Estimation of genetic variability and identification of regions under selection based on runs of homozygosity in Beijing-You Chickens

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ABSTRACT The genetic composition of populations is the result of a long-term process of selection and adaptation to specific environments and ecosystems. Runs of homozygosity (ROHs) are homozygous segments of the genome where the 2 haplotypes inherited from the parents are identical. The detection of ROH can be used to describe the genetic variability and quantify the level of inbreeding in an individual. Here, we investigated the occurrence and distribution of ROHs in 40 Beijing-You Chickens from the random breeding population (BJY_C) and 40 Beijing-You Chickens from the intramuscular fat (IMF) selection population (BJY_S). Principal component analysis (PCA) and maximum likelihood (ML) analyses showed that BJY_C was completely separated from the BJY_S. The nucleotide diversity of BJY_C was higher than that of BJY_S, and the decay rate of LD of BJY_C was faster. The ROHs were identified for a total of 7,101 in BJY_C and 9,273 in BJY_S, respectively. The ROH-based inbreeding estimate (FROH) of BJY_C was 0.079, which was significantly lower than that of BJY_S (FROH = 0.114). The results were the same as the estimates of the inbreeding coefficients calculated based on homozygosity (FHOM), the correlation between uniting gametes (FUNI), and the genotypic relationship matrix (FGRM). Additionally, the distribution and number of ROH islands in chromosomes of BJY_C and BJY_S were significantly different. The ROH islands of BJY_S that included genes associated with lipid metabolism and fat deposition, such as CIDEA and S1PR1, were absent in BJY_C. However, GPR161 was detected in both populations, which is a candidate gene for the formation of the unique five-finger trait in Beijing-You chickens. Our findings contributed to the understanding of the genetic diversity of random or artificially selected populations, and allowed the accurate monitoring of population inbreeding using genomic information, as well as the detection of genomic regions that affect traits under selection.

Key words: biodiversity, ROH, selection signature, chickens

INTRODUCTION

Common metrics used to assess genetic diversity include nucleotide diversity, effective allele frequency, polymorphic information content, and genotypic heterozygosity (Liu et al., 2020). The value of heterozygosity reflects the level of variation in the population and usually ranges between 0 and 1 (Madilindi et al., 2020). Higher heterozygosity represents richer genetic diversity (Kaviriri et al., 2020). Natural populations usually showed the large heterogeneities and high genetic diversities (Hoban et al., 2020). It was found that sheep populations deviated from Hardy-Weinberg equilibrium when the actual heterozygosity was lower than desired heterozygosity, suggesting the occurrence of selection or inbreeding (Windig et al., 2019). In addition, the most direct indicator for assessing the genetic diversity of a population is the inbreeding coefficient (Kalashnikov et al., 2020). Due to the rapid development of sequencing technologies, the use of resequencing data to calculate the true inbreeding coefficients of genomes is gradually becoming a trend.

The run of homozygosity (ROH) information can be used as an analytical indicator to better study the genetic diversity of livestock populations (Meyermans et al., 2020). In addition, common ancestry analyses based on ROHs can provide a more effective means of reducing inbreeding decline in breeding programs (Zhang et al., 2015). A previous study reported a
MATERIALS AND METHODS

Ethics Statement

The animal experiments were carried out in accordance with the Guidelines for Experimental Animals established by the Ministry of Science and Technology (Beijing, China). The study was approved by the Animal Management Committee of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (Beijing, China). Ethical approval regarding animal survival was given by the animal ethics committee of IASCAAS (approval number: IASCAAS-AE20140615).

Animals and Sample Collection

Blood samples were collected from forty 120-day-old Beijing-You Chickens (17 males and 23 females) of a random breeding population (BJY_C) and 40 Beijing-You Chickens (22 males and 18 females) of a selection population (BJY_S) for DNA extraction. The BJY_S population was selected for the relative content of intramuscular fat for 5 yr, and 40 males were selected each year to be matched with 160 females.

