Whole-genome Resequencing Provides Insights into the Population Structure and Domestication Signatures of Ducks in Eastern China

Peishi Feng

Zhejiang University of Technology <https://orcid.org/0000-0001-7981-4514>

Tao Zeng

Zhejiang Academy of Agricultural Sciences

Yang Hua

Zhejiang Academy of Agricultural Science

Guohong Chen

Yangzhou University Animal Science and Technology College

Jinping Du

Hubei Academy of Agricultural Science

Li Chen

Zhejiang Academy of Agricultural Sciences

Junda Shen

ZheJiang Academy of Agricultural Sciences

Zhongrong Tao

Zhejiang Academy of Agricultural Sciences

Ping Wang (✉ wangping45@zjut.edu.cn)

Zhejiang University of Technology, Hangzhou <https://orcid.org/0000-0002-6716-4451>

Lin Yang

South China Agricultural University College of Animal Science

Lizhi Lu

zhejiang academy of agricultural sciences

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Abstract

Background: Ducks are an ancient domesticated animal with high economic value, used for its meat, eggs, and feathers. However, the origin of indigenous Chinese ducks remains elusive. To address this question, we performed whole-genome resequencing to explore the genetic relationships among the domestic ducks and wild ducks in eastern China and to identify the genomic differences shaped by artificial selections.

Results: The whole genomes of 60 ducks from eastern China were sequenced, with an average effective sequencing depth of $\sim 6\times$ per individual. The results of population and demographic analysis indicate that all individuals from the same breed gather together, and domestic ducks form a cluster separating from wild ducks. By applying selective sweep analysis, we identified that several candidate genes, important pathways and GO categories associated with artificial selection were functionally related to cellular adhesion, type 2 diabetes, lipid metabolism, the cell cycle, liver cell proliferation, and muscle functioning in domestic ducks.

Conclusion: Genetic structure analysis showed that the domesticated ducks were not direct descendants of the mallard or Spot-billed duck and probably originated from an unknown population. During the long history of artificial selection, domesticated ducks have developed a complex biological adaptation to captivity.

Background

Domestication is the process of animal adaptation to humans and the captive environment by genetic changes across many generations [1]. Evolutionary biology is centered on the processes of variation, heredity, and selection driven by domestication. The phenotypic and genetic variation among breeds in many species may be influenced by domestication or captivity, such as in pig [2], chickens [3], dogs [4] and yaks [5].

Chinese domestic ducks are among the earliest domesticated fowl in the world dating back to 3000 years before present (YBP) [6]. China is famous for its abundance of waterfowl breeds, there are 31 recognized domestic duck breeds. However, the diversity and origin of Chinese domestic ducks are still debated. Some suggest that Chinese domestic ducks originated from wild mallards (*Anas platyrhynchos*) [7, 39], while others argue for a hybrid originated wild mallards and spot-billed duck (*Anas poecilorhyncha*) [8].

Domestic ducks differ from their wild ancestors in morphology [9, 10] and behavior [11] because they are bred for egg production and meat production. The economic importance of the domestic ducks has driven research to decode the duck genome. Thus it is necessary to pinpoint the genetic basis of domesticated ducks.

In this study, we sequenced the genomes of 60 individuals from two wild populations, the spot-billed ducks (*Anas poecilorhyncha*) and mallards (*Anas platyrhynchos*), and four indigenous Chinese breeds

(Fenghua (FH), Shaoxing (SX), Shanma (SM), and Cherry Valley Pekin (CV) ducks). The Fenghua duck is used for egg or meat production. Shaoxing and Shanma ducks are Chinese dominant layer breeds, characterized by small bodies size, early maturity and high productivity. The Cherry Valley Pekin duck is famous for its fast growth, high lean rate and high feed conversion ratio. We used whole-genome resequencing approach and transcriptome sequencing methods to explore the genetic relationships among wild and domestic ducks and identified the genomic footprints of selection during the domestication of native ducks.

