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Verification of specific selection SNPs between broiler and layer chicken in Chinese indigenous chicken breeds

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ABSTRACT. The direction of production for indigenous chicken breeds is currently unknown and this knowledge, combined with the development of chicken genome-wide association studies, led us to investigate differences in specific loci between broiler and layer chicken using bioinformatic methods. In addition, we analyzed the distribution of these seven identified loci in four Chinese indigenous chicken breeds, Caoke chicken, Jiuyuan chicken, Sichuan mountain chicken, and Tibetan chicken, using DNA direct sequencing methods, and analyzed the data using bioinformatic methods. Based on the results, we suggest that Caoke chicken could be developed for meat production, while Jiuyuan chicken could be developed for egg production. As Sichuan mountain chicken and Tibetan chicken exhibited large polymorphisms, these breeds could be improved by changing their living environment.

Key words: Molecular marker; Polymorphic site; Indigenous breeds; Breeding direction

INTRODUCTION

Specialized commercial populations, which originate in foreign countries, occupy the majority of the chicken market in china. Therefore, it is necessary to take full advantage of indigenous breeds that have large genetic resources and play an important role in maintaining biological polymorphism, as well as having enhanced production performance, such as being well-adapted, resistant to foraging, and stress tolerant, etc. Although, these indigenous may also have some disadvantages, such as low production performance. The first limiting factor for the development of indigenous breeds is the unknown direction of production, which is the first and foremost requirement when studying indigenous breeds.

Reproduction and growth traits are controlled by quantitative trait loci and a genome-wide scan is a powerful approach that can be used to gain an understanding of these complex traits. Recently, many investigations have used genome-wide association studies (GWAS) to identify special loci used during chicken domestication (van Kaam et al., 1999; Useche et al., 2001; Akey et al., 2002; Reich et al., 2003; Rubin et al., 2010; Gu et al., 2011; Xie et al., 2012; Li et al., 2012, 2013). Rubin et al. (2010) used whole-genome re-sequencing methods to analyze chicken selection loci and the results revealed that there are 7,000,000 single nucleotide polymorphisms (SNPs), including 1300 deficiency loci, and during chicken domestication specific loci are involved in specialization into broiler (meat-producing) and layer (egg-producing) chicken. A follow-up study indicated that the high-growth and low-growth lines of broiler were distinguished by an 18,961-bp deficiency. Using a GWAS, Gu et al. (2011), found that the chicken chromosome 4 (GGA4) region, approximately 8.6 Mb in length (71.6 to 80.2 Mb), had a large number of significant SNP effects for late growth during weeks 7 to 12. Furthermore, Xie et al. (2012), using a GWAS, found a narrow 1.5 Mb region (173.5 to 175 Mb) of chicken (*Gallus gallus*) chromosome (GGA) 1 to be strongly associated with chicken growth.

Previous research indicates that mutations developed during chicken domestication are relatively fixed for different traits. In this study, molecular biological techniques are used to explore the breeding direction of Sichuan indigenous breeds by detecting the specific loci in each breed.

MATERIAL AND METHODS

Birds and management

A total of 193 chickens belonging to four different Chinese indigenous breeds were sampled for this study: Tibetan chicken, Caoke chicken, Jiuyuan chicken, and Sichuan mountain chicken. All of these breeds are famous Chinese indigenous chicken breeds and are mainly distributed in Sichuan Province, China. The selected chickens were all from unimproved breeds. The distribution of sampled chickens and the number of samples collected are shown in Table 1.

All birds were reared under the same condition: cage free (density of <35 chicken/100 m^2) and standard conditions of temperature (20° to 22°C), relative humidity (55 to 60%), and ventilation were maintained. The chickens were fed the same professional breeder diet and had free access to feed and water during the entire rearing period. Birds were managed with full consideration of bird welfare. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of the National Institute of Agrobiological Sciences.

Table 1. Distribution and number of experimental chicken sampled for each Chinese indigenous breed.

