Signatures of selection reveal candidate genes involved in production traits in Chinese crossbred buffaloes

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ABSTRACT

Identification of selection signature is important for a better understanding of genetic mechanisms that affect phenotypic differentiation in livestock. However, the genome-wide selection responses have not been investigated for the production traits of Chinese crossbred buffaloes. In this study, an SNP data set of 133 buffaloes (Chinese crossbred buffalo, $n = 45$; Chinese local swamp buffalo, $n = 88$) was collected from the Dryad Digital Repository database (<https://datadryad.org/stash/>). Population genetics analysis showed that these buffaloes were divided into the following 2 groups: crossbred buffalo and swamp buffalo. The crossbred group had higher genetic diversity than the swamp group. Using 3 complementary statistical methods (integrated haplotype score, cross population extended haplotype homozygosity, and composite likelihood ratio), a total of 31 candidate selection regions were identified in the Chinese crossbred population. Here, within these candidate regions, 25 genes were under the putative selection. Among them, several candidate genes were reported to be associated with production traits. In addition, we identified 13 selection regions that overlapped with bovine QTLs that were mainly involved in milk production and composition traits. These results can provide useful insights regarding the selection response for production traits of Chinese crossbred buffalo, as identified candidate genes influence production performance.

Key words: crossbred buffalo, milk performance, signature of selection

INTRODUCTION

Buffaloes (*Bubalus bubalis*) are an important source of milk and meat production. Buffalo milk provides

more than 5% of the world's milk supply (Windsor et al., 2021). Buffaloes are generally divided into 2 types, river [diploid number ($2n$) = 50] and swamp ($2n = 48$), which differ in morphology, behavior, and the number of chromosomes (Di Berardino and Iannuzzi, 1981). River buffaloes are mainly used for milk and meat production, whereas swamp-type buffaloes are used for draft power and meat production (Zhang et al., 2020). Extensive efforts have been performed on the genetic improvement of dairy swamp buffaloes using the crossbreeding system in the southeast Asia. Over the last half-century in China, it is well known that crossbreds of the introduced river and Chinese local swamp buffalo breeds yield more milk than that of the Chinese local buffalo. Evidence shows that the river buffalo yield approximately 2,200 to 2,400 kg/year of milk, whereas the swamp buffalo yield only 500 to 700 kg/yr of milk (Shi et al., 2012); the crossbred buffaloes yield an average of ~1,700 kg of milk per lactation (Deng et al., 2019). It was reported that the milk production of Murrah crossbreds in the first and second generation respectively reached 1,240.5 kg and 1,423.3 kg, and those for Nili-Ravi crossbreds in the first and second generation respectively reached 2,041.2 kg and 2,351.3 kg as a result of a good heterosis, whereas the milk yield of the triple crossbreds and their offspring respectively reached 2,294.6 kg and 1,994.9 kg (Yang et al., 2013), as still expected by heterotic effects. Thus, the crossbred buffaloes have played an important role in meeting the Chinese rising demand for milk, making it a potential resource for the Chinese dairy market.

Today, SNP array technology is a popular and powerful tool for the assessment of genetic diversity and signature of selection in composite breeds (Kim and Rothschild, 2014; Cheruiyot et al., 2018; Singh et al., 2020). Thus far, in buffaloes, genetic improvement has been carried out by classical breeding programs and by marker-assisted selection for dairy traits such as higher concentrations of caseins (Zicarelli et al., 2020; Paucullo et al., 2021) and milk fat (Gu et al., 2019, 2020). However, since the use of the Axiom Buffalo Genotyping Array (Iamartino et al., 2017), the information on