Results

We selected 60 individuals from six breeds (spot-billed duck, mallard duck, Fenghua duck, Shaoxing Duck, Shanma Duck and Cherry Valley Pekin Duck) (Fig. 1A). Using the Illumina Genome Analyzer platform, we generated a total of 397.88 GB of clean data with an average of 6.63 GB per individuals (Supplementary Table S1). These reads mapped to 95.09% of the reference genome assembly with 6.52-fold average depth (Supplementary Table S2). We called 2,809,077 high-quality single nucleotide polymorphic sites (SNPs) for 60 ducks (Supplementary Table S3), 63.92% (1.8 million) of the high-quality SNPs were located in the intergenic regions, and only 1.94% (0.55 million) were located in the exonic regions (Supplementary Table S4). We identified 42,463 synonymous SNPs and 12,084 nonsynonymous of exons, for a nonsynonymous/synonymous ratio of 0.28. There were 838,413 SNPs shared between the breeds (Supplementary Fig. S1).

Population Genetic Structure

To explore relatedness among the domestic ducks, we conducted a principal component analysis (PCA) based on genome wide SNP data. The laying duck breeds (Shaoxing and Shanma ducks) and meat duck breeds (Cherry Valley Pekin duck) were separated by different clusters that were also distinct from the wild populations (spot-billed duck and mallard) and Fenghua duck (Fig. 1A, supplementary Fig. S2). The neighbor-joining (NJ) tree revealed that the individuals from Chinese indigenous breeds were clustered into a subclade, suggesting they have a closer genetic relationship and potentially derive from a common ancestor (Fig. 1B). To estimate different ancestral proportions, we further performed a population structure analysis with FRAPPE by assuming K ancestral populations (Fig. 1C). When $K=2$, a clear division was observed between the wild ducks and domesticated breeds with slight shared ancestry sequences between these two groups. Moreover, we observed 59% wild population ancestry of in Fenghua duck. When $K=5$, there was a division between each group except Shaoxing and Shanma ducks.

To examine the population structure with admixture, we inferred the duck population tree for ducks using TreeMix (Fig. 2). One of the three included migration events showed strong gene flow from Fenghua duck into mallard, and the two other migration events estimates a weaker gene flow from Shaoxing duck into spot-billed duck and Shanma duck into Cherry Valley Pekin duck.

Patterns of Genomic Variation and Linkage Disequilibrium

The genome-wide average genomic diversity (θ_π) values were 5.949×10^{-4} for mallard, 5.862×10^{-4} for spot-billed duck, 5.815×10^{-4} for Fenghua duck, 5.303×10^{-4} for Shaoxing duck, 5.462×10^{-4} for Shanma duck and 4.694×10^{-4} for Cherry Valley Pekin duck (Supplementary Table S5). These values were much lower than in other animals (Supplementary Table S6). The wild duck had the greatest θ_π and θ_w , suggesting that domestication reduces genetic diversity. Additionally, Linkage disequilibrium (LD) also showed that the wild ducks had a faster decay of the pairwise correlation coefficient (r^2) than the domestic duck (Fig. 1D).

Demographic History

We employed the pairwise sequentially Markovian coalescent (PSMC) method [12] to infer fluctuations in the ancestral effective population sizes (N_e) of each breed in response to Quaternary climatic change (Fig. 3A). From 1 million to 10 thousand years, all of the domestic breeds (SX, SM, CV and FH) exhibited similar demographic trajectories with a peak in ancestral N_e at 50-60 thousand years ago followed by distinct declines (Supplementary Fig. S3). The decline occurred ~ 60 thousand years ago, coinciding with the beginning of the Last Glacial Maximum [37]. The effective population sizes of mallard and spot-billed duck appear to have increased rapidly after ~40 and ~20 thousand years ago, respectively (Supplementary Fig. S3).