Whole-Genome Resequencing

Blood collected from the veins of all animals was extracted using the standard phenol/chloroform extraction method. The quantity and quality of DNA extracted from whole blood samples were determined using a Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA) and agarose gel electrophoresis was used to visually assess DNA integrity. Samples that passed the quality test were sent to the Beijing Compass Biotechnology Company for 10G whole-genome sequencing.

After sequencing, paired-end libraries were generated for each eligible sample using standard procedures. The average insert size was 300 to 500 bp, and the average read length was PE150 bp (150 bp paired-end reads). All libraries were separately sequenced on a HiSeq X Ten sequencer to average raw read sequence coverage of 10×.

The filtered raw reads were compared to the reference genome (version: ftp://ftp.ensembl.org/pub/release-101/fasta/gallus_gallus/dna/) using the MEM mode of BWA software (version 0.7.12). Picard (version 1.119) and SAMtools (version 1.9) were used to obtain the sorted BAM files. Before detecting mutations, the basic quality score recalibration (BQSR) was recalibrated, which involved 2 steps. In the first step, Base Recalibrator, the Picard tool and stool were used to obtain the classified BAM file for analysis. In the second step, Apply BQSR, the calibration table file obtained in the first step was used to readjust the basic quality value in the original BAM file. The new quality value was then re-exported to a new BAM file. GATK (version 4.0.2.1) was used to select GATK variants for filtering (Poplin et al., 2018). The hard filter standards used for single nucleotide polymorphisms (SNPs) were QualByDepth (QP) < 2.0, RMSMappingQuality (MQ) < 40, FisherStrand (FS) > 60.0, StrandOddsRatio (SOR) > 3.0, and MappingQualityRankSumTest (MQRankSum) < -12.5.

The data were first filled with Beagle v5.0 software. We first filled SNP genotypes with Beagle v5.0 (Ayres et al., 2012) for all SNP loci, and the filled SNP locus was quality-controlled using the Plink v1.9 software and then quality-controlled the SNPs using Plink v1.9 (Purcell et al., 2007), according to the following criteria: 1) the call rate was higher than 0.9; 2) the minor allele frequency (MAF) was higher than 0.05; and 3) SNPs were filtered to exclude loci assigned to unmapped contigs and sex chromosomes. After quality control, 80 chickens and 6,252,214 variants were retained. The clean DNA sequencing data reported in this paper have been deposited in the Genome Sequence Archive (Wang et al., 2017) in the BIG Data Center (2019) under accession number CRA004519, and can be publicly accessed at http://bigd.big.ac.cn/gsa.

Genetic Characterization

Different approaches and software were used to disclose the genetic structure of Beijing-You Chickens:

a) Wright’s statistics, including observed heterozygosity (\(H_0\)), expected heterozygosity (\(H_E\)), and inbreeding coefficients. Genomic inbreeding based on homozygous SNPs was determined using PLINK v1.9 software. The inbreeding coefficient for an individual
(F\textsubscript{HOM}) was computed as $F_{\text{HOM}} = (O - E)/(L - E)$, where $O$ is the number of observed homozygotes, $E$ is the number of homozygotes expected by chance, and $L$ is the number of genotyped autosomal SNPs. Genomic SNP-by-SNP inbreeding coefficient ($F_{\text{GRM}}$) estimates were calculated using GCTA software. The $F_{\text{GRM}}$ was calculated as $F_{\text{GRM}} = \frac{1}{m} \sum_{i=1}^{N} \left( \frac{(p_i - E_i)^2}{2p_i(1-p_i)} - 1 \right)$, where $x_i$ is the number of copies of the reference allele for the $i$\textsuperscript{th} SNP, $m$ is the number of SNPs, and $p_i$ is the frequency of the reference allele. Genomic inbreeding coefficients were also estimated based on ROHs ($F_{\text{ROH}}$). The $F_{\text{ROH}}$ for each animal was calculated as $F_{\text{ROH}} = \frac{\sum_{i=1}^{N} L_{\text{ROH}i}}{L_{\text{auto}}}$, where $L_{\text{ROH}i}$ is the length of ROH$^i$ of individual $I$, and $L_{\text{auto}}$ is the autosomal genome length covered by the SNPs included on the chip. Genomic inbreeding coefficients were estimated based on uniting gametes ($F_{\text{UNI}}$). The $F_{\text{UNI}}$ for each animal was calculated as $F_{\text{UNI}} = \frac{x_i^2 + 2p_i x_i + 2p_i^2}{2p_i(1-p_i)}$, where $x_i$ is the number of copies of the reference allele for the $i$\textsuperscript{th} SNP, and $p_i$ is the frequency of the reference allele.

