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Microsatellite Analysis of Genetic Diversity in Three Populations of Japanese Quail (*Coturnix coturnix japonica*) from Kazakhstan

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Abstract: Three Japanese quail lines A-C were examined genetically using 13 micro-satellite markers to detect genetic diversity. The studied loci on average produced 5 alleles locus^{-1} (range: 2-8). The mean observed Heterozygosity (H_o) was 0.609 and ranged across loci from 0.00-0.967 whereas the mean expected Heterozygosity (H_e) was 0.636 and ranged between 0.139 and 0.802. The Polymorphic Information Content (PIC) values varied among loci and ranged between 0.346 for locus GUJ0010 and 0.814 for locus GUJ0087 with overall mean 0.644. Differentiation among populations was moderate but highly significant (F_{ST} = 0.10, R_{ST} = 0.17; $p<0.0001$) however, within populations differentiation accounted for 3.61 and -0.73% of the total nuclear microsatellite variation under Infinite Allele Model (IAM) and Stepwise Mutation model (SSM), respectively. Cluster analysis based on Nei's genetic distance indicated that the studied populations formed two main groups. The 1st group included line A and the 2nd group harboured lines B and C. These results reflect that the set of studied markers can be used effectively to capture the magnitude of genetic variability in different Japanese quail populations.

Key words: Japanese quail, micro-satellites, genetic diversity, cluster analysis, variability, Egypt

INTRODUCTION

Quail considered an economically important avian species and provide an alternative to the more commonly used chicken. They require less space and low initial investment and have noble export potential. Quail are in the genus Coturnix, family Phasianidae and order Galliformes (Sharma *et al*., 2000). The Japanese quail is now a well-established animal model in biology and a bird used for intensive egg and meat production mainly in Asia and Europe but also in the Middle East and America (Minvielle, 2004).

Characterization of the genetic diversity of indigenous animal populations is a prerequisite for providing needed information for the conservation of useful genotypes against future uncertainties in the face of daunting global challenges such as climate change, emerging diseases, population growth and rising consumer demands (Kayang *et al*., 2010). Continued genetic improvement of livestock is dependent on the fact that substantial genetic variation exists within individual breeds allowing them to respond to selection for different traits (Sruoga *et al*., 2007). Quantitative assessment of genetic diversity within and between populations is an important tool for decision making in genetic conservation plans (Davila *et al*., 2009). Recent advances in molecular technology have opened up new horizons for estimating genetic relatedness between and within animal populations and molecular markers may serve as an important initial guide to develop conservation strategies (Davila *et al*., 2009). The genetic diversity of a breed is mainly evaluated through its nuclear diversity (Berthouly-Salazar *et al*., 2010). Genetic diversity measures using micro-satellites yield reliable estimations of variability within and genetic relationships among chicken populations and was extensively studied (Takezaki and Nei, 1996; Vanhala *et al*., 1998; Ponsuksill *et al*., 1999; Zhou and Lamont, 1999; Kaiser *et al*., 2000; Weigend and Romanov, 2001; Zhang *et al*., 2002a, b; Delany, 2003; Chen *et al*., 2004; Osman *et al*., 2006; Takahashi and Nakamura, 2007; Roushdy *et al*., 2008; Kayang *et al*., 2010).

Micro-satellites were used to examine gene flow between species, population structure within species and relatedness between individuals within sympatric social groups (Gee *et al*., 2003). Expected heterozygosity varied between 0.7 and 0.95 in California quail and between 0.48 and 0.90 in Gambel's quail. Significant deficiencies

