWS line. More than one haplotype continues to segregate in the HWS line within this region, but they all share a common fixed haplotype between 9.5 and 10.5 Mb in the first region of differentiation indicated by the F_{ST}.

abdominal fat weight, and shank length and circumference in an F_2 cross between Huiyang Beard chickens and a commercial broiler line (Sheng et al., 2013). Indeed, this particular differentiated region on chromosome 1 (169,271,500 to 170,269,500 bp) frequently arises in association and QTL mapping studies for production traits, including growth (Liu et al., 2008; Podisi et al., 2013; Abdalhag et al., 2015), digestive system anatomy (Gao et al., 2009), feed intake (Yuan et al., 2015), and egg yolk weight (Wolc et al., 2014), suggesting that this region has important functions during development. Finally, a candidate functional locus was identified in the HWS population as part of a larger chicken sequencing study: a deletion in the gene *SH3RF2* (SH3 domain containing ring finger 2), which was significantly associated with increased growth and located within the *Growth12* region on chromosome 13 (Rubin et al., 2010).

In our study, pooled genome resequencing revealed that the haplotype complexity varied across the QTL regions (Table 2). A majority of differentiated regions overlapping QTL showed fixation for a single haplotype within one line, while the other line still segregates for multiple, alternative haplotypes. These signatures most likely reflect genetically variable founders, contributing multiple haplotypes with effects of different sizes (Sheng et al., 2015). For example, if a variant with large positive selective benefit in one line was present at low frequency in the founder population, once selection was imposed, a large region would become fixed in the line due to linkage. The hypothetical variant would have a reciprocally negative selective coefficient in the other line, which could quickly remove the haplotype this variant occurs on from the line. This would leave other haplotypes segregating in the line under neutrality, or if there was another variant with a positive effect in that line, it would increase in frequency, depending on its selection coefficient, and thus increase the frequency of all haplotypic backgrounds on which it occurs. Although we can speculate on these relative contributions to the observed genomic signatures, DNA

Table 2. Description of haplotypic complexity within body weight QTL regions as revealed in pooled genome resequencing data in generation 55 of high (HWS) and low (LWS) selected Virginia body weight lines samples.

QTL[1]	Chromosome	Coordinates (Mb)[2]	LWS55[3]	HWS55[4]	Figure[5]
Growth1	1	169 to 174	Fixed for one LWS haplotype	Multiple haplotypes differentiated from LWS	S1
Growth2	2	58 to 63	Fixed for one LWS haplotype	Close to fixation for one haplotype differentiated from LWS	S2
Growth3	2	108 to 117	Multiple haplotypes differentiated from those in HWS	Haplotypes share fixed regions 108 to 112 and 115.8 to 116.5 Mb differentiated from LWS	S3
Growth4	3	32.5 to 35	Multiple haplotypes differentiated from those in HWS	Haplotypes share fixed regions 32.5 to 33.2 and 33.9 to 34.7 Mb differentiated from LWS	S4
Growth6	4	4 to 14	Haplotypes share fixed regions 8 to 9 Mb and 12 to 14 Mb differentiated from HWS	Haplotypes share fixed regions 8 to 12 Mb differentiated from LWS	S5
Growth9	7	23 to 25	Multiple haplotypes differentiated from those in HWS	Fixed for one HWS haplotype	S6
Growth12	20	12 to 13	Fixed for one LWS haplotype	Multiple haplotypes differentiated from LWS	S7

[1]QTL name as in Jacobsson et al. (2005).
[2]Position as in galGal5 genome assembly.
[3]LWS55: low body weight selected line, generation 55.
[4]HWS55: high body weight selected line, generation 55.
[5]Supplementary figure providing detailed information on the QTL region.

samples prior to generation 40 are lacking, which precludes empirically tracing the selection history of the haplotypes. They do, however, demonstrate the breadth of standing genetic variation present in the founder population.

Concluding Remarks

Demonstrated here is the advantage of applying pooled whole-genome resequencing to selected populations, revealing more regions of differentiation and their underlying haplotypic structure than SNP-based methodologies. The pooled genome sequencing approach underscores previous research, again demonstrating that response to bidirectional selection in the Virginia high and low weight lines is highly polygenic and acts on standing genetic variation across many genomic regions. Furthermore, in-depth genomic analysis revealed regions in which multiple haplotypes have contributed to the response to selection for high or low 8-week BW. This selection experiment continues to be a valuable resource to increase our understanding of polygenic genetic architectures contributing to long-term selection responses. By integrating different study designs and analytical methods, it is evident that the extreme divergence in 8-week BW in these lines has relied on abundant standing genetic variation across the genome, functioning as multiple haplotypes, linked loci, and epistatic interactions.

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SUPPLEMENTARY DATA

Supplementary data are available at *Poultry Science* online.