b) Linkage disequilibrium within 500 kb was calculated using PopLDdecay software (Zhang et al., 2019), and then, the genome-wide LD distribution was mapped based on marker spacing and $r^2$ values. 

c) Principal component analyses (PCA) were performed using GCTA 64 software. The PCA of pairwise individual genetic distances was performed based on the allele frequencies of pruned SNPs. 

d) ADMIXTURE v.1.3.0 software was used to infer the most probable number of ancestral populations based on the SNP data (Alexander et al., 2009). ADMIXTURE was run from $K = 1$ to $K = 12$, and the optimal number of clusters (K-value) was determined as the one having the lowest cross-validation error. Each inferred chicken population structure was visualized using an R script, as suggested in the ADMIXTURE procedure. 

e) A maximum likelihood (ML) tree was created and graphically represented using RAxML and FigTree version 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree) software, respectively. 

f) ROH analysis was performed for each individual (complete SNP dataset = 6,252,214) using Plink software. The ROH was defined by (Yuan, et al., 2022): 1) a minimum of 200 kb in size and 100 homozygous SNPs; 2) one heterozygous SNP was permitted in the ROH, so that the length of the ROH was not disrupted by an occasional heterozygote; 3) one missing SNP was allowed in the ROH; 4) the maximum gap between SNPs of 40 kb was predefined to ensure that the SNP density did not affect the ROH. According to the nomenclature reported by other authors (Zavarez, et al., 2015), the ROHs were grouped into 5 classes of length: <0.5 Mb, 0.5-0.1 Mb, 1-2 Mb, 2-4 Mb, and >4 Mb. Number, total length, and the average ROH length were calculated across individuals within the chicken populations. In addition, the percentage of the total genome length affected by the ROH was also estimated.

### RESULTS

#### Genetic Diversity Analysis

A comparison of the nucleotide polymorphisms of the 2 populations revealed that the nucleotide diversity ($\Pi$) of BJY$-\_C$ (0.0027) was slightly higher than that of BJY$-\_S$ (0.0025) (Table 1). The observed heterozygosity ($H_O$) and expected heterozygosity ($H_E$) of BJY$-\_C$ ($H_O = 30.26\%$, $H_E = 31.48\%$) were significantly higher than that of BJY$-\_S$ ($H_O = 26.19\%$, $H_E = 29.51\%$), and the difference between the $H_O$ and $H_E$ of BJY$-\_S$ (3.31\%) was higher than that of BJY$-\_C$ (1.22\%) (Table 1). In addition, the mean inbreeding coefficient of BJY$-\_C$ ($F_{\text{HOM}} = 0.0065$, $F_{\text{GRM}} = 0.0062$, $F_{\text{UNI}} = 0.0637$, $F_{\text{ROH}} = 0.079$) was significantly lower than that of BJY$-\_S$ ($F_{\text{HOM}} = 0.1868$, $F_{\text{GRM}} = 0.1384$, $F_{\text{UNI}} = 0.1646$, $F_{\text{ROH}} = 0.114$) (Table 1).