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of heterozygotes were observed (Cc25 and Cc93 in *C. californica*, Cc1 and Cc3 in *C. gambelii*) (Gee *et al*., 2003). In East China, three micro-satellite loci were used to investigate genetic diversity of four quail populations with no genetic relationship resources. The results demonstrated that gene diversity among loci ranged from 0.468-0.635 with an average gene diversity observed in the populations were in the increasing order of 0.468, 0.515, 0.555 and 0.635, respectively (Olajide *et al*., 2006). Allele frequencies of eight micro-satellite loci were estimated for four strains of Japanese quail in Iran, two loci were monomorphic in Panda and Texedo. In the same study, the expected heterozygosity varied between 0.708 and 0.849. All locus-strain combinations deviated from Hardy-Weinberg equilibrium except GUJ0041 in Pharach strain, GUJ0021, GUJ0034, GUJ0041 and GUJ0097 in Panda strain and GUJ0034, GUJ0049 and GUJ0070 in Golden strain ($p<0.001$) (Amirinia *et al*., 2007). Kim *et al*. (2007) analyzed information from pedigrees and from microsatellite markers to estimate inbreeding in a line of Japanese quail. Data from analysis of 14 micro-satellite markers in the inbred and QO lines were used to calculate the population differentiation (F_{ST}) of the lines caused by inbreeding. The mean expected Heterozygosity (H_e) for the randombred line was 0.48 compared with 0.24 for the inbred line, resulting in F_{IS} values for the randombred and inbred lines of 0.10 and 0.13, respectively. They estimated the population differentiation (F_{ST}) as 0.50 and the inbreeding coefficient (F_{IT}) as 0.57.

The objective of the current study is to assess genetic diversity between closely related populations of Japanese quail from Kazakhstan using nuclear micro-satellite markers.

MATERIALS AND METHODS

Quail sampling and DNA extraction: A total of 25 individual quail representing three lines (A; selected for high body weight, B; selected for high egg number until 90 days of age and C; control line) were used. Approximately, 2 mL of blood was drawn from the jugular vein for each individual quail in tubes treated with K_3-EDTA (FL medical, Italy). DNA was extracted using Biospin Whole Blood Genomic DNA extraction kit (Precision Biotek Instruments Pvt. Ltd., Japan). To ensure DNA purity and to determine DNA quantity, all DNA samples were checked against 1 kilobase (kb) molecular size standard (GeneDirex, China) on agarose 2%.

Micro-satellite genotyping: Each individual quail was genotyped for 13 nuclear micro-satellite loci that were chosen based on their degree of polymorphism and its relation to selected traits (Kayang *et al*., 2002; Minvielle *et al*., 2005, 2006) (Table 1). Polymerase Chain Reactions (PCR) amplifications were performed in a 20 μL reaction volume containing 30 ng genomic DNA, 130 μM of dNTP mix, 2.5 μL 10× reaction buffer (with 15 mM $MgCl_2$), 0.3 μM of forward and reverse primer and

Table 1: Primer name, sequence and annealing temperature of the 13 micro-satellites used in the current study

Primers	GenBank AN	Repeat array		Primer sequence (5′-3′)	TA (°C)
GUJ010	AB035820	(CA) 15	F	TTCCTTCTGGGTGCTGCTCA	60
			R	CATAGACACATCCCTCCCTC	
GUJ027	AB035837	(CA) 15	F	TTCACAGATGACAATCTAGC	55
			R	CTGCAAGTAACAGAAGGTAA	
GUJ033	AB035843	(CA) 13	F	TCTGCTCTCACAGCAGTGCA	60
			R	GCATAGAGCCCAGCAGTGTT	
GUJ049	AB035859	(CA) 11	F	GAAGCAGTGACAGCAGAATG	58
			R	CGGTAGCATTTCTGACTCCA	
GUJ052	AB063120	(CA) 12	F	AAACTACCGATGTAAGTAAG	54
			R	ATGAGATATATAAGGAACCC	
GUJ054	AB063122	(CA) 7	F	GTGTTCTCTCACTCCCCAAT	56
			R	ATGTGAGCAATTGGGACTG	
GUJ055	AB063123	(CT) 12 (CA) 11	F	GCATACTGCAATATACCTGA	56
			R	TTGACATACTTGGATTAGAGA	
GUJ059	AB063127	(CA) 10	F	GACAAAGTTACAGCTAGGAG	56
			R	TAGGTGCGAAAATCTCTGAC	
GUJ063	AB063131	(CA) 7CT (CA) 2CT (CA) 7	F	GCTCAGGTTCTCAGCTGATG	55
			R	GGGAGAGATCAAGGGAACAG	
GUJ071	AB063139	(CA) 8	F	AGATCCTGCTCCTGGAATTG	58
			R	CAGCTGCACTTAATACAGGC	
GUJ085	AB063153	(GT) 14	F	ACAACCACTTCTCCAGCTAC	55
			R	GCTTGTGCTGCTGTTGCTAA	
GUJ087	AB063155	(CT) 12AA (CA) 11	F	CATGCCGGCTGCTATGACAG	55
			R	AAGTGCAGGGAGCGAGGAAG	
GUJ097	AB063165	(CA) 14	F	GGATGCTCAGTGTGGAAAAG	55
			R	GAGCAAGAGGTGAGTGTTTC	

