ABSTRACT

The Jianchang duck is mainly distributed in Southwest China, and has the characteristics of fast growth rate and strong abilities in lipid deposition in the liver. In order to investigate the effects of domestication process on formation of the unique characteristics of Jianchang duck, the whole genome of sixteen individuals and three pooling of Jianchang duck were re-sequenced, and genome data of 70 mallards and 83 domestic ducks from thirteen different places in China were obtained from NCBI. The population stratification and evolution analysis showed gene exchanges existed between the Jianchang and other domestic duck populations, as well as Jianchang ducks and mallards. Genomic comparison between mallards and Jianchang ducks showed genes, including CNTN1, CHRNA9, and SHANK2, which is involved in brain and nerve development, experienced strong positive selection in the process of Jianchang duck domestication. The genomic comparison between Jianchang and domestic duck populations showed that HSD17B12 and ESM1, which affect lipid metabolism, experienced strong positive selection during the domestication process. FST analysis among populations of Jianchang duck with different plumage colors indicated that MITF was related to the phenotype of a white feather, while MC1R was related to the phenotype of hemp feather. Our results provided a base for the domestication process of Jianchang duck and the genomic genes for unique traits.

KEYWORDS

Jianchang duck domestication molecular phylogeny selection signature plumage coloration

Domestic ducks are domesticated from mallards since about 2,200 years ago in China as a single domestication event (Qu et al. 2009; Zhang et al. 2018). Under the impacts of directional artificial selection, the ducks were classified into three types depending on the usage purpose, including egg type, meat type, and dual-purpose type (Su et al. 2007). During the domestication process of duck genes that affect the brain and neuron development, which undergone a strong positive selection effect (Zhang et al. 2018). And the genes that refer to plumage color were selected as well, e.g., the alleles of MITF genotypes were already fixed in Pekin duck, a famous white plumage meat-type duck breed (Zhou et al. 2018).

Jianchang ducks, mainly distributed in Southwest China (Liangshan, Sichuan province), have four populations according to their feather color phenotypes, including white feather, light hemp feather, deep hemp feather and white chest black feather (Figure 1A-D) (Yang et al. 1996). Compared with other local duck breeds in China, Jianchang ducks grow fast and easily deposit lipid in the liver (Jiansheng 1987; Wang et al. 2018). These characteristics may be related to natural living environments and long-term artificial selection. The natural environment and geographical isolation in Southwest China, have led to genetic changes of many species (Li et al. 2013; Wang et al. 2015; Lan et al. 2018). Particularly, the high altitude and low temperature in Liangshan areas (Liu et al. 2017) may improve the ability of Jianchang ducks
duck to resist the cold temperature through increasing fat deposition and body size. Besides that, the Hui nationality people living in Liangshan area for hundreds of years (Kongshao 2005), prefer to take the belly fat of stuffed salted duck as cooking oil, which may also lead to an indirect selection on the enhancement of fat deposition ability of Jianchang duck.

The long-term domestication process will produce genetic footprints on the genomes of animals (Franz and Larson 2018; Guo et al. 2018; Wilkinson and Wiener 2018). We suspected that there would be genetic changes underlying formation of the characteristics of Jianchang duck during long-term domestication process. Analysis of microsatellite and mtDNA markers demonstrated that the genetic background of Jianchang duck was significantly different from other local breeds (Qu et al. 2009). In order to investigate the effects of domestication process on formation of the unique characteristics of Jianchang duck, it is necessary to further investigate the genetic footprints of Jianchang duck at a nuclear genome view basing on genome re-sequencing. These analyses could provide a basis for further evaluation of the unique genetic foundation of Jianchang duck.