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genetic diversity, population structure, domestication, and migration of river- and swamp-type buffalo has also increased (Colli et al., 2018; Lu et al., 2020; Macciotta et al., 2021). This enables us to understand the resources and characteristics of buffalo breeds across the world. Moreover, identification of recent selection signatures in domesticated animals could provide genomic information of the selection response in production traits, assisting the design of more efficient selection schemes (Pan et al., 2016; Pavlidis and Alachiotis, 2017; Friedrich and Wiener, 2020). The loci and genomic regions subject to positive selection are widely reported in different domestic animals (Jahuey-Martínez et al., 2019; Manzari et al., 2019; Yu et al., 2020). There are a few reported results on the selection signature of other buffalo breeds, such as Murrah, Nili-Ravi, and crossbred breeds. Mokhber et al. (2018) identified several candidate genes associated with economically important traits in Iran Azeri and Khuzestani buffalo breeds using the buffalo SNP array. In Chinese crossbred buffalo, there is no documented information on the selective sweeps. Therefore, the current study aimed to identify selection signatures in the genome of the Chinese crossbred buffalo using 3 complementary approaches. The integrated haplotype score (**iHS**) was used to detect within-population selection signatures. Then, the composite likelihood ratio (**CLR**) test was performed to detect the selective sweep in crossbred buffalo using site frequency spectrum patterns of SNPs. Further, the crossbred buffalo were compared with swamp breeds by cross-population extended haplotype homozygosity (**XP-EHH**) to discover the genomic regions that correlated with the divergence of the composite from each of its parental populations since the Admixture. These identified candidate genes will help in accreting the genetic program of buffalo molecular breeding.

MATERIALS AND METHODS

Ethics Statements

Animal care and use committee approval was not required for this study because the data were obtained from an existing database.

Data Source

In this study, 2 SNP data sets of 133 buffaloes from 7 Chinese local buffalo breeds and 1 crossbred breed were collected. The 7 Chinese local buffalo breeds were Enshi ($n = 15$), Fulin ($n = 15$), Guizhou ($n = 11$), Binhu ($n = 15$), Wenzhou ($n = 3$), Haizhi ($n = 14$), and Yibin ($n =$

15) buffalo breeds, respectively. The Chinese crossbred buffaloes ($n = 45$) were the Mediterranean $\times$ Jiangnan $\times$ Nili-Ravi, Murrah $\times$ Nili-Ravi $\times$ Xilin, and Murrah $\times$ Nili-Ravi $\times$ Fuzhong buffalo breeds. Of the 133 buffaloes, 88 individuals were reported in Colli et al. (2018), and 45 animals were tested in a previous study (Deng et al., 2019). All genotyped data were generated by using the 90K Affymetrix Buffalo SNP Array (Affymetrix/Thermo Fisher Scientific). The raw data were converted to the input files of PLINK v1.9 software (Purcell et al., 2007), and a data set of 63,524 SNPs was generated using the bcftools v1.12 (Danecek et al., 2021) software. Then, Beagle v5.2 software (Browning et al., 2018) was used to phase haplotypes and impute missing genotypes of the merged SNPs. The quality of imputed SNP data was controlled according to the following parameters: (1) SNP call rates >0.99 ; (2) individual with call rates >0.99 ; (3) SNPs with minor allele frequencies >0.05 ; (4) removal of a significant ($P < 1E-6$) deviation from Hardy-Weinberg equilibrium; (5) discarding of all unmapped SNPs and those on sex chromosomes. The related individuals were further removed by using the IBD method implemented in PLINK v1.9 (Purcell et al., 2007) software. In the end, a set of 35,562 SNPs from 131 buffaloes were used for the following analyses.

Population Structure

To investigate the genetic relationship between the individuals, principal component analysis (**PCA**) was performed using the GCTA v1.93.2 software (Yang et al., 2011). Then the first 2 principal components were plotted via the ggplot2 package in R (<https://www.r-project.org/>). Further, the MEGA-X software (version 10.2.6; Kumar et al., 2018) was used to construct the neighbor-joining trees of individuals to confirm the phylogenetic relationships between the breeds. Finally, the Admixture v1.30 software (Alexander and Lange, 2011) was used for the estimation of breed composition in the tested populations.

Expected heterozygosity (**He**), observed heterozygosity (**Ho**), effective population size (**Ne**), and allelic richness (**A_R**) were calculated to evaluate the genetic diversity of the tested buffalo populations, including the crossbred buffalo (**CB**; $n = 44$) and swamp buffalo (**SB**; $n = 87$) populations. The PLINK v1.9 (Purcell et al., 2007) was used to calculate the H_e and H_o between alleles at 2 separate SNP loci with the default setting. The SNeP v1.1 software (Barbato et al., 2015) was used to estimate the effective population size (N_e) in the test populations.