Genome-Wide Selective Sweep Test

To accurately detect the genomic footprints of selection, we pooled the domestic duck samples (SX, SM and CV) and compared them to the wild duck (MA and SB), which are geographically close. Using the top 5% the F_{ST} values and θ_π ratio cutoffs ($F_{ST} > 0.13$ and $\log_2(\theta_\pi \text{ ratio } (\theta_{\pi, \text{wild duck}} / \theta_{\pi, \text{domestic duck}}) \geq 0.84)$), we identified 665 candidate domestication regions (CDRs) containing 387 genes under selection in the domestic ducks (Fig. 4, Supplementary table S7). In addition, ten candidate genes (*Cmip*, *Tmem132b*, *Mphosph6*, *Smg7*, *Lyst*, *Zbtb37*, *Serpinc1*, *Npl*, *Tmem132c* and *Plcg2*) ranking within the top 10 F_{ST} values with $\log_2(\theta_\pi \text{ ratio } (\theta_{\pi, \text{wild duck}} / \theta_{\pi, \text{domestic duck}}) \geq 0.84$ were functionally involved in cellular adhesion function, type 2 diabetes, lipid metabolism, cell cycle, liver cell proliferation and muscle functioning [13-18] (Table 1).

Table 1
Positively selected genes with top 10 FST values in domestic ducks.

Gene ID	Gene name	Gene symbol	Fst	Description
ENSAPLT00000011005	c-Maf inducing protein	Cmip	0.496	associating with language and reading, type 2 diabetes, obesity, lipid metabolism, breast and gastric cancer, negatively regulating T cell signaling [40-45]
ENSAPLT00000011847	transmembrane protein 132B	Tmem132b	0.468	associating with excessive daytime sleepiness [46]
ENSAPLT00000002396	M-phase phosphoprotein 6	Mphosph6	0.464	regulating cell cycle and ovary development, recruiting the exosome to the pre-rRNA, associating with coronary artery disease, IgA nephropathy and leukocyte telomere length [47-51]
ENSAPLT00000016529	nonsense mediated mRNA decay factor	Smg7	0.463	regulating DNA damage response and nonsense-mediated mRNA decay [52]
ENSAPLT00000006672	lysosomal trafficking regulator	Lyst	0.458	associating with Chediak-Higashi syndrome [53]
ENSAPLT00000005034	zinc finger and BTB domain containing 37	Zbtb37	0.424	involving in aryl hydrocarbon receptor in hematopoietic stem cell functional regulation [54]
ENSAPLT00000005105	serpin family C member 1	Serpinc1	0.424	associating with antithrombin deficiency and ovarian cancer [55,56]
ENSAPLT00000004292	N-acetylneuraminate pyruvate lyase	Npl	0.421	regulating the cellular concentrations of sialic acid which is essential for muscle function [57]
ENSAPLT00000012003	transmembrane protein 132C	Tmem132c	0.412	associating with pulmonary function, breast cancer, insulin secretion impairment, body weight [58-61]
ENSAPLT00000011198	phospholipase C gamma 2	Plcg2	0.391	involving in inherited immune disorders, promoting liver cell proliferation [62-63]

To identify the active pathways in the domestication of ducks, the positively selected genes in domestic ducks were mapped to the canonical reference pathways in the KEGG database. The top three enriched pathways were “pantothenate and CoA biosynthesis” (2 genes, $P = 0.02667$), “FoxO signaling pathway” (6 genes, $P = 0.03002$), and “inositol phosphate metabolism” (4 genes, $P = 0.03511$) (Supplementary Fig. S4, Supplementary Table S8). The positively selected genes of domestic ducks that were successfully annotated to 47 categories of Gene Ontology (GO), belonging to three parts: cellular components, molecular function and biological processes (Supplementary Fig. S5, Supplementary Table S9). Of these, the categories that were most represented in the “biological process” principal category were “cellular process” (137 genes), followed by “single-organism process” (123 genes). In the principal category of “cellular component”, the two categories most represented were “cell” (149 genes) and “cell part” (149 genes). Within the “molecular function” principal category belonged to the “bind” (107 genes).

