EVALUATION OF GENETIC DIVERSITY WITHIN AND BETWEEN THE QUAIL BREEDS IN PAKISTAN

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ABSTRACT: *Quail has been declared as model poultry specie and large number of breed/varieties of quail exists in different parts of the world. However, the Japanese quail is the most famous worldwide from commercial production point of view. The present study was conducted to evaluate the genetic diversity between Japanese quail (imported in 1970s and maintained by employing inbreeding) and native wild quail. In this study 10 birds of each of Wild and Japanese quail were used and tested for 07 microsatellite markers. The analysis of data revealed that the observed number of alleles varied from 1 to 3 for the wild quail and 1 to 2 for the Japanese quail. In case of Wild quail ADL0024 and UBC0001 loci showed PIC values up to 0.38 while in case of Japanese quail, ADL0024, ADL0036 and UBC0005 showed PIC value up to 0.38. Expected average heterozygosity of wild quail was 0.7301 whereas, for the Japanese quail it was 0.225. The average Nei genetic distance of wild quail was 0.25 while in case of Japanese quail, it was 0.21. It is observed that different genetic sites in these breeds are showing rich genetic diversity and high selection potentials and, therefore, may also act as genetic markers to find out the location of these two breeds. However, it is suggested that in future this study should be conducted by testing more number of birds and for greater number of microsatellite markers.*

KEYWORDS Quail, genetic diversity, microsatellite markers

1. NTRODUCTION

Domestic quail is derived or originated from the wild Japanese quail (*Coturnix coturnix joponica*) and are also being used for eggs, meat, and for experimental purposes as well [1]. However, it has been reported that Japanese quail is of considerable economic importance in a number of countries [2]; mainly due to less need for housing space and feed allowance compared with other poultry species including chicken and turkey [3]. Moreover, due to their low mortality percentage, owing to better disease resistance, and high value of meat and eggs, the quail farming have become a profitable business [3].

Domestication of the quail was started mainly as a pet song bird in around the 11th century [4, 5]; however, even at that time it is also valued for eggs and meat. Currently, several varieties of quail are reported to be present in different parts of the world including European quail, African quail and some varieties of Asian quail. However, the East Asia is the origin of Japanese quail which is mostly found in China, Mongolia, Siberia, Japan, Korea, and Kuye Island, ranging from 100-150 degree on east longitude and 17-55 degree on north latitude [1]. Conservation of the genetic variability is important to determine genetic individuality in a given population which may be detected from the genetic variability [6]. The genetic diversity within and between breeds and the genetic admixtures among breeds can also be estimated even if they are closely related [7]. According to FAO recommendation the latest method is the use of polymorphic microsatellite markers to measure the genetic diversity [8,9] and [10] reported that genetic markers in Japanese quail are twice as polymorphic as compared to chicken [11]. In Pakistan, some successful efforts have been made to improve the body weight of Japanese quail stocks, imported about 4 decades earlier. In addition to these imported stocks of Japanese quail, a huge population of wild or native quail also exists in Pakistan which is also being reared, up to some extent, by fanciers mostly as pets or for game purposes. Therefore, the present study was designed to assess the genetic diversity between the quail breeds present in Pakistan and to find out any potential relationship between local/wild quail and commercial lines of Japanese quail.

2. MATERIALS AND METHODS

Selection of birds and blood sampling

The birds of Japanese quail were selected from Avian Research and Training (ART) center, UVAS, Lahore whereas, the birds of wild quail collected from rural areas of Punjab. Blood samples were collected from a total of 20 quail birds, 10 each of Wild and Japanese quail. From each bird 1 ml blood sample was taken by slaughtering the bird. Blood samples were collected in a 15ml falcon tube having 60ul of anti-coagulant (EDTA). Finally, blood samples were stored at 4°C for subsequent DNA extraction.

