Poultry Science Journal

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GUASNR

Evaluation of Genetic Diversity in Japanese and English White Quail Populations Using Microsatellite Markers

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Poultry Science Journal 2013, 1 (1): 53-62

Article history:
Received: August 29, 2012
Accepted: February 12, 2013
Available online: March 1, 2013

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Keywords:
Japanese quail
Heterozygosity
Genetic variation
Microsatellite marker

Abstract

The Japanese and English White quails are widespread strains and belongs to the Galliformes order, Phasianidae family, Coturnix genus and Japonica species. These birds are likely to be well-adapted to the hard conditions and resistance to diseases as it has attained economic importance as an agricultural species. In the current study, the genetic variation of Japanese and English White quail populations were studied. Frequency of polymorphic loci, polymorphic information content, heterozygosity, Shannon's information index, number of observed and effective alleles were assessed using 4 microsatellite markers with high polymorphic information content value (GUJ0034, GUJ0049, GUJ0080 and GUJ0097). The Blood samples were collected randomly from 50 Japanese quails and 50 English White quails rearing in the research center of Gorgan University of Agricultural Sciences and Natural Resources. The genomic DNA was extracted using DIAtom DNA Prep 100 kit, and its quality and quantity were determined using electrophoresis gel and spectrophotometery methods. The PCR reactions were successfully performed with four microsatellite markers. The results based on the chi-square and likelihood ratio tests showed a significant deviation from Hardy-Weinberg equilibrium. The means of genetic diversity parameters such as number of effective alleles, the number of observed alleles, the expected and observed heterozygosity, Shannon's information index and PIC in quail populations were 4.78±0.37, 7.50±0.57, 0.79±0.02, 0.60±0.16, 1.73±0.05 and 0.76±0.02 respectively. The results of the current study showed that the investigated quail populations have a relatively high genetic diversity with respect to the applied microsatellite markers and confirmed prior study's findings on the ability of microsatellite markers in investigating genetic diversity.

Please cite this article as: Mamizade N, Ahani Azari M, Zerehdaran S, Khan Ahmadi AR & Naghavian S. 2013. Evaluation of genetic diversity in Japanese and English White quail populations using microsatellite markers. Poult. Sci. J. 1 (1): 53-62.

Introduction

Japanese quail belongs to the Galliformes order, Phasianidae family, Coturnix genus and Japonica species and English White quail are known as colored strain of Japanese quail. The scientific designation for Japanese quail is *Coturnix japonica* which is different from the common quail "*Coturnix coturnix*" (Mizutani, 2003; Hassan *et al*., 2003). These birds originally domesticated around the 11th century as a pet song bird (Howes, 1964; Crawford, 1990) and has gained in value as a food animal since 1910 (Wakasugi, 1984).

The Japanese quail is valued for its uniquely flavored egg and meat. In the mean time, it is also used widely for laboratory researches because of its small body size (80–300 g), rapid generation turnover, resistance to diseases and high egg production (Padgett and Ivey, 1959). It has been considered as a suitable model for poultry research (Wilson *et al*., 1961). From the phylogenetic point of view the Japanese quail is closely related to the chicken (Stock and Bunch, 1982). Both species have similar karyotypes of 2n=78 chromosomes and a genome length of 1.2×10^9 bp, consisting of morphologically distinct macrochromosomes (1–8 and the ZW sex chromosomes) and cytologically indistinguishable microchromosomes (Shibusawa *et al*., 2001). Conservation genetics for preservation of species has received increasing attention in the recent years (Allendorf and Luikart, 2007; Frankham, 2003). In this field of genetics, knowledge of the relatedness between animals is very important in extended breeding programs that prevent incestuous matings in order to minimize inbreeding depression and the loss of genetic variation (Frankham *et al.*, 2002). Microsatellite markers that are tandem repeat loci with a core motif of 1 to 6 bp repeated several times, are used extensively in genetic diversity studies. They are highly polymorphic (Tautz, 1989) and considered to be evenly distributed in the genome. It is proven that they are very useful to determine genetic diversity and phylogenies of organisms, especially between populations of the same species (Buchanan *et al*., 1994; Mac Hugh *et al*., 1994). In this study, the initial molecular characterization of Japanese and English White quail populations are screened using microsatellite markers to explore the genetic diversity.