Breed	No. of samples	Distribution
Tibetan chicken (TC)	42	Xiangcheng Garze
Caoke chicken (CK)	54	Simian County, Sichuan
Jiuyuan black chicken (JY)	48	Wangyuan County, Sichuan
Sichuan mountain chicken (SM)	49	Junlian County, Sichuan

Sample collection

Approximately 2 mL blood was collected from the brachial vein of each individual according to the requirements of animal welfare of Sichuan Agricultural University, and stored at -20°C. The next day, genomic DNA was isolated by phenolic extraction. The quality and integrity of DNA was assessed using the $A_{260/280}$ ratio and agarose gel electrophoresis.

Objective gene screening

Firstly, we undertook a literature review to determine the genes related to animal reproduction traits, and then we identified the genes on the local chromosome using NCBI (http://www.ncbi.nlm.nih.gov/). Secondly, we determined the variable loci within the genes, the amount and type of mutation in specialized layer and broiler chicken using chicken VD data (http://chicken.genomics.org.cn/), and then assessed if there were differences in the loci between specialized layer and broiler chicken. Thirdly, the primers for the candidate differential gene loci were designed and verified on specialized layer and broiler chickens to exclude primers with a false position. Finally, we selected loci on three genes as the objective gene loci to detect genetic variation in the four Chinese indigenous chicken breeds.

Analysis of genetic variation

Following confirmation of the objective genes, the primers were designed using Primer Primer 5.0, DNA sequence fragments were amplified, and genetic variation was analyzed using the direct sequencing method by Beijing Liuhe Genomics Biological Technology Co., Ltd. (Beijing, China).

Sequence and data analysis

Chromas version 1.45 was used to obtain the original sequence data and DNA Star was used to inspect, edit, and proof the sequences to ensure data accuracy. To determine whether the sequences obtained were correct, an NCBI nucleotide BLAST search was conducted (http://blast.ncbi.nlm.nih.gov/). MEGA4.1 and DnaSP were used to analyze variation in the loci and identify haplotypes.

The genetic variation within each indigenous breed was evaluated and compared. The total number of alleles at each locus, the respective allele frequency, observed and expected (H_E) heterozygosities, and polymorphism information content (PIC) value for each breed across the loci were calculated using GenAlEx 6.4 (Peakall and Smouse, 2012) and CERVUS ver. 3.0.3 (Kalinowski et al., 2007).

RESULTS

Genetic screening and primer design

The study identified 7 SNPs on three genes as the differential genes between specialized layer and broiler chicken. The 7 SNPs included three loci specific to layer chicken, chr24:5748784A>G, chr24:5748832T>C, and chr5:52922938A>G, and four loci specific to broiler chicken, chr3:7519896A>G, chr3:7519918G>A, chr5:52926659T>A, and chr5:52926664T>C. These 7 SNPs are located on the *DRD2*, *LHCGR*, and *ESR2* genes, respectively (Table 2), all of which are reproductive trait-related genes. The primers were designed according to the gene loci (GenBank accession No. NC_006092.3, NC_006090.3, NC_006111.3), as shown in Table 2.

Table 2. Sequence, fragment size, and annealing temperature of primer pairs used in PCR to amplify seven SNPs in four Chinese indigenous chicken breeds.

Gene	SNP locus	Primer	Sequences of primer	Amplification fragment (bp)	Annealing temperature (°C)
ESR2	Chr5:52922914A>G	1	Forward: 5'-CCAGATAGTGGACCAAGCC-3' Reverse: 5'-CCTCGTAAACAGCCATTCC-3'	705	58.2
	Chr5:52926659A>T Chr5:52926664C>T	2	Forward: 5'-GATTTGATGCCCGATGAG-3' Reverse: 5'-CTGCTACACTGGGAAGACC-3'	517	54.1
LHCGR	Chr3:7519896G>A Chr3:7519918G>A	3	Forward: 5'-CAAGAGGCAGATGTAAAGACT-3' Reverse: 5'-TTAGACCCAGGATGTGAGAA-3'	960	53.2
DRD2	Chr24:5748832T>C Chr24:5748784A>G	4	Forward: 5'-ACATCGGCTATTTCCAGAC-3' Reverse: 5'-CAACTGCTTGCTCTTCTCA-3'	555	55.2