Positively Selected Genes Involved in Insulin Signaling Pathway

Using the top 5% of the F_{ST} values and $\theta\pi$ ratio cutoffs based on sliding 40 kb windows for the Shaoxing group compared to the mallard group, we identified 497 candidate domestication regions (CDRs) containing 311 genes with both high F_{ST} values and a high $\theta\pi$ ratio (fig. 5A). Six genes exhibiting strong selective sweep signals were significantly over-represented in insulin signaling pathway, including ectonucleotide pyrophosphatase /phosphodiesterase-1 (*Enpp1*), ectonucleotide pyrophosphatase/phosphodiesterase-3 (*Enpp3*), SHC adapter protein 4 (*Shc4*), SOS Ras/Rac guanine nucleotide exchange factor 1 (*Sos1*), neuroblastoma RAS viral oncogene homolog (*Nras*) and protein kinase cAMP-dependent type II regulatory subunit beta (*Prkar2b*).

Notably, we observed much higher F_{ST} values (fig. 5C) and lower Tajima’s D values (fig. 5D) for the target gene *Enpp1* compared to those in the adjacent genomic regions, providing further support that the candidate genes were reliable. We also used transcriptome sequencing to investigate the molecular signatures of domestication and identified significantly downregulation *Enpp1* expression in the muscle and liver tissues of Shaoxing ducks compared to mallards (fig. 5B).

Discussion

Population structure and demographic history

China has the largest number of ducks in the world and was one of the earliest to domesticate wild ducks [19,20]. However, the origin of Chinese domestic ducks is still controversial. Here, we advance a new hypothesis that the domesticated ducks were not direct descendants of the mallard or Spot-billed duck and probably originated from an unknown population on the basis of the whole-genome sequences.

In this study, we carried out whole-genome resequencing of 60 individuals to explore the genetic relationships among the domestic ducks and wild ducks in eastern China. The PCA and NJ tree results suggest that mallard and spot-billed duck cluster more closely with each other and have a closer genetic relationship. This finding raised a question as to whether Chinese domestic ducks originate from mallard and spot-billed duck. In order to solve this problem, we perform population genetic structure analysis.. A surprising result was the clear division between wild ducks and domestic breeds in the clustering analysis when $K=2$, and spot-billed duck and mallard were separated only from $K=5$. This suggests that the split of mallard and spot-billed duck are later than that of Chinese domestic ducks. Using TreeMix to infer migration patterns among the different populations, we did not find any gene flow among wild ducks, Shaoxing duck, Shanma duck and Cherry Valley Pekin duck, but this analysis show the strong gene flow from Fenghua duck into mallard. It is speculated that the locations where Fenghua duck and mallard were collected contribute to this gene flow.

In the PSMC model, we observed the similar trajectories for four Chinese domestic ducks with an apparent expansion during the Penultimate Glaciation and the Last Interglaciation, and a decline between 50 and 60 thousand years ago. In addition, mallard and spot-billed duck population reached their pinnacle between 20 and 40 thousand years ago. This is consistent with our population genetic structure results that the ancestor of Chinese domestic ducks is different from the ancestors of mallard and spot-billed duck.

Selection for domestication

The *Enpp1*, *Enpp3*, *Shc4*, *Sos1*, *Nras* and *Prkar2b* are related to the insulin signaling pathway and showed signals of positive selection in Shaoxing duck. *Enpp1* is the subtypes of ENPP family, which directly interacts with the insulin receptor and blocks the insulin signaling pathway [21], serving as a gatekeeper of insulin action. ENPP3 is positive associated with the serum ATP concentration, facilitating lipid deposition [22]. The protein encoded by *Prkar2b* is a regulatory subunits of the protein kinase A (*PKA*) and is involved in insulin resistance [23]. *Shc4* (also known as ShcD) serves as a phosphotyrosine adapter molecule that induces Ras GTPase and mitogen activated protein kinase (MAPK) activation [24]. Also, the *SOS1* and *NRAS* provide protein-making instructions that are involved in regulating the activation of the Ras/MAPK signaling pathway, which helps to control insulin signaling (fig. 6).

These genes may have played a crucial role in duck domestication by altering glucose and lipid metabolism. Differences in glucose and lipid metabolism between wild and domesticated animals due to directional selection were also observed in pigs [25] and chickens [3]. *Enpp3* and *Prkar2b* were identified as the targets of selection during the domestication of Pekin ducks and other indigenous Chinese ducks [26,27].

