Whole-genome selective sweep analyses identifies the region and candidate gene associated with white earlobe color in Mediterranean chickens

Ying Guo [*,†,‡,§] Carl-Johan Rubin,[‡] Tilman Rönneburg [‡] Shouzhi Wang, [#,||,¶] Hui Li,[#,||,¶] Xiaoxiang Hu,*,†,1 and Örjan Carlborg [‡]

*State Key Laboratory of Animal Biotech Breeding, China Agricultural University, Beijing, China; †National Engineering Laboratory for Animal Breeding, China Agricultural University, Beijing, China; ‡Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden; §Yazhouwan National Laboratory, Sanya, China; #Key Laboratory of Chicken Genetics and Breeding, Ministry of Agriculture and Rural Affairs, Harbin, China; ||Key Laboratory of Animal Genetics, Breeding and Reproduction, Education Department of Heilongjiang Province, Harbin, China; and ¶College of Animal Science and Technology, Northeast Agricultural University, Harbin, China

ABSTRACT We compared the genomes of multiple domestic chicken breeds with red and white earlobes to identify the differentiated regions between groups of breeds differing in earlobe color. This was done using a selective sweep mapping approach based on whole-genome sequence data. The most significant selective sweep was identified on chromosome 11, where the white earlobe chicken breeds originated from Mediterranean share a common haplotype, and where multiple candidate genes are located. The most plausible functional candidate gene is the *Melanocortin 1 Receptor* (***MC1R***), a receptor known to regulate pigmentation in the skin and hair, and it is also the gene with the strongest positional support from the haplotype-based analyses. It, however, still needs to be explored experimentally to identify effects also on chicken earlobe color variation. Our study is the first exploration of the genetic basis of white earlobe color in Mediterranean chickens using a selective sweep mapping method based on whole-genome sequencing data and shows its value for identifying likely functional genes mediating the pigmentation in earlobe. It also indicates a potential novel role of *MC1R* in birds and exemplifies how selection on fancy traits has influenced the genome during formation of the modern chicken breeds.

Key words: Mediterranean chicken, earlobe color, selection, *MC1R*

INTRODUCTION

The earlobe is a tissue on the face of chicken located next to the ear. Chicken breeders have selected populations based on color of this morphologic character, like for comb and feather color, often making it one of the breed defining traits. Across breeds, it however displays many different color and size variations resulting from the long-term selection on these earlobe traits. Currently, the most common colors in the world-wide domestic populations are red and white while other colors including yellow, turquoise, purple-blackish exist but in a smaller number of breeds. For some breeds the color of eggshell has been identical with the color of earlobe, but there is no genetic relationship between the color of earlobe and eggshell. Few studies exist on the inheritance of earlobe color, but its genetic basis has been reported to be complex and earlier studies have reported different patterns of association in different breeds (Wragg et al., 2012; Nie et al., 2016). Sex-linked dominant inheritance has been observed in some studies (Wragg et al., 2012; Nie et al., 2016), whereas other studies have reported that white earlobe color in breeds like leghorn is mainly autosomally inherited with at least 2 contributing loci (Warren, 1928).

Using genetic mapping, many loci contributing to trait variations have been identified in the last 20 yr (Kijas et al., 1998; Mundy, 2005; Gunnarsson et al., 2007; Guo et al., 2016). For instance, studies of pigmentation have successfully identified many genes with key roles in determining the color of, for example, the coat (Dunn, 1922; Kijas et al., 1998; Kerje et al., 2004), skin (Våge and Boman, 2010; Dorshorst et al., 2011), and

[1]Corresponding author: huxx@cau.edu.cn

eggshell (Wang et al., 2013; Chen et al., 2020) in animals. Most of these studies have used genome wide association and linkage mapping analyses. Recently, high-density genotyping and whole-genome sequencing analyses have provided new opportunities to screen for selection signals across the genome and these have been widely used in population genomic studies to pin-point signals that in many cases overlap with known causal gene regions (Qanbari et al., 2019; Guo et al., 2021). These results have thus on one side validated the likely contributions of variants identified in earlier mapping to selection responses in populations and further showed its potential as a powerful approach to unravel the genetic basis of selected traits.

This study aims to identify the genetic basis for the white earlobe color variations in the Mediterranean-origin chicken including White Leghorn and Black Minorca using a selective sweep based mapping method. By contrasting whole-genome sequence data from multiple red and above 2 white earlobe breeds highlighted multiple putative signals of selection in the genome. One promising functional candidate pigmentation gene is located in the most strongly selected region, suggesting its potential involvement in the regulation of white earlobe coloration.

