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Evaluation of Eight Microsatellite Loci Polymorphism in Four Japanese Quail (*Coturnix japonica*) Strain in Iran

[1]C. Amirinia, [1]H. Emrani, [2]M.A. Radjaee Arbabe, [2]R.Vaez Torshizi and [3]A. Nejati Javaremi
[1]Department of Biotechnology, Animal Science Research Institute of Iran, Karaj, Iran
[2]Faculty of Agriculture,Tarbiat Modares University, Tehran, Iran
[3]Department of Animal Science, Faculty of Agriculture, Tehran University, Karaj, Iran

Abstract: Allele frequencies of eight microsatellite loci as GUJ0001, GUJ0021, GUJ0034, GUJ0041, GUJ0049, GUJ0059, GUJ0070 and GUJ0097 was estimated for four strain of Japanese quail in Iran. Whole blood samples were collected from 200 individuals belonging to four strain (Pharach, Panda, Tuxedo and Golden).Total Genomic DNA was extracted by the GUSN-Silica Gel kit. The extracted DNA was amplified through Polymerase Chain Reaction (PCR). Of the eight microsatellite loci used in this study, two loci (GUJ0001 and GUJ0041) were monomorphic in Panda and Texedo, respectively. The highest and the lowest PIC values belonged to GUJ0059 in Golden (0.815) and GUJ0041 in Panda strain (0.427), respectively. 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 ($p<0.001$). Results suggest the effectiveness of this set of loci for testing genetic relatedness.

Key words: Japanese quail, polymorphism, microsatellite, Iran

INTRODUCTION

Programs of animal breeding and selection needs to rely on a precise assessment of parentage, for example, in the elimination of undesirable recessive alleles in early selection for future breeding, or to ensure accurate determination of relatedness and efficient control of pedigree registration. Microsatellites, segments of the nuclear genome composed of tandem repeat of short-sequence motifs, have become excellent candidate markers for this kind of studies (Queller *et al*., 1993), since they are numerous, highly variable and easy to score. A large number of highly polymorphic microsatellites have been characterized and mapped in poultry, including chicken, quail and other phasianidae family (Inoue-Murayama *et al*., 2001; Pang *et al*., 1999; Kayang *et al*., 2000, 2002, 2003, 2004; Kikuchi, 2005), facilitating the use of these markers in parentage testing. Acceptance of this kind of test must provide a high degree of certainty in the assignment of parentage, which can be evaluated with the Exclusion Probability (Weir and Bruce, 1996). It requires a previous knowledge of allele frequencies in the population of interest and certainty about the assumptions of the model, like independence of the markers and Hardy-Weinberg equilibrium.

Japanese quail is valued for its eggs and meat. It is also a valuable laboratory species because of its small body size, rapid generation interval and high prolificacy (Mills *et al*., 1997).

There are four strains of Japanese quail in Iran: Panda, Pharach, Golden and Tuxedo. Strains classification is according to plumage color. Panda have white feather with irregular black and rust colored markings. Pharach or wild-type have a mix of several colors, but black and numerous shades of brown predominant on the dorsal side. Phenotype of Tuxedo is white colored in face and breast feathers and brown colored occurs on the back and hackle feathers.

In this study we analyzed the polymorphism of a set of eight polymorphic microsatellites marker in Japanese quail and have evaluated their usefulness for relatedness testing in Japanese quail strains

MATERIALS AND METHODS

Sampling and DNA extraction: Japanese quail strains, breeds at Bonab Research Center; east of Iran, were used as the experimental animals. Whole blood samples were collected from 200 individuals belonging to four strains: 70 individuals from Pharach strain, 40 individuals from

Corresponding Author: C. Amirinia, Department of Biotechnology, Animal Science Research Institute of Iran (ASRI), First Dehghan Villa, Shahid Beheshti St., Karaj, P.O. Box 1483-31585, Iran
Tel: +98-(0)261-4464226 Fax: +98-(0)261-4464230