Genetic diversity analysis

Table 3 shows the results of the genetic diversity analysis for each chicken breed. The Chr5:52922938A>T locus was homozygous and the H_E values were zero in the Sichuan mountain chicken breed, while this locus had low polymorphism (PIC < 0.25) in the Caoke chicken breed, and moderate polymorphism (0.25 < PIC < 0.5) in the Tibetan chicken and Jiuyuan black chicken breeds. The Chr5:52926659T>A and Chr5:52926664C>T loci exhibited moderate polymorphism in all of four chicken breeds, except for Chr5:52926664C>T, which exhibited low polymorphism and reached Hardy-Weinberg equilibrium (P > 0.05) in the Jiuyuan black chicken breed. Apart from the Jiuyuan black chicken breed, which has low polymorphism, the other three breeds all have moderate polymorphism at the Chr3:7319896G>A and Chr3:7519918G>A loci. The Chr3:7319896G>A locus was in Hardy-Weinberg equilibrium (P > 0.05) in Sichuan mountain chicken, while the Chr3:7519918G>A locus was in Hardy-Weinberg equilibrium (P > 0.05) in three of the four breeds, except the Caoke chicken breed. The Chr24:5748832 T>C locus was homozygous in the Caoke chicken breed, while the other three breeds exhibited moderate polymorphism for this locus. All of the experimental breeds exhibited moderate polymorphism for the Chr24:5748784A>G locus.

Genotype and allele analysis

Table 4 shows the genotypes and allele frequencies for the four chicken breeds. The Chr5:52922938A>G locus has three genotypes (GG, AA, and AG). Each genotype has a dif-

ferent frequency of occurrence: Jiuyuan black chicken only have the AG genotype, Sichuan mountain chicken only have the GG genotype, Tibetan chicken have both the AA and GG genotypes, while Caoke chicken has all three genotypes. The Chr5:52922938A>T locus has three genotypes (AA, AT, and TT). The three genotypes occur with varying frequency in each of the four breeds (Table 4). The Chr5:52926664C>T locus is composed of three genotypes (CC, CT, and TT), which occur with varying frequency in the four chicken breeds, except the TT genotype, which is not present in Jiuyuan chicken (Table 4). The GG genotype of the Chr3:7319896G>A locus is mainly observed in Caoke chicken and Jiuyuan black chicken, with probabilities of 52 and 88%, respectively, while the AA genotype is mainly observed in Tibetan chicken and Sichuan mountain chicken, with probabilities of 74 and 55%, respectively.

Table 3. Genetic diversity for each of the four Chinese indigenous chicken breeds for each of the seven SNP loci.