Conclusion

In conclusion, we performed whole-genome resequencing to characterize the evolutionary origin of ducks in eastern China and the genome-wide signatures of artificial selection associated with domestication. Several candidate genes, important pathways and GO categories associated with artificial selection were functionally related to cellular adhesion, type 2 diabetes, lipid metabolism, the cell cycle, liver cell proliferation, and muscle functioning in domestic ducks. We found strong genomic evidence for the involvement of the insulin signaling pathway in the domestication of Shaoxing duck. These results advance our understanding of the genetic relationships between domestic and wild ducks, reveals the genetic footprints of domestication and shed light on the genetic mechanisms underlying species adaptation to captivity.

Methods

Sampling

The blood samples from all the 60 individual ducks (10 per breed) were collected from the wing vein using vacuum tubes containing EDTA-K₂ as an anticoagulant. The spot-billed ducks, mallards and Fenghua ducks were captured in Fenghua City, Zhejiang Province, China (29°35' N, 121°24' E). The Shaoxing and Shanma ducks were collected in Zhuji City, Zhejiang Province, China (29°38' N, 120°10' E), and the Cherry Valley Pekin duck were raised in Huzhou City, Zhejiang Province, China (30°41' N, 120°19' E). From Shaoxing ducks and mallards, 3 randomly selected ducklings were killed by rapid decapitation and sterile dissection, and muscle and liver tissues were sampled and immediately snap frozen in liquid nitrogen.

Sequencing and Quality Control

A total of 60 ducks, which were sampled from Eastern China, were sequenced on the Illumina HiSeq 2000 platform (Illumina, San Diego CA, USA). We generated a total of 401.491 Gb of raw sequence data (supplementary table S1).

To make sure reads reliable and without artificial bias in the following analyses, raw reads of fastq format were firstly processed through a series of quality control (QC) procedures in-house C scripts. The low-quality reads were filtered out based on the following [28]:

- (1) Reads with $\geq 10\%$ unidentified nucleotides (N);
- (2) Reads with $> 50\%$ bases having phred quality < 5 ;
- (3) Reads with > 10 nt aligned to the adapter, allowing $\leq 10\%$ mismatches;
- (4) Putative PCR duplicates generated by PCR amplification in the library construction process (read 1 and read 2 of two paired-end reads that were completely identical).

Consequently, 387.88 Gb were retained for assembly, of which the quality of 96.06% and 91.52% of the bases were $\geq Q20$ and $\geq Q30$, respectively.

Reads Mapping and SNP Calling

The remaining high quality reads were mapped to the mallard (*Anas platyrhynchos*) reference genome [29] using Burrows-Wheeler Aligner (Version: 0.7.8) [30] with the command line was 'BWA mem -t 4 -k 32 -M'. SAMtools was used to remove the duplicated reads to reduce mismatch generated by PCR amplification before sequencing.

After alignment, we used SAMtools [31] to carry out SNP calling. The 'mpileup' command was used to identify SNPs with the parameters as '-q 1 -C 50 -S -D -m 2 -F 0.002'. The following filtering steps were applied in order to obtain high quality SNPs:

(1) Quality score ≥ 20 .

(2) Coverage depth ≥ 2 and ≤ 1000 .

Annotation of Genetic Variants

Using the ANNOVAR package [32], 2,809,077 high-quality SNPs were annotated according to the genome. Based on the genome annotation, SNPs were classified into several categories, such as exonic regions, intronic regions, splicing sites, upstream and downstream regions and intergenic regions. The SNPs in coding exons were fall into two categories: synonymous SNPs and nonsynonymous SNPs.

Principal Component Analysis

The software GCTA [33] was used for PCA with the population scale SNPs. The significance level of the eigenvectors was determined using the Tracey-Widom test to clarify the phylogenetic relationship among 60 individuals. The first three significant components were plotted (supplementary fig. S2), and the discrete points to a degree reflect the real structure of population.