Table 1: Primer information of eight microsatellite markers

Locus name	Gen bank accession No.	Repeat array	Forward and revers primers (5' → 3')		TA (°C)
GUJ0001	AB035652	(CA)7TG(CA)13	GAAGCGAAAGCCGAGCCA	F	56
			CAGCACTTCGGAGCACAGGA	R	
GUJ0021	AB035831	(CA)11	GAGCATTTCTAGTCTGTCTC	F	62
			GATCAATACACAGGCTAAGG	R	
GUJ0034	AB035844	(CA)9CG(CA)2	CGTAACGGTCCAATATGGAT	F	55
			TCCACGATGCAGAGGTATTT	R	
GUJ0041	AB035851	(CA)11	AAAATGTCTGCAAAATGGGC	F	55
			TGAAACATACCTGAGTGCTA	R	
GUJ0049	AB035859	(CA)11	GAAGCAGTGACAGCAGAATG	F	55
			CGGTAGCATTTCTGACTCCA	R	
GUJ0059	AB063127	(CA)10	GACAAAGTTACAGCTAGGAG	F	50
			TAGGTGCGAAAATCTCTGAC	R	
GUJ0070	AB063138	(CA)9	AAACCCCAAAGAAGCTGTCC	F	54
			ACGTTGTCACCATCAGCTTG	R	
GUJ0097	AB063165	(CA)14	GGATGCTCAGTGTGGAAAAG	F	55
			GAGCAAGAGGTGAGTGTTTC	R	

TA = Annealing temperature

Panda strain, 50 individuals from Tuxedo strain and 40 individuals from Golden strain, The analysis were carried out separately for four sub samples as well as on the combined dataset.

Genomic DNA was extracted by the GUSN-Silica Gel kit (Boom *et al*., 1989).

Microsatellite primers: The Eight microsatellite with high PIC value recommended by Kayang *et al*. (2002) were used. Primers were synthesized by TIBMOLBIOL Company (Germany). Information of 8 microsatellite markers are given in Table 1.

PCR amplification: All PCR reactions contained the following components: 200 μM dNTPs, 3.5 mM $MgCl_2$, 0.25 μM each of primer, 1U Taq polymerase, 100-200 ng DNA. Amplification was carried out in a total volume of 15 μL. Reactions were run on a thermal cycler (Biometra) using an initial 2.5 min denaturation at 95°C, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 50-62°C for 30 sec, extention at 72°C for 30 sec and a final extention step at 72°C for 5 min. PCR products were electrophoresed on 8% nondenaturing polyacrylamide gel and bands visualized by rapid silver staining (Sanguinetti *et al*., 1994). The allele and genotypic frequencies were directly estimated from the gel.

Statistical analysis: Hardy-Weinberg Equilibrium (HWE) based on likelihood ratio (Weir and Bruce, 1996) determined for different locus-population combinations by POPGENE software (Yeh *et al*., 1999). observed heterozygosity and expected heterozygosity (Nei, 1978) were calculated by POPGENE software. Average expected theoretical heterozygosity from Hardy-Weinberg assumptions was calculated using formula of (Hedrick, 1999).

$$H_e = 1 - \sum_{i=1}^{n} p_i^2$$

Where:

P_i is the frequency of the ith allele. Polymorphism Information Content (PIC) was calculated using the formula of (Botestein *et al*., 1980), by HET software (Ott, 1989).

$$PIC = 1 - (\sum_{i=1}^{n} p_i^2) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

Where P_i, P_j are frequencies of corresponding alleles. Effective number of alleles (ne) was calculated using the formula of (Hedrick, 1999) by POPGENE software.

$$n_e = 1 \Big/ \sum_{i=1}^{n} p_i^2$$

RESULTS AND DISCUSSION

Allele length, allele frequencies, Hobs, HExp, PIC, effective number of alleles and number of individuals per locus are give in Table 2. Most of the loci were polymorphic in four Japanese quail strains but GUJ0001 and GUJ0041 were monomorphic in the panda and Tuxedo, respectively. Number of alleles per locus varied between 5 and 9. Maximum number of alleles(9 alleles) were observed at GUJ0059 locus and minimum number of alleles (5 alleles) were observed at GUJ0001. The GUJ0001 locus in Panda strain was monomorphic. A few alleles were found in four strains that haven't been previously reported in the same loci for other Japanese quail that was studied by Kayang *et al*. (2002). Observed heterozygosity of the four strains varied from 0.113 to 0.654. The effective

