Evidence of balancing selection in multiple indigenous chicken populations

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ABSTRACT. Microsatellite DNA markers, which are assumed to drift, have been widely used to assess genetic diversity in all major domestic animal species. These markers provide insight into the arrival and dispersion history of a species, with regard to their content or management history. However, no direct evidence supports current standard microsatellite markers falling under this assumption.

Therefore, the objective of this study was to investigate the effect and divergence of microsatellites under different types of selection on genetic patterns and population diversity. A total of 192 birds (*Gallus gallus*) from eight different geographic locations were investigated using 20 microsatellites that are classified into different groups by their selective effect (neutral, positive selection, and balancing selection) by the FDIST2 outlier test. The results showed that most polymorphisms were in the balancing selection marker group, the expected heterozygosity was 0.70, the observed heterozygosity was 0.65, and the mean number of alleles was 6.91. AMOVA revealed that the balancing group contributed the lowest amount of variance among groups, which was -0.60%, the highest variance contributed within the population being 92.28% in comparison with that of other groups. A similar pattern of population genetics was revealed following Slatkin linearized F_{ST}, principal component factor analysis, and population structure by Bayesian clustering. In conclusion, balancing selective markers offer high polymorphism for estimating genetic diversity but reduced genetic divergence between populations.

Key words: Genetic diversity; Chicken; Artificial selection; Microsatellite

INTRODUCTION

Microsatellite DNA markers, also known as simple sequence repeats or short tandem repeats (Turnpenny and Ellard, 2005), are variable number of tandem repeats and have been extensively studied to assess the diversity of populations of multiple species. In particular, the Food and Agriculture Organization, FAO (2011) recommend the use of microsatellite systems to identify diversity in domestic animals, and a large number of studies have been performed in multiple species including chickens (Tadano et al., 2014; Abebe et al., 2015), cattle (Pelayo et al., 2015; Sharma et al., 2015), pigs (Choi et al., 2014; Revidatti et al., 2014), sheep (Agaviezor et al., 2012; Yilmaz et al., 2014; Ćurković et al., 2016), and goats (Nomura et al., 2012; Wei et al., 2014). Most previous studies have shown that the breeds cluster according to their geographic location, and vary in the degree of diversity depending on the breeding and management histories of the breeds using these types of marker systems, which were typically co-dominant and assumed to be drifting (Granevitze et al., 2009). However, no evidence is available to fully support the latter notion.

In addition, studies have revealed the genomic linkage regions of quantitative traits using microsatellites (Yoo et al., 2014; Warrington et al., 2015), indicating that artificial selection changes the genotype frequency of some microsatellites. Of note, Mwacharo et al. (2013) identified four microsatellites that were under positive selection at 30 loci in African village chickens, which revealed that not all microsatellite loci are subject to neutral selection or genetic drift.

In the current study, we used a range of chickens to classify microsatellites according to different types of selection (neutral, positive, and balancing selection) using a relative genetic algorithm. Then, we comprehensively compared data on the pattern of population genetics and diversity of breeds between different marker systems to reveal the genetic contributions of microsatellites under different types of selection.

MATERIAL AND METHODS

Samples and genotype test

Blood samples were collected from 192 birds from eight populations of three geographic mainland locations: three indigenous populations from four countries in Africa (Uganda, Ethiopia, and Egypt), three local populations from China (Beijing You, DeHua, and Fujian Silk), and one indigenous population from Cambodia (Ca), as well as an indigenous breed from Fiji, which is located in the south Pacific Ocean. Genomic DNA was extracted using a standard phenol-chloroform method (Sambrook and Russell, 2001).