MATERIALS AND METHODS

Sample Information

In total, 14 chicken populations were included in this study, 13 domestic breeds and the Red Junglefowl. All samples were whole-genome sequenced and the details of how this was done are available in our previous studies (Guo et al., 2019, 2021). Among the 14 analyzed populations, 55 chickens were from the White Leghorn ($n = 41$) and Black Minorca ($n = 14$) breeds having white earlobes. In total, 219 chickens with red earlobes were included from 11 breeds: Black Cochin ($n = 10$), Buff Cochin ($n = 9$), Partridge Cochin ($n = 4$), Dominique ($n = 10$), Langshan ($n = 32$), LangshanUS ($n = 10$), Light Brahma ($n = 21$), Liyang ($n = 32$), Recessive White ($n = 32$), and 2 White Plymouth Rock ($n = 59$) derived selection lines (the Virginia low- and high-weight selected lines; **LWS** and **HWS**). All samples were sequenced individually. The average genomic coverage for the sequence data is 8×.

Population Comparisons

The population difference was compared based on allele frequency differences between the white ($n_{breeds} = 2$) and red ($n_{breeds} = 11$) earlobe groups using *VCFtools* (Danecek et al., 2011). The statistics used to compare the populations were the fixation index (F_{st}), Pi-diversity, Tajima's D and the allele frequency. The window size for the whole-genome Fst calculations was 20 kb. For the regional analyses with Pi-diversity and Tajima's D, a window size of 5 kb was used to acquire higher resolution in the target region.

Selection signatures were also sought on all chromosomes using the haplotype-based approach implemented in *HapFLK* (Fariello et al., 2013). Red Junglefowl was used as the outgroup. The *hapFLK* scores were calculated for each population and whole-genome P values were calculated and plotted in R (R Core Team, 2013). The haplotype cluster frequencies for the most significant region on chromosome 11 were calculated and plotted using R.

Variant Screening in Candidate Selective Sweep Regions

Putative causal variants were evaluated in the candidate region on chromosome 11 by filtering the 66,111 SNPs identified in the whole-genome sequencing based on the allele frequency in the groups of breeds with white and red earlobes. Only SNPs with allele frequency above 0.95 in 1 group, and below 0.05 in the other were kept. The results were shown in Table S1. Structural variations scanning using the bam files from chromosome 11 was done using the *Breakdancer* (Fan et al., 2014) with the options "-a -q 20 -r 6."

RESULTS

We estimated the genomic divergence, and screened for signals of selection, between red and white earlobe chicken populations using multiple analytical approaches. The results from these are presented in the sections below.

Estimating Population Divergence by the Fixation Index (F_{st})

The fixation index (F_{st}) was estimated between red ($n_{breeds} = 11$; $n_{individuals} = 55$) and white ($n_{breeds} = 2$; $n_{individuals} = 55$) earlobe chickens using available whole-genome sequencing data. Undersampling here was implied to reduce the size by randomly selecting equal number chickens from each red earlobe population. The strongest F_{st} signal was observed on Chromosome 11 (Figure 1) where the 2 White Plymouth Rock derived selection lines (the Virginia LWS and HWS lines) and white earlobe chickens displayed a high degree of fixation.

A Haplotype-Based Selective Sweep Analysis Identifies a Strong Selective Sweep on Chromosome 11