Supplementary figure 1. Genomic signatures on chicken chromosome 1 in region of *Growth1*. Upper panel: FST in 1,000 bp windows between HWS and LWS at generation 55 represented by gray dots; region of differentiation highlighted with orange line above the plot; SNP positions with significant association to body weight in AIL F15 generation (Zan et al. bioRxiv) represented in blue above plot.

Middle panel: adjusted allele frequency in LWS55 represented by blue dots (allele frequency across region adjusted against LWS55; median over 200 bp windows).

Lower panel: adjusted allele frequency in HWS55 (allele frequency across region adjusted against LWS55; median over 200 bp windows).

Supplementary figure 2. Genomic signatures on chicken chromosome 2 in region of *Growth2*. Upper panel: FST in 1,000 bp windows between HWS and LWS at generation 55 represented by gray dots; region of differentiation highlighted with orange line above the plot; SNP positions with significant association to body weight in AIL F15 generation (Zan et al. bioRxiv) represented in blue above plot.

Middle panel: adjusted allele frequency in LWS55 represented by blue dots (allele frequency across region adjusted against HWS55; median over 2,000 bp windows).

Lower panel: adjusted allele frequency in HWS55 (allele frequency across region adjusted against HWS55; median over 2000 bp windows).

Supplementary figure 3. Genomic signatures on chicken chromosome 2 in region of *Growth3*. Upper panel: FST in 1,000 bp windows between HWS and LWS at generation 55 represented by gray dots; region of differentiation highlighted with orange line above the plot; SNP positions with significant association to body weight in AIL F15 generation (Zan et al. bioRxiv) represented in blue above plot.

Middle panel: adjusted allele frequency in LWS55 represented by blue dots (allele frequency across region adjusted against LWS55; median over 1,000 bp windows).

Lower panel: adjusted allele frequency in HWS55 (allele frequency across region adjusted against LWS55; median over 1000 bp windows).

Supplementary figure 4. Genomic signatures on chicken chromosome 3 in region of *Growth4*. Upper panel: FST in 1,000 bp windows between HWS and LWS at generation 55 represented by gray dots; region of differentiation highlighted with orange line above the plot; SNP positions with significant association to body weight in AIL F15 generation (Zan et al. bioRxiv) represented in blue above plot.

Middle panel: adjusted allele frequency in LWS55 represented by blue dots (allele frequency across region adjusted against HWS55; median over 500 bp windows).

Lower panel: adjusted allele frequency in HWS55 (allele frequency across region adjusted against HWS55; median over 500 bp windows).

Supplementary figure 5. Genomic signatures on chicken chromosome 4 in region of *Growth6*. Upper panel: FST in 1,000 bp windows between HWS and LWS at generation 55 represented by gray dots; region of differentiation highlighted with orange line above the plot; SNP positions with significant association to body weight in AIL F15 generation (Zan et al. bioRxiv) represented in blue above plot.

Middle panel: adjusted allele frequency in LWS55 represented by blue dots (allele frequency across region adjusted against HWS55; median over 500 bp windows).

Lower panel: adjusted allele frequency in HWS55 (allele frequency across region adjusted against HWS55; median over 500 bp windows).

Supplementary figure 6. Genomic signatures on chicken chromosome 7 in region of *Growth9*. Upper panel: FST in 1,000 bp windows between HWS and LWS at generation 55 represented by gray dots; region of differentiation highlighted with orange line above the plot; SNP positions with significant association to body weight in AIL F15 generation (Zan et al. bioRxiv) represented in blue above plot.

Middle panel: adjusted allele frequency in LWS55 represented by blue dots (allele frequency across region adjusted against HWS55; median over 500 bp windows).

Lower panel: adjusted allele frequency in HWS55 (allele frequency across region adjusted against HWS55; median over 500 bp windows).

Supplementary figure 7. Genomic signatures on chicken chromosome 20 in region of *Growth12*. Upper panel: FST in 1,000 bp windows between HWS and LWS at generation 55 represented by gray dots; region of differentiation highlighted with orange line above the plot; SNP positions with significant association to body weight in AIL F15 generation (Zan et al. bioRxiv) represented in blue above plot.

Middle panel: adjusted allele frequency in LWS55 represented by blue dots (allele frequency across region adjusted against LWS55; median over 200 bp windows).

Lower panel: adjusted allele frequency in HWS55 (allele frequency across region adjusted against LWS55; median over 200 bp windows).

Supplementary Table 1. List of differentiated regions between Low Weight Selected (LWS) and High Weight Selected (HWS) Virginia body weight chicken lines at selected generation 40, including chromosome, start and end positions, the number of SNP within the region, and the length.

Supplementary Table 2. List of differentiated regions between Low Weight Selected (LWS) and High Weight Selected (HWS) Virginia body weight chicken lines at selected generation 50, including chromosome, start and end positions, the number of SNP within the region, and the length.

Supplementary Table 3. List of differentiated regions between Low Weight Selected (LWS) and High Weight Selected (HWS) Virginia body weight chicken lines at selected generation 55, including chromosome, start and end positions, the number of SNP within the region, and the length.

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