SNP	Breeds	H_O	H_E	N_A	PIC	χ^2	P
Chr5: 52922938							
A>G	CK	0.698819	0.301181	1.430986	0.134979	1.239669	0.265535
	JY	0.5	0.5	2	0.375	48	<0.0001
	SM	1	0	1	0	-	-
	TC	0.529744	0.470256	1.887704	0.359686	15.90533	<0.0001
	Total	0.502269	0.497731	1.99096	0.373863	1.540589	0.2145
Chr5: 52926659							
T>A	CK	0.549554	0.450446	1.819657	0.348995	27.39184	<0.0001
	JY	0.536675	0.463325	1.863324	0.35599	0.739498	0.389822
	SM	0.51020	0.489796	1.96	0.369846	16.67361	<0.0001
	TC	0.502551	0.497449	1.989848	0.373721	18.57106	<0.0001
	Total	0.53491	0.465086	1.86946	0.356934	2.282563	0.130836
Chr5: 52926664							
T>C	CK	0.520748	0.479252	1.920316	0.364411	6.357222	0.01169
	JY	0.712665	0.287335	1.403183	0.246054	2.556213	0.109861
	SM	0.610162	0.389838	1.638908	0.313851	1.292205	0.255642
	TC	0.518141	0.481859	1.929978	0.365765	9.771903	0.001772
	Total	0.569586	0.430414	1.755662	0.337786	0.119836	0.729212
Chr3: 7519896							
G>A	CK	0.625	0.375	1.6	0.304688	6.828283	0.008973
	JY	0.847222	0.152778	1.180328	0.141107	9.917355	0.001637
	SM	0.583299	0.416701	1.714388	0.329881	3.450571	0.06323
	TC	0.69161	0.30839	1.445902	0.260838	12.10089	0.000504
	Total	0.504349	0.49565	1.9827	0.372816	54.30615	<0.0001
Chr3: 7519918							
G>A	CK	0.53858	0.46142	1.856734	0.354966	5.683225	0.017128
	JY	0.736328	0.263672	1.35809	0.22891	1.646091	0.199492
	SM	0.546855	0.453145	1.828637	0.350475	3.825476	0.050479
	TC	0.547902	0.452098	1.825142	0.349901	1.407982	0.235392
	Total	0.501342	0.498658	1.994645	0.374328	6.193828	0.01282
Chr24: 5748832							
T>C	CK	1	0	1	0		
	JY	0.670139	0.329861	1.492228	0.275457	0.005319	0.941863
	SM	0.567472	0.432528	1.762202	0.338988	11.33585	0.00076
	TC	0.613379	0.386621	1.630314	0.311883	42	<0.0001
	Total	0.611157	0.388843	1.63624	0.313243	84.62	<0.0001
Chr24: 5748784							
A>G	CK	0.654321	0.345679	1.528302	0.285932	4.408163	0.035767
	JY	0.635634	0.364366	1.573233	0.297985	11.3098	0.000771
	SM	0.510204	0.489796	1.96	0.369846	5.444444	0.019631
	TC	0.637188	0.362812	1.569395	0.296996	22.84406	<0.0001
	Total	0.500483	0.49951	1.998069	0.374758	59.32124	<0.0001

CK = Caoke chicken; JY = Jiuyuan black chicken; SM = Sichuan mountain chicken; TC = Tibetan chicken; H_O = observed heterozygosity; H_E = expected heterozygosity; N_A = number of alleles; PIC = polymorphism information content; χ^2 and its associated P value indicate significant deviations from Hardy-Weinberg equilibrium.

Table 4. Genotype and allele frequencies of the seven SNP loci in four Chinese indigenous chicken breeds.