Table 2: Different statistical parameters for strain and the whole strains

Locus		Allele frequencies				
		Whole pop	Pharach	Panda	Tuxedo	Golden
Sample size (n)	Allele size (Bp)	200	70	40	50	40
GUJ0001	231	0.148	0.148	0.000	0.364	0.000
	235	0.435	0.463	1.000	0.272	0.385
	237	0.287	0.24	0.000	0.364	0.385
	239	0.093	0.111	0.000	0.000	0.154
	247	0.037	0.037	0.000	0.000	0.077
	Ne	3.34	3.37	1.000	2.95	3.07
	PIC	0.647	0.641	0.000	0.586	0.613
	H_{obs}	0.113	0.23	0.000	0.000	0.000
	H_{exp}	0.704	0.717	0.000	0.676	0.687
GUJ0021	149	0.204	0.158	0.000	0.357	0.166
	153	0.231	0.158	0.000	0.143	0.417
	155	0.306	0.342	0.333	0.321	0.25
	157	0.13	0.158	0.000	0.143	0.111
	161	0.074	0.158	0.000	0.000	0.055
	165	0.018	0.000	0.166	0.036	0.000
	173	0.037	0.026	0.500	0.000	0.000
	Ne	4.63	4.48	2.570	3.66	3.58
	PIC	0.756	0.751	0.535	0.68	0.677
	H_{obs}	0.352	0.526	0.666	0.428	0.055
	H_{exp}	0.787	0.798	0.666	0.727	0.73
GUJ0034	217	0.073	0.000	0.125	0.000	0.178
	221	0.073	0.066	0.250	0.062	0.035
	225	0.037	0.000	0.000	0.062	0.071
	231	0.012	0.000	0.000	0.000	0.036
	237	0.061	0.033	0.000	0.000	0.143
	241	0.22	0.166	0.250	0.125	0.321
	245	0.268	0.266	0.125	0.437	0.214
	249	0.256	0.466	0.250	0.312	0.000
	Ne	4.95	3.100	4.570	3.2	4.78
	PIC	0.769	0.627	0.745	0.636	0.761
	H_{obs}	0.39	0.200	0.750	0.25	0.571
	H_{exp}	0.703	0.690	0.883	0.709	0.805
GUJ0041	106	0.185	0.125	0.000	0.000	0.406
	110	0.444	0.571	0.687	1.000	0.031
	114	0.213	0.160	0.000	0.000	0.437
	118	0.055	0.053	0.125	0.000	0.031
	122	0.055	0.071	0.000	0.000	0.062
	126	0.046	0.018	0.187	0.000	0.031
	Ne	3.5	2.65	1.910	1.000	2.75
	PIC	0.675	0.59	0.427	0.000	0.568
	H_{obs}	0.277	0.321	0.250	0.000	0.25
	H_{exp}	0.717	0.629	0.491	0.000	0.646
GUJ0049	225	0.036	0.000	0.111	0.000	0.041
	229	0.122	0.150	0.111	0.150	0.083
	233	0.085	0.200	0.055	0.000	0.083
	237	0.134	0.400	0.000	0.050	0.083
	239	0.231	0.050	0.389	0.100	0.375
	241	0.207	0.050	0.166	0.300	0.291
	249	0.183	0.150	0.166	0.400	0.042
	Ne	5.82	4.000	4.260	3.500	4.000
	PIC	0.805	0.715	0.735	0.668	0.715
	H_{obs}	0.561	0.600	0.666	0.400	0.583
	H_{exp}	0.833	0.769	0.787	0.733	0.766
GUJ0059	199	0.046	0.000	0.000	0.000	0.143
	203	0.07	0.000	0.000	0.083	0.143
	207	0.116	0.000	0.200	0.000	0.286
	211	0.023	0.000	0.000	0.042	0.036
	215	0.116	0.041	0.000	0.208	0.143
	217	0.046	0.083	0.000	0.000	0.071
	219	0.186	0.208	0.400	0.208	0.071
	227	0.221	0.458	0.200	0.250	0.000
	231	0.051	0.208	0.200	0.208	0.107
	Ne	6.75	3.27	3.570	4.960	6.03
	PIC	0.832	0.65	0.762	0.767	0.815
	H_{obs}	0.186	0.333	0.000	0.25	0.071
	H_{exp}	0.854	0.709	0.757	0.815	0.849