#### Genetic Characterization

The degree of LD was attenuated with increasing marker distance, and the decay rate of LD was also decreased gradually. The LD decay analysis showed that BJY$-\_C$ had a faster decay rate of LD than that of BJY$-\_S$ (Figure 1). In the PCA analysis, BJY$-\_C$ was completely separated from the BJY$-\_S$, and there were no outlying samples (Figure 2A).

The ADMIXTURE program was run for $K$ values from 2 to 3 (Figure 2B and 2D). The lowest cross validation error was found at $K = 2$, and represents the number of ancestors in Beijing-You Chickens (Figure 2C). A number

|        | $\Pi$  | $H_O$ | $H_E$ | $F_{\text{HOM}}$ | $F_{\text{GRM}}$ | $F_{\text{UNI}}$ | $F_{\text{ROH}}$ |
|--------|--------|-------|-------|-----------------|-----------------|-----------------|-----------------|
| BJY$-\_C$ | 0.002717 | 0.302681 | 0.314877 | 0.0605 | 0.0062 | 0.0637 | 0.079 |
| BJY$-\_S$ | 0.002552 | 0.261985 | 0.295136 | 0.1868 | 0.1384 | 0.1646 | 0.114 |

Note: BJY$-\_C$ and BJY$-\_S$ represent a random breeding population of Beijing-You Chickens and a selection population of Beijing-You Chickens based on intramuscular fat, respectively. $\Pi$, $H_O$ and $H_E$ represent the nucleotide diversity, heterozygosity, and heterozygosity, respectively. $F_{\text{HOM}}$ represents the inbreeding coefficient for an individual, $F_{\text{GRM}}$ represents the genomic SNP-by-SNP inbreeding coefficient, and $F_{\text{UNI}}$ and $F_{\text{ROH}}$ represent the genomic inbreeding estimates based on the correlation between uniting gametes and ROHs, respectively.
of K greater than 2 did not produce a larger number of ancestor contributions in Beijing-You Chickens, as is shown in Figure 2B and 2D. When K = 2, the samples had the best segregation (Figure 2C), and segregation occurred between BJY_C and BJY_S. A phylogenetic tree for BJY_C and BJY_S was constructed using probabilistic methods of phylogenetic inference (Figure 3). All the samples of BJY_C were clustered together, as did the samples of BJY_S, with no outliers.

Summary of Runs of Homozygosity

The total number of ROHs detected on all chromosomes is shown in Tables 2 and 3. A total of 7,101 runs in BJY_C (Table 2) and 9,273 runs in BJY_S (Table 3) were identified. The results showed that the number of ROHs in BJY_S increased by 2172 compared to BJY_C. The ROHs less than 0.5 Mb and 0.5−1 Mb accounted for a relatively high proportion of the total ROH in both populations (BJY_C: 76%, 20%, BJY_S: 70%, 23%, respectively). The results also showed that the percentage of the ROHs less than 0.5 Mb of BJY_C was higher than that of BJY_S, but with lengths between 0.5 and 1 Mb was less. In addition, there were no ROHs with lengths greater than 4 Mb in BJY_C, but 2 were detected in the selected population, located on chromosomes 15 and 18, respectively. It is worth mentioning that we did not find any ROH on Chr31 in any of the 80 chickens.

Except for 2 chickens in BJY_C, the average length of ROHs in other individuals was greater than 0.35 Mb in both populations, and the number of ROHs in most individuals ranged from 150 to 250. The length of most ROHs per individual ranged (as mean values) from 0.35 Mbp to 0.5 Mbp (Figure 4).