The average sample size was 24 birds per population. Individuals were genotyped at 20 microsatellite loci selected from the 30 loci suggested for use in biodiversity studies in chickens (FAO, 2011) (Table 1). Approximately 1-2 μL PCR product was diluted in 10 μL autoclaved distilled water for use in DNA genotyping. Two-microliters of diluted PCR products was added to 7.75 μL Hi Di™ Formamide, and 0.25 μL Gene Scan-500 LIZ™. The mixtures were heated at 94°C for 5 min and then immediately chilled using ice for 2 min. Genotyping was performed on a Genetic Analyzer 3130 xl (AB Applied Bio Systems).

Data analysis

Locus F_{ST} (pairwise difference) values across populations were used to test the hypothesis of diversifying selection acting at each locus. The FDIST2 outlier test (Beaumont and Nichols, 1996) was implemented in LOSITAN (Antao et al., 2008) with 100,000 simulations and a cut-off probability value of 0.99. Genetic diversity [expected (H_E), observed (H_O) heterozygosities, mean number of alleles (N_A), and polymorphism information content] were estimated from the allele frequencies using FSTAT 2.9.3.2 (Goudet, 2001). Matrix of Slatkin linearized F_{ST} (Slatkin, 1995) of populations and AMOVA were displayed using the Arlequin software 3.5.1.3 (Excoffier and Lischer, 2010).

Bayesian clustering was reconstructed using STRUCTURE 2.3.3 (Pritchard et al., 2000; Falush et al., 2003) to infer population structure. In this study, the number of clusters (K) varied between $2 \leq K \leq 8$ or 7, using a burn-in of 50,000 followed by 100,000 Markov Chain Monte Carlo in 50 iterations. STRUCTURE_Harvester (Earl and vonHoldt Bridgett, 2012) was used to generate a graphical display of the simulated results and the most optimal K with $\Delta K = m|L'(K)|/s|L(K)|$. We further generated additional information to assist in the interpretation of results from STRUCTURE and to correctly infer the underlying genetic structure. Principal component factor analysis (PCA) was performed with the MultiVariate Statistical Package (MVSP) Version 3.13 m software (Kovach and Services, 2004), which was conducted in a zoological and genetic study (Rosa et al., 2007).

RESULTS

Polymorphism and diversity of each classifying locus group

First, 20 microsatellites were tested using the FDIST2 outlier test (Beaumont and

Nichols, 1996) implemented in LOSITAN (Antao et al., 2008) with all individuals. Five loci subject to positive selection were indicated, including MCW0216, MCW0222, MCW0014, MCW0067, and MCW0081. Four loci subject to balancing selection included LEI0094, LEI0166, MCW0034, and MCW0183. The remaining 11 loci were found to be under neutral selection in this study. Therefore, 20 microsatellites were classified into three marker systems: the neutral group, balancing selection group, and positive marker group (Figure 1 and Table 2). Finally, the diversity of eight indigenous breeds was described by each classified group marker, and across all 20 microsatellite markers.

Table 1. Microsatellite marker information for PCR amplification.