Signature of Selection

Three methods, the iHS, CLR, and XP-EHH tests, were used to detect the selection signatures. First, the iHS test was used to compare the EHH between derived and ancestral alleles within the crossbred buffalo population (Voight et al., 2006). The iHS score was calculated for each autosomal SNP in the CB group using the *selscan* v1.3 software (Szpiech and Hernandez, 2014). The normalized iHS values were further analyzed by the *norm* program with the default parameters provided by *selscan*. The top 1% of the empirical distributions of the normalized iHS ($iHS > 2.41$) were considered as potential selection scans.

The CLR test uses information from allele frequencies to detect selective scans for the determination of skews in the allele spectrum to bias rare and frequent alleles (Nielsen et al., 2005). The CLR value was calculated for each autosomal SNP in the crossbred buffalo population using SweeD v4.0.0 software (Pavlidis et al., 2013). The potential selected regions were deemed as the 99.5th percentile of the empirical genome-wide distribution of CLR, as discussed by Wang et al. (2018).

Unlike within-population tests (iHS and CLR), the XP-EHH test could detect the ongoing or approximately fixed selection signatures across the populations through comparison of the frequencies of the selected haplotypes (Sabeti et al., 2007). Here, the crossbred and the swamp buffalo populations were used as test and reference populations, respectively. The XP-EHH scores were estimated and standardized between CB and SB groups using *selscan* v1.3 software (Szpiech and Hernandez, 2014). The normalized XP-EHH values in the top 1% of the empirical distribution ($XP-EHH > 2.31$) are defined as the selection signatures.

Candidate selection sweep regions were detected as regions having the identified SNPs with a window size of 50 kb and overlapping regions with at least 2 different statistics. The selected window size was in accordance with a previous study that suggested that linkage disequilibrium in crossbred buffaloes does not exceed 50 kb (Deng et al., 2019). Genes within the candidate regions of the selection signatures were annotated by the gene annotation files of the buffalo genome (*UOA_WB_1*; Low et al., 2019). For the above annotated genes, the protein-coding genes were used for further analysis. The Animal Quantitative Trait Loci database (Animal QTLdb; <https://data.nal.usda.gov/dataset/animal-quantitative-trait-loci-database-animal-qtl-db>; Hu et al., 2019) was used to predict the potential phenotypic traits for the candidate genes. Meanwhile, the bovine genome coordinates corresponding to the selection regions in buffalo were generated by the LiftOver (version

1.1.11; Hinrichs et al., 2006) software, which was used to identify overlapping QTL described in CattleQTLdb v. 45 (Hu et al., 2019). In addition, the published literature was used to infer the potential function of the candidate genes affecting the traits. Moreover, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis for the candidate genes was performed using the KOBAS 3.0 (Bu et al., 2021) software with the *Bos taurus* as the background. Biological significance was considered if the number of input genes was >2 and $P < 0.05$.

RESULTS

Population Structure of Chinese Buffalo Breeds

To investigate the breed separation among the studied populations, we analyzed the phylogenetic relationship, PCA, and population structure. The phylogenetic relationship analysis revealed that there was a distinct separation between the crossbred buffalo and swamp-type buffalo (Figure 1A). In addition, the first principal component accounted for 23.74% of the total variation and separated crossbred breeds from swamp breeds (Figure 1B). The PCA further supported the results of phylogenetic analysis. A key quantity in the analysis of structured populations is the parameter K, which describes the number of subpopulations that make up the total population. Admixture results for $K = 1$ to $K = 4$ showed that the $K = 2$ represented the most appropriate population number for the present data set (Figure 1C; Supplemental Table S1; <https://doi.org/10.7910/DVN/Z4JNVQ>; Deng, 2021). These results suggested that these buffaloes can be divided into 2 groups (CB and SB) for further analysis.

