Artificial Selection Response due to Polygenic Adaptation from a Multilocus, Multiallelic Genetic Architecture

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Abstract

The ability of a population to adapt to changes in their living conditions, whether in nature or captivity, often depends on polymorphisms in multiple genes across the genome. In-depth studies of such polygenic adaptations are difficult in natural populations, but can be approached using the resources provided by artificial selection experiments. Here, we dissect the genetic mechanisms involved in long-term selection responses of the Virginia chicken lines, populations that after 40 generations of divergent selection for 56-day body weight display a 9-fold difference in the selected trait. In the F15 generation of an intercross between the divergent lines, 20 loci explained >60% of the additive genetic variance for the selected trait. We focused particularly on fine-mapping seven major QTL that replicated in this population and found that only two fine-mapped to single, bi-allelic loci; the other five contained linked loci, multiple alleles or were epistatic. This detailed dissection of the polygenic adaptations in the Virginia lines provides a deeper understanding of the range of different genome-wide mechanisms that have been involved in these long-term selection responses. The results illustrate that the genetic architecture of a highly polygenic trait can involve a broad range of genetic mechanisms, and that this can be the case even in a small population bred from founders with limited genetic diversity.

Key words: genetic variation, epistasis, multiallelic, genetic architecture, multilocus, polygenic adaptation.

Introduction

The genetics of adaptation has directed a wide field of research for decades (Olson-Manning et al. 2012; Pardo-Díaz et al. 2015). Studies in natural populations have, for example, provided insights to geographical adaptations in Arabidopsis thaliana (Fournier-Level et al. 2011; Barboza et al. 2013; Shen et al. 2014; Forsberg et al. 2015), beak size in the Darwin finch (Lamichhaney, Berglund, et al. 2015), and alternative reproductive strategies in ruff (Lamichhaney, Fan, et al. 2015). In the pursuit of understanding the genetics and genomic underpinnings, the field has seen examples of adaptation based on few large effect QTLs (Nadeau and Jiggins 2010), accompanied with successful dissection of the molecular mechanisms underlying traits (color polymorphism in the peppered moth (Hof et al. 2016); armor plating in sticklebacks (Colosimo 2005); coat color in mice in different environmental substrates (Steiner et al. 2007; Linnen et al. 2013)). Thoroughly dissected adaptive loci usually have large individual effects (Rockman 2012; Pardo-Díaz et al. 2015), however, in other cases adaptation relies on many loci with small effect (Nadeau and Jiggins 2010) that are particularly found when considering fitness traits in e.g. mice, drosophila, human (Flint and Mackay 2009) and maize (Buckler et al. 2009). Local adaptation conferred by polygenic quantitative traits in such cases is difficult to characterize and identifying the genetic mechanisms and interactions that govern variation in such traits is challenging (Savolainen et al. 2013). We therefore still know little about how these mechanisms together contribute to natural and artificial polygenic adaptations: Are many of them likely to involve closely linked genes or multiallelic and/or epistatic loci? And/or will most minor loci contribute additively?

Complex trait genetics is particularly relevant for evolutionary adaptation, as many fitness-related phenotypes are genetically complex and most traits under selection are polygenic (Flint and Mackay 2009). Polygenic traits have the advantage that they can respond rapidly to change using the available standing variation (Teotónio et al. 2009; Orozco-Wengel et al. 2012; Burke et al. 2014). This may not even
require fixation, such that a population may reach a new adaptive optima via allele frequency changes across multiple loci (Pritchard and Di Rienzo 2010; Graves et al. 2017). Theoretically, this would preserve genetic diversity, readily available for the population to respond to future challenges (Whitlock 2015). Mutations with small effects are relatively difficult to detect, but are necessary to reach fitness optima (Collins et al. 2007; Kopp and Hermisson 2009) and adaptation can rely exclusively on these (Yeaman 2015). Another level of standing variation available for adaptation is that held in epistatic loci (Phillips 2008; Paaby and Rockman 2014). Although the relative contributions of epistatic loci to adaptation is poorly understood, the abundance of epistasis would suggest it is likely a contributing mechanism of the evolutionary process (Phillips 2008), and simulations show that epistasis could prolong the response to selection (Paixão and Barton 2016).

Experimentally adapted populations are powerful resources for dissecting the genetic basis of selection responses (Hill 2005). These closed populations, subjected to long-term artificial selection for clearly defined adaptive traits, will accumulate beneficial genetic variations at a more rapid rate than natural populations within controlled conditions. By mapping individual loci contributing to selection responses in these populations, we can get a better understanding of the genetic architecture (the number of loci and how they combine their effects) underlying complex polygenic traits and their contributions to adaptation and evolution. Modern genomics allows cost-efficient, in-depth characterization of genetic variation across many loci, or entire genomes, in large populations. In this way, populations obtained in long-term selection experiments have provided valuable insights to for example the genetics of growth adaptation in mice (Allan et al. 2005), chickens (Jacobsson et al. 2005; Johansson et al. 2010; Besnier et al. 2011), and seed protein and kernel oil level in maize (Laurie et al. 2004; Lucas et al. 2013).

Reported here is our most recent progress in the dissection of the genetic architecture contributing to differentiation in the single complex trait used to select individuals when breeding the Virginia body weight chicken lines. This population provides a model system to study the response of complex traits in higher order vertebrates to substantial selection pressure. These lines have been under bi-directional selection for 56-day body weight since 1957 (Dunnington and Siegel 1996; Márquez et al. 2010; Dunnington et al. 2013). In the current generation (selected generation 59) the lines display >16-fold difference in body weight, with earlier work having revealed many loci contributing to this difference (Johansson et al. 2010; Sheng et al. 2015), as well as allelic heterogeneity (Brandt et al. 2017) and epistasis (Pettersson et al. 2011).