Phylogenetic Tree

We also inferred an individual-based neighbor-joining (NJ) tree in TreeBeST (<http://treesoft.sourceforge.net/treebest.shtml#inno>) based on the p -distance. The bootstrap was set to 1,000 times to evaluate the reliability of branch..

Population Structure and TreeMix analysis

The population genetic structure of 60 individuals was inferred by FRAPPE [34]. We set the number of cluster (K) from 2 to 6 and ran analysis with 10,000 iterations. Migration events among the populations were inferred using TreeMix [35]. We converted plink output for TreeMix by plink2treemix module in TreeMix software. We set the number of migration events from 1 to 3. For each number of migration events, we ran 100 random input orders.

Linkage Disequilibrium Analysis

We compared the pattern of linkage disequilibrium (LD) among 6 breeds using the 2.8 million high-quality SNPs. To estimate LD decay, we calculated the squared correlation coefficient (r^2) between pairwise SNPs using the software Haploview [36]. The average r^2 value was calculated for pairwise markers in a 500-kb window and averaged across the whole genome.

Effective Population Size

We used a hidden Markov model (HMM) of pairwise sequentially Markovian coalescence (PSMC) to reconstructed demographic history of 60 individuals. Firstly, we called genotype each individual using the package SamTools [30] based on the command 'mpileup' with the parameter '-C 50 -D -S -m 2 -F 0.002'. Then, we performed the program 'fq2psmcfa' with the parameter '-N30, -t15, -r5 and -p '4+25*2+4+6'' to convert the consensus sequence to the required input format. A mutation rate (μ) of 1.6×10^{-9} per bp per generation [7] and a generation time of 1 year were used for analysis. In addition, we applied a bootstrapping approach, repeating sampling 100 times on two samples to estimate the variance of simulated results.

Selective Sweep Analysis

The nucleotide diversity (θ_π), population-differentiation statistic (F_{ST}), Tajima's D statistic and Watterson estimator (θ_W) were calculated with sliding windows of 40 kb that had 20 kb overlap between adjacent windows. The putative genomic regions under positive selection during domestication were extracted based on being the highest differences in genetic diversity ($\log_2(\theta_\pi \text{ ratio})$) and the top 5% of F_{ST} . We identified a total of 665 potential selective-sweep regions overlapping with 387 candidate genes in merging domestic ducks and 491 potential selective-sweep regions overlapping with 311 candidate genes in Shaoxing ducks, which would be used for subsequent analysis and discussion.

Functional Enrichment Analysis

Gene Ontology term enrichment analysis was processed with those selective genes by goseq packages in R software. We used the GOSeq R package, in which gene length bias was corrected, to perform GO and

functional pathway analysis on the candidate genes. The Gene ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways with a Benjamini adjusted P -values less than 0.05 were considered significantly enriched.

RNA-seq and Gene Expression Analysis

To infer whether the genes under selection could also affecting gene expression between Shaoxing ducks and mallards, we compared gene expression in breast muscle, liver and cerebellum between this two groups. 3 females from Shaoxing ducks and mallards respectively were selected for transcriptomics analysis.

All samples were individually sequenced by Illumina HiSeq 4000 sequencing platform. Perl scripts was used to ensure the quality of raw data. The reference genomes and the annotation file were downloaded from ENSEMBL database (<http://www.ensembl.org/index.html>). We used Bowtie/Bowtie 2 to build the genome index and TopHat v2.0.12 to map clean data to reference genome. And HTSeq v6.0 was used to count the number of fragments for each gene in each sample. The expression level of genes in each sample was estimated by FPKM (Fragments Per Kilobase Per Million Mapped Fragment). We used DEGseq v1.18.0 to analyze differential gene expression between Shaoxing and Shanma ducks. Genes with $q \leq 0.05$ and $|\log_2 \text{Ratio}| \geq 1$ are identified as differentially expressed genes.