MATERIALS AND METHODS

Sampling and DNA isolation
All the experimental procedures, described below, were approved by the Animal Ethics Monitoring Committee of Sichuan Agriculture University and carried out in accordance with Guideline of Animal Welfare China. Whole blood samples were taken from the duck wing vein using standard venipuncture, and genomic DNA was extracted using standard phenol/chloroform extraction method. Subsequently, the concentration and purity of the extracted DNA were detected using an ultraviolet spectrophotometer. In individual sequencing, sixteen Jianchang duck individuals were selected. Total sixteen individuals were selected from the four populations for resequencing, i.e., deep hemp feather (n = 4), shallow hemp feather (n = 4), white feather (n = 4), and white chest black feather (n = 4). Each feather color population includes half male and half female. In pooling sequencing, total three pooling DNA were prepared and each pooling was mixed with DNA from twenty individuals, definitely, hemp feather pooling includes ten deep hemp and ten shallow hemp individuals, white feather pooling includes twenty white feathers individuals, white chest black feather pooling includes twenty white chest black feathers individuals. Each pooling includes half males and half females, and a similar amount of DNA for each individual was mixed (Supplementary Tables S1). The total DNA concentration was above 100ng / ul, and the total DNA content was above 20 ug.

Library preparation, high-throughput sequencing and download sequenced data
For each sample, two paired-end libraries (150 bp) were constructed according to the manufacturer protocols (Illumina) and sequenced on the Illumina Hiseq 2500 sequencing platform. The sixteen individuals were sequenced at 5× coverage, and the three pooling DNA samples were sequenced at 20× coverage (Supplementary Tables S2). Clean data of genome 153 duck individuals from thirteen places in China were downloaded from NCBI (https://www.ncbi.nlm.nih.gov) and their information was provided in supplementary data (Supplementary Tables S3). These data were included Banzui duck (BZ; n = 2), Longsheng duck (LS; n = 2), Wansui duck (W; n = 2), Sansui duck (SS; n = 2), Putian duck (PT; n = 2), J’an duck (GA; n = 2), Taiwan duck (TW; n = 2), Youxian duck (YX; n = 2), Gaoyou duck (GY; n = 10), Jinding duck (JD; n = 10), Shanma duck (SM; n = 10), Shaoxing duck (SX; n = 37) and Mallard (MD; n = 70).

Sequence filtering, read alignment and variation detection
The raw reads were filtered using NGS QC (v2.3.3) Toolkit (Corkins et al. 2017) with default parameters. The filtered reads were mapped to the Anas platyrhynchos genome (duckbase.refseq.v4; (Yu et al. 2018) using BWA (v0.7.12; (Jo and Koh 2015) with default parameters. After aligning the reads, SNPs and small indels (1-50 bp) were called used GATK (v4.0.11.0; (Nielsen et al. 2011) with the parameter of minimum quality score of 20 on the mapping result. Finally, all SNPs were retained and filtered using GATK with including parameters: -T SelectVariants -selectType SNP -select " AF < 0.95 ", and high-quality SNPs were retained in the form of VCF.

Population stratification analysis
Neighbour-joining phylogenetic tree was then built using the snphyl tool (v2.0) with default parameters through the VCF file (Lee et al. 2014). And the iTOL website (http://itol.embl.de/index.shtml) was used to beautify the phylogenetic tree (Chen et al. 2018).

GCTA (v1.25) was used for Principle Component Analysis (PCA) to extract the genetic relationship matrix of the first four eigenvectors generated with default parameters (Yang et al. 2011; Sliker 2018). Then admixture (v1.3.0) was used to analyze the population genetic structure (Peter 2016). Presume ancestral populations (K) between two and six were run with 10,000 iterations.

Population evolutionary analysis
TreeMix (v1.13) with default parameters except for the “ -root BZ -k 500 -m 3 ” option was used for gene flow analysis (Klimova et al. 2018). Pairwise Sequentially Markovian Coalescence (PSMC) model was used to infer population history dynamics though bam files by mapping (Nadachowska-Brzyska et al. 2015). The history of population size over time was reconstructed by applying the distribution of the nearest common ancestor between the two alleles in the sample. Parameters of PSMC analysis are set as “-n25-t15-r5-p ” and “-g 1-u 0.2e-08 ”, respectively.

Selective sweep and functional enrichment analyses
In order to define the selection sweep characteristics associated with Jianchang duck and the candidate regions for the targeted selection of Jianchang duck during domestication, we used Vcftools (v0.1.13) to calculate the fixation statistics (FS) and population nucleotide diversity ratio θπ (other / Jianchang) and θπ (wild / Jianchang) (Liu et al. 2018 preprint), and two complementary methods were used to identify areas that may be affected by long-term selection. The average FS and θπ were calculated in 20kb windows with a 10kb shift. The logarithmic function was used to transform the θπ ratio. The FS and θπ logarithmic advantage ratio of the top 5% of windows were considered as putative selection target regions. The resulting sites were genetically annotated by Bedtools (v2.17.0; (Quinlan and Hall 2010), and the selected genes were subjected to GO analysis and KEGG analysis using DAVID (v6.8) and KOBAS (v3.0) to annotate the function of the selected genes (Yang et al. 2018).