GenBank AN = GenBank Accession Number; TA = Temperature of Annealing

one unit of AmpliTaq DNA polymerase (BioReady rTaq, BioFlux). For the 13 micro-satellites (SSR) used for genotyping, amplifications were performed using Techne® TC-3000 thermal cycler (Bibby Scientific Ltd., UK) with the following temperature profile; initial denaturation at 95°C for 9 min, 30 cycles of 95°C for 30 sec, 54-60°C (optimized per marker B) (Table 1) for 1 min and 72°C for 1 min and 5 min final extension at 72°C. Fragments were separated on 8% polyacrylamide gel on vertical plates (with 5 V cm^{-1}) according to Maniatis *et al*. (1975). Allelic sizes were estimated using the GelAnalyzer (2010) software.

Microsatellite DNA polymorphism and deviation from Hardy-Weinberg equilibrium: The number of alleles per locus, allelic richness corrected for unequal sample size using rarefaction approach (El-Mousadik and Petit, 1996) as implemented in MSA 4.05 (Dieringer and Schlotterer 2003), observed Heterozygosity (H_o) and Nei's unbiased estimates of expected heterozygosity (H_e; Nei, 1987) were calculated using MSA 4.05. The information content of each locus was calculated using the Polymorphism Information Content (PIC) (Lynch and Walsh, 1998). This estimate provides an estimate of the discriminating power of a locus by taking into account not only the number of alleles that are expressed but also their relative frequencies. PIC values were estimated using program Cervus 3.0.3 (Kalinowski *et al*., 2007). Departures from Hardy-Weinberg equilibrium were assessed across populations and for each locus based on exact tests as implemented in GENEPOP 4.0 (Raymond and Rousset, 1995) under the assumption of heterozygote excess and heterozygote deficit. Also, deviation from HWE at each locus in each population was assessed using Genealex 6.4 (Peakall and Smouse, 2006). The estimates of fixation indices F_{IS} (Wright, 1965) were obtained using MSA 4.05.

Analysis of Molecular Variance (AMOVA): To quantify the extent of molecular variation, locus-by-locus Analysis of Molecular Variance (AMOVA) was performed using two different models of SSR evolution. The 1st model based on the Infinite Allele Model (IAM) where the allelic differentiation is thought to result from non-direct random mutation that might alter the allele size. In this model, the F-statistics (Wright, 1965; Weir and Cockerham, 1984) reflects differences in allele classes regardless the allele size difference. The 2nd model based on the stepwise mutation model (Ota and Kimura, 1973; Kimura and Ohta, 1978) where forward and reverse mutation of SSR causes changes of allele size involving one or two repeat unit. In this model R-statistics (Slatkin, 1995) reflects repeat length variations. In the current study both F_{ST} and R_{ST} were used to determine the potential differences between the two statistics. F- and R-statistics were obtained using AMOVA approach as implemented in ARLEQUIN 3.5 (Excoffier and Lischer, 2010).

Cluster analysis: To determine population groupings; cluster analysis was performed using the Unweighted Paired Group Arithmetic Average (UPGMA) implemented in POPTREE2 (Takezaki *et al*., 2010) based on Nei's genetic distance (Nei *et al*., 1983). To test for the robustness of UPGMA tree, bootstrap tests (Felsenstein, 1985) were performed using 1000 replications. In the bootstrap test, the loci were resampled with replacement in POPTREE2. The phylogenetic tree is constructed with the distance values calculated from the same number of resampled loci similar to that of original input dataset in each replication.