Abbreviations

YBP: years before present; SB: spot-billed duck, MA: mallard, FH: Fenghua duck, SX: Shaoxing duck, SM: Shanma duck, CV: Cherry Valley Pekin duck, SNP: single nucleotide polymorphic site, PCA: principal component analysis, NJ tree: neighbor-joining tree, PSMC: pairwise sequentially Markovian coalescent, GO: gene ontology, CDR: candidate domestication region, Enpp1: ectonucleotide pyrophosphatase /phosphatidylserase-1, Enpp3: ectonucleotide pyrophosphatase/phosphatidylserase-3, Shc4: SHC adapter protein 4, Sos1: Ras/Rac guanine nucleotide exchange factor 1, Nras: neuroblastoma RAS viral oncogene homolog, Prkar2b: protein kinase cAMP-dependent type II regulatory subunit beta.

Declarations

Ethics approval and consent to participate

The animal care and protocol used in this study were reviewed and approved by the Institutional Animal Care and Use Committee of the Zhejiang Academy of Agricultural Sciences and South China Agricultural University, and performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals (SCAU-10564).

Consent for publication

Not applicable

Avalability of data and material

The raw sequence data files discussed in this experiment have been deposited in SRA and BioProject ID is PRJNA599025 (<https://dataview.ncbi.nlm.nih.gov/object/PRJNA599025>)

Competing interests

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Founding

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Authors' contributions

PF analyzed main content of the data and performed the experiment with the help of HY, PW and LC. TZ collected samples with the help of JD, JS, ZT and GC. The whole work is guided by LY and LL. All authors read and approved the final manuscript.

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Figures

Figure 1

Phylogenetic and population genetic analyses of wild and domestic ducks. (A) Principal component plot of 60 individuals. (B) Unrooted neighbor-joining tree constructed using the p-distances between individuals. (C) Population structure of 60 ducks ($K = 2-6$). The y-axis represents the proportion of the individual's genome from inferred ancestral populations, and x-axis represents the different populations. (D) Genome-wide linkage disequilibrium of ducks.

Figure 2

TreeMix analysis with (A) one, (B) two and (C) three migration events.

Figure 3

Demographic history of the duck populations. (A) Dynamic changes in the effective population sizes (N_e) of six duck breeds inferred by PSMC. The gray-shaded area (from left to right) refers to the Last Glaciation, the Penultimate Glaciation and the Naynyxungla Glaciation [37]. (B) The temperature from 10 KYA to 1000 KYA [38] (C) Sea level changed from 10 KYA to 1000 KYA [38].

Figure 4

Identification of the genomic regions with strong selective sweep signals in domestic ducks. (A) Distribution of F_{ST} values and $\log_2(\theta_\pi \text{ ratio})$ calculated in 40-kb sliding windows with 20-kb overlap between the domestic groups and the wild groups. The data points in blue are genomic regions under selection in wild groups, and the data points in green are genomic regions under selection in the domestic groups. (B) Distribution of Tajima's D values for the whole genome and selected genes of domestic ducks. (C) Box plots of Tajima's D values for the whole genome and selected genes of domestic ducks. *Indicates a significantly elevated Tajima's D relative to the whole genes (Mann-Whitney U test $P < 0.05$).

Figure 5

Genomic regions with strong selective signals in the Shaoxing duck and mallard. (A) Distribution of F_{ST} values and $\log_2(\theta\pi \text{ ratio})$ calculated in 40-kb sliding windows with 20-kb overlap between Shaoxing duck and mallard. The data points in red are genomic regions under selection in the SX duck. (B) Volcano plot of the differentially expressed genes in the breast muscle and liver of the Shaoxing duck and mallard. Orange splashes indicate significantly upregulated genes. Blue splashes indicate significantly downregulated genes. Grey splashes indicate genes without significant different expression. (C) F_{ST} values around the Enpp1 locus. (D) Tajima's D values around the Enpp1 locus. The blue and orange lines represent the mallard and Shaoxing duck, respectively. (E) Allele frequencies of eight SNPs within the Enpp1 gene across 6 groups.

Figure 6

Selection of the insulin signaling pathway [64, 65]. The candidate genes under selection in duck domestication are shown in red.

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