Materials and Methods

To establish a quail breeding and genetics research station in Gorgan university of Agricultural Sciences and Natural resources in 2009, Japanese and English White quail herds comprised of 400 and 250 birds were obtained from the main populations in Tehran and Kashan cities, respectively. The whole blood samples were randomly collected from 50 birds in F_1 progeny of each population. About 75 µL of blood per bird was collected in 0.5 mM EDTA (pH=8), and transferred to the laboratory. The genomic DNA was extracted by the DIAtom DNA prep 100 kit. Both

spectrophotometery and agarose gel electrophoresis for DNA quality and quantity determination were used. Four microsatellite markers (GUJ0034, GUJ0049, GUJ0080 and GUJ0097) with high polymorphic information content (PIC) value, recommended by Kayang *et al*. (2002) were used in the present study, as shown in Table 1. The PCR reaction mixture with a final volume of 12 µL contained 100 ng of template DNA, 6 µLof Master Mix and 1 µL of 10 pmol/µL for each forward and reverse primers. ddH_2O were added to the volume of 12 µL. The amplification conditions for PCR were: 2.5 min denaturing at 95°C followed by 30 cycles of denaturation at 95°C for 1 min, annealing for 30 sec at 57 to 61°C (as optimized for each marker), and extension at 72°C for 30 sec. This was followed by a final extension of 72°C for 5 min. The PCR products were then separated on 6% nondenaturing polyacrylamide gels with a molecular weight marker (pBR322 DNA/*Msp*I), on an electrophoresis system, at 160 V for 4.5 to 5.5 h. Then the bands were visualized after rapid silver staining method (Tahmoorespoor, 2009). The amplified patterns for the loci were visualized on a UV transilluminator and were photographed. The allelic and genotypic frequencies were directly estimated from the gel banding patterns, which determined the size of alleles in each bird. Hardy-Weinberg equilibrium based on likelihood ratio and Chi-square tests was evaluated for different locus-population combinations using Pop Gene software Version 1.31 (Yeh *et al.*, 1999). The observed and expected heterozygosity (Nei, 1978) were calculated using Pop Gene software. The average expected theoretical heterozygosity from Hardy-Weinberg assumptions was calculated using the following formula (Hedrick, 1999):

$$H_0 = 1 - \sum_{i=1}^{n} P_i^2$$

Where P_i is the frequency of the i^{th} allele. Polymorphism Information Content (PIC) was calculated using the formula of Botstein *et al.*(1980), by HET software (Ott, 2001):

$$PIC = 1 - \left(\sum_{i=1}^{n} P_i^2\right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_i^2 P_j^2$$

Where P_i and P_j are frequencies of corresponding alleles. Effective number of alleles (ne) was calculated using following formula (Hedrick, 1999):

$$n_e = 1 \,/ \sum_{i=1}^{n} P_i^2$$

Table 1. Characteristics of microsatellite markers used for the analysis of the investigated quail Populations

Locus Name	Chromo-some number	GenBank accession number		Primer sequences 5'→ 3'	Repeat array	T_A* (◦C)
GUJ0034	7	AB035844	F	CGTAACGGTCCAATATGGAT	(CA)9CG (CA)2	57°C
			R	TCCACGATGCAGAGGTATTT		
GUJ0049	5	AB035859	F	GAAGCAGTGACAGCAGAATG	(CA)11	57°C
			R	CGGTAGCATTTCTGACTCCA		
GUJ0080	9	AB063148	F	TTGAAGGGACATAGGGAAGC	(CA)9	61°C
			R	GAAAACGGTGAAGTCTGGTG		
GUJ0097	14	AB063165	F	GGATGCTCAGTGTGGAAAAG	(CA)14	58°C
			R	GAGCAAGAGGTGAGTGTTTC		

*T_A= Annealing temperature.

Results

PCR for all of the microsatellite loci was performed successfully. The results of banding patterns of two microsatellite loci (GUJ0034 and GUJ0049) after electrophoresis on polyacryleamide geles are shown in figures 1 and 2, respectively. All investigated loci were complete (100%) polymorphic in whole populations. Alleles size, effective (ne) and observed (na) number of alleles, PIC, Shannon's information index (I) of each locus and heterozygosity value**s** are presented in Tables 2-4. The number of alleles per locus varied between 7 and 8. The effective number of alleles per locus varied from 4.42 (GUJ0034) to 5.15 (GUJ0097). Shannon's information index estimations varied from 1.67 (GUJ0080) to 1.81 (GUJ0049). The maximum and minimum PIC values belonged to GUJ0097 (0.78) and GUJ0034 loci (0.74), respectively. The expected heterozygosity from 0.78 (GUJ0034) to 0.81 (GUJ0097), while the observed heterozygosity varied from 0.50 to 0.84 (Table 4). All loci deviated from the Hardy-Weinberg equilibrium ($P<0.05$). Comparison of genetic diversity parameters of the two populations are presented in Table 5.