A selective sweep analysis using a haplotype-based method (Fariello et al., 2013) was also performed based on all available individuals from the red ($n_{breeds} = 11$; $n_{individuals} = 219$) and white ($n_{breeds} = 2$; $n_{individuals} = 55$) populations. And the results showed a strong selection signal on chromosome 11 (Figure 2). We then further looked into the most strongly associated region on
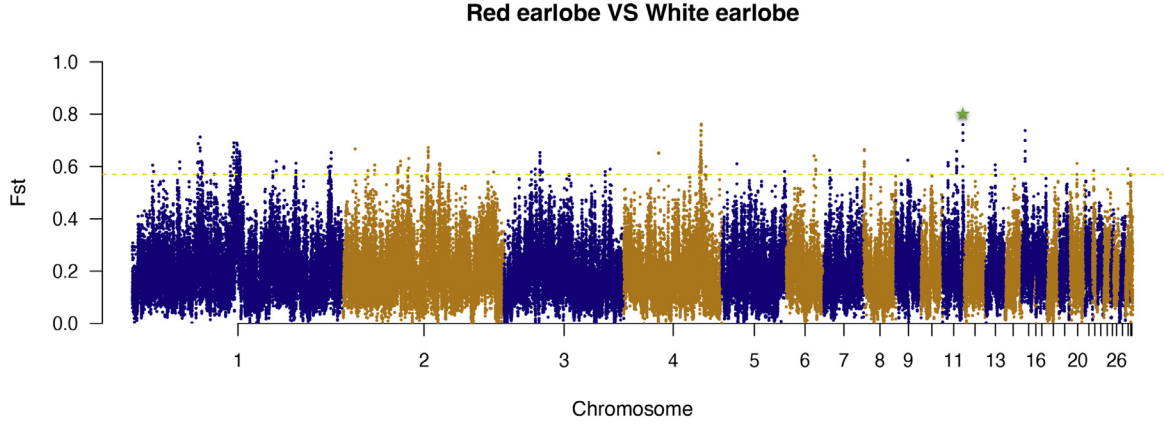
Figure 1. Estimated genome-wide divergence between red and white earlobe chicken breeds. The genome-wide divergence was estimated as Fst in whole-genome sequence data between red ($n = 55$) and white earlobe ($n = 55$) chickens. The horizontal golden dashed line is the 97.5th percentile.
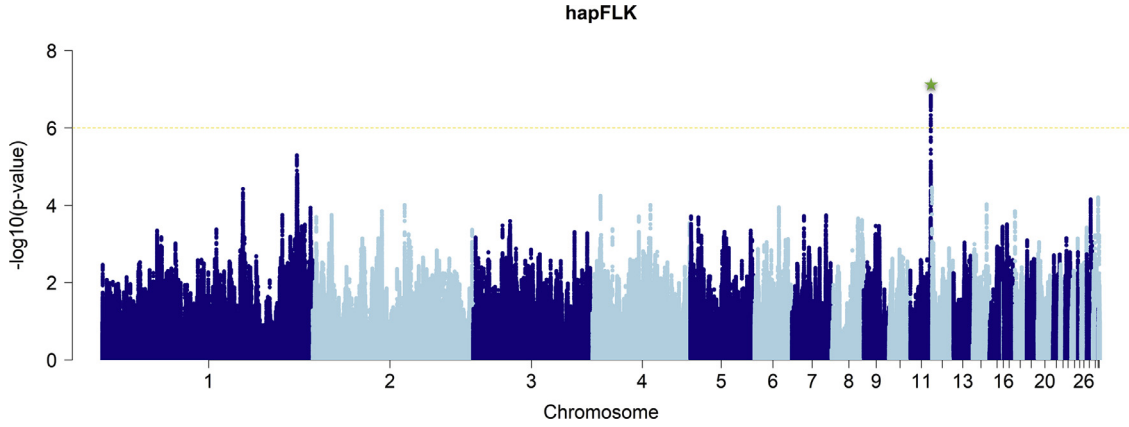

Figure 2. Whole-genome selection scan using the haplotype-based method $hapFLK$. The x-axis represents the position on the genome, while the y-axis represents the $-\log10(P$ value). A fixed P value threshold of 1×10^{-6} is utilized to identify loci with strong associations.
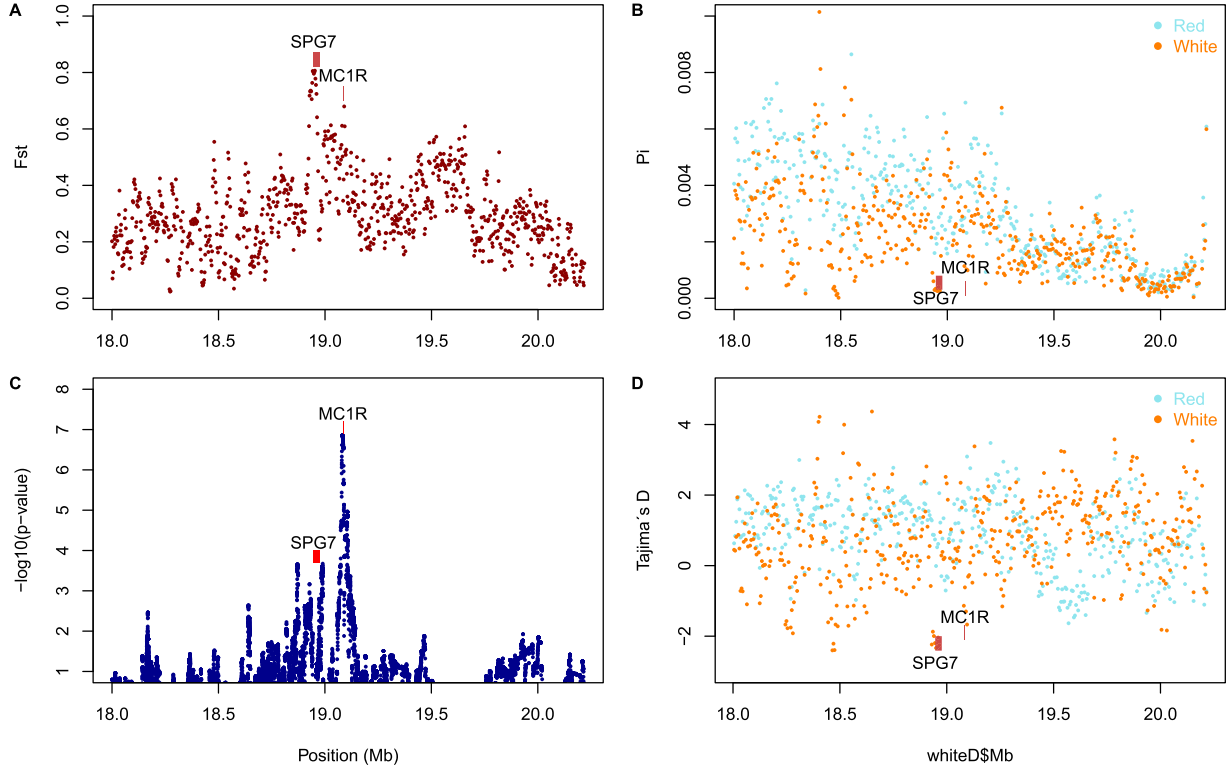