In this study, we performed a multilocus fine-mapping analysis across nine earlier identified QTL in this population (Jacobsson et al. 2005; Besnier et al. 2011; Brandt et al. 2017), using data from the F15 generation of the Advanced Intercross Line (AIL) formed from high and low line founders of generation 40. Novel insights are provided to the range of genetic mechanisms contributing to the long-term, polygenic adaptation of the divergently selected lines. In particular, we show how major loci contributing to the selection response contain tightly linked loci, multiple adaptive haplotypes and are involved in epistatic interactions. This demonstrates the importance to consider a broad range of genetic mechanisms to further our understanding about adaptation even in relatively small, closed populations.

Results

The Virginia Chicken-Lines: An Experimental Population to Study Polygenic Adaptation

We employed a multilocus backward-elimination analysis to scan for independent associations across 216 markers in nine targeted QTL and an additional 218 markers from selective-sweeps located elsewhere in the genome (Sheng et al. 2015) to control for genetic background effects outside the QTL. In total, 24 SNP markers with statistically independent associations to 56-day body weight were found at 20% False Discovery Rate (FDR) within 20 independent loci (fig. 1). Of these, 13 were located in seven of the nine focal QTL (supplementary table S1, Supplementary Material online), and 11 in selective-sweeps elsewhere in the genome (supplementary table S2, Supplementary Material online). Two additional loci that did not have significant marginal effects were found to be epistatic (fig. 1 and supplementary fig. S3, Supplementary Material online).

Genetic Architectures of the Adaptive QTL

We tested for associations to each of the SNP markers within the nine QTL to be fine mapped in the AIL F15 generation, and compared these profiles to those seen in the F2 (Wahlberg et al. 2009). This provides insight to how common linkages between independent loci were in the major adaptive loci in the genome. All markers detected at 20% FDR in the multilocus bootstrapping analysis were fitted as fixed effects to account for the polygenic genetic architecture of 56-day body weight in this population. Two QTL, Growth2 on GGA2 and Growth3 on GGA2 (Jacobsson et al. 2005), fine mapped to single, bi-allelic loci (fig. 1; supplementary fig. S1 and table S1, Supplementary Material online). More complex genetic architectures involving multiple linked loci and segregation of multiple alleles in at least one of the founder lines were revealed in the other five QTL (Growth1 on GGA1, Growth4 on GGA3, Growth6 on GGA4, Growth9 on GGA7 and Growth12 on GGA20; fig. 1; supplementary table S1 and fig. S2, Supplementary Material online).

Most QTL Covered Multiple Adaptive Loci Highlighting the Importance of Linkage among Standing Variants for Selection Response

The GGA1 QTL, Growth1, detected in the F2 generation of our intercross (Jacobsson et al. 2005; Wahlberg et al. 2009) fine mapped to three loci (two revealed in the backward elimination analysis and one in the single marker QTL scan) in a 7 Mb region in the F15 generation (fig. 1; supplementary fig. S2A and table S1, Supplementary Material online). Similarly, the
GGA20 F2 QTL, Growth12 (Jacobsson et al. 2005; Wahlberg et al. 2009) fine-mapped to two linked loci (one in the backward elimination analysis (radial bars) and in addition to this a pair with an epistatic interaction (circles connected by a yellow line). All loci had small individual additive effects on body weight (height of bar illustrate effect size in gram; coloured bars in QTL and black in sweeps; negative estimates are transgressive; supplementary tables S2 and S3, Supplementary Material online). Seven QTL (G1–G12; G: Growth, numbers as in Jacobsson et al. 2005) contained markers significant at a 20% FDR threshold. Four QTL fine-mapped to multiple SNPs (green bars). Some SNPs were so closely linked that they more likely represent base-generation haplotypes than independent associations (stars). Two loci were epistatic and for these no marginal effects were reported (highlighted as golden diamonds). In total, the additive genetic variance contributed by the 20 loci with marginal effects ($V_{A20}$) was ~60% of the total additive genetic variance, with the remaining variance attributed to polygenic effects ($V_{AP}$). The central circle diagram illustrates that the total additive genetic variance ($V_A = V_{A20} + V_{AP}$) amounts to nearly half the residual phenotypic variance in the F15 generation of the Advanced intercross line ($V_e = \text{residual variance not explained by additive genetic or fixed effects}$).

GGA4 F2 QTL, Growth6 (Jacobsson et al. 2005; Wahlberg et al. 2009) (supplementary fig. S2C, Supplementary Material online) was fine-mapped to two loci, ~10 Mb apart in the F15 (fig. 1; supplementary fig. S2E and table S1, Supplementary Material online). Since one of the loci in each of these QTL was not significant in the bootstrap-based, backward-elimination analysis, we explore the hypothesis that epistasis could explain this in a separate section below.