DNA Extraction and Selection of Microsatellite Markers

The blood samples were subjected to DNA extraction by using following protocol the developed by Sambrook and Russel (2001). In the present study, a set of 7 microsatellite markers (MS) were used, selected from the FAO recommended (MoDAD) list, and from previously reported markers for quails developed by University of British Columbia (UBC), Canada (Table 1). Some of these markers were already used in different studies to determine the genetic diversity of chicken [12]. Primer set for these markers was designed by retrieving the DNA sequences from ArkDB database (http://www.thearkdb.org/arkdb/) of the Roslin Institute and from the NCBI (http://www.ncbi.nlm.nih.gov/) [13].

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Table 1 Information of microsatellite markers used in the present study

Locus	Chromosome no.	Gene Bank Accession no.	Repeat	References
ADL 0023	5	L23905	(ca)5(cg)4(ca)9	[12, 13]
ADL 0024	3	L23906	(a)10(n)66(ca)6	[14, 15]
ADL 0366	1	G29072	(ac)14	[13, 16]
UBC 0001	1	AF121113	(cag)3N9(ca)3ta(ca)5	[13, 17, 18]
UBC 0002	6	AF`121114	(at)3t(ct)11a(ac)7	[13, 17]
UBC0004	4	AF`121115	(tg)10(ta)5	[13, 18]
UBC0005	3	AF`121117	(ac)9	[13, 18]

ADL USDA ARS Avian Disease and Oncology Laboratory
UBC University of British Columbia

Table 2 Primer sequence and annealing temperature for different microsatellite markers

Primers	Sequence	Annealing temperature
ADL0023	5'-TCGCACTTCTATCCTGGGCTTCTGA-3' 3'-GCAACACATACACAGCCAGGGTCAG-5'	56
ADL0024	5'-GTTTTTGAAGCAAAAACCCAGCAAG-3' 3'-ACCTCACTCTGTAATGGAACAGTTC-5'	46
ADL0366	5'-TGTCAAGCCCATCGTATCAC-3' 3'-CCACCTCCTTCTCCTGTTCA-5'	53
UBC0001	5'-TCTCTAAAATCCAGCCCTAA-3' 3'-AGCTCCTTGTACCCTATTGC-5'	48
UBC0002	5'-CAGCCAATAGGGATAAAAGC-3' 3'-CTGTAGATGCCAAGGAGTGC-5'	50
UBC0004	5'-TCCTTGGGCAGTAGTTTCAA-3' 3'-CTCCCATGTTGCTTCTTTAG-5'	38
UBC0005	5'-GGAACATGTAGACAAAAGC-3' 3'-AGTAGTCCATTTCCACAGCCA-5'	57

Table 3 Summary of Genetic variation (observed no. of alleles, Effective no. of alleles, and Shannon's information index) of all markers in population of wild quail

Marker Name	No. of genotype	Na*	Ne*	I*	Matching probability	Power of exclusion	Power of Discrimination	PIC
ADL0023	10	3.00	1.52	0.639	0.54	0.03	0.46	0.31
ADL0024	10	2.00	2.00	0.693	1.00	1.00	-	0.38
ADL0366	10	1.00	1.00	0.00	1.00	-	-	0.00
UBC0001	10	2.00	2.00	0.693	1.00	1.00	-	0.38
UBC0002	10	2.00	1.84	0.647	0.58	0.42	0.41	0.35
UBC0004	10	1.00	1.00	0.00	1.00	-	-	0.00
UBC0005	10	1.00	1.00	0.00	1.00	-	-	0.00
Mean		1.714	1.479	0.382				
St. Dev		0.075	0.047	0.035				

*na = Observed number of alleles
* ne = Effective number of alleles
* I = Shannon's Information index

Table 4 Summary of Heterozygosity Statistics for All markers in for wild quail

Marker Name	No. of genotype	Obs. Homo	Obs Het	Exp hom*	Exp Het*	Nei**
ADL0023	10	0.2	0.8	0.3579	0.6421	0.34
ADL0024	10	0	1	0.4737	0.5263	0.5
ADL0366	10	1	0	1	0	0
UBC0001	10	0	1	0.4737	0.5263	0.5
UBC0002	10	0	1	0.4737	0.5263	0.455
UBC0004	10	1	0	1	0	0
UBC0005	10	1	0	1	0	0
Mean		0.457	0.543	0.2699	0.7301	0.2564
St. Dev		0.047	0.034	0.025	0.02	0.03