Primer	Chromosome	Primer sequence (5'-3')	Annealing temperature (°C)	GenBank accession No.	Allele range (bp)
ADL0268	1	F: CTCCACCCCTCTCAGAACTA	60	G01688	102-106
		R: CAACTTCCCATCTACCTACT			
ADL0278	8	F: CCAGCAGTCTACCTTCCTAT	60	G01698	114-126
		R: TGTCATCCAAGAACAGTGTG			
MCW0216	13	F: GGGTTTTACAGGATGGGACG	60	AF030586	139-149
		R: AGTTTCACTCCCAGGGCTCG			
MCW0248	1	F: GTTGTTCAAAAGAAGATGCATG	60	G32016	205-225
		R: TTGCATTAACTGGGCACTTTC			
MCW0034	2	F: TGCACGCACTTACATACTTAGAGA	60		212-246
		R: TGTCCTTCCAATTACATTCATGGG			
MCW0069	E60C04W23	F: GCACTCGAGAAAACTTCCTGCG	60		158-176
		R: ATTGCTTCAGCAAGCATGGGAGGA			
MCW0081	5	F: GTTGCTGAGAGCCTGGTGCAG	60		112-135
		R: CCTGTATGTGGAATTACTTCTC			
MCW0222	3	F: GCAGTTACATTGAAATGATTCC	60	G31996	220-226
		R: TTCTCAAAACACCTAGAAGAC			
MCW0295	4	F: ATCACTACAGAACACCCTCTC	60	G32052	88-106
		R: TATGTATGCACGCAGATATCC			
LEI0234	2	F: ATGCATCAGATTGGTATTCAA	60	Z94837	216-364
		R: CGTGGCTGTGAACAAATATG			
MCW0206	2	F: CTTGACAGTGATGCATTAAATG	60	AF030579	221-249
		R: ACATCTAGAATTGACTGTTCAC			
LEI0166	3	F: CTCCTGCCCTTAGCTACGCA	60	X85531	354-370
		R: TATCCCCTGGCTGGGAGTTT			
MCW0111	1	F: GCTCCATGTGAAGTGGTTTA	60	L48909	96-120
		R: ATGTCCACTTGTCAATGATG			
MCW0330	17	F: TGGACCTCATCAGTCTGACAG	60	G32085	256-300
		R: AATGTTCTCATAGAGTTCCTGC			
MCW0183	7	F: ATCCCAGTGTCGAGTATCCGA	58	G31974	296-326
		R: TGAGATTTACTGGAGCCTGCC			
MCW0014	6	F: TATTGGCTCTAGGAACTGTC	58		164-182
		R: GAAATGAAGGTAAGACTAGC			
MCW0067	10	F: GCACTACTGTGTGCTGCAGTTT	60	G31945	176-186
		R: GAGATGTAGTTGCCACATTCCGAC			
MCW0037	3	F: ACCGGTGCCATCAATTACCTATTA	64		154-160
		R: GAAAGCTCACATGACACTGCGAAA			
LEI0094	4	F: GATCTCACCAGTATGAGCTGC	60	X83246	247-287
		R: TCTCACACTGTAACACAGTGC			
MCW0016	3	F: ATGGCGCAGAAGGCAAAGCGATAT	60		
		R: TGGCTTCTGAAGCAGTTGCTATGG			162-206

Twenty microsatellite DNA markers selected based on ISAG-FAO recommendations (FAO, 2011) were used in this study.

In the balancing selection group, the H_E ranged from 0.64 (Fujian Silk) to 0.78 (Fiji), the H_O ranged from 0.56 (Cambodia) to 0.76 (Uganda), and the mean N_A ranged from 4.50 (Fujian Silk) to 8.75 (Egypt). In the neutral loci group, the H_E ranged from 0.59 (Beijing You) to 0.75 (Cambodia), the H_O ranged from 0.50 (Ethiopia) to 0.71 (DeHua), and the

mean N_A ranged from 4.45 (Beijing You) to 6.91 (Cambodia). In the group containing all 20 microsatellites, the H_E ranged from 0.54 (Fujian Silk) to 0.70 (Cambodia), the H_O ranged from 0.48 (Ethiopia) to 0.63 (DeHua), and the mean N_A ranged from 4.15 (Fujian Silk) to 6.85 (Cambodia). However, in the positive selection group, the H_E ranged from 0.31 (Fujian Silk) to 0.65 (Uganda), the H_O ranged from 0.21 (Fujian Silk) to 0.61 (Uganda), and the mean N_A ranged from 2.80 (Fujian Silk) to 5.60 (Cambodia); detailed information is presented in Table 3. Therefore, as the result of heterozygosity and the mean N_A among those breeds, the highest degree of polymorphism was represented by the balancing selection group; the second highest was observed in the neutral group and in the group containing 20 microsatellites; and the lowest amount of polymorphism was found in the positive selection group.