The GGA4 F2 QTL Growth6 (Jacobsson et al. 2005; Wahlberg et al. 2009) (supplementary fig. S2C, Supplementary Material online) was fine-mapped to two loci, ~10 Mb apart in the F15 (fig. 1; supplementary fig. S2D and table S1, Supplementary Material online). The backward-elimination analysis detected associations to two markers <1 Mb apart in each of these loci (supplementary fig. S2D and table S1, Supplementary Material online). Both markers in the first locus, and the first marker in the second locus, segregated in HWS at generation 40 (supplementary table S1, Supplementary Material online). We explore the hypothesis that the associations to the physically close markers (<1 Mb apart) tag multiple segregating base-generation haplotypes with different effects on body weight in a separate section below.
Four out of seven F2 QTL (fig. 1) fine-mapped to two or more independent loci (>2.5 Mb apart) in the F15 generation. In three of them, associations were also detected to even closer markers (<1 Mb apart) in the backward elimination analysis. Because most of the selection response in the Virginia lines is from standing variation (Sheng et al. 2015) and the QTL were detected due to the HWS and LWS carrying alternative variants of them (Jacobsson et al. 2005), this demonstrates the importance of the linkage phase between the adaptive polymorphisms (i.e. the presence of multiple haplotypes) in multiple loci across the genome in the base-population for the response to selection.

The Role of Epistatic Interactions in Adaptation

Epistasis has earlier been found to contribute to selection response in this population (Carlberg et al. 2006; Pettersson et al. 2011). Although the current study was not designed to study genetic interactions, epistasis could explain why some associations detected in the QTL-scan are not detected in our bootstrap-based backward-elimination analysis. We were limited in our epistasis analysis, but tested for and found significant epistatic interactions ($P = 8.9 \times 10^{-5}$) between the pair of loci in Growth1 and Growth12 that were detected in the single marker QTL-scan but not in the backward-elimination analysis (fig. 1). As the allele-frequency at the epistatic marker in Growth1 (rs14924102) had drifted from 0.5 in the AIL ($P = 0.13$ in AIL F15), only a small number of individuals carried the two-locus genotype-classes including the minor-allele (A) at this locus ($n = [3,118]$ for the [AAAA, AATA, AATT] genotypes). There is need for caution in interpreting this result, because, the significance of the epistatic interaction is due to the few individuals with the minor-allele homozygote genotype at this locus having opposite effects to those in the more frequent genotypes at this locus (AAAA and AATT; supplementary fig. S3, Supplementary Material online).


The proximal locus in the Growth9 QTL on GGA7 fine-mapped to two SNP markers 593 kb apart (supplementary table S1, Supplementary Material online) that segregated for two alleles in HWS. Identified were three haplotypes across these two loci in the F15 population that segregated at frequencies > 0.1 ($freq_{AA} = 0.35$, $freq_{CA} = 0.10$, and $freq_{GG} = 0.55$). In the founders of this pedigree, the LWS was fixed for GG, whereas HWS segregated for AA and GG at frequencies $freq_{AA} = 0.65$, $freq_{AG} = 0.175$ and $freq_{GG} = 0.175$, respectively (supplementary fig. S4 and table S4, Supplementary Material online). Fitting a two-locus haplotype-ANOVA to these markers (supplementary table S4, Supplementary Material online) did not reveal any significant associations between these multimarker genotypes and 56-day body weight in the F15 ($P = 0.08$). These marker haplotypes do not, however, fully capture the allelic complexity in the HWS and LWS lineages at generation 40 and 53 (supplementary fig. S4, Supplementary Material online), which suggests that the region needs to be evaluated further using more informative markers to understand whether the haplotype divergence contributes to the multimarker association at this locus.

The distal locus in the Growth9 QTL fine-mapped to two SNPs located 102 kb apart on GGA7 (supplementary table S1, Supplementary Material online) where the first marker segregates for two alleles in HWS. The other SNP is fixed for alternative alleles between HWS and LWS. Three haplotypes segregate at frequencies > 0.1 in the AIL F15 population ($freq_{AA} = 0.40$, $freq_{GA} = 0.10$, and $freq_{CT} = 0.48$). In the founder lines at generation 40, LWS is fixed for the CT haplotype, whereas HWS segregates for CT and AA at $freq_{CT} = 0.125$ and $freq_{AA} = 0.875$, respectively (supplementary fig. S5 and table S4, Supplementary Material online). The CA haplotype in the F15, which was not observed in the founders, is likely a recombinant between the CT and AA haplotypes. The two-locus haplotype-ANOVA detected a significant association to 56-day body weight in the F15 AIL population at this locus ($P = 0.008$). However, the CT haplotype tags three different haplotypes at this locus in LWS at generation 40 (supplementary fig. S5 and table S4, Supplementary Material online), and few individuals in the F15 carried each tagged haplotype. This reduced the power to test the effects of individual haplotypes and additional data are needed to explore in further detail the allelic heterogeneity at this locus.