Figure 3. Region on chromosome 11 with the strongest selection signal in the genome-wide analyses. The Y-axis represents the Fst (A), Pi (B), the $-\log10(P$ value) from the HapFLK analysis (C) and Tajima's D (D) in chickens with red and white earlobe color. The X-axis gives the location on chromosome 11 in Mb.

chromosome 11 (Figure 3A), where the highest peak is located around 19 Mb. Two genes, *SPG7* and *MC1R*, are located in this region and both overlap with the highest signal in the Fst, and the HapFLK, analyses (Figure 3C). *MC1R* is known to control hair color (Valverde et al., 1995; Raimondi et al., 2008) in humans and coat/plumage color in animals (Kijas et al., 1998; Kerje et al., 2003; Mundy, 2005).

The Same Candidate Region on Chromosome 11 Supported Also by Analyses Using Tajima's D and Pi

Selection in the population was further explored via a measure of nucleotide diversity in the populations (Pi) and an index based on difference of the genetic diversity (Tajima's D). These analyses were performed to explore the robustness of the selection signals to the assumption of the analyses and provide further insights to the genomic basis of the selection signals in the genome related to the selection for earlobe color. To this end, the allele frequency changes, nucleotide diversity, and Tajima's D was evaluated for the candidate regions on chromosome 11 (Suppl. Figure 1, Figure 3B and D), and chromosome 4 (Suppl. Figure 2). The lack of Pi diversity and lower values (<0) of Tajima's D at the target locus around 19 Mb in the white earlobe chicken breeds suggests a selective sweep there. A comparison of the differences between the Fst, HapFLK, Pi and Tajima's D between red and white earlobe color chickens on chromosome 4 around the 72 Mb region was also performed. Here, the signal appears driven by fixation in the red earlobe breeds (Suppl. Figure 2C). However, the selection signature is not as strong as on chromosome 11 and there is no clear evidence of haplotype selection around the same region.

Haplotype Divergence Among Red and White Earlobe Breeds in the Selected Chromosome 11 Region

The haplotype-based selection analysis identified a slightly different shape of the peak than the Fst analysis (Figure 3A and C). The haplotype analysis method screened for selection signals among all included populations without dividing them into red and white earlobe breeds. Thus, further analyses of the haplotypes in this region within and across the populations would help clarifying the basis for the detected selection signal at the haplotype level. As illustrated in Figure 4, one major haplotype (Figure 4, red color) was only detected and present at high frequencies in the 2 white earlobe breeds White Leghorn and Black Minorca, suggesting a selective sweep around the *MC1R* gene region. In the *SPG7* gene region, 2 haplotypes (Figure 4, green and dark green) were at high frequency in the 2 white earlobe breeds, but were also present in red earlobe breeds including the Dominique and the White Plymouth Rock derived HWS and LWS selection lines. This haplotype

distribution among the breeds suggests the *MC1R* gene as the main positional candidate for the earlobe color.