RESULTS AND DISCUSSION

Micro-satellite DNA polymorphism and deviation from Hardy-Weinberg equilibrium: The mean number of alleles per locus for the studied loci was 5.385 alleles $locus^{-1}$ (range: 2-8; Table 2). This average could be informative for such studies according to Barker (1994) who suggested that the average number of alleles per

Table 2: No. of alleles, allele size range and heterozygosities of 13 SSRs in three quail populations

Locus	k[a]	Size range (bp)	H_o[b]	H_e[c]	PIC
GUJ0010	2.000	150-154	0.000	0.139	0.346
GUJ0027	6.000	176-221	0.917	0.802	0.759
GUJ0033	6.000	189-215	0.676	0.764	0.719
GUJ0049	7.000	211-254	0.845	0.787	0.795
GUJ0052	4.000	104-115	0.328	0.366	0.363
GUJ0054	7.000	125-157	0.318	0.724	0.717
GUJ0055	6.000	174-203	0.967	0.784	0.775
GUJ0059	6.000	207-253	0.819	0.787	0.788
GUJ0063	2.000	227-251	0.803	0.491	0.371
GUJ0071	6.000	175-205	0.875	0.799	0.768
GUJ0085	3.000	242-249	0.056	0.354	0.404
GUJ0087	7.000	145-176	0.776	0.800	0.814
GUJ0097	8.000	145-175	0.544	0.672	0.747
Mean	5.385	-	0.609	0.636	0.644

k = The number of alleles per locus; H_o = The observed Heterozygosity; H_e = The unbiased estimate of expected Heterozygosity (Nei, 1987), PIC = Polymorphic Information Content

locus in studies of genetic distances must be >4 to reduce the standard error in the estimation of genetic distances. Only three loci had a number of alleles <4 (Table 2). Kayang *et al.* (2002) reported lower mean number of alleles per locus as 3.7 (range 1-6 alleles) in a set of 100 micro-satellite marker in Japanese quail randombred population. On another study by Kim *et al.* (2007), they reported mean number of alleles of three and two in QO and inbred Japanese quail lines, respectively reflecting a reduction in genetic diversity. On the other hand, a higher value of 18 alleles locus^{-1} was observed in a wild quail collected from France as reported by Chazara *et al.* (2010). In chicken, Kaiser *et al.* (2000) reported mean number of alleles per locus 2.8 and 2.9 for two broiler populations L and C, respectively, Emara *et al.* (2002) reported values of 3.5, 2.8 and 3.1 allele per locus for three commercial broiler lines. In a study of genetic variation within and between 52 populations from a wide range of chicken types mean number of alleles per locus was 3.5, 1.3 and 5.2 for the average, the least and the most polymorphic population, respectively (Hillel *et al.*, 2003). Higher values for average number of alleles per locus was reported, 9.2 in 19 native Chinese chicken breeds (Yu-Shi *et al.*, 2005) and 11.4 in 64 population of chickens from different continents (Granevitze *et al.*, 2007) and 7.5 for five sub-populations of Turkish native chicken breeds (Kaya and Yildiz, 2008); 4.9 for Egyptian native breeds (Fayoumi and Dandarawi) and commercial laying hens (brown Hy-line) by Roushdy *et al.* (2008) and 6.25 in 13 Spanish breeds of chickens (Davila *et al.*, 2009). Finally, for 6 different populations of chickens from Ghana, Benin and Japan, three vulturine guinea fowls were included as outgroup, Kayang *et al.* (2010) noted average number of alleles per locus as 11.0.