The estimation of genetic diversity for the studied populations is listed in Table 1. Compared with the SB group, the CB group displayed a comparably high level of genetic diversity, as measured by H_e (mean $\pm$ SD; 0.388 ± 0.120 vs. 0.163 ± 0.195) and H_o (0.392 ± 0.139 vs. 0.162 ± 0.196). In addition, the CB group ($A_R = 1.992 \pm 0.087$) had higher A_R values than the SB group ($A_R = 1.556 \pm 0.465$). Moreover, the N_e values for the most recent generation (13) were 124 and 528 for CB and SB groups, respectively.

Within-Population Selection Signatures in the Crossbred Population (iHS and CLR)

The iHS approach was used to identify the selection sweeps based on the recent selection history in the crossbred buffalo population. The results revealed that 108 potential regions were ($iHS \geq 2.41$) distrib-

Figure 1. Phylogenetic relationship and population structure of buffalo breeds. (A) Neighbor-joining representation of the pairwise Nei's D genetic distances among populations. (B) Principal component (PC) analysis plots display the individual relationships of 131 buffaloes. (C) Population structure of 131 buffaloes inferred by model-based clustering using Admixture software. K = cluster number. CB = crossbred buffaloes; SB = swamp buffaloes.

uted across 18 autosomes of the crossbred population (Figure 2A, Supplemental Table S2; <https://doi.org/10.7910/DVN/Z4JNVQ>; Deng, 2021). The strongest signal of iHS (3.7205) was found on the buffalo (*Bubalus bubalis*) chromosome 3 (BBU3) (163.00–163.05 Mb). The top 10 regions with their iHS values and genes are shown in Supplemental Table S3 (<https://doi.org/10.7910/DVN/Z4JNVQ>; Deng, 2021). We observed that a total of 13 genes were annotated into these regions.

Alternatively, the CLR test was used to detect selective scans in the crossbred buffalo population. The findings demonstrated that 148 potential regions (CLR ≥ 4.00) were distributed across 23 autosomes of the studied population (Figure 2B, Supplemental Table S4; <https://doi.org/10.7910/DVN/Z4JNVQ>; Deng, 2021). The strongest signature was located in the BBU2 (77.81–77.84 Mb). The top 10 regions with their CLR values and genes are represented in Supplemental Table

Table 1. Sample description (mean $\pm$ SD) for the studied buffalo population

Parameter ¹	Crossbred	Swamp
Nu	44	87
Ne	124	528
He	0.388 ± 0.120	0.163 ± 0.195
Ho	0.392 ± 0.139	0.162 ± 0.196
A_R	1.992 ± 0.087	1.556 ± 0.465

¹ Nu = the number of samples; Ne = effective population size; He = expected heterozygosity; Ho = observed heterozygosity; A_R = allelic richness.

S5 (<https://doi.org/10.7910/DVN/Z4JNVQ>; Deng, 2021). A total of 5 genes were found within these regions.

Across Population Selection Signatures Based on XP-EHH

Manhattan plots of pairwise XP-EHH analysis between CB and SB groups are presented in Figure 3. The detailed information on the 135 potential regions is displayed in Supplemental Table S6 (<https://doi.org/10.7910/DVN/Z4JNVQ>; Deng, 2021). These regions were distributed across 19 autosomes; among them, the strongest XP-EHH signal occurred at BBU3. The top

Figure 2. Manhattan plot of (A) the integrated haplotype score (iHS) and (B) composite likelihood ratio (CLR) values for the Chinese crossbred buffalo. The black line indicates the threshold level of the top 1% iHS and top 0.5% CLR values.

Figure 3. The cross population extended haplotype homozygosity (XP-EHH) plots for comparisons of crossbred buffaloes with swamp buffaloes. The blue line indicates the threshold level of the top 1% XP-EHH values.

10 regions with their XP-EHH values and genes are shown in Table 2. Results showed that 3 genes were annotated to the potential regions mentioned above.