Screening the Suggested Selective Sweep Region on Chromosome 11 for Positional Candidate Causal Variants

The candidate selective sweep region on chromosome 11 was screened for putative functional variants that might cause the red to white earlobe color change. In total, 66,111 SNPs were detected in this region (18,000,048−20,218,607 bp). Of these, 4 SNP alleles were found to be near fixation (derived allele frequency >95%) in the 2 white earlobe breeds while at very low frequencies (derived allele frequency <5%) in the red earlobe breeds (Table S1). None of the SNPs was found to be near fixation in red while presenting low derived allele frequency in the white. A screen for structural variants was performed using *Breakdancer* (Fan et al., 2014), but none of those detected were unique to either of the 2 color groups.

DISCUSSION

Extensive variation exists for coat, plumage and skin color among breeds of domestic animals. This makes them useful models to study the effects of individual and combinations of genes on pigmentation within and across species. Many contributing genes, and causal mutations in these, have been discovered making use of the recent innovations in both whole-genome sequencing and bioinformatics. One of the central pigmentation genes is *MC1R*, which controls coat color in dog (Anderson et al., 2020), pig (Kijas et al., 1998), sheep (Klungland et al., 1999), horse (Marklund et al., 1996), as well as the feather color in chicken (Kerje et al., 2003), Japanese quail (Nadeau et al., 2006), and Eleonora's falcon (Gangoso et al., 2011).

Most domestic breeds of chicken have red earlobes, while white earlobes are found in some breeds including those with Mediterranean origin. One reason for this divergence in color is that the trait has been selected by breeders to give particular breeds a distinct appearance. The coloring/no-color distinction in the earlobe is often consistent with the coloring/no-color feature of the egg shell, but no evidence has yet been provided to show that these 2 characters are genetically linked (Warren, 1928). The co-occurrences of these 2 color-related traits might therefore be more related to the origin of the breeds (Warren, 1928).

Previous studies have explored the genetics of earlobe color in several breeds. In the Rhode Island Red, it was reported to be a polygenic and sex-linked trait in GWAS using a 600K SNP-chip (Luo et al., 2018). In the Qingyuan Partridge chicken, multiple associations were detected both on the autosomes, and the Z chromosome, using a Reduced-Representation Genome Sequencing approach (Nie et al., 2016). No overlapping loci, genes or variants were detected in these studies, which might
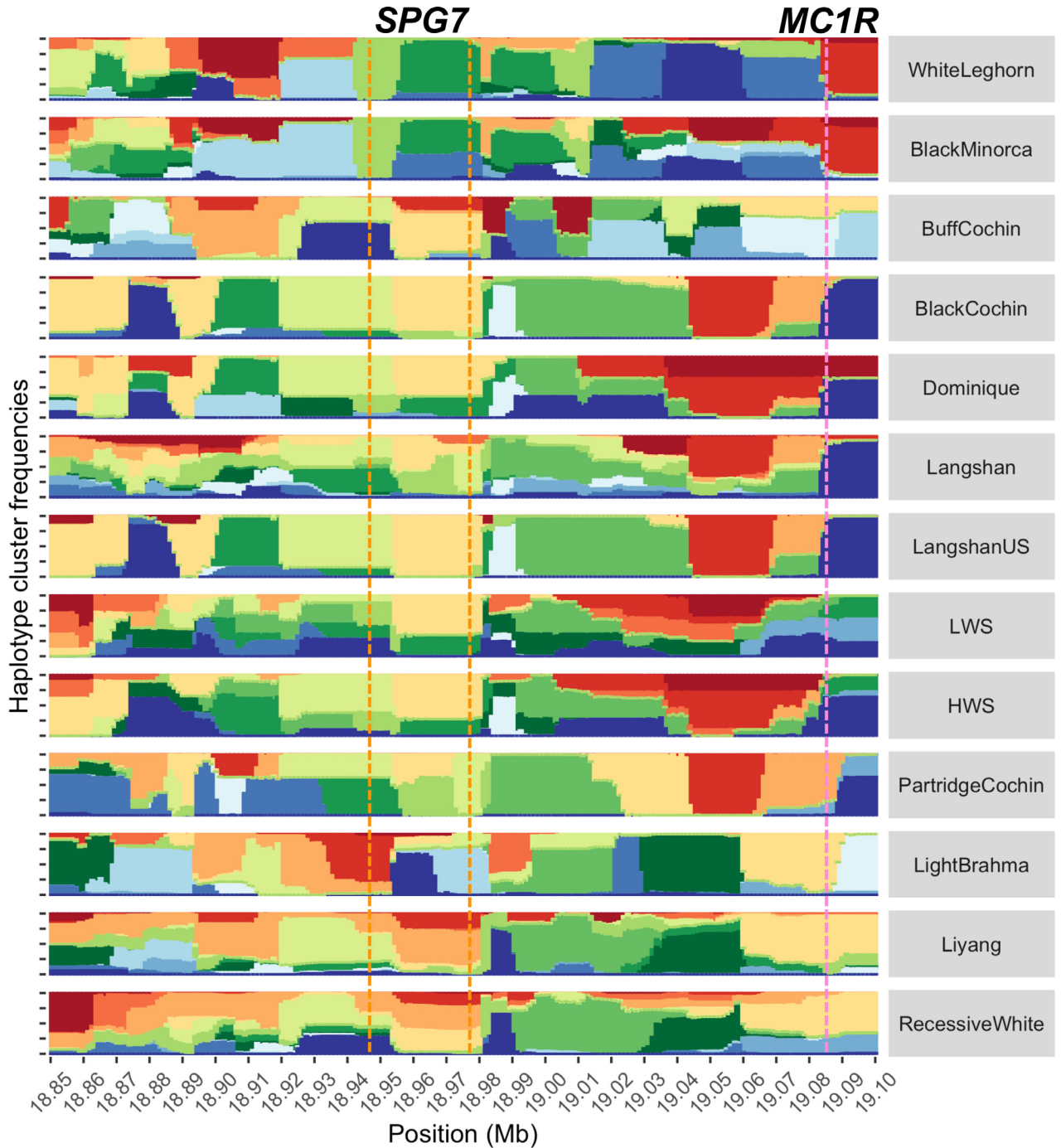
Figure 4. Haplotype cluster frequencies in the selective sweep peak region on chromosome 11. The x-axis provides the chromosomal location and the y-axis the frequency (0−1) of each haplotype cluster. The 2 breeds that have white earlobe are listed on the top. The region of gene *SPG7* is pointed by the orange bar while the location of *MC1R* gene is pointed by pink bar.