Pooling data for FS analysis
Popoolation2 (Kofler et al. 2011) was used to calculate FS values between different feather color pooling VCF files after filtering. The FS values were averaged over SNPs in a 5 kb sliding window with a 2.5 kb step size for each comparison groups.
RESULTS

Population structure

Genome-wide population stratification analysis was conducted to show the population genetic structure and relationships of Jianchang duck (Figure 1A–D) with other duck populations (Figure 1E). The neighbor-joining (NJ) of pairwise genetic distances based on whole genome-wide SNPs (Figure 2A) showed that Chinese duck breeds can be mainly classified into wild and domesticated groups. From the categories of domesticated ducks, Jianchang duck was present in an independent branch. PCA (Figure 2B) analysis revealed that the whole duck population was divided into three groups: wild, Jianchang and other domesticated. Further population structure analysis (Figure 2C) showed that when K = 2, all individuals were divided into two groups: wild and domesticated ducks; when K = 3, a clear division existed between Jianchang and other domesticated populations in the domesticated population, and the Cross-Validation error is the lowest.

Gene flow and demographic history analysis

Gene flow analysis (Figure 3A) showed that Jianchang duck (designated as JC) was the first group of ducks to be separated from domestic ducks, which is the closest relative to Longsheng duck. There were genetic exchanges between the Jianchang and other domestic populations, as well as between Jianchang and wild populations. PSMC analysis showed (Figure 3B) that the development trend of the quantitative kinetics of Jianchang duck population was similar to that of other domestic duck population, but far from that of the wild duck population. The trend of the effective ancestor population size of these three groups increased in the interglacial periods and decreased in the Pleistocene.

Data availability

The raw data files obtained in this study by HiSeq XTen sequencing have been submitted to the Sequence Read Archive Database of the National Center for Biotechnology Information. The BioProject accession number is PRJNA548668 and the BioSample accession numbers of sequence read archive runs of sixteen samples are SAMN12046286, SAMN12046287, SAMN12046288, SAMN12046289, SAMN12046290, SAMN12046291, SAMN12046292, SAMN12046293, SAMN12046294, SAMN12046295, SAMN12046296, SAMN12046297, SAMN12046298, SAMN12046299, SAMN12046300, SAMN12046301. Genomic data selected from published local breeding genomic data of mallards, respectively. We filtered all genes located in the top 5% regions with a high FST and pairwise diversity ratio (θπ) among two populations (Supplementary Tables S4). We identified 370 genes in a comparison group of Jianchang and other domestic (Figure 4A, Supplementary Tables S5), and 867 genes in the comparison group of Jianchang and wild (Supplementary Tables S6) (Figure 4C, Supplementary Tables S7).

Selective sweep and functional enrichment analyses

The genome-wide variations of Jianchang duck were compared with other domestic duck breeds (BZ, LS, MW, SS, PT, GA, TW, YX, YG, JD, SM, and SX) and mallards, respectively. We filtered all genes located in the top 5% regions with a high FST and pairwise diversity ratio (θπ) among two populations (Supplementary Tables S4). We identified 370 genes in a comparison group of Jianchang and other domestic (Figure 4A, Supplementary Tables S5), and 867 genes in the comparison group of Jianchang and wild (Supplementary Tables S6) (Figure 4C, Supplementary Tables S7).