The Growth9 QTL on GGA3 fine-mapped to two SNP markers 260 kb apart (supplementary table S1 and fig. S2B, Supplementary Material online). The first marker was nearly fixed for alternative alleles in HWS and LWS at generation 40 and the second segregated for two alleles in LWS (supplementary table S1, Supplementary Material online). Three haplotypes segregated at this locus in the F15 generation at frequencies > 0.1 ($freq_{AG} = 0.59$, $freq_{GA} = 0.29$, and $freq_{GG} = 0.12$). The AG haplotype was almost fixed in HWS ($P = 0.98$), whereas GA and GG segregated in LWS ($freq_{GA} = 0.61$ and $freq_{GG} = 0.29$, respectively, supplementary table S5, Supplementary Material online) at generation 40. The two-locus haplotype ANOVA detected a significant association to 56-day body weight in the F15 generation ($P = 0.001$) and the individual haplotypes have different effects on 56-day body weight in the F15 (supplementary table S5, Supplementary Material online), with the major LWS haplotype (GA) decreasing body weight by 14.8 g ($P = 0.039$; fig. 2A and supplementary table S5, Supplementary Material online) compared with the nearly fixed HWS haplotype (AG). The minor LWS haplotype (GG) decreased 56-day body weight even more ($-44.3$ g; $P = 0.0003$; fig. 2B and supplementary fig. S6, Supplementary Material online). The high-density SNP haplotypes in the HWS and LWS lineages across the region of Growth9 (GGA3: 32.9–34.6 Mb) with the two significantly associated linked SNP markers were then studied in detail. The major two-locus HWS haplotype (AG) and the minor LWS haplotype (GG) at generation 40 each tag well-defined haplotypes across the region (fig. 2B and supplementary fig. S6, Supplementary Material online). The major two-marker LWS-haplotype (GA) at generation 40,
however, tags two different haplotypes. During selection for decreased body weight in LWS from generation 40–53, the frequency of the haplotype with the lowest effect on 56-day body weight (GG) increased (fig. 2B and supplementary fig. S6, Supplementary Material online). By generation 53, it had become the major haplotype in the LWS line (supplementary table S5 and fig. S6, Supplementary Material online). This suggests an ongoing selection at this QTL at generation 40, leading to fixation of the haplotype with the least effect on 56-day body weight by generation 53.

The backward-elimination analysis thus detected associations to pairs of physically close markers in three loci in two QTL. They were located so closely (0.1–0.6 Mb) that they could not be tested independently using data from the 15th generation of the deep intercross line and hence they were useful for tagging the alternative haplotypes that accumulated during selection in the divergent lines (fig. 2; supplementary figs. S4–S6 and table S5, Supplementary Material online).

A Single Fine-Mapped Locus Identified in Two QTL

The multilocus bootstrap-based backward-elimination analysis revealed one 56-day body weight associated marker in each of the two QTL on GGA2 (Growth2, Growth3; supplementary table S1, Supplementary Material online). These results were consistent with that of the QTL-scan across Growth2 that identified a well-defined region with a peak at 60.5 Mb (galGal4) to the marker retained in the backward-elimination analysis (supplementary fig. S1A, Supplementary Material online). The fine-mapped association signal in the F15 is located ~5 Mb away from, and slightly outside of, the...
Variance Explained by the Fine-Mapped Loci

We used a linear model to estimate how much of the additive genetic variance for 56-day body weight in the F15 that could be explained by the associated markers in the fine-mapped QTL (supplementary table S1, Supplementary Material online) and those identified in selective-sweep regions elsewhere in the genome (Sheng et al. 2015) (supplementary table S2, Supplementary Material online; see Materials and Methods). Together 24 markers, representing 20 loci, retained in the backward-elimination analysis (20% FDR) explained 27.8% of the residual phenotypic variance, corresponding to 60.5% of the additive genetic variance in the population (fig. 1).

Discussion

The Virginia chicken lines provide a unique population for studying the genetic mechanisms underlying extreme responses to long-term directional selection in vertebrates (Hill 2005). With over 50 generations of selection in constant, controlled conditions and 15 generations of deep intercrossing, these lines have been valuable in increasing our understanding of polygenic adaptation. They demonstrate that many loci all have made small contributions to the long-term response (Dunnington and Siegel 1996; Jacobsson et al. 2005; Johansson et al. 2010; Besnier et al. 2011; Dunnington et al. 2013; Sheng et al. 2015). Here we studied data from the F15 generation advanced intercross line where accumulation of recombination events over many generations has facilitated the fine-mapping of multiple QTL to single Mb resolution. Twenty-four significantly associated SNP markers in 20 loci explain nearly two-thirds of the additive genetic variance in this population. Two additional SNP markers were found to be epistatic. Despite the 9-fold body weight difference between the selected lines, none of the associated alleles at the individual loci contributed >27 g to 56-day body weight. Seven loci were fine-mapped in detail and most of them were found to rely on contributions by multiple linked loci, multiple haplotypes, and epistasis.

Our mapping approach employs an adaptive step-down procedure to restrict the full SNP data set to a set of multiple, significantly associated SNP markers (Abramovich et al. 2006; Gavrilov et al. 2009). We successfully implemented this approach in our previous studies (Sheng et al. 2015; Brandt et al. 2017; Lillie et al. 2017) that is suitable for polygenic traits, because it considers all SNPs simultaneously unlike classic locus-by-locus GWAS methods. Four QTL were fine mapped into two tightly linked loci within the larger individual QTL. This observation of a single QTL fractionating into multiple, closely linked QTL is common, and has been seen in studies on growth in mice (Christians et al. 2006), longevity in Drosophila (Wilson et al. 2006), domestication traits in maize (Lemmon and Doebely 2014) and more. We also observed that the tightly linked SNPs in Growth1 were opposite in effects (supplementary tables S2 and S3, Supplementary Material online). This locus is differentially fixed in the founders (i.e. selected generation 40), implying that as selection favored some functional variant, the linkage block became fixed before recombination could decouple the unfavorable variant. Within the AIL, however, recombination had successfully broken up this linkage and we saw increased genetic variance explained from F2 to F15 at this locus.