* Expected homozygosty and heterozygosity were computed using Levene (1949)

** Nei's (1973) expected heterozygosity

Table 5 Summary of Genetic variation (observed no. of alleles, Effective no. of alleles, and Shannon's information index) of all markers in population of Japanese quail

Marker Name	No. of genotype	Na*	Ne*	I*	Matching probability	Power of exclusion	Power of Discrimination	PIC
ADL0023	10	1.000	1.000	0.000	1.00	-	-	0.00
ADL0024	10	2.000	2.000	0.693	1.00	1.00	-	0.38
ADL0366	10	2.000	2.000	0.693	1.00	1.00	-	0.38
UBC0001	10	1.000	1.000	0.000	1.00	-	-	0.00
UBC0002	10	1.000	1.000	0.000	1.00	-	-	0.00
UBC0004	10	1.000	1.000	0.000	1.00	-	-	0.00
UBC0005	10	2.000	2.000	0.693	1.00	1.00	-	0.38
Mean		1.429	1.429	0.297				
St. Dev		0.053	0.053	0.037				

*na = Observed number of alleles

* ne = Effective number of alleles

* I = Shannon's Information index

Table 6 Summary of Heterozygosity Statistics for All markers in population of Japanese quail

Marker Name	No. of genotype	Obs. Homo	Obs Het	Exp hom*	Exp Het*	Nei**
ADL0023	10	1	0	1	0	0
ADL0024	10	0	1	0.4737	0.5263	0.5
ADL0366	10	0	1	0.4737	0.5263	0.5
UBC0001	10	1	0	1	0	0
UBC0002	10	1	0	1	0	0
UBC0004	10	1	0	1	0	0
UBC0005	10	0	1	0.4737	0.5263	0.5
Mean	10	0.5714	0.4286	0.7744	0.2256	0.2143
St. Dev		0.053	0.053	0.02813	0.0200	0.026

* Expected homozygosty and heterozygosity were computed using Levene (1949)

** Nei's (1973) expected heterozygosity

Table 8 Nei's Unbiased Measures of Genetic Identity and Genetic distance

Population	Wild quail	Japanese quail
Wild quail	-	0.55
Japanese quail	0.58	-

PCR Amplification

After the extraction of DNA, the microsatellite loci were amplified though polymerase chain reaction (PCR) using primer set (Table 3) for selected microsatellite markers. For each PCR reaction a 25µL reaction mixture was prepared which included 20 pmol of each primer, 100µM deoxynucleoside triphosphate, 10X reaction buffer (100 mM Tris-HCl, 15 mM $MgCl_2$, 50 mM KCl, 0.01%)

(SIGMA),Taq DNA Polymerase (SIGMA) and genomic DNA as a template. An amount of 25 µl reaction mixture was pipetted out in the labelled PCR tubes and loaded in the PCR machine. For PCR amplification of different samples following procedure was followed. Initial denaturation at 94°C for 5 minutes, and then for each of 35 cycles following procedure was used, denaturation at 95°C for 30 seconds, annealing at primer specific temperature (Table 2) for 45 second, 72°C for 90 second for extension process that followed by a final extension at 72°C for 10 minutes

After PCR amplification a 10ul of the subsequent PCR product for each sample was loaded onto the 3% agarose gel after mixing it with dye and allowed to run on the gel on 100 volts for 20-30 min. The stained gels were scanned for observing the bands of different loci by Gel-Doc machine.