The mean observed Heterozygosity (H_o) was 0.609 and ranged across loci from 0.00-0.967 whereas the mean expected Heterozygosity (H_e) was 0.636 and ranged between 0.139 and 0.802. The slight difference between the observed compared to the expected heterozygosity probably due to one or more reasons. This might reflect slight inbreeding, selection against heterozygotes and/or Wahlund effect. Also, the nature of markers used in the current study might also contribute to the observed level of heterozygosity as a result of non-detection of homozygotes from heterozygotes due to presence of null alleles. The results were in a good agreement with those obtained by Kayang *et al.* (2002) whose found that the observed and expected heterozygosities ranged from 0.00-0.95 (mean 0.423) and 0.00-0.77 (mean 0.527), respectively. On the other hand, expected heterozygosity varied between 0.7 and 0.95 in California quail and between 0.48 and 0.9 in Gambel's quail in a study to examine gene flow between species, population structure within species and relatedness between individuals within sympatric social groups by Gee *et al.* (2003). Eight microsatellite loci were used to study genetic relatedness in four strain of Japanese quail in Iran, the expected heterozygosity varied between 0.708 and 0.849 (Amirinia *et al.*, 2007). Kim *et al.* (2007) reported observed heterozygosity from analysis of the microsatellite markers of the QO and inbred Japanese quail lines was 0.43 and 0.21, respectively. They found that mean expected Heterozygosity (H_e) for the randombred line was 0.48 compared with 0.24 for the inbred line. The genetic variability of various local chicken populations derived from Bolivia, India, Nigeria and Tanzania was evaluated with 22 micro-satellites, results showed that all populations showed high levels of heterozygosity with the lowest value of 45% for the population named Aseel from India and the highest value of 67% for Arusha from Tanzania (Wimmers *et al.*, 2000). For three commercial broiler pure lines, the observed heterozygosity ranged between 0.0 and 89% in the studied lines (Emara *et al.*, 2002). Thirty micro-satellite markers with medium to high polymorphisms were selected to detect the genetic diversity of 8 indigenous chicken breeds in Sichuan. The results showed that mean heterozygosity of 8 chicken breeds was on average 0.50, the highest was observed for Luning chicken (0.681) and the lowest was recorded for Jiuyuan Dark chicken (Tu *et al.*, 2005). In 19 Chinese native chicken breeds, Yu-Shi *et al.* (2005) found that the range of heterozygosity was 0.582~0.743 and the heterozygosity of Zang chicken was the highest (0.743) compared to the lowest (0.582) obtained for Beier chicken. A total of 720 individuals of 12 indigenous chicken populations, geographically localized in Southern China genetically studied using 30 micro-satellite markers and the average expected Heterozygosity (H_e) was 0.669 while the average observed Heterozygosity (H_o) was 0.764 (Ya-Bo *et al.*, 2006). The genetic diversity of the Turkish native chicken breeds Denizli and Gerze was evaluated with 10 micro-satellite markers (Kaya and Yildiz, 2008) and H_e was 0.665. The observed heterozygosity was lower than the expected heterozygosity for all studied loci, the mean values was 0.461 and 0.637, the observed and expected heterozygosity ranged from 0.003-0.735 and 0.181-0.863, respectively (Davila *et al.*, 2009).

The Polymorphic Information Content (PIC) among loci ranged between 0.346 for locus GUJ0010 and 0.814 for locus GUJ0087 with general mean of 0.644. The majority of the studied microsatellite loci used in this study was highly revealing (Table 2). According to classification of Botstein *et al.* (1980), the highly informative markers have PIC values >0.50, the reasonably informative markers have

PIC value between 0.25-0.50 and the slightly informative markers have PIC value <0.25. While four markers in the current study have reasonably informative PIC values 0.346, 0.363, 0.371 and 0.404 (GUJ0010, GUJ0052, GUJ0063 and GUJ0085, respectively). Kayang *et al.* (2002) reported lower values than those obtained in the experiment and ranged between 0.00-0.729 with mean value of 0.4769 while Amirinia *et al.* (2007) found that PIC in values ranged between 0.427 in Panda strain (lowest) and 0.815 in Golden strain (highest) for four strains of Japanese quail. On the other hand in chickens, values of PIC varied in many studies on, native 19 Chinese chicken breeds 0.523-0.702 (Yu-Shi *et al*., 2005) in 12 indigenous chicken populations in Southern China 0.560-0.641 (Ya-Bo *et al*., 2006) in Turkish native chicken breeds 0.426-0.599 (Kaya and Yildiz, 2008) in 13 Spanish chicken breeds, tester line and white Leghorn population (mean value = 0.591) ranging from 0.172-0.847 (Davila *et al*., 2009).