Multiple haplotypes underlying QTLs (or allelic heterogeneity) have been shown to significantly impact traits, including human disease (McClellan and King 2010), expression profiles in Drosophila heads (King et al. 2014), trace metal uptake in Arabidopsis thaliana (Forsberg et al. 2015), and agronomic traits in rice (Yano et al. 2016). Combining our backward-elimination approach with haplotype information from the pedigreed founders allowed us to discern multihaplotypic loci in this population. Markers within these loci tag segregating haplotypes with distinct effects on body weight in the Virginia lines. The changing haplotype frequencies observed among generations in the selected lines further support that they are under selective pressure in this population. Most selection response in the lines was from standing variation (Sheng et al. 2015), and long divergent segments between the lines are likely to reflect rapid fixation events (Johansson et al. 2010; Sheng et al. 2015). The multiple haplotypes revealed across multiple loci probably have entered the Virginia lines upon its founding from their common base population, which consisted of seven moderately inbred White Plymouth Rock lines. Although the power in this study is limited to distinguish the phenotypic effects of individual haplotypes due to their low allele frequencies, the results still show it likely that allelic heterogeneity in the base population made important contributions to the phenotypic variation and selection response contributed by multiple loci.

Five from seven QTL fractionated into multiple loci, or contained multiple alleles in this study, which is a finding consistent with what has been reported in earlier QTL-based studies of adaptation where several linked loci with minor effects appear as single large QTL (Knight et al. 2001; Orgogozo et al. 2006; McGregor et al. 2007). The remaining two QTL fine-map to narrow association peaks, although the
resolution in the F_{15} generation does not allow testing of whether they are due to individual or multiple-tightly linked loci. Further work based on additional markers and subsequent later generation intercross individuals are needed before it can be ruled out with confidence that these confined QTL do not contain multiple tightly linked adaptive alleles.

Important contributions of epistasis towards phenotypic traits have been demonstrated in other populations (Shindo et al. 2005; Flint and Mackay 2009; Forsberg et al. 2017) and extensive epistasis has been identified in the Virginia lines (Carlberg et al. 2006; Pettersson et al. 2011). These earlier reports have illustrated that epistatic interactions are likely to have a major effect on both the rate of response, as well as our ability to predict response from either individual loci or the level of additive variance at different time points in the selection process, in the Virginia lines (Carlberg et al. 2006; Le Rouzic and Álvarez-Castro 2008; Pettersson et al. 2011). The radial epistatic network architecture in this population (Carlberg et al. 2006; Pettersson et al. 2011) resembles that recently shown in studies with a large experimental yeast cross population (Forsberg et al. 2015), and further research on epistasis in the Virginia lines should reveal if high-order interactions similar to those in yeast (Forsberg et al. 2017) could be involved also in animals. Here, we were able to show one epistatic interaction between Growth1 and Growth12 in the F_{15}, but in general our data set lacks the statistical power to extensively explore epistatic interactions due to drift of allele frequencies in the AIL and the limited number of animals.

Our work herein demonstrates how response to selection on the polygenetic trait—56-day body weight—in the Virginia lines has been predominantly achieved by small-effect variants. Most moderate effect QTL in the F_{2} fractionated further into smaller effect loci or segregating haplotypes and as a result, the contribution by each associated locus was even smaller than originally thought. Furthermore, the multiple linked variants within associated regions in the genome could have affected the capacity of the population to respond to selection by slowing the initial rate of selection response and providing greater opportunities for long-term response as unfavorable linkages are broken up. Genetic samples prior to generation 40 do not exist, so it is not possible to quantify the extent of these effects, but because most major QTL were affected by such linkages it is reasonable to believe that they will not only have been of marginal influence. Earlier in-depth dissections of individual adaptive loci also revealed complex loci that involve linked loci (Moose et al. 2004; Forsberg et al. 2015), multiple alleles (Kroymann and Mitchell-Olds 2005; Li et al. 2014; Forsberg et al. 2015), and nonadditive epistatic interactions (Kroymann and Mitchell-Olds 2005; Stylianou et al. 2006; Li et al. 2014). In the Virginia lines, we here have observed all these evolutionary genomic processes contributing to the selection response and stark phenotypic differences between the lines, highlighting the complexity of the genetic mechanisms involved also for minor effect loci contributing to polygenic adaptation.

It would valuable to obtain measures for the contribution, and possible constraints, for the different mechanisms revealed here to the selection response. It is, however, not possible to obtain the sufficient precision in the estimated contributions by each locus to the polygenic trait as their individual contributions are so small, the population size is limited and the allele frequencies have drifted in the AIL population (minor allele frequencies range from 0.03 to 0.5). Evaluations of the effect of selection in these loci, and attempts to estimate their contributions to selection response, will instead be performed in subsequent studies where larger populations have been more thoroughly genotyped in the associated regions. Critically, many more small effect loci may be present in this population and further epistatic and haplotypic complexity remain to be seen, by fully studying the multilocus, multihaplotypic and potentially epistatic complexities underlying adaptation in the Virginia lines.

In conclusion, this study illustrates that the strong responses to long-term divergent selection in the Virginia body weight lines rely on a highly polygenic and complex genetic architecture, with contributions from many genomic regions, containing multiple linked loci, multiple haplotypes and epistatic loci. This valuable experimental population has facilitated the mapping of many minor effect loci contributing to the polygenic adaptation and revealed that they affect the phenotype via different genetic mechanisms. Although we have dissected this trait to considerable depth, it is likely that there are still many loci with minor effect or epistatic interactions contributing to the long-term selection responses. The Virginia lines clearly demonstrate that, even in relatively small, closed populations, long-term polygenic selection responses are likely to involve a wide variety of complex mechanisms.