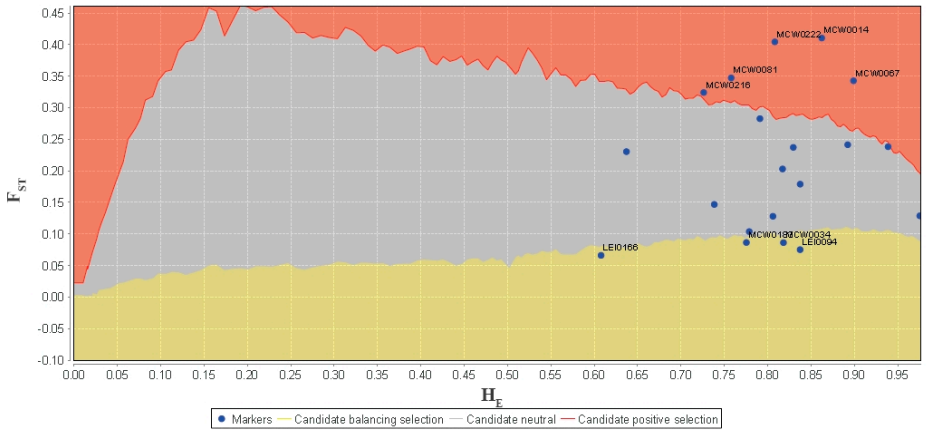

Figure 1. Distribution of classified markers using FDIST2.

Table 2. Selective test using LOSITAN for selection and Fisher test for Hardy-Weinberg equilibrium at each loci.

Locus	H_E	F_{ST}	P (Simul F_{ST} < sample F_{ST})	FDR	HWE (P value)
ADL0268	0.778754	0.103662	0.046031	-	0.09375
LEI0094	0.837402	0.075039	0.002578	Outlier	0.06545
LEI0166	0.607809	0.065976	0.011291	Outlier	0.05656
MCW0034	0.818204	0.086203	0.016783	Outlier	0.21722
MCW0069	0.892299	0.241317	0.912927	-	0.00605*
MCW0216	0.726200	0.324037	0.979665	Outlier	0.00821*
MCW0248	0.637106	0.230334	0.738828	-	0.58664
ADL0278	0.829551	0.237096	0.852127	-	0.00008*
LEI0234	0.975466	0.128687	0.413606	-	0.68647
MCW0016	0.938942	0.238169	0.956818	-	0.0095*
MCW0037	0.806056	0.127913	0.13485	-	0.43762
MCW0222	0.808267	0.404324	0.999869	Outlier	0.45184
MCW0295	0.837488	0.178828	0.499164	-	0.71929
MCW0014	0.862383	0.410386	0.999972	Outlier	0.00118*
MCW0067	0.899011	0.342634	0.999213	Outlier	0.78113
MCW0081	0.757921	0.347172	0.990639	Outlier	0.35447
MCW0111	0.791066	0.282621	0.947723	-	0.00863*
MCW0183	0.775437	0.086394	0.013872	Outlier	0.57109
MCW0206	0.817181	0.202901	0.674529	--	0.43517
MCW0330	0.738447	0.146668	0.249702	--	0.89697

Names of positively selected loci are indicated by red font color; balancing selection loci are in blue, and neutral loci are black. H_E is the expected heterozygosity of each loci, and F_{ST} is the mean population difference for each loci. FDR is the confidence interval (P < 0.1, is the balancing selection; P > 0.9, is the positive selection and 0.1 < P < 0.9, is the neutral drift).

Table 3. Comparison of genetic parameters of diversity with different marker systems within each population.