SNP	Genotype	CK number/frequency		JY number/frequency		SM number/frequency		TC number/frequency	
Chr5: 52922938									
A>G	AA	46	0.851852	0	0	0	0	32	0.761905
	AG	7	0.12963	48	1	0	0	0	0
	GG	1	0.018519	0	0	49	1	10	0.238095
	A		0.916667		0.5		0		0.761905
	G		0.083333		0.5		1		0.238095
Chr5: 52926659									
A>T	AA	32	0.592593	18	0.375	23	0.469388	16	0.380952
	AT	7	0.12963	25	0.520833	10	0.204082	7	0.166667
	TT	15	0.277778	5	0.104167	16	0.326531	19	0.452381
	A		0.657407		0.635417		0.571429		0.464286
	T		0.342593		0.364583		0.428571		0.535714
Chr5: 52926664									
C>T	CC	24	0.444444	30	0.625	28	0.571429	10	0.238095
	CT	17	0.314815	18	0.375	16	0.326531	30	0.714286
	TT	13	0.240741	0	0	5	0.102041	2	0.047619
	C		0.601852		0.8125		0.734694		0.595238
	T		0.398148		0.1875		0.265306		0.404762
Chr3: 7319896									
G>A	GG	28	0.518519	42	0.875	7	0.142857	5	0.119048
	GA	10	0.185185	4	0.083333	15	0.306122	6	0.142857
	AA	16	0.296296	2	0.041667	27	0.55102	31	0.738095
	G		0.611111		0.916667		0.295918		0.190476
	A		0.388889		0.083333		0.704082		0.809524
Chr3: 7519918									
G>A	AA	18	0.333333	0	0	24	0.489796	20	0.47619
	AG	33	0.611111	15	0.3125	16	0.326531	15	0.357143
	GG	3	0.055556	33	0.6875	9	0.183673	7	0.166667
	A		0.638889		0.15625		0.653061		0.654762
	G		0.361111		0.84375		0.346939		0.345238
Chr24: 5748832									
T>C	TT	0	0	2	0.041667	10	0.204082	31	0.738095
	CT	0	0	16	0.333333	11	0.22449	0	0
	CC	54	1	30	0.625	28	0.571429	11	0.261905
	T		0		0.208333		0.316327		0.738095
	C		1		0.791667		0.683673		0.261905
Chr24: 5748784									
A>G	AA	0	0	32	0.666667	13	0.265306	30	0.714286
	AG	24	0.444444	9	0.1875	16	0.326531	4	0.095238
	GG	30	0.555556	7	0.145833	20	0.408163	8	0.190476
	A		0.222222		0.760417		0.428571		0.761905
	G		0.777778		0.239583		0.571429		0.238095

CK = Caoke chicken; JY = Jiuyuan black chicken; SM = Sichuan mountain chicken; TC = Tibetan chicken.

The AG genotype of the Chr3:7519918G>A locus occurs mainly in Caoke chicken, the GG genotype occurs mainly in Jiuyuan black chicken, while the AA genotype occurs mainly in Sichuan mountain chicken and Tibetan chicken. The homozygous genotypes of the Chr24:5748832 T>C and Chr24:5748784A>G loci are observed in all four breeds (Table 4).

SNP locus phylogenetic analysis

Phylogenetic analysis of the 7 SNPs in the four chicken breeds was undertaken and the results show that the internal genetic distance was 0.0122 in Caoke chicken, 0.0139 in Jiuyuan black chicken, 0.014 in Sichuan mountain chicken, and 0.0134 in Tibetan chicken. Tables 5, 6, and 7 detail the results of the genetic distance among the four chicken breeds.

Table 5. Disparity and genetic distance within the four breeds of Chinese indigenous chicken.

Index	CK	JY	SM	TC
Disparity index	0.0042	0.0016	0.0018	0.0054
Distance within groups	0.0122	0.014	0.0139	0.0134

CK = Caoke chicken; JY = Jiuyuan black chicken; SM = Sichuan mountain chicken; TC = Tibetan chicken.

Table 6. Mean genetic distance between Chinese indigenous chicken breeds and layer and broiler chicken.

Breed	Layer	Broiler	CK	Gallus	SM	JY
Layer	-					
Broiler	0.0416	-				
CK	0.0307	0.0104	-			
Gallus	0.0234	0.0174	0.0225	-		
SM	0.0145	0.0264	0.0196	0.0245	-	
JY	0.0163	0.0247	0.0191	0.0243	0.0153	-
TC	0.0158	0.0252	0.0230	0.0125	0.0194	0.0180

CK = Caoke chicken; JY = Jiuyuan black chicken; SM = Sichuan mountain chicken; TC = Tibetan chicken.

Table 7. Average composition genetic distance between Chinese indigenous chicken breeds and layer and broiler chicken.