Comparative Analysis of Selective Sweep

The distribution of overall potential selected regions detected by 3 complementary methods (iHS, CLR, and XP-EHH) has been shown in Figure 4. Among them, serval potential overlapping regions were observed on 11 different chromosomes, including the BBU1, BBU2, BBU3, BBU4, BBU6, BBU8, BBU9, BBU11, BBU13, BBU15, and BBU20. The detailed information on the overlapping regions, which were detected by at least 2 methods, is listed in Table 3. In total, 31 regions were weighed as the final candidate regions of positive selection. A total of 25 genes were annotated into these

selective sweeps. The detailed information on their GO and KEGG enrichment analyses is listed in Supplemental Table S7 (<https://doi.org/10.7910/DVN/Z4JNVQ>; Deng, 2021). The GO analysis showed that these genes were enriched into 10 GO terms, including the synaptic membrane adhesion (GO:0099560), modulation of chemical synaptic transmission (GO:0050804), and PDZ domain binding (GO:0030165), among others. The KEGG enrichment analysis revealed that genes present in these regions enriched 2 pathways including the cell adhesion molecules and Axon guidance.

Further, we compared the regions under selection with the cattle QTL database, and the results are present in Supplemental Table S8 (<https://doi.org/10.7910/DVN/Z4JNVQ>; Deng, 2021). We observed that a total of 61 reported QTLs were related to milk production and composition traits. Relatively concentrated areas

Table 2. List of the top 10 regions identified by cross population extended haplotype homozygosity (XP-EHH) and the genes within them

BBU ¹	Start (Mb)	End (Mb)	XP-EHH	P-value	Gene	Trait
1	83.80	83.85	3.1736	0.0011	<i>LOC112583749</i>	
1	174.43	174.48	3.1700	0.0011	—	
3	155.21	155.26	3.9211	7.81E-05	—	
3	100.27	100.32	3.5467	0.0003	—	
3	155.15	155.20	3.3761	0.0006	—	
3	100.30	100.35	3.3707	0.0006	—	
3	92.17	92.22	3.1606	0.0011	<i>FREM1</i>	Reproduction (Wiradjaja et al., 2013)
8	84.32	84.37	3.1362	0.0012	—	
8	37.57	37.62	3.1053	0.0014	<i>PCLO</i>	Maternal calving difficulty (Purfield et al., 2015)
15	75.94	75.99	3.1692	0.0011	—	

¹BBU = buffalo chromosome.

related to milk production and composition traits were noted on BBU1, BBU6, BBU9, BBU11, and BBU13.

DISCUSSION

Chinese crossbred buffaloes are the consequence of crossbreeding program between Chinese local swamp buffaloes and river buffaloes to improve production performance over several decades ago. The performance of the resulting crossbreds significantly improved as the milk yield increased to 1,200 to 2,000 kg (Yang et al., 2007). Therefore, investigation of the genomic background of Chinese crossbred buffalo leads to better understanding of the major genes affecting milk production in buffaloes, which can be taken into account for future breeding programs. In the present study, a genotypic data set of 131 buffaloes (44 Chinese crossbred buffalo data included) were collected from a public database. The phylogenetic analysis results showed that animals were located into 2 groups (CB vs. SB breeds). Both PCA and Admixture analysis results further supported these results. Moreover, we observed that the CB group displayed a comparably high level of genetic diversity [H_e (0.388 ± 0.120 vs. 0.163 ± 0.195) and H_o (0.392 ± 0.139 vs. 0.162 ± 0.196)] compared with the SB group. A similar result has been reported by Colli et al. (2018).

Production traits as quantitative traits are affected by many genes. Identification of causative genes or variants has a key role in uncovering the genetic architecture underlying polygenic traits in livestock such as buffaloes (Gu et al., 2019; Zicarelli et al., 2020; Paucullo et al., 2021). Nowadays, several strategies are used to identify the causative genes or variants associated with the traits of interest, such as the candidate-gene approach (Gu et al., 2020), GWAS (Liu et al., 2018), runs of homozygosity (ROH) island (Macciotta et al., 2021), and signature of selection (Mokhber et al., 2018). In addition, the identification of causative mutations associated with the quantitative traits is still a challenge because most of these variants are located in noncoding regions of the genome. In this study, genomic regions that were under putative selection in Chinese crossbred buffaloes were investigated. According to previous studies, combined assessments of selection signatures can provide an unbiased criterion to identify variants under selection more precisely (Grossman et al., 2010; Ma et al., 2015; Ghoreishifar et al., 2020). Accordingly, 3 complementary statistical methods (iHS, CLR, and XP-EHH) were used to conduct the selective sweep analysis in the Chinese crossbred buffalo population. Using the iHS method, 81 potential regions ($iHS \geq 2.41$) across 18 autosomes were identified within the crossbred population (Figure 2). Focusing on the top 10