The studied populations did not show significant deviations from HW expectation for the combined loci however, individual loci showed significant deviation from HW. Across populations, only GUJ0055 and GUJ0063 loci showed significant deviations under the heterozygote excess assumption whereas GUJ0010, GUJ0033, GUJ0054 and GUJ0085 loci showed significant deviations under heterozygote deficit assumption. Moreover when assessing deviations from Hardy-Weinberg Equilibrium (HWE) for each individual locus in each population, individual loci showed significant deviations which agrees well with the results on observed heterozygosity. In line A, locus GUJ0010 was monomorphic, all other 12 loci deviated significantly from HWE except four loci (GUJ0027, GUJ0033, GUJ0052 and GUJ0055) they were in Hardy-Weinberg equilibrium. In line B we found that all studied loci deviated significantly from HWE except GUJ0049, GUJ0054, GUJ0055 and GUJ0085 they were in HWE. On the other hand, line C showed no departure from HWE in 11 out of 13 studied loci, GUJ0010 and GUJ0085 were monomorphic, in addition to GUJ0054 and GUJ0097 they were significantly deviated from HWE. These results reflected that line A and line B were generally not in HWE according to the history of the studied lines, they were under artificial selection for different economic traits and thus researchers concluded that the set of studied markers can be used to determine the genetic variation in different populations of Japanese quail effectively. The findings were in agreement with Amirinia *et al.* (2007) they reported that for four strains of Japanese quail studied using 8 microsatellite markers in Iran, all locus-strain combinations deviated from Hardy-Weinberg equilibrium except GUJ0041 in Pharach strain, GUJ0021, GUJ0034, GUJ0041 and GUJ0097 in Panda strain and GUJ0034, GUJ0049 and GUJ0070 in Golden strain ($p<0.001$) (Table 3). Davila *et al.* (2009) also noted that some Spanish chicken breeds showed significant deviations from the Hardy-Weinberg equilibrium, suggesting that these breeds have been selected for years for morphological traits such as plumage, shank and egg colors and comb and earlobe sizes although the presence of null alleles or genotyping error could also be the reason. Furthermore, no departure from Hardyb-Weinberg equilibrium was observed for 64 chicken populations from different continents (Granevitze *et al*., 2007).

Analysis of Molecular Variance (AMOVA): AMOVA results based on both the Infinite Alleles Model (IAM) and the Stepwise Mutation Model (SMM) indicated that approximately ~85% of the microsatellite variation resided within individuals (Table 4). Differentiation among populations was moderate but highly significant (F_{ST} = 0.10, R_{ST} = 0.17; $p<0.0001$) however within populations differentiation accounted for 3.61 and -0.73% of the total nuclear micro-satellite variation under IAM and SSM, respectively. Except within individual's variance under SMM and among populations' variances under IAM and SMM, all variance components were highly significant ($p<0.0001$). While higher F_{ST} from microsatellite marker analysis (0.50) was recorded using two lines of Japanese quail (randombred and inbred) by Kim *et al.* (2007). The results were similar to those obtained by microsatellite marker analyses of Indian birds which indicated an average F_{ST} of 0.126 within *G.g. murghi* and 0.154 w ithin *G.g. domesticus* while it was >0.2 between the two groups. The average F_{ST} was 0.126 within *G. g. murghi* while it was 0.154 within

Table 3: Nuclear SSR polymorphism and diversity in three quail populations. Standard errors across populations are shown in parentheses unless otherwise indicated

Data set	n	A	r (10)	H_o	H_e	F_{IS} (95% CI)
All populations	25	5.38 (0.11)	3.76 (0.06)	0.609 (0.02)	0.636 (0.01)	0.105 (-0.137-0.354)
Line A	10	4.08 (0.17)	3.32 (0.10)	0.605 (0.03)	0.628 (0.02)	0.000 (0.000-0.000)
Line B	11	4.54 (0.14)	3.62 (0.09)	0.678 (0.03)	0.703 (0.03)	0.000 (0.000-0.000)
Line C	4	3.00 (0.16)	2.85 (0.16)	0.545 (0.06)	0.578 (0.04)	0.000 (0.000-0.000)

n = number of genotyped quails; A = Average number of alleles per locus detected in each population; r (10) = Allelic richness, defined as the number of alleles that would have been detected if 8 alleles (i.e., 4 quails; rare fraction cut-off) had been sampled in each population; H_o = Observed Heterozygosity; H_e = Unbiased estimate of expected Heterozygosity (Nei, 1987); F_{IS} = Fixation index measuring the correlation of alleles within individuals relative to that within populations (Wright, 1965); 95% confidence intervals based on bootstrapping over loci are shown in parentheses