Breed	Broiler	Layer	CK	Gallus	SM	JY
Broiler	-					
Layer	0.0057	-				
CK	0.0173	0.0110	-			
Gallus	0.0171	0.0057	0.0210	-		
SM	0.0105	0.0111	0.0152	0.0246	-	
JY	0.0136	0.0135	0.0138	0.0293	0.0117	-
TC	0.0144	0.0105	0.0171	0.0171	0.0169	0.0176

CK = Caoke chicken; J = Jiuyuan black chicken; SM = Sichuan mountain chicken; TC = Tibetan chicken.

DISCUSSION

Research by Li et al. (2012) suggested that genes related to chicken resistance traits are located on chromosome 16, and at the same time, they also located both the egg laying performance and growth performance genes. In our study, the objective genes were *LHCGR*, *ESR2*, and *DRD2,* located on chromosomes 3, 5, and 24, respectively, and our results agree with those of Li et al. (2013). Several studies have revealed that these three genes are related to reproduction traits in animals and humans (Greene et al., 1986; Maxwell et al., 1987; Duan et al., 2003; Kossack et al., 2008; Xu et al., 2010; Yang et al., 2012; Yu et al., 2012). As the results of the objective gene screening show, the loci are mainly located in non-coding regions, where proteins are not encoded, indicating that the mutation site does not change the gene function through changes to the protein. It may be that the mutation site plays a role by reducing the coding region mutation, as demonstrated in previous research (Akey et al., 2002; Matukumalli et al., 2006; Groenen et al., 2011). The function of the mutation site requires further investigation.

Locus polymorphism, a form of genetic polymorphism, is the result of both natural and manual selection; however, natural selection plays the main role and three levels of poly-

morphism are assumed: low (PIC < 0.25), moderate (0.25 < PIC < 0.5) and high (PIC > 0.5) (Akey et al., 2002). Based on the results of the analyses conducted in the current study, we found that loci specific to layer chicken exhibited low polymorphism in the Caoke chicken breed, indicating that Caoke chicken has low levels of egg performance traits under natural selection. While the Jiuyuan black chicken breed exhibited moderate polymorphism for loci specific to layer chicken. In order to improve genetic progress, manual selection should be consistent with natural selection, thus, when we determine the breeding direction it should agree with natural selection. As the results show, some loci deviated from Hardy-Weinberg equilibrium, especially in the Tibetan chicken breed. Such deviations from Hardy-Weinberg equilibrium can be a result of sampling error caused by genetic drift. Deviations from Hardy-Weinberg equilibrium for loci in the Tibetan chicken breed may be a consequence of their living environment, where the altitude is 2500 m. Further sampling of this population would help to eliminate the sampling error caused by genetic drift in future research. At the same time, we infer that the Tibetan chicken breed has diverged as a subspecies due to its geographical distribution and living environment.

Based on the genetic distance analysis, the Caoke chicken breed has the smallest genetic distance and largest coefficient of variation compared to the other three breeds, which indicates that Caoke chicken has high consistency and may have been introduced to the other breed's blood. The genetic distance between each breed indicates that genetic distance was greatest between layer and broiler chicken breeds. Of the experimental groups, the genetic distance between the Caoke chicken breed and broiler chicken was smaller than between the Caoke chicken breed and layer chicken, while the genetic distance between the Jiuyuan black chicken breed and broiler chicken was greater than between the Jiuyuan black chicken breed and layer chicken. The Sichuan mountain chicken and Tibetan chicken breeds exhibited no significant differences.

CONCLUSION

Variation observed in the Chr24:5748784A>G, Chr24:5748832T>C, and Chr5:52922938A>G loci was specific to layer chicken, while variation in the Chr3:7519896A>G, Chr3:7519918G>A, Chr5:52926659T>A, and Chr5:52926664T>C loci was specific to broiler chicken.

Following analysis of the data, we suggest the Caoke chicken breed could be suitable for development in meat production, while the Jiuyuan chicken breed could be suitable for development in egg production. As the Sichuan mountain chicken and the Tibetan chicken exhibited large polymorphisms, these breeds could be improved by changing their living environment.

Conflicts of interest

The authors declare no conflict of interest.

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