Figure 4. Genomic distribution of selection signature detected by 3 complementary methods (iHS, XP-EHH, and CLR). The red, blue, and green colors represent the iHS, XP-EHH, and CLR signatures, respectively. iHS = integrated haplotype score; XP-EHH = cross population extended haplotype homozygosity; CLR = composite likelihood ratio.

regions with the iHS values, we found 13 genes located in the potential selected regions. Among them, the *LPAR1* (Ye and Chun, 2010), *COX7C* (Selvaraju et al., 2018), *CADPS* (Galliou et al., 2020), and *ADAM3* (Yamaguchi et al., 2009) genes have been confirmed to be associated with reproductive traits. The *CADPS* gene has also been reported to be a candidate gene affecting BW (Yang et al., 2019). As for the selection signature within the population, the CLR test found 148 potential regions ($CLR \geq 4.00$) across 23 autosomes in the crossbred buffalo population. Notably, we observed that 5 genes were annotated into the top 10 regions with the CLR values. Among them, the *NAALADL2* gene was reported to be associated with milk CLA content in cattle (Strillacci et al., 2014). By the XP-EHH method, 135 potential regions were discovered in the crossbred population. For the top 10 selected regions, we only observed the *FREM1* gene annotated to the regions

(92.16~92.21 Mb) of BBU3. It has been reported to have a reproductive role (Wiradjaja et al., 2013).

By 3 methods (iHS, CLR, and XP-EHH), 31 significant signals were found in the Chinese crossbred buffalo population. Twenty-five genes (*ROBO1*, *CCDC50*, *UST2B*, *NLGN1*, *ARL6IP6*, *NTNG1*, *ITGB3BP*, *LR-RFIP1*, *LOC112585195*, *LOC112584709*, *EFCAB7*, *EXOC4*, *OR2T27*, *OR2T1*, *OR2T2*, *MDGA2*, *SCG5*, *TMCO5B*, *ARHGAP11A*, *FMN1*, *KIF23*, *PAQR5*, *PIBF1*, *KHDRBS3*, and *CHD2*) overlapped by at least 2 methods. The *ROBO1* (Nadesalingam et al., 2001) and *KHDRBS3* (Jiang et al., 2019) genes were reported to be associated with milk production traits in cattle, respectively. Using the cattle QTL database, we found that 13 selection regions in crossbred buffalo population were overlapped with the bovine QTL regions (Supplemental Table S8). The corresponding regions in the bovine genome contain 61 reported QTLs

Table 3. Selection sweeps identified by at least 2 tests and annotated genes in these regions using 3 methods [cross population extended haplotype homozygosity (XP-EHH), composite likelihood ratio (CLR), and integrated haplotype score (iHS)]