**Materials and Methods**

**Ethics Statement**

All procedures involving animals used in this experiment were carried out in accordance with the Virginia Tech Animal Care and Use Committee protocols.

**Animals, Phenotyping and DNA Extraction**

The chickens used in this study were from the F_{15} generation of an Advanced Intercross Line bred from the Virginia high (HWS) and low (LWS) body weight selected lines. The HWS and LWS lines were founded in 1957 from a common base population obtained by crossing seven partially inbred lines of White Plymouth Rock chickens. They have since then been subjected to bi-directional selection for high or low 56-day body weight, respectively. For further details on the Virginia lines, see (Dunnington and Siegel 1996; Márquez et al. 2010; Dunnington et al. 2013). After 40 generations of selection, the High (HWS) and Low (LWS) weight selected lines differed 9-fold for the selected trait. An Advanced Intercross Line (AIL) was founded from generation 40 of the HWS and LWS, where the sex-average 56-day body weights were 1,412 g (SE: ± 36 g) and 170 g (SE: ± 5 g), respectively (Jacobsson et al. 2005). This AIL has previously been used in the fine-mapping of QTL using the F_{2}–F_{8} AIL generations (Besnier et al. 2011;
of mapping results from the F2 (Wahlberg et al. 2009) and the regions (Sheng et al. 2015). The Nov 2011 (galGal4) chicken (galGal4), of which 170 Mb represent targeted QTL regions (in the 434 markers covered the HWS/LWS F0 founders. By using this coding, positive to affect body weight in the F2 (Jacobsson et al. 2005; Carlborg et al. 2006; Wahlberg et al. 2009) and F2–F8 intercross populations (Pettersson et al. 2011; Brandt et al. 2017). Together the 434 markers covered ~300 Mb of the chicken genome (galGal4), of which 170 Mb represent targeted QTL regions (in total 216 markers), and 130 Mb (218 markers) selective-sweep regions (Sheng et al. 2015). The Nov 2011 (galGal4) chicken genome assembly was used for comparing physical locations of mapping results from the F2 (Wahlberg et al. 2009) and the F15 intercross generations.

Coding of SNP Marker Alleles in the AIL F15 Generation
The GoldenGate assay reports SNP marker alleles on a [A, T, C, G] basis. Before the statistical analysis, we re-coded the marker genotypes in individuals as [−1, 0, 1]. The coding −1/1 was used to represent homozygotes for the most common alleles in the LWS/HWS founders, respectively. The code 0 was used to represent heterozygotes. The LWS/HWS origin was determined by comparing the F15 genotypes to those of the HWS/LWS F0 founders. By using this coding, positive estimates of additive effects in the association analysis indicate that predominant allele in LHS increased weight, and negative additive effects that the predominant allele in LWS increased weight.

Multilocus Association Analysis Using a Backward-Elimination Strategy with Bootstrapping to Correct for Population Structure
The statistical analyses were designed to simultaneously fine map nine QTL regions contributing to 56-day body weight in the Virginia body weight lines, while also accounting for the effects of other regions across the genome. This was to appropriately account for the highly polygenic genetic architecture of body weight in this population (Jacobsson et al. 2005; Johansson et al. 2010; Besnier et al. 2011; Pettersson et al. 2013; Sheng et al. 2015), where many of the genotyped markers both in and outside the QTL regions should contribute to the trait. As all individuals in the AIL were progeny of dams of the same age, hatched on the same date, and reared separate from their parents, the environmental contributions to between-family means in the F15 population should be minimal. We therefore assume that a large portion of the differences in family means are due to the joint effects of the markers in the genotyped QTL and selective sweep regions, rather than nongenetic effects. Population structure might, however, still be of concern in a deep-intercross population (Peirce et al. 2008; Cheng et al. 2010), as there might be a confounding between allelic effects and family effects. We therefore also validate our results using a bootstrap-based approach developed to account for the possible effects of population structure in general deep-intercross populations, including AILs (Valdar et al. 2009).

Not known in advance how many genetic markers in the fine-mapped QTL contributed to 56-day body weight in the AIL F15 generation. Based on previous findings (Besnier et al. 2011; Brandt et al. 2017), we expected that at least some QTL would contain multiple linked loci. The number of loci in the final model could therefore vary substantially. Traditional model selection criterion, such as AIC or BIC, are based on prior assumptions on the most appropriate size of the final model. To account for the fact that the final model size is here unknown, we implement the analysis with an adaptive model selection criterion controlling the False Discovery Rate (FDR) (Abramovich et al. 2006; Gavrilov et al. 2009), developed for this purpose. Our previous analysis in the same data set showed that this method can successfully control the FDR while replicating and refining evaluated loci with prior evidence of being involved in adaptation (Sheng et al. 2015). Starting with the 434 genotyped SNP markers, we then selected a final set of markers using a two-step backward-elimination strategy applied on a standard linear model with 56-day body weight as response variable. A 20% FDR among the selected loci was used as the termination criterion.

In the first step, a linear model including the SNP markers to be tested, together with the fixed effects of sex of the individual and the genotypes of the 16 sweeps that were earlier found to be associated with 56-day body weight in this population (Sheng et al. 2015). The 16 earlier mapped sweeps were included in the model to capture the major polygenic effect in this population. To avoid over-parameterizing the linear model, the 434 markers were divided into eight pre-screening sets including approximately 50 markers each. These sets were selected to, as far as possible, include markers from the same chromosomes. A backward-elimination analysis with the adaptive FDR criterion was performed on each set separately. All markers that reached the 20% FDR in either of these analyses were kept for the further joint analyses described in the next section.