	Positive			Balancing			Neutral			All loci (20 microsatellites)		
Breed	H_E	H_O	N_A	H_E	H_O	N_A	H_E	H_O	N_A	H_E	H_O	N_A
Fujian Silk	0.3083	0.2083	2.80	0.6385	0.5833	4.50	0.6061	0.5909	4.64	0.5382	0.4938	4.15
Beijing You	0.5364	0.5304	3.40	0.6283	0.6209	5.25	0.5914	0.5958	4.45	0.5851	0.5845	4.35
DeHua	0.5466	0.4667	3.80	0.6815	0.6341	6.25	0.7267	0.7111	6.73	0.6726	0.6346	5.90
Cambodia	0.5567	0.5083	5.60	0.7232	0.5625	8.25	0.7497	0.6533	6.91	0.6961	0.5989	6.85
Egypt	0.5823	0.4750	4.80	0.7644	0.7500	8.75	0.6514	0.6439	6.27	0.6567	0.6229	6.40
Ethiopia	0.3948	0.3587	3.80	0.6461	0.5833	6.75	0.5651	0.5038	5.55	0.5387	0.4834	5.35
Uganda	0.6502	0.6083	5.40	0.7394	0.7604	7.25	0.6858	0.5758	5.55	0.6876	0.6208	5.85
Fiji	0.5312	0.4833	4.00	0.7755	0.7396	8.25	0.7352	0.6515	6.18	0.6922	0.6271	6.05
Total	0.5133	0.4549	4.20	0.6996	0.6543	6.91	0.6639	0.6158	5.79	0.6334	0.5833	5.61

Population structure of microsatellite groups under different types of selection

The results of AMOVA with 20 autosomal microsatellites showed that the amount of genetic variance among populations, within groups, and within populations was 18.86 and 77.65%, respectively, and 3.49% among the groups. In the neutral marker group, the contribution to genetic variance among populations within groups and within popwulations was 17.59 and 80.21%, respectively, and 2.20% among the groups. The results for the positive selection locus showed that the contribution to genetic variance among populations within groups and within populations were 29.12 and 61.37%, respectively, and 9.51% among the groups. Detailed information is presented in Table 4. However, across the balancing selection locus, the percentage of genetic variant contributions among populations within groups and within populations were 8.32 and 92.28%, respectively. However, -0.60% among groups indicated genetic differentiation, which was found mainly within populations using balancing markers.

Table 4. AMOVA using different marker systems.

	Source of variation	Among groups	Among populations within groups	Within populations	Total
Positive marker	Sum of squares	105.658	151.514	479.188	736.359
	Variance components	0.19760	0.60476	1.27443	2.07679
	Percentage variation	9.51%	29.12%	61.37%	100%
Balancing markers	Sum of squares	12.848	37.177	524.703	574.727
	Variance components	-0.00898	0.12614	1.39961	1.51678
	Percentage variation	-0.60%	8.32%	92.28%	100%
Neutral markers	Sum of squares	105.885	208.181	1358.583	1672.648
	Variance components	0.09918	0.79214	3.61325	4.50457
	Percentage variation	2.20%	17.59%	80.21%	100%
All markers	Sum of squares	224.921	399.302	2370.905	2995.128
	Variance components	0.28494	1.53890	6.33525	8.15909
	Percentage variation	3.49%	18.86%	77.65%	100%

A similar genetic pattern as found for F_{ST} values was found for the neutral locus group and for the group containing 20 microsatellites; however, a higher degree of genetic differentiation was observed in the neutral group. In addition, the difference-pattern of the balancing selective locus and positive selective locus exhibited large divergence from that of the neutral locus group and the group containing 20 microsatellites. Detailed numerical values for the Slatkin linearized F_{ST} are presented in Tables 5-8, and the visual pattern of F_{ST} for each group of markers is shown in Figure 2.

Table 5. Matrix of Slatkin linearized F_{ST} as t/M = F_{ST} / (1 - F_{ST}) analysis with 20 microsatellites.