Functional Annotation of Genes

IMF in breast muscle (IMF_{br}, %) (dry weight basis) of BJY_S (BJY_S: IMF_{br}= 2.85 ± 0.90) was significantly higher than that of BJY_C (BJY_C: IMF_{br}= 2.60 ± 0.78, P < 0.05) due to the artificial selection (Liu et al.,...
To identify candidate genes associated with the selected trait, the incidence of ROH fragments across the genome was presented as a Manhattan plot (Figure 5). The genomic distribution of ROH segments was non-uniform across chromosomes in BJY_C and BJY_S. The threshold used to define ROH islands was 37.5 and 47.5% in BJY_C and BJY_S, respectively. We selected the top 10 ROH islands in the top 1% of SNPs most commonly observed in the ROHs of the 2 populations. We annotated the associated genes based on the autosomal chicken gene list (Gallus gallus, GRCg6a.101.gff3) (Tables 4 and 5). In BJY_C, there were seven genes in the ROH islands on chromosome 1, two genes on chromosomes 5 and 15, and one gene on chromosome 17. Additionally, there were 17 genes in ROH islands on chromosome 1, nine genes on chromosome 2, and eight genes on chromosome 8 in BJY_S. Multiple ROH islands were identified on chromosome 1 in both populations. Among them, 6 of the top ten ROH islands in BJY_S were located on chromosome 1, while 5 of the top 10 ROH islands were located on chromosome 1 in BJY_C. Three common genes, GPR161, ST3GAL6 and COL8A1, were observed in multiple overlapping regions on chromosome 1 in both populations. Moreover, the ROH islands on chromosome 1 of BJY_S also harbored genes associated with lipid metabolism and fat deposition, such as CIDEA and S1PR1, however, such genes were absent in BJY_C.

**DISCUSSION**

In this study, the patterns of variation in high-density SNPs were used to detect genetic variability between conserved and selected populations of Beijing-You Chickens. Through ROH analyses of resequencing information, the findings of this study highlighted changes in the population structure and variant genes of Beijing-You Chickens. Nucleotide diversity is a commonly used measure of diversity within or between populations, and is a quantitative indicator of genetic variation (Tatarinova et al., 2016; Adhikari et al., 2020). Nucleotide diversity is also often associated with other values that measure population diversity, such as the expected heterozygosity (Selvam et al., 2017; López-Cortegano et al., 2019).

With the widespread use of SNP gene chips and resequencing technologies for livestock, ROH studies based on genomic information are becoming increasingly prevalent (Rodríguez-Ramilo et al., 2019). Population structure and population evolution studies based on ROHs have also been performed in cattle (Bos taurus) (Xu et al., 2019), pigs (Xie et al., 2019; Shi et al., 2020), horses (Grilz-Seger et al., 2019), sheep (Deniskova et al., 2019; Gorssen et al., 2020), goats (Nandolo et al., 2018), and chickens (Talebi, et al., 2020). Our results showed that BJY_S, with relatively homogeneous genetic diversity, had smaller Pi values compared to BJY_C. These
findings were in agreement with the results of another study (Kijas et al., 2019) where selection led to a decrease in population diversity. The effective number of polymorphic SNPs (considered as the number of SNP in which at least one heterozygous individual was identified) represents the 99.9% of the total loci (Zhang et al., 2017). The moderately high values of \( H_O \) and \( H_E \) of the inbreeding coefficient were consistent with the two populations in this study (Yang et al., 2019; Smaragdov and Kudinov, 2020). Thus, the collective results of this study indicated that the genetic backgrounds of the 2 populations of Beijing-You Chickens were different.

Traditionally, the inbreeding coefficient is estimated using pedigree data (\( F_{PED} \)). However, \( F_{PED} \) could not completely reveal the relatedness among individuals in the population, especially the missing parent or incorrect parent information. With the development of high-throughput genotyping technologies, genomic inbreeding coefficients, such as \( F_{R0H}, F_{HOM}, F_{UNI}, \) and \( F_{GRM} \) can be computed using molecular information. In fact, the inbreeding coefficients calculated using different