In the comparison group of Jianchang and other domestic, all 370 genes located in the top 5% FST and θπ regions were used for the GO and KEGG analysis, resulting in a total of 54 GO terms and 76 KEGG enrichment terms. Among the top 10 enriched GO categories (Supplementary Tables S8), three are related to Growth and development, including dystrophin-associated glycoprotein complex, Wnt signaling pathway involved in dorsal/ventral axis specification and coreceptor activity involved in Wnt signaling pathway, and two GO categories are associated with Skeletal phylogeny, including osteoblast development and actin cytoskeleton reorganization. Moreover, two GO categories are associated with Physiological processes, including sialyltransferase activity and peptidase activity. Among the top 10 enriched pathways (Supplementary Tables S9), lipid metabolism displayed the greatest enrichments, followed by nutrition metabolism, with four genes (ESM1, HSD17B12, PTPRJ and GLI2) showed strong signs of selective sweeps. The HSD17B12 gene (Figure 4B), which is involved in the biosynthesis of fatty acids (Zhang et al. 2017), was located in the prominent position of FST and θπ ratio. Total twelve homozygous or heterozygous SNP sites in five introns of HSD17B12 were identified in part of other domestic duck individuals, and no plausible SNPs were detected in these sites for the Jianchang duck individuals.

In the comparison group of Jianchang and wild, all 867 genes located in the top 5% FST and θπ regions were used for the GO and KEGG analysis, and finally 121 GO enrichment terms and 119 KEGG enrichment terms were enriched. Among the top 10 enriched categories in GO functional analysis (Supplementary Tables S10), eight GO categories were related to neuromodulation, including receptor complex, positive regulation of cell-substrate adhesion, positive regulation of cell migration, response to endoplasmic reticulum stress, positive regulation of positive chemotaxis, dopamine metabolic process, transmembrane receptor protein tyrosine kinase activity and positive regulation of actin filament polymerization. Among the top 10 KEGG pathways (Supplementary Tables S11), neural related processes displayed the greatest enrichments, and in neural-related
processes three genes (CNTN1, CHRNA9, and SHANK2) showed strong signals of selective sweeps. The CNTN1 gene (Figure 4D) was located in a significant position in $F_{ST}$ and $\theta\pi$ ratio, which is involved in neural processes of cerebellar development and retinal axon regeneration (Haenisch et al. 2005). Total thirteen homozygous or heterozygous SNP sites in nine introns of CNTN1 were identified in part of mallards, and no plausible SNPs were detected in these sites for the Jianchang duck individuals.

**Genes related to feather color in Jianchang duck populations**

When all colored plumage duck populations were compared with the white ones, it was found that one significant selection signals existed on chromosome 13 (Supplementary Figure 5, Figure 5A), whereas, there was no signal when it was compared among the colored plumage duck groups. Further analysis showed that the candidate chromosomal segment under the significant signals on chromosome 13 harbored one gene encompassing MITF ($F_{ST} = 0.72$). Moreover, in the intronic region of the MITF gene, we identified nine homozygous SNP sites which have already fixed in hemp plumage breeds ($n = 8$) and nine homozygous or heterozygous SNP sites present in white chest black plumage breeds ($n = 4$), and these sites in white plumage breeds ($n = 4$) were consistent with the reference genome (Supplementary Figure 5, Figure 5A). When comparing colored plumage ducks of the white chest black plumage ducks with the hemp ones, we also observed one significant signal existed on chromosome 12 (Figure 5B). Further analysis showed that MC1R was distributed in the candidate chromosomal region ($F_{ST} = 0.64$). And nine homozygous or heterozygous SNP sites, in the intronic region of the MC1R gene, were identified in the hemp plumage breeds ($n = 8$), and no plausible SNPs were detected in these sites for the white chest black plumage breeds ($n = 4$) (Figure 5B).

**DISCUSSION**

Comparing with mallards and other domestic ducks, Jianchang ducks have great differences in morphology, physiology and behavior (Xiangdong et al. 2001) (Jiansheng 1987; Wang et al. 2018). Our current results basing on the population stratification analysis, showed that Jianchang ducks can be regarded as an independent branch from mallards and other domestic ducks. Our results are consistent with the findings basing on the analysis of estimate genetic
diversity and genetic structure by microsatellite markers (Li et al. 2006). These analyses revealed different genetic backgrounds of Jianchang duck from other domesticated ducks and mallards, which reflect the genomic basis of unique characteristics of Jianchang duck.