be due to i) there being different causal variants in the breeds (Warren, 1928), ii) mutations in different genes/pathways might lead to the trait or iii) that the density and information of the genetic markers were too low in the previous studies to explore the key loci in sufficient detail.

Here, we aimed to explore the genetic basis of earlobe color variation by comparing the full extent of genetic variation between white earlobe breeds of Mediterranean origin with multiple other red earlobe breeds. Previous research utilizing a cross between White Leghorn and Jersey Black Giant breeds suggested that the primary

factors influencing earlobe color are located on autosomal chromosomes (Warren, 1928). Therefore, our study solely focused on analyzing the autosomal chromosomes to elucidate the genetic basis of earlobe color variation. The selective sweep mapping approach used in the current study is conceptually different from the genome-wide association approach taken in the earlier studies mentioned above (Nie et al., 2016; Luo et al., 2018). Putative selected regions were here identified as those that were highly divergent between the evaluated breeds, accounting for the population structure by combining multiple breeds with different origins, but

with similar earlobe colors, in 2 groups, and then using different population-genetic approaches to manage and compare the genomic structures among the populations. A strong signal, that was stable in most analyses, was found in a region on chromosome 11. There, white and red earlobe breeds were highly differentiated, but despite this no obvious causative mutation was detected in the target region or genes. Two candidate functional genes (Figure 3), *SPG7* and *MC1R*, overlap the peak. Genetic variation in *SPG7* has been reported be associated with hereditary spastic paraplegia in human (Sánchez-Ferrero et al., 2013) while no studies have linked it with pigmentation. For *MC1R*, a well-known connection with coloration has been established in many studies in animals (Kijas et al., 1998; Everts et al., 2000; Kerje et al., 2003; Mundy, 2005). Our results indicate that *MC1R* is also a key gene for regulating chicken earlobe color.

A second putative selective sweep was detected on chromosome 4 (Suppl. Figure 2) and this overlapped with a weakly associated region reported in previous study (Wragg et al., 2012). In that study 10 white vs. 47 red earlobe chickens were contrasted by using genotypes obtained from the 60K SNP array analyzed for a sliding window size of 20 kb. Few SNP markers ($n = 1,649$) located on chromosome 11 were present on that 60K SNP array with no markers located on/near *MC1R* and only 3 close to *SPG7*. Hence, discovering the selection signal is likely to have been more difficult using such a sparse SNP dataset. In the putative sweep region on chromosome 4, the selective sweep is due to fixation in many of the red earlobe breeds (Suppl. Figure 2), supported by the low Tajima's D and high Fst detected at around 71.8 Mb on this chromosome. In a 1 Mb region surrounding this selection signal (70.8−72.8 Mb), there is only 1 well-annotated protein coding gene (***PCDH7***), which has no known function related to pigmentation.