| Chr | <0.5 MB | 0.5–1 MB | 1–2 MB | 2–4 MB | >4 MB | Total |
|-----|---------|---------|--------|--------|------|-------|
| 1   | 0.96    | 17      | 0      | 0      | 1    | 32.5  |
| 2   | 0.92    | 20      | 0      | 0      | 1    | 32.5  |
| 3   | 0.85    | 25      | 0      | 0      | 1    | 28    |
| 4   | 0.83    | 22      | 0      | 0      | 1    | 46.8  |
| 5   | 0.75    | 29      | 0      | 0      | 1    | 74.2  |
| 6   | 0.73    | 39      | 0      | 0      | 1    | 74.2  |
| 7   | 0.72    | 36      | 0      | 0      | 1    | 72    |
| 8   | 0.69    | 31      | 0      | 0      | 1    | 74.2  |
| 9   | 0.69    | 36      | 0      | 0      | 1    | 72    |
| 10  | 0.63    | 32      | 0      | 0      | 1    | 64    |
| 11  | 0.63    | 30      | 0      | 0      | 1    | 60    |
| 12  | 0.67    | 39      | 0      | 0      | 1    | 78    |
| 13  | 0.73    | 43      | 0      | 0      | 1    | 86    |
| 14  | 0.72    | 47      | 0      | 0      | 1    | 94    |
| 15  | 0.57    | 39      | 0      | 0      | 1    | 78    |
| 16  | 0.46    | 25      | 0      | 0      | 1    | 50    |
| 17  | 0.66    | 25      | 0      | 0      | 1    | 50    |
| 18  | 0.67    | 35      | 0      | 0      | 1    | 70    |
| 19  | 0.77    | 40      | 0      | 0      | 1    | 80    |
| 20  | 0.83    | 39      | 0      | 0      | 1    | 78    |
| 21  | 0.71    | 32      | 0      | 0      | 1    | 64    |
| 22  | 0.72    | 36      | 0      | 0      | 1    | 72    |
| 23  | 0.82    | 43      | 0      | 0      | 1    | 86    |
| 24  | 0.78    | 40      | 0      | 0      | 1    | 80    |
| 25  | 1.00    | 22      | 0      | 0      | 1    | 44    |
| 26  | 0.83    | 30      | 0      | 0      | 1    | 60    |
| 27  | 0.88    | 25      | 0      | 0      | 1    | 50    |
| 28  | 0.89    | 28      | 0      | 0      | 1    | 56    |
| 29  | 0.97    | 30      | 0      | 0      | 1    | 60    |
| 30  | 1.00    | 33      | 0      | 0      | 1    | 66    |
| 31  | 0       | 0       | 0      | 0      | 0    | 0     |
| 32  | 0       | 0       | 0      | 0      | 0    | 0     |
| 33  | 0.84    | 2       | 0      | 0      | 1    | 3     |
| 34  | 0.94    | 2       | 0      | 0      | 1    | 3     |

\(^1\)Proportion calculated as number of ROH per class over the total number of ROH.

Figure 4. Relationship between the number and average length of ROHs in each individual in BJY_C and BJY_S. BJY_C and BJY_S represent a random breeding population of Beijing-You Chickens and a selection population of Beijing-You Chickens based on intramuscular fat, respectively. Abbreviation: ROHs, runs of homozygosity.
methods in different buffalo populations were similar, and all were representative of inbreeding (Ghoreishifar et al., 2020). FHOM may overestimate inbreeding levels because it cannot distinguish IBD alleles from IBS alleles (Wang et al., 2014). In addition, FHOM and FGRM values can be negative for some individuals. Thus, FROH may be a more accurate method for quantifying animal inbreeding levels, which can alleviate the issues mentioned above (Liu et al., 2020). Also, the report showed that there were clear variations in the inbreeding of ROH arising from the selection pressure experienced by the chickens (Zhang et al., 2020). In this study, we calculated the different genomic inbreeding coefficients of BJY_S and BJY_C. The FHOM, FUNI, FGRM, and FROH showed the same results, that the inbreeding coefficient of BJY_S was higher than that of BJY_C. Additionally, the FHOM, FUNI, FGRM, and FROH were similar in the BJY_C population, while FHOM was the highest (0.1868) and FROH was the lowest (0.114) in the BJY_S population.