Statistical Analysis

Each PCR product was genotyped and Gel documentation data was analyzed to work out the standard parameters of genetic diversity among quail breeds, under study. Allele sizes were estimated by using standard ladder DNA marker. However, polymorphic information contents (PIC), matching probability, Power of exclusion and Power of discrimination values were calculated by using Power Stat 2.1 software. POPGENE 1.32 computer package was used to find out the values of observed and expected heterozygosity, observed and effective number of alleles, F- statistics, Shannon's information index and Nei's genetic distance [12].

3. RESULTS AND DISCUSSION

In the present study 7 microsatellite markers (ADL0023, ADL0024, ADL0366, UBC0001, UBC0002, UBC0004, and UBC0005) were used to find out the genetic diversity within two quail breeds i.e wild quail and Japanese quail. Out of the total seven loci, six were found polymorphic but marker UBC0004 was the only locus that was found monomorphic for both populations. Summary of the genetic variation in terms of observed no. of alleles, Effective no. of alleles, Shannon's information index, Matching probability, Power of exclusion, Power of discrimination and Polymorphic information contents (PIC) of all markers for Wild and Japanese quail are given in Table 3 and 5 respectively. Similarly, summary statistics of Heterozygosity including Observed homozygosity, Observed Heterozygosity, expected homozygosity, expected Heterozygosity, Nei genetic distances for wild and Japanese quail are given in Table 4 and 6 respectively. The analysis of data revealed that the observed number of alleles varied from 1 to 3 in wild quail, with maximum number of allele for marker ADL0023 and minimum for marker ADL0366, UBC0004, and UBC0005 (Table 4). While in case of Japanese quail maximum 2 number of alleles was observed for any locus, specifically for ADL0024, ADL0366, and UBC0005 (Table 6).

Present results are in accordance with the finding of [19] who found genetic co-adaptability of wild quail in china thorough microsatellite markers. They observed up to 6 numbers of alleles per locus. However, the current results are in contrast with the findings of [12] who studied genetic diversity in four different varieties of Aseel chicken and reported a maximum of 14 alleles per locus. One of the reasons for this variation might be the difference in the genome of chicken and quail. In wild population ADL0024 and UBC0001 loci showed the highest PIC values while in case of Japanese quail, ADL0024, ADL0036 and UBC0005 showed PIC value. In total, it is concluded that these sites were highly polymorphic in both population and the remaining sites were lowly polymorphic. In the current study the two markers which showed maximum PIC values were chicken specific

This distance explained how much these two breeds of quail are different from each other. Although the genetic difference between these two populations is less i.e about 0.4. Other possible reasons for this result could be the common origin and evolutionary progress, time of species formation, gene flow between different species, crosses between different lines, mixing, and human activities. Due to non-availability of genome sequence of quail, less genetic information is available about quail. In agreement to this, [1] investigated the genetic distance of domestic quail and the two wild quails and found that the genetic variance level of WSH (domestic) was close to that of YJQ (wild).

markers and UBC005 is the only quail marker which showed PIC values equivalent to chicken specific markers. In contrast to present study, [20] assessed the genetic variation and established the relationship amongst breeds and strains using 15 chicken specific microsatellite markers, and founded that highest PIC value was up to 0.87. Same is the case with [21] who studied genetic diversity of 14 indigenous grey goose breeds in China based on microsatellite markers, and indicated the highest PIC was in the Xupu up to 0.69.

Moreover, the average Heterozygosity, in case of wild quail, was observed up to 0.543. In case of wild quail, 100% heterozygosity was observed in case of ADL0024, UBC0001 and UBC0002. While, the marker ADL0023 showed heterozygosity up to 80% (Table 2). However, for the Japanese quail the average value of observed Heterozygosity was 0.429 and for the markers individually it was highest (100%) for ADL0024, ADL0366, and UBC0005 (Table 4). On overall basis, two chicken specific markers (ADL0024 and ADL0036) and two quail specific markers (UBC0001 and UBC0005) showed maximum Heterozygosity in both quail population; reflecting that these sites were showing rich genetic diversity and high selection pressure. Therefore, they may also act as genetic markers to find out the genetic diversity between these two breeds. The results of