	Fujian Silk	Beijing You	DeHua	Cambodia	Egypt	Ethiopia	Uganda	Fiji
Fujian Silk	0.00000							
Beijing You	0.27723	0.00000						
DeHua	0.15645	0.20246	0.00000					
Cambodia	0.27925	0.32803	0.19169	0.00000				
Egypt	0.40627	0.34577	0.28229	0.15869	0.00000			
Ethiopia	0.54407	0.44176	0.37832	0.29034	0.16158	0.00000		
Uganda	0.42245	0.39280	0.28422	0.25305	0.24741	0.36421	0.00000	
Fiji	0.29731	0.30762	0.20797	0.07111	0.05722	0.19249	0.24779	0.00000

Table 6. Matrix of Slatkin linearized F_{ST} as t/M = F_{ST} /(1 - F_{ST}) analysis with neutral microsatellites.

	Fujian Silk	Beijing You	DeHua	Cambodia	Egypt	Ethiopia	Uganda	Fiji
Fujian Silk	0.00000							
Beijing You	0.26056	0.00000						
DeHua	0.12883	0.19917	0.00000					
Cambodia	0.22042	0.27909	0.12822	0.00000				
Egypt	0.31917	0.29314	0.24760	0.17716	0.00000			
Ethiopia	0.36933	0.34997	0.29202	0.25753	0.14268	0.00000		
Uganda	0.39343	0.38945	0.27894	0.26989	0.28057	0.34859	0.00000	
Fiji	0.20673	0.22310	0.14829	0.06951	0.07182	0.14437	0.26973	0.00000

Table 7. Matrix of Slatkin linearized F_{ST} as t/M = F_{ST} / (1 - F_{ST}) analysis with balancing selected microsatellites.

	Fujian Silk	Beijing You	DeHua	Cambodia	Egypt	Ethiopia	Uganda	Fiji
Fujian Silk	0.00000							
Beijing You	0.12162	0.00000						
DeHua	0.12593	0.14180	0.00000					
Cambodia	0.11001	0.13270	0.04783	0.00000				
Egypt	0.12890	0.11507	0.03294	0.06582	0.00000			
Ethiopia	0.17007	0.22576	0.10570	0.07965	0.09825	0.00000		
Uganda	0.09461	0.09742	0.02911	0.05143	0.02001	0.07456	0.00000	
Fiji	0.10087	0.10901	0.02981	0.04867	0.00312	0.06908	0.02444	0.00000

Table 8. Matrix of Slatkin linearized F_{ST} as t/M = F_{ST} / (1 - F_{ST}) analysis with positive selected microsatellites.

	Fujian Silk	Beijing You	DeHua	Cambodia	Egypt	Ethiopia	Uganda	Fiji
Fujian Silk	0.00000							
Beijing You	0.51682	0.00000						
DeHua	0.28802	0.26924	0.00000					
Cambodia	0.69423	0.65266	0.52170	0.00000				
Egypt	1.02637	0.70413	0.62754	0.20597	0.00000			
Ethiopia	1.74571	0.93237	0.94633	0.63615	0.28755	0.00000		
Uganda	0.88552	0.67359	0.53722	0.40467	0.39022	0.71721	0.00000	
Fiji	0.88037	0.74743	0.57578	0.10061	0.07708	0.49527	0.41886	0.00000

The genetic pattern of PCA in different marker groups is shown in Figure 3. As expected, clear geographic separation was found to exist in the group containing 20 microsatellites, and in the neutral and positive locus groups, respectively, but not in the balancing locus group. The genetic structure of 192 individuals assessed using the STRUCTURE software revealed an increasing value for *K* (from 2-7 or 8), and we estimated the most optimal *K* to be 3 (**Tables S1, S2, S3** and **S4**) in all four marker groups (20 microsatellite, neutral locus, positive selection locus, and balancing selection locus). A similar genetic pattern was shown by the group containing 20 microsatellites, and by the neutral locus, and positive selection locus groups; however, the balancing selection locus was found to have a blurry pattern (Figure 4).