All domesticated animals are originated from common ancestor wild animals (Li et al. 2010; vonHoldt and Driscoll 2016; Evin et al. 2017). We compared the genomic differences between mallard and jianchang duck, to check what genes have been selected during...
domestication process of jianchang duck. Based on 867 genes in the top 5% of \( F_{ST}\) and 9\% region comparing between them, the GO terms were significantly enriched in energy metabolism and neural processes. Genes related to neural processes, such as \textit{CNTN1}, \textit{CHRNA9}, and \textit{SHANK2}, showed particularly strong signals of selective sweeps presumably. \textit{CNTN1} is involved in retinal axon regeneration, and \textit{CHRNA9} affect inner ear development (Järvelä 2018). \textit{CNTN1} and \textit{SHANK2} are related to synapses formation and transmission regulation, which can affect memory by regulating neurotransmission (Haenisch et al. 2005; Lim et al. 2017; Monteiro and Feng 2017). The current study identified thirteen SNPs in the intron of \textit{CNTN1}, and they were observed to have segregated between mallards and

---

**Figure 5** MITF and MC1R show different genetic signature between different plumage color ducks. (A) \( F_{ST}\) plot around the MITF locus. The MITF gene is in the shaded area. (B) \( F_{ST}\) plot around the MC1R locus. The MC1R gene is in the shaded area. SNPs were named according to their position on the scaffold. Blue represents the mutation site and gray represents the reference genome. WCB represents the white chest black plumage ducks, W represents the white plumage ducks, DH represents the deep hemp plumage ducks, SH represents the shallow hemp plumage ducks and Hemp represents the hemp plumage ducks.
Jianchang ducks, suggesting CNTN1 was under selection pressures during domestication of Jianchang duck. It is widely known that genes associated with neural processes were initially under selection pressures during the domestication of animals (Alberto et al. 2018; Lan et al. 2018; Zhang et al. 2018). Our findings were also consistent with the behavioral characteristics of animals at the primary stage of domestication. During this period, animals gradually adapted to a domesticated environment and became more docile and less responsive to environmental stimulation (Price 1984; Johnsson 2017). In fact, domestic ducks were less alert than mallards, and showed significant differences in brain morphology (Gille and Salomon 2000; Söderquist et al. 2017). Similarly with other domesticated ducks, in the early domestication process from mallards, the genes under big selection pressures in jianchang duck are also associated with neural processes.

Compared with other domestic duck breeds in China, Jianchang duck has the characteristics of fast growth rate and strong ability of lipid deposition in liver (Jiansheng, W. H. L., 1987 Comparative Study of Restriction Maps of Duck mtDNAs. Acta Genet. Sin. 3: 230–236. doi.org/10.1111/j.1469-7998.2000.tb00635.x). MC1R related to white feather, and fat deposition in Jianchang ducks. Thus they may contribute to the characteristics of strong abilities in fat deposition in Jianchang ducks. MITF is an important gene locus with complex regulatory mechanisms, which are involved in pigmentation and melanocyte development, proliferation and survival in some vertebrates (Levy et al. 2006; Vachtenheim and Borovansky 2010). Zhou et al., (2018) have shown that a novel intronic insertion most possibly leads to a splicing change in MITF accounted for white duck down feathers. Total nine SNPs distributed in the intron of MITF have been identified to be segregated between colored plumage duck populations and white ones. Additionally, in the hair color study of quail, llamas, and pig (Wu et al. 2016; Kageyama et al. 2018; Lan et al. 2018), MC1R is related to the formation of brown hair. It has been well known that MC1R occurs in the regulation of eumelanin (black/brown) synthesis within the mammalian melanocyte (Kijas et al. 1998). By comparing the genomic variations of hemp plumage ducks and white breast black plumage ones, total nine SNPs located in the intron of MC1R were found to be differentiated, implying that MC1R is related to the hemp plumage of duck.

CONCLUSION

We draw a conclusion that in the early domestication process of ducks, genes associated with neural processes were under selection pressures. Jianchang duck seems to have evolved earlier than other local breeds in China, and have genetic exchanges with other domestic and wild populations. The genes related to lipid metabolism were mainly selected and segregated from other local breeds, thus they may contribute to the characteristics of strong abilities in fat deposition in Jianchang ducks. MITF was identified to be related to white feather, and MC1R was related to hemp feather of Jianchang duck.

ACKNOWLEDGMENTS

This study was supported by National Natural Science Foundation of China (31872345), Key Technology Support Program of Sichuan Province (2016NYZ0044), China Agricultural Research System (CARS-43-6) and National Key R&D Program of China (2018YFD0501503-3).