Table 4: Analysis of Molecular Variance (AMOVA) in three quail populations under infinite allele model and stepwise mutation model

	Variance component (%)	
Level of variation	IAM[a]	SMM[b]
Among populations	10.23[c]	17.61[c]
Among individuals within populations	3.61[d]	-0.73[d]
Within individuals	86.16[c]	83.12[d]

[a]AMOVA under the infinite alleles model; [b]AMOVA under the stepwise mutation model; [c]p<0.0001; [d]p>0.05

G.g. domesticus. The AMOVA estimation showed a significant (p = 0.01) within population variation (66% in 7G and 69% in 2G population) (Kanginakudru *et al*., 2008). In 13 Spanish chicken breeds, a tester line and a White Leghorn population using 24 micro-satellite markers, mean fixation index of each population (F_{ST}) was 0.244 (Davila *et al*., 2009). Furthermore, a total of 232 helmeted guinea fowls (*Numida meleagris*) sampled from three populations in Ghana, one population in Benin and two populations in Japan were genotyped across six autosomal microsatellite loci. The indigenous West African populations (Ghana and Benin) were more genetically diverse but less differentiated (F_{ST} = 0.162) compared to the non-indigenous populations in Japan (F_{ST} = 0.389) (Kayang *et al*., 2010). On the opposite trend, Wright's F-statistics revealed negligible genetic differentiation (F_{ST}) in local Ghanaian chicken populations (Osei-Amponsah *et al*., 2010). The observed moderate genetic differentiation in the studied populations is indicative of high genetic variation within compared to among populations which is not surprising since mating relatives was attempted during breeding these populations.

Cluster analysis: Cluster analysis based on Nei's genetic distance indicated that the studied populations formed two main groups (Fig. 1). The 1st group included line A and the 2nd group harboured lines B and C with 100% bootstrap value. The number of replications in which the branch appeared is counted and the proportion of this number in the total replications is shown in percent on the branch of the tree (POPTREE2 user guide). The phylogenetic trees was constructed in many studies for different populations (Zhou and Lamont, 1999) for White Leghorn (Wimmers *et al*., 1999) for 22 local chicken populations derived from Bolivia, India, Cameroon, Nigeria and Tanzania (Yu-Shi *et al*., 2005) and (Ya-Bo *et al*., 2006) for Chinese chicken breeds, (Kanginakudru *et al*., 2008) for Indian birds (Kaya and Yildiz, 2008) for Turkish native chickens and finally by (Davila *et al*., 2009) for 13 Spanish chicken breeds, a tester line and a White Leghorn population. The results obtained by these studies and also the study provided

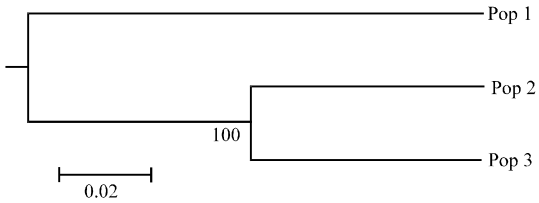

Fig. 1: Dendrogram of three lines of Japanese quail (Pop 1 = line A, Pop 2 = line B and Pop 3 = line C) produced by UPGMA clustering based on Nei's genetic distance using 13 micro-satellite loci

evidence of the applicability of microsatellite markers to determining the genetic relatedness among different poultry populations and evaluating their genetic variability.

CONCLUSION

Thirteen variable nuclear micro-satellite markers were sufficient to differentiate among three domesticated quail populations from Kazakhstan. Within-population variation was high compared to among-population variation. Two main groups were identified based on cluster analysis. The studied populations followed HW expectation although deviation from HWE was observed at individual loci. The possible cause for this deviation is probably due to one or more effect including presence of null alleles, Wahlund effect and selection. Differentiation among population was moderate but measurable. Results from the current study are useful in determine genetic variability in quail populations as well as designing prospective breeding programs for different traits.

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