Table 2: Continued

Locus		Allele frequencies				
		Whole pop	Pharach	Panda	Tuxedo	Golden
Sample size (n)	Allele size (Bp)	200	70	40	50	40
GUJ0070	188	0.034	0.066	0.000	0.00	0.000
	196	0.164	0.183	0.083	0.05	0.250
	198	0.129	0.15	0.083	0.15	0.083
	200	0.25	0.233	0.25	0.2	0.333
	202	0.172	0.183	0.25	0.2	0.083
	204	0.112	0.1	0.000	0.3	0.042
	206	0.086	0.000	0.333	0.1	0.167
	216	0.051	0.083	0.000	0.00	0.042
	Ne	6.26	6.04	4	4.88	4.570
	PIC	0.82	0.813	0.707	0.765	0.750
	H_{obs}	0.397	0.3	0.333	0.6	0.500
	H_{exp}	0.844	0.841	0.782	0.815	0.797
GUJ0097	137	0.096	0.214	0.062	0.115	0.000
	141	0.096	0.143	0.062	0.000	0.147
	145	0.173	0.107	0.187	0.269	0.147
	149	0.25	0.285	0.062	0.038	0.470
	153	0.221	0.214	0.437	0.269	0.088
	159	0.086	0.000	0.187	0.115	0.088
	161	0.076	0.036	0.000	0.192	0.058
	Ne	5.77	4.84	3.66	4.76	3.520
	PIC	0.804	0.762	0.69	0.758	0.685
	H_{obs}	0.654	0.5	0.625	0.846	0.647
	H_{exp}	0.83	0.807	0.75	0.805	0.727

Ne: effective number of alleles, PIC: polymorphism information content, H_{Obs}: observed heterozygosity, H_{Exp}: expected heterozygosity

number of alleles per locus varied from 3.35 (GUJ0001) to 6.76 (GUJ0059) and expected heterozygosity from 0.708 (GUJ0001) to 0.849 (GUJ0059). 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 ($p<0.001$).

The highest and the lowest PIC values belonged to GUJ0059 in Golden (0.815) and GUJ0041 in Panda strain (0.427), respectively. Based on the classification of Botstein *et al.* (1980) PIC>0.5 is highly informative, 0.25<PIC<0.5 is middle informative and PIC<0.25 is slightly informative. In this research, all of the loci were highly informative (PIC>0.5), except GUJOO41 (PIC = 0.427) in panda strain. The average PIC of 8 microsatellite loci in 4 strains is 0.764 and is close to the result of analysis of genetic polymorphisms of microsatellite loci in Japanese quail by Kayang *et al.* (2002).

Comparing hetrozygosity with PIC; all PIC values were less than their related hetrozygosity. It seems that these two parameters are closely related. The Polymorphic Information Content (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 PIC is a good standard for evaluating genetic markers. The PIC values in this breed were also lower than their related heterozygosities.

Effective number of alleles is a reciprocal of gene homozygosity (Hartel and Clerk, 1989). The most and the least average effective number of alleles were in GUJ0059 locus (6.76) and in GUJ0001 locus (3.35), respectively, which is close to the result of Kayang *et al.* (2002).

The Hardy-Weinberg equilibrium tests showed several deviant locus in four strain. Of the 8 microsatellites typed in this study, two loci deviated from equilibrium in all strain and six loci were found to be in disequilibrium in at least two strains ($p<0.001$). There are many causes for disequilibrium such as selection, migration, mutation and inbreeding. It is possible that such deviations from Hardy-Weinberg equilibrium may result from population substructure and the presence of null alleles.

CONCLUSIONS

Average alleles number, average heterozygosity, average polymorphism information content and average effective number of alleles of every strain in this study are all very high and there is the better compatibility between them and shows that variation of 8 microsatellite loci in every Japanese quail strain is relatively high and have rich genetic polymorphisms.

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