LITERATURE CITED

Alberto, F. J., F. Boyer, P. Orozco-Lara, K. Streeter, B. Servin et al., 2018 Convergent genomic signatures of domestication in sheep and goats. Nat. Commun. 9: 813. https://doi.org/10.1038/s41467-018-03206-y

Balavenkatraman, K., E. Jandt, K. Friedrich, T. Kautenburger, B. Pool-Zobel et al., 2006 DEP-1 protein tyrosine phosphatase inhibits proliferation and migration of colon carcinoma cells and is upregulated by protective nutrients. Oncogene 25: 6319–6324. https://doi.org/10.1038/sj.onc.1209647

Chen, S., Z. Xu, Y. Zhao, X. Zhong, C. Li et al., 2018 Structural characteristic and phylogenetic analysis of the complete chloroplast genome of Dianthus Caryophyllus. Mitochondrial DNA B Resour. 3: 1131–1132. https://doi.org/10.1080/23802359.2018.1521313

Corkins, M. E., S. Wilson, J.-C. Cocuron, A. P. Alonso, and A. J. Bird, 2017 The gluconate shunt is an alternative route for directing glucose into the pentose phosphate pathway in fission yeast. Journal of Biological Chemistry. MI17. 798488.

Engelen, M., M. J. Schackmann, R. Ofman, R.-J. Sanders, I. M. Dijkstra et al., 2012 Bezafibrate lowers very-long-chain fatty acids in X-linked adrenoleukodystrophy fibroblasts by inhibiting fatty acid elongation. J. Inherit. Metab. Dis. 35: 1137–1145. https://doi.org/10.1007/s10545-012-9471-4

Ervin, A., K. Dobney, and T. Cucchi, 2017 A history of pig domestication: new ways of exploring a complex process in Ecology, Conservation and Management of Wild Pigs and Pecaries, Cambridge University Press. 10: 39–48. https://doi.org/10.1017/9781131694123.006

Frantz, L. A., and G. Larson, 2018 A genetic perspective on the domestication continuum in Hybrid Communities: Biosocial Approaches to Domestication and Other Trans-species Relationships, Taylor & Francis. https://doi.org/10.4324/9781315179988-2

Gille, U., and F.-V. Salomon, 2000 Brain growth in mallards, Pekin and Muscovy ducks (Anatidae). J. Zool. (Lond.) 252: 399–404. https://doi.org/10.1111/j.1469-7998.2000.tb00635.x

Guo, J., H. Tao, P. Li, L. Li, T. Zhong et al., 2018 Whole-genome sequencing reveals selection signatures associated with important traits in six goat breeds. Sci. Rep. 8: 10405. https://doi.org/10.1038/s41598-018-28719-w

Haensch, C., H. Diekmann, M. Klinger, G. Gennarini, J. Y. Kuwada et al., 2005 The neuronal growth and regeneration associated Ctnn1 (F3/F11/ Contactin) gene is duplicated in fish: expression during development and retinal axon regeneration. Mol. Cell. Neurosci. 28: 361–374. https://doi.org/10.1016/j.mcn.2004.04.013

Järvelä, I., 2018 Genomics studies on musical aptitude, music perception, and practice. Ann. Y. Acad. Sci. 1423: 82–91. https://doi.org/10.1111/nyas.13620

Ji, H., and G. Koh, 2015 Faster single-end alignment generation utilizing multi-thread for BWA. Biomed. Mater. Eng. 26: S1791–S1796. https://doi.org/10.1111/bme.12359

Kageyama, M., A. Takenouchi, K. Kinoshita, Y. Nakamura, and M. Tsudzuki, 2018 The “Extended Brown” Plumage Color Mutant of Blue-Breasted Quail (Coturnix chinesis) is Associated with a Mutation in the Melanocortin 1-Receptor Gene (MC1R). J. Poult. Sci. 55: 233–238. https://doi.org/10.4324/9781315179988-2

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

Klimova, A., A. Ortega-Rubio, D. L. Vendramin, and J. I. Hoffman, 2018 Genotyping by sequencing reveals contrasting patterns of population structure, ecologically mediated divergence, and long-distance evolution.