In the second step of the analysis, all markers selected in the pre-screening were analyzed jointly using the bootstrap based method of Valdar et al. (2009). This analysis was used to identify the loci that contribute to 56-day body weight in this population and that was robust to the possible effects of population structure. Here, a RMIP (Resample Model Inclusion Probability) was calculated for each marker tested.
A final model was selected where only markers with RMIP > 0.46, the threshold suggested for an AIL generation F18 (Valdar et al. 2009), was included. All of these analyses were implemented in custom scripts using the statistical software R (R Core Team 2015). We have made the backward elimination script publicly available as a R package named BE on Github (https://github.com/yanjunzan/BE; last accessed July 12, 2017).

Estimation of the Additive Effects and Significances of the Markers Associated with 56-Day Body Weight in the F15 Population

The general linear model used to estimate additive effects and significances of the locus associated with 56-day body weight in this population can be formulated as

\[ Y = X_1 \beta_1 + e \]  

(1)

Here, \( Y \) is a column vector containing the 56-day body weight of the 825 F15 individuals. \( \beta_1 \) is a vector with the estimate of the fixed effect of sex and the additive effect of all markers tested. \( X_1 \) is the design matrix including the coding for the sex of the birds and the genotypes of the markers coded as \([-1,0,1]\). \( e \) is the normally distributed residual. The markers included to control for the background genetic effects will vary depending on the analysis as described in the sections below.

Analyses of Markers Selected in the Backward-Elimination Analysis

There are few recombination events within each QTL in the AIL pedigree which limits the resolution in the fine mapping analysis. Therefore, in the backward-elimination analysis, we consider markers located closer than 1 Mb from each other as representing the same fine mapped locus. The effects of all markers in these loci were estimated using the model described earlier (model 1) by defining \( X_1 \) to include only the genotypes of the tested marker from that locus and the SNPs in the remaining loci. This meant that when estimating the effect of a marker located inside a fine mapped locus tagged by more than 1 marker, the remaining markers within this locus were excluded from the analysis. The significance for each marker was obtained using a likelihood ratio test comparing regression models (model 1) with and without the tested marker.

Single Marker Association Scans in the Replicated QTL

To obtain statistical support profiles for the QTL with significant associations in the backward-elimination analysis, we performed single marker association scans across all markers in these QTL. The same regression model (model 1) was used, but \( X_1 \) includes the genotype of the tested marker together with the sex effect and the additive effects of all SNPs selected in the backward-elimination analysis.

Effects of the QTL Detected in the F2 Population

The additive effects and significances for QTL identified in the F2 population were extracted at the location of the marker closest to the reported QTL peak from the results in (Wahlberg et al. 2009).

Calculation of Founder Line Allele Frequencies for the Markers Associated with Body Weight in the F15 Population

Previously, we genotyped 20 HWS and LWS individuals from generation 40 using a 60k SNP chip (Johansson et al. 2010). Some of the SNPs evaluated here were included in that study and data for those SNPs were used to calculate the allele frequencies in the founder lines. For the remaining SNPs, the allele frequencies in the founder lines were estimated using sequence data (~30x coverage) from two pools including 29 HWS founders and 30 LWS founders, respectively (Lillie et al., in preparation).

Testing for Pairwise Epistatic Interaction between Loci

Previous studies of the Virginia body weight selected populations revealed and replicated extensive epistatic interactions among QTL alleles (Carlborg et al. 2006; Le Rouzic et al. 2008). Unfortunately, the allele frequencies were too imbalanced and the number of individuals too small in this F15 data set to provide sufficient statistical power for an extensive exploration of epistasis. Therefore, we limited testing of epistasis to explore the possibility that two of the loci found to be sensitive to the genetic background in the bootstrap analysis are involved in an interaction. It is possible that these association peaks significant in the scan across the QTL in the original data, but not in the bootstrap-based backward-elimination analysis, could be due to sensitivity to allelic background at other loci (i.e. epistasis). We therefore tested for epistasis between these peaks by evaluating whether removing the pairwise interactions in model 2 affecting the overall fit using likelihood ratio test.

\[ Y = \mu + a_1 x_1 + a_2 x_2 + d_1 \delta_1 + d_2 \delta_2 + a_1 a_2 l_{12} + a_1 d_2 l_{1d} + a_2 d_1 l_{2d} + d_1 d_2 l_{dd} + e, \]  

(2)

where \( Y \) is the residual from a regression model (model 1) fitting all significant markers selected in the bootstrap-based backward-elimination analysis together with the sex of the chickens. \( \mu \) is the reference point, \( x_1 \) and \( x_2 \) \((\delta_1 \) and \( \delta_2 \) are the additive allele-substitution effects (dominance deviations) at the two loci, \( l_{12} \) \( l_{1d} \) \( l_{2d} \) and \( l_{dd} \) are the additive by additive, additive by dominance, dominance by dominance and dominance by dominance interaction effects, and \( e \) is the normally distributed residual. The \( a \) - and \( d \) - are the NOIA indicator regression variables for the two loci. These indicator regression variables are allele frequency weighted codings of the genotypes at the evaluated loci and calculated as detailed in (Alvarez-Castro and Carlborg 2007). Using the orthogonal statistical NOIA parameterization of the model, we tested for epistasis by comparing the full model (model 2) to the reduced model without the interaction effects using a likelihood ratio test. All models were fitted using the \textit{lm} function in R with design matrices created by the \textit{multilinearRegression} function in \textit{noia} R-package (Alvarez-Castro and Carlborg...
Haplotype Estimation in Individuals from the Founder-Lines and All $F_{15}$ Generation