BBU ¹	Start (bp)	End (bp)	Detection method	Gene	Trait
1	71,853,981	71,935,290	XP-EHH, CLR	<i>ROBO1</i> ²	Milk traits (Nadesalingam et al., 2001)
1	120,339,661	120,389,661	iHS, CLR	—	—
1	120,846,630	120,896,630	iHS, CLR	<i>CCDC50</i> , <i>UTS2B</i>	—
1	121,102,139	121,193,883	XP-EHH, CLR	<i>LOC112585195</i>	—
1	137,786,832	137,836,832	iHS, CLR	<i>NLGN1</i> ²	Growth traits (Zhuang et al., 2020)
2	95,054,201	95,104,201	XP-EHH, CLR	—	—
2	95,953,056	96,003,056	XP-EHH, CLR	—	—
2	95,995,991	96,020,991	XP-EHH, CLR	<i>ARL6IP6</i>	—
3	100,022,187	100,354,732	XP-EHH, CLR	—	—
4	158,354,717	158,404,717	iHS, CLR	<i>LOC112584709</i>	—
4	159,553,844	159,603,844	XP-EHH, CLR	—	—
6	36,001,246	36,051,246	iHS, XP-EHH	<i>NTNG1</i> ²	Inseminations per conception (Galliou et al., 2020); BW (Cole et al., 2014)
6	81,364,748	81,414,748	XP-EHH, CLR	<i>ITGB3BP</i> , <i>EFCAB7</i>	—
6	116,680,610	116,730,610	XP-EHH, CLR	<i>LRRFIP1</i> ²	Fat deposition (Stambuk et al., 2020)
8	2,339,059	2,389,059	iHS, CLR	—	—
8	97,067,483	97,117,483	XP-EHH, CLR	<i>EXOC4</i>	—
9	25,485,987	25,535,987	iHS, CLR	—	—
9	69,127,414	69,177,414	XP-EHH, CLR	<i>OR2T27</i> , <i>OR2T1</i> , <i>OR2T</i>	—
11	61,891,319	61,941,319	iHS, CLR	—	—
11	62,608,836	62,934,032	XP-EHH, CLR, iHS	<i>MDGA2</i>	—
11	63,715,423	63,765,423	iHS, CLR	—	—
11	73,239,962	73,289,962	XP-EHH, CLR	—	—
11	73,288,129	73,338,129	XP-EHH, CLR	<i>ARHGAP11A</i> , <i>SCG5</i>	—
11	73,987,833	74,037,833	iHS, CLR	<i>FMN1</i> ² , <i>TMCO5B</i>	Age at second calving (Buzanskas et al., 2017)
11	86,353,809	86,512,307	XP-EHH, CLR	<i>KIF23</i> , <i>PAQR5</i>	—
13	39,474,821	39,524,821	iHS, CLR	—	—
13	43,132,835	43,182,835	iHS, CLR	<i>PIBF1</i> ²	Embryonic development (Tao et al., 2021)
13	44,092,925	44,142,925	iHS, CLR	—	—
15	75,778,026	75,888,166	XP-EHH, CLR	<i>KHDRBS3</i> ²	Milk traits (Rutten et al., 2013)
15	75,939,189	760,162,96	XP-EHH, CLR	—	—
20	55,503,444	55,553,444	iHS, CLR	<i>CHD2</i> ²	Sperm quality (Gòdia et al., 2020)

¹BBU = buffalo chromosome.

²Genes found in the literature to be associated with specific traits related to livestock breeding.

that are related to the milk production and composition traits. Relatively concentrated areas related to milk production and composition traits were noted on BBU1, BBU6, BBU9, BBU11, and BBU13. The results suggested that these candidate genes (including the *ROBO1*, *LRRFIP1*, *ARHGAP11A*, *SCG*, *FMN1*, *TMCO5B*, *PIBF1*, and *KHDRBS3*) within the regions might play vital roles in milk production traits in buffaloes. Moreover, 4 genes were reported to be associated with reproduction traits in different farm animals, such as the *NTNG1* gene with inseminations per conception in cattle (Galliou et al., 2020), the *FMN1* gene with age at second calving in cattle (Buzanskas et al., 2017), the *PIBF1* gene with embryonic development in sheep (Tao et al., 2021), and the *CHD2* gene with sperm quality in pig (Gòdia et al., 2020). In addition, Cole et al. (2014) also found that *NLGN1* was the candidate gene influencing the BW in pigs. Moreover, our findings showed that 3 genes (*OR2T27*, *OR2T1*, *OR2T2*) belonged to members of the olfactory receptor gene family (Olender et al., 2008). The olfactory receptors have vital roles in the regulation of appetite in humans and rodents, which can affect animal production efficiency (Connor et al., 2018). Therefore, the 3 olfactory receptor genes could have some function in the production performance in buffalo.

CONCLUSIONS

In the present study, we investigated the population structure and genetic diversity of Chinese crossbred buffalo. Using 3 complementary statistical methods, 31 candidate selection regions were detected in the Chinese crossbred population. Among them, several candidate genes were reported to be associated with production traits and adaptation. In addition, we identified 13 selection regions that overlapped with bovine QTLs that were mainly involved in milk production and composition traits. These results provided useful insights into the selection responses for production traits in Chinese crossbred buffalo and identified several candidate genes influencing production performance.

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