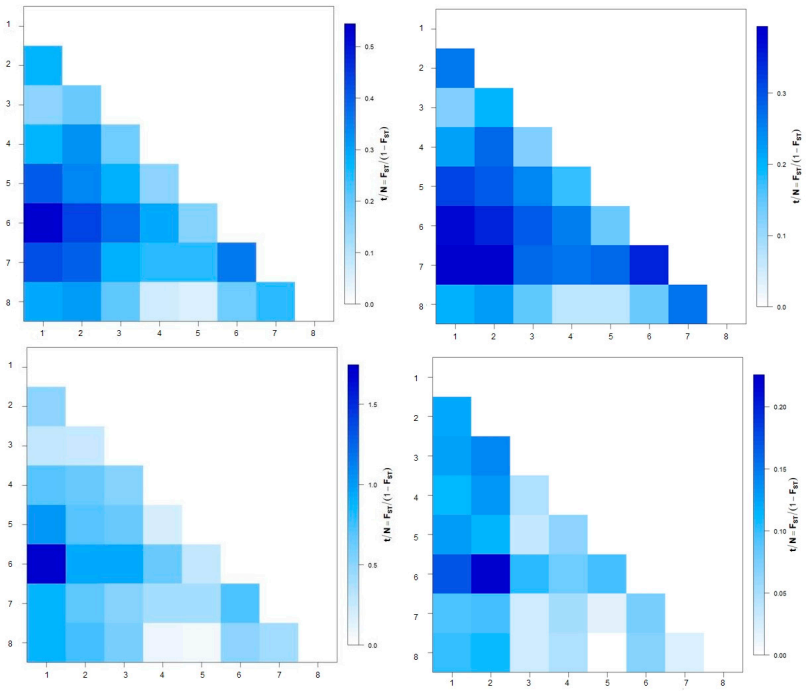

Figure 2. Matrix showing Slatkin linearized F_{ST} as t/M = F_{ST} / (1 - F_{ST}) analysis with different marker systems. 1 = Fujian Silk, 2 = Beijing You, 3 = DeHua, 4 = Cambodia, 5 = Egypt, 6 = Ethiopia, 7 = Uganda, and 8 = Fiji.

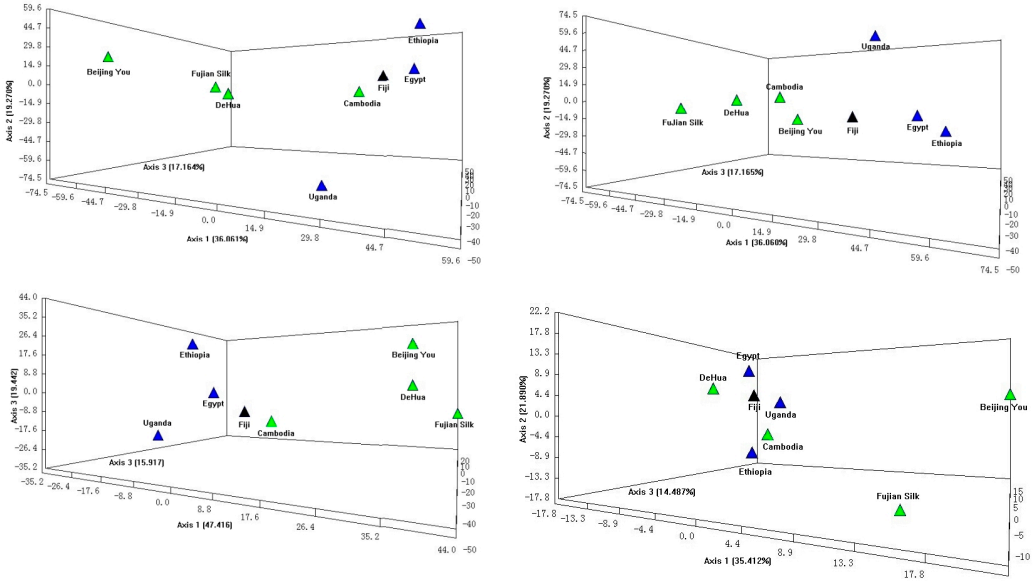

Figure 3. Phylogenetic clusters of eight chicken breeds using factorial correspondence analysis (FCA).