CONCLUSIONS

Herein, to detect selection signatures for earlobe color in the domestic chicken, we used whole-genome sequencing to perform a selective sweep mapping analysis. A robust signal was detected on chromosome 11 in Mediterranean-origin breeds, overlapping the key pigmentation gene *MC1R*, making this gene a key candidate for regulating pigmentation of the chicken earlobe.

ACKNOWLEDGMENTS

Ethics Statements: All procedures involving animals were carried out in accordance with the Virginia Tech Animal Care Committee animal use protocols.

Data Archiving Statement: All whole-genome sequence data are available from NCBI with BioProject numbers: PRJNA597842 and PRJNA552722.

DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2023.103232.

REFERENCES

Anderson, H., L. Honkanen, P. Ruotanen, J. Mathlin, and J. Donner. 2020. Comprehensive genetic testing combined with citizen science reveals a recently characterized ancient MC1R mutation associated with partial recessive red phenotypes in dog. Canine Med. Genet. 7:1–11.

Chen, L., X. Gu, X. Huang, R. Liu, J. Li, Y. Hu, G. Li, T. Zeng, Y. Tian, and X. Hu. 2020. Two cis-regulatory SNPs upstream of ABCG2 synergistically cause the blue eggshell phenotype in the duck. PLoS Genet. 16:e1009119.

Danecek, P., A. Auton, G. Abecasis, C. A. Albers, E. Banks, M. A. DePristo, R. E. Handsaker, G. Lunter, G. T. Marth, S. T. Sherry, G. McVean, and R. Durbin. 2011. The variant call format and VCFtools. Bioinformatics 27:2156–2158.

Dorshorst, B., A.-M. Molin, C.-J. Rubin, A. M. Johansson, L. Strömstedt, M.-H. Pham, C.-F. Chen, F. Hallböök, C. Ashwell, and L. Andersson. 2011. A complex genomic rearrangement involving the endothelin 3 locus causes dermal hyperpigmentation in the chicken. PLoS Genet. 7:e1002412.

Dunn, L. C. 1922. A gene for the extension of black pigment in domestic fowls. Am. Nat. 56:464–466.

Everts, R. E., J. Rothuizen, and B. A. Van Oost. 2000. Identification of a premature stop codon in the melanocyte-stimulating hormone receptor gene (MC1R) in Labrador and Golden retrievers with yellow coat colour. Anim. Genet. 31:194–199.

Fan, X., T. E. Abbott, D. Larson, and K. Chen. 2014. BreakDancer: identification of genomic structural variation from paired-end read mapping. Curr. Protoc. Bioinform. 45:15–16.

Fariello, M. I., S. Boitard, H. Naya, M. SanCristobal, and B. Servin. 2013. Detecting signatures of selection through haplotype differentiation among hierarchically structured populations. Genetics 193:929–941.

Gangoso, L., J. M. Grande, A. Ducrest, J. Figuerola, G. R. Bortolotti, J. A. Andrés, and A. Roulin. 2011. MC1R-dependent, melanin-based colour polymorphism is associated with cell-mediated response in the Eleonora's falcon. J. Evol. Biol. 24:2055–2063.

Gunnarsson, U., A. R. Hellström, M. Tixier-Boichard, F. Minvielle, B. Bed'Hom, S. Ito, P. Jensen, A. Rattink, A. Vereijken, and L. Andersson. 2007. Mutations in SLC45A2 cause plumage color variation in chicken and Japanese quail. Genetics 175:867–877.

Guo, Y., X. Gu, Z. Sheng, Y. Wang, C. Luo, R. Liu, H. Qu, D. Shu, J. Wen, R. P. M. A. Crooijmans, Ö. Carlborg, Y. Zhao, X. Hu, and N. Li. 2016. A complex structural variation on chromosome 27 leads to the ectopic expression of HOXB8 and the muffs and beard phenotype in chickens. PLoS Genet. 12:e1006071.

Guo, Y., M. Lillie, Y. Zan, J. Beranger, A. Martin, C. F. Honaker, P. B. Siegel, and Ö. Carlborg. 2019. A genomic inference of the White Plymouth Rock genealogy. Poult. Sci. 98:5272–5280.

Guo, Y., J. Ou, Y. Zan, Y. Wang, H. Li, C. Zhu, K. Chen, X. Zhou, X. Hu, and Ö. Carlborg. 2021. Researching on the fine structure and admixture of the worldwide chicken population reveal connections between populations and important events in breeding history. Evol. Appl. 15:553–564.