We inferred the haplotypes for $n = 20$ individuals from each of generations 40 (founders) and 53 from the HWS and LWS lines (in total 80 birds), and all 825 birds with phenotypes from the All $F_{15}$ generation. This was done using the software fastPHASE (Scheet and Stephens 2006) with default parameters “fastPHASE – Z input-file”. For this, we used 60k SNP chip genotypes generated for the generation 40 and 53 HWS and LWS individuals in two earlier studies (Johansson et al. 2010; Pettersson et al. 2013) and the new 434 SNP markers genotypes in the All $F_{15}$ individuals. Some of the SNP markers genotyped in the All $F_{15}$ were not included on the 60k SNP chip and could therefore not be unambiguously assigned to HWS or LWS haplotypes based on this haplotyping. Instead, they were assigned to a founder haplotype based on the agreement between their allele-frequencies, estimated from the pooled whole-genome re-sequencing data described earlier, and those of a nearby SNP from the 60k SNP-chip genotype data.

Haplotype-Based Association Analysis

A haplotype based association analysis was performed in regions of the QTL where the bootstrap based backward elimination analysis, and the haplotype estimation, suggested that multiple alleles were segregating in one, or both, of the founder lines. These analyses used a one-way ANOVA (model 1) where $Y$ is a column vector including the residuals from a regression model fitted on the phenotypes of all individuals with the genotypes of all markers selected in the backward-elimination analysis located outside of the evaluated locus and sex as fixed effects. $X$ is the design matrix with one column for the reference point and multiple columns containing the haplo-genotypes. $\beta$ is a column vector with the estimates for the reference point and the deviations from the reference point for each haplo-genotype, and $e$ is the normally distributed residual.

Estimation of Genetic Effects of Individual Haplotypes

For loci where the haplotype based association analysis indicated that multiple haplotypes were segregating in one, or both, of the founder lines, we also performed an analysis to disentangle the effects of the individual haplotypes. For a locus with multiple haplotypes (denoted here as A, B, C . . . N), we first identified the common haplotypes (haplotype frequency > 0.1) and only included them in the analysis. Second, a strata was formed that only contained homozygous and heterozygous individuals for particular haplotype combinations (e.g. AA, AB, BB). Third, using these strata we estimated the effects of the individual haplotypes, in turn (e.g. the effects of A and B were estimated in the strata containing individuals with the haplotype combinations AA, AB and BB) using a regression model (model 1) parameterized as follows.

Y here contains the residual for the birds in an analyzed strata from a regression model where the genotype of all markers located outside of the tested locus, and sex, were fitted as fixed effects in a regression on the 56-day body weight. Here, $X$ is the design matrix where the haplo-genotype for individuals homozygous for the LWS derived haplotype, heterozygous for LWS/HWS derived haplotype and homozygous for the HWS derived haplotype are coded as $[0,1,2]$, respectively. $e$ is the normally distributed residual. This analysis was repeated for all the major haplotypes for which sufficiently large numbers of individuals were genotyped and phenotyped ($n > 50$) in at least two of the three haplo-genotype classes in the All $F_{15}$ generation. The significances for the associations obtained as the $P$ value from a Wald test calculated using the Im function in R.

Visualization of Results

Figure 1 was generated using the R package circlize (Gu et al. 2014).

Estimation of the Variance Explained by the Mapped Loci

The variance explained by the mapped loci was estimated by fitting a linear model (1). Here, $Y$ is a column vector containing the residuals after fitting sex to the 56-day body weight of the 825 $F_{15}$ individuals. $\beta$ is a vector with the estimate of the additive effect of all markers tested. $X$ is the design matrix including the coding for the genotypes of the markers as $[-1,0,1]$. $e$ is the normally distributed residual. $R^2$ from this model was used as estimates for the proportion of the residual phenotypic variance explained by the mapped loci ($h^2_{VAP}$). The proportion of the total additive genetic variance explained was calculated as $h^2_{VA20}/h^2_{VA}$, where $h^2_{VA}$ is the heritability estimated using a standard mixed model, with the same response and a kinship estimated using all the genotyped makers using the software GenABEL with the ibs function with default settings (Aulchenko et al. 2007). The remaining portion of the additive genetic variance attributed to polygenic genetic effects ($h^2_{VA20}$) was calculated by deducting $h^2_{VA}$ from $h^2_{VA20}$.

Supplementary Material

Supplementary data are available at Molecular Biology and Evolution online.

Author Contributions

Ö.C. and P.B.S. initiated the study and designed the project; P.B.S. developed the Virginia chicken lines; P.B.S. designed, planned and bred the Virginia Advanced Intercross Line; P.B.S. and C.F.H. designed, planned, bred, phenotyped, and extracted DNA from the $F_{15}$ Virginia Advanced Intercross Line population; Z.S. performed the quality control of the genotype data; Ö.C. and L.R. designed the statistical analyses; Z.S., Y.Z., and Ö.C. contributed analysis scripts; Ö.C., Y.Z., M.L., and Z.S. performed the data analyses and summarized the results. Ö.C., M.L., and Y.Z. wrote the manuscript. All authors read and approved the final manuscript.
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References

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