ROHs are not randomly distributed throughout the genome, and most ROHs appear in the selected regions, where they cluster as ROH islands. Compared to other regions of the genome, ROH islands have low genetic diversity and high homozygosity. Therefore, ROH islands contain many candidate genes related to specific traits (Zhang et al., 2018). We identified a large number of ROH islands in the BJY_S and BJY_C populations, and the number and distribution of which were significantly different. GPR161, ST3GAL6, and COL8A1 were the common genes in the top 10 ROH islands of the 2 populations.

A previous study showed that GPR161, a G protein coupled receptor, negatively regulated the sonic hedgehog pathway (SHH) through cAMP signaling (Mukhopadhyay et al., 2013). SHH is involved in limb development in vertebrates (Lin et al., 2020), and is a candidate gene responsible for the polydactylous mouse mutant Sasquatch phenotype (Sharpe et al., 1999). Thus, GPR161 may also be a candidate gene for the formation of the unique five-finger trait in Beijing-You chickens.

Some genes associated with lipid metabolism and fat deposition, such as CIDEA and S1PR1, were identified on the ROH islands of BJY_S, but were absent in BJY_C. CIDEA was identified to bind lipid droplets and regulate their enlargement, thereby restricting lipolysis and favoring lipid storage (Jash et al., 2019). Additionally, single-locus and multilocus genome-wide association studies for IMF in duroc pigs showed that S1PR1 was associated with IMF based on its potential functional roles in lipid metabolism (Ding et al., 2019). The formation of these ROH islands with genes related to fat metabolism may be related to the artificial selection of IMF in Beijing-You chickens.

We used ROH to estimate the inbreeding coefficient in Beijing-You Chickens. The results showed that inbreeding was higher in the BJY_S (Table 1). We found significant differences in the total number of ROHs in individuals from the 2 different populations, and the

Table 4. The 10 top 1% ROH identified on BJY_C autosomes by PLINK.

| ROH_ID | Chr | Start   | End     | Length   | Genes                  |
|--------|-----|---------|---------|----------|------------------------|
| S1     | 1   | 166960573| 167004023| 43.451   | *                      |
| S2     | 6   | 19191553 | 19303357 | 111.805  | *                      |
| S3     | 1   | 8525533  | 85300605 | 45.467   | GPR161                 |
| S4     | 1   | 85300879 | 85461940 | 160.962  | ST3GAL6, COL8A1        |
| S5     | 17  | 10341476 | 10589614 | 248.139  | ALC, LMX1B, ZBTB43, ZBTB34, RALGPS1 |
| S7     | 1   | 85556245 | 85559292 | 19.048   | *                      |
| S8     | 1   | 85556811 | 85602505 | 45.695   | *                      |
| S9     | 15  | 9478785  | 9515894  | 37.11    | MLEC, CABB1            |
| S10    | 5   | 39087143 | 39247885 | 160.743  | ANGEL1, IRF2BP1        |

Note: The threshold of 1% is determined by the incidence of SNPs in ROH.
average length of ROHs was longer in BJY_S, thus, also suggesting that selection and a reduction in population size was more likely to form ROH. Those findings were also in agreement with the results of ROH studies in different breeds of cattle (Cendron, et al., 2021). ROH islands are subject to stress selection when the candidate genes in such regions undergo adaptive selection (Mastrangelo, et al., 2017), and thus, the candidate genes obtained in this study based on the ROH islands can be used to distinguish changes in genetic levels following selection in Beijing-You Chickens.

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**DISCLOSURES**

The authors declare no conflict of interest.

**SUPPLEMENTARY MATERIALS**

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.102342.

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