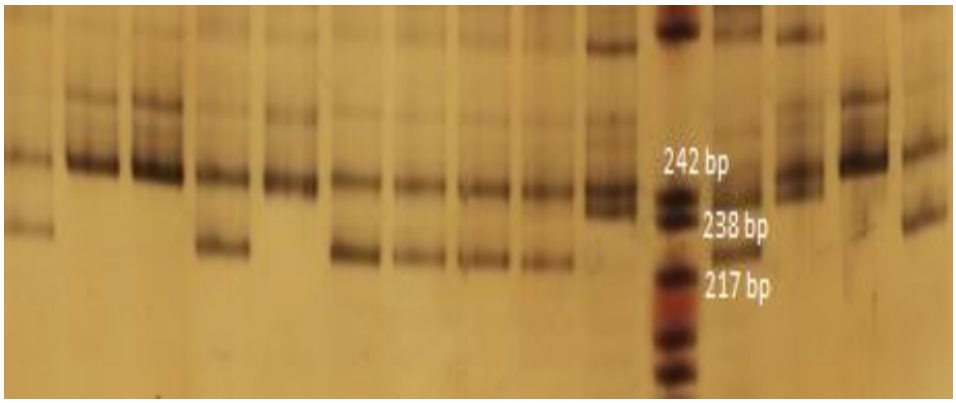

Figure 1. Example of banding patterns for the GUJ0034 locus.

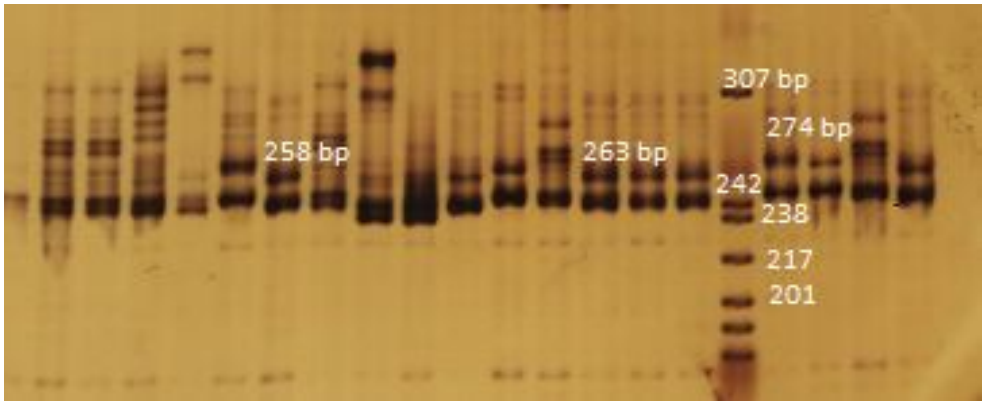

Figure 2. Example of banding patterns for the GUJ0049 locus.

Table 2. Alleles and their size (bp) for the investigated loci

Locus / Allele	GUJ0034	GUJ0049	GUJ0080	GUJ0097
A	219	237	151	131
B	221	239	155	137
C	225	241	157	141
D	231	245	160	145
E	237	249	164	149
F	241	258	167	153
G	245	263	171	161
H	249	274	-	-

Table 3. Estimated genetic diversity indicator parameters for the microsatellite loci

Locus	Sample Size	na[1]	ne[2]	I[3]	PIC[4]
GUJ0034	100	8	4.42	1.70	0.74
GUJ0049	100	8	5.06	1.81	0.77
GUJ0080	100	7	4.51	1.67	0.75
GUJ0097	100	7	5.15	1.73	0.78
Mean	100	7.50	4.78	1.73	0.76
St. Dev	-	0.57	0.37	0.05	0.02

[1]na= Observed number of alleles; [2]ne= Effective number of alleles; [3]I= Shannon's Information index; [4]PIC= Polymorphic information content.

Table 4. The heterozygosity values for the investigated loci

Locus	Obs-Hom[1]	Exp-Hom[2]	Obs-Het[3]	Exp-Het[4]	Nei Exp-Het[5]	Ave-Het[6]
GUJ0034	0.45	0.22	0.55	0.78	0.77	0.76
GUJ0049	0.49	0.19	0.51	0.80	0.80	0.74
GUJ0080	0.50	0.22	0.50	0.78	0.78	0.71
GUJ0097	0.16	0.19	0.84	0.81	0.81	0.80
Mean	0.40	0.20	0.60	0.79	0.79	0.76
St. Dev	0.16	0.02	0.16	0.02	0.01	0.04

[1]Observed homozygosity; [2]Expected homozygosity; [3]Observed heterozygosity; [4]Expected heterozygosity was computed using Levene (1949); [5]Nei's (1973) expected heterozygosity and [6]Average heterozygosity.