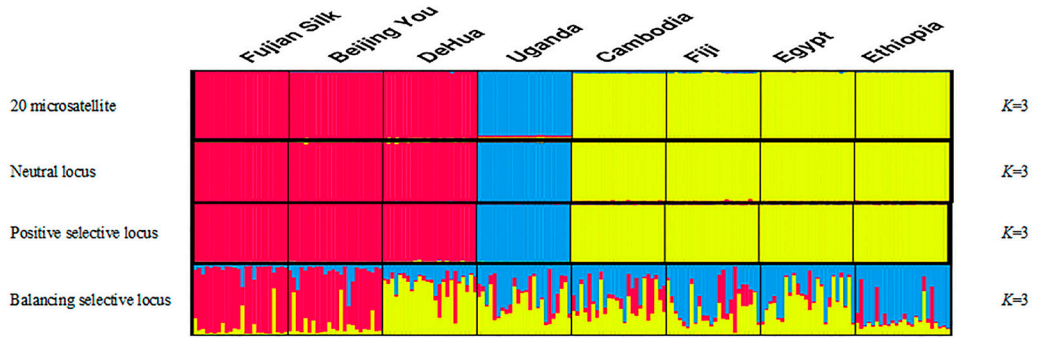

Figure 4. Cluster of 192 individuals of eight populations using different marker systems.

DISCUSSION

In the present study, information on genetic diversity obtained through H_E, H_O, and N_A values for the four marker groups, revealed that the neutral marker group possesses a higher level of polymorphism than the positive selection group and the group containing 20 microsatellites, with the lowest level of polymorphism observed for the balancing group. This finding indicates that the balancing locus possessed the highest level of polymorphism and that the polymorphism of the neutral locus was midway between that of the positive and balancing loci.

Balancing selection usually occurs when heterozygotes for alleles under consideration have a higher adaptive value than the homozygotes, thus, conserving genetic polymorphism. In addition, heterozygote advantage is the main mechanism of balancing selection, which means an individual who is heterozygous at a particular gene locus has a greater fitness than an individual homozygous at the same locus (Eugenie, 1978). Studies have shown that high genetic variance and heterozygote advantage can support a high survival rate, particularly in immune regions of genes (Daum et al., 2012; Eizaguirre et al., 2012; Coppage et al., 2013).

In addition, increasing numbers of studies have focused on identifying the effect of positive selection on the genomes of different species (Sabeti et al., 2007; Drummond and Suchard, 2008; Huard-Chauveau et al., 2013). Under this type of directional selection, advantageous alleles accumulate in an ongoing manner due to the high survival rate; however, dominant/recessive alleles will eventually become fixed (Molles and Cahill, 1999). Therefore, the main power of positive selection is attributed to the divergence between domestic animal populations or breeds (Parker et al., 2004).

The result of AMOVA revealed that the positive selection group contributed the most to genetic variance (9.51% among the group), and the balancing selection group contributed the least (-0.59%). The contribution to genetic variance indicated that the positive locus supported a higher difference in the mating population genetic structure as well as balancing locus to dilute this difference compared with that of the neutral locus. In addition, PCA and population structure analysis using a Bayesian clustering algorithm revealed that balancing selection may reduce the divergence between populations due to the high heterozygosis and increasing frequency of different alleles.

Conflicts of interest

The authors declare no conflict of interest.

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Supplementary material

Table S1. *K* value estimation using 20 microsatellites.

Table S2. *K* value estimation using 11 neutral locus.

Table S3. Estimated *K* values using fine positive selective locus.

Table S4. Estimated *K* value using four balancing selective locus.