Kerje, S., Ö. Carlborg, L. Jacobsson, K. Schütz, C. Hartmann, P. Jensen, and L. Andersson. 2003. The twofold difference in adult size between the red junglefowl and White Leghorn chickens is largely explained by a limited number of QTLs. Anim. Genet. 34:264–274.

Kerje, S., P. Sharma, U. Gunnarsson, H. Kim, S. Bagchi, R. Fredriksson, K. Schütz, P. Jensen, G. Von Heijne, and R. Okimoto. 2004. The Dominant white, Dun and Smoky color

variants in chicken are associated with insertion/deletion polymorphisms in the PMEL17 gene. Genetics 168:1507–1518.

Kijas, J. M. H., R. Wales, A. Törnsten, P. Chardon, M. Moller, and L. Andersson. 1998. Melanocortin receptor 1 (MC1R) mutations and coat color in pigs. Genetics 150:1177–1185.

Klungland, H., K. H. Røed, C. L. Neø, K. S. Jakobsen, and D. I. Våge. 1999. The melanocyte-stimulating hormone receptor (Mci-R) gene as a tool in evolutionary studies of artiodactyles. Hereditas 131:39–46.

Luo, W., J. Xu, Z. Li, H. Xu, S. Lin, J. Wang, H. Ouyang, Q. Nie, and X. Zhang. 2018. Genome-wide association study and transcriptome analysis provide new insights into the white/red earlobe color formation in chicken. Cell. Physiol. Biochem. 46:1768–1778.

Marklund, L., M. J. Moller, K. Sandberg, and L. Andersson. 1996. A missense mutation in the gene for melanocyte-stimulating hormone receptor (MCIR) is associated with the chestnut coat color in horses. Mamm. Genome 7:895–899.

Mundy, N. I. 2005. A window on the genetics of evolution: MC1R and plumage colouration in birds. Proc. R. Soc. B Biol. Sci. 272:1633–1640.

Nadeau, N. J., F. Minvielle, and N. I. Mundy. 2006. Association of a Glu92Lys substitution in MC1R with extended brown in Japanese quail (Coturnix japonica). Anim. Genet. 37:287–289.

Nie, C., Z. Zhang, J. Zheng, H. Sun, Z. Ning, G. Xu, N. Yang, and L. Qu. 2016. Genome-wide association study revealed genomic regions related to white/red earlobe color trait in the Rhode Island Red chickens. BMC Genet. 17:115.

Qanbari, S., C.-J. Rubin, K. Maqbool, S. Weigend, A. Weigend, J. Geibel, S. Kerje, C. Wurmser, A. T. Peterson, and I. L. Brisbin Jr. 2019. Genetics of adaptation in modern chicken. PLoS Genet. 15:e1007989.

Raimondi, S., F. Sera, S. Gandini, S. Iodice, S. Caini, P. Maisonneuve, and M. C. Fargnoli. 2008. MC1R variants, melanoma and red hair color phenotype: a meta-analysis. Int. J. Cancer 122:2753–2760.

R Core Team. 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Sánchez-Ferrero, E., E. Coto, C. Beetz, J. Gamez, A. I. Corao, M. Diaz, J. Esteban, E. Del Castillo, G. Moris, and J. Infante. 2013. SPG7 mutational screening in spastic paraplegia patients supports a dominant effect for some mutations and a pathogenic role for p. A510V. Clin. Genet. 83:257–262.

Våge, D. I., and I. A. Boman. 2010. A nonsense mutation in the beta-carotene oxygenase 2 (BCO2) gene is tightly associated with accumulation of carotenoids in adipose tissue in sheep (Ovis aries). BMC Genet. 11:1–6.

Valverde, P., E. Healy, I. Jackson, J. L. Rees, and A. J. Thody. 1995. Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. Nat. Genet. 11:328–330.

Wang, Z., L. Qu, J. Yao, X. Yang, G. Li, Y. Zhang, J. Li, X. Wang, J. Bai, and G. Xu. 2013. An EAV-HP insertion in 5′ flanking region of SLCO1B3 causes blue eggshell in the chicken. PLoS Genet. 9:e1003183.

Warren, D. C. 1928. Inheritance of earlobe color in poultry. Genetics 13:470.

Wragg, D., J. M. Mwacharo, J. A. Alcalde, P. M. Hocking, and O. Hanotte. 2012. Analysis of genome-wide structure, diversity and fine mapping of Mendelian traits in traditional and village chickens. Heredity (Edinb.) 109:6.