Table 5. Estimated genetic diversity indicator parameters for strain-locus combinations

Strain	Japanese quail				English White quail			
Locus	na[1]	Obs-Het[2]	I[3]	PIC[4]	na[1]	Obs-Het[2]	I[3]	PIC[4]
GUJ0034	8	0.46	1.63	0.72	6	0.64	1.63	0.75
GUJ0049	7	0.54	1.69	0.75	8	0.48	1.59	0.68
GUJ0080	5	0.52	1.36	0.64	7	0.48	1.51	0.69
GUJ0097	7	0.88	1.76	0.78	6	0.80	1.69	0.79
Mean	6.75	0.60	1.61	0.72	6.75	0.60	1.61	0.72
St. Dev	1.26	0.19	0.18	0.06	0.96	0.15	0.07	0.42

[1]na=Observed number of alleles; [2]Obs-Het=Observed heterozygosity; [3]I=Shannon's Information index; [4]PIC=Polymorphic information content.

Discussion

The maximum number of alleles (8) were observed for GUJ0034 and GUJ0049 loci and the minimum number of alleles (7) were observed for GUJ0080 and GUJ0097 loci, respectively. New alleles consisting 258, 263, 274 and 171 bp were found on GUJ0049 and GUJ0080 loci, which were not reported previously in studies by Kayang *et al*. (2002) and Amirinia *et al.* (2007). Allele of 171 bp was observed only in English White quails. The maximum (0.84) and minimum (0.50) observed heterozygosity were estimated at GUJ0097 and GUJ0080 loci, respectively, which is close to the results of Kayang *et al*. (2002) and more than values reported by Amirinia *et al*. (2007). The highest PIC (0.78) was observed at GUJ0097 loci. Based on the classification of Botstein *et al*. (1980), PIC>0.5 is highly informative, 0.25<PIC<0.5 is moderate informative and PIC<0.25 is slightly informative. In the current study, all of the investigated loci were highly informative (PIC>0.5). The average PIC among 4 microsatellite loci in population was 0.76 that is in close agreement with the results of Kayang *et al*. (2002) and Amirinia *et al.* (2007).

Comparing heterozygosity with PIC showed that all PIC values were less than their related heterozygosity. These two parameters are closely related, because PIC is calculated as the expected heterozygosity minus a factor derived from the allele frequencies. Thus, PIC must always be less than expected heterozygosity (Botstein *et al*., 1980). The effective number of alleles is a reciprocal of gene homozygosity (Hartel and Clerk, 1989). The highest (5.15) and the lowest (4.42) average effective number of alleles were at GUJ0097 and GUJ0034 loci respectively, which is close to the results of Kayang *et al*. (2002) and Amirinia *et al*. (2007). All the investigated loci showed deviations from Hardy-Weinberg equilibrium in these populations. There are many causes for disequilibrium such as selection, migration, mutation and inbreeding. Such deviations from Hardy-Weinberg equilibrium may result from population substructure and the presence of null alleles.

In comparison of two strains, the lowest observed number of alleles (5) was at GUJ0080 locus in Japanese quail. The highest (0.88) and the lowest (0.46) observed heterozygosity were at GUJ0097 and GUJ0034 loci in Japanese quail respectively. Observed heterozygosity in English White quail ranged from 0.48 to 0.80, but average observed heterozygosity (0.60) was similar for both strains (Table 5). The average observed heterozygosity for English White quail was 0.95 in the study of Mohammadifar *et al*. (2010). The lowest Shannon's Information index (1.36) and the lowest PIC (0.64) were at GUJ0080 locus in Japanese quail that was expected due to the low (5) number of observed alleles. The mean of PIC (0.72) was similar in Japanese and English White quails, which is higher (0.52) than the result of Mohammadifar *et al.* (2010). Therefore, it can be concluded that genetic variation is almost identical in two strains. This fact is confirmed by similar average observed heterozygosity (0.60) in two strains.

Finally, results showed that the Japanese and English White quail strains have relatively high and similar genetic diversity with respect to the studied microsatellite markers. Furthermore in agreement with the results of the most prior studies, it is confirmed that microsatellite markers are powerful tools in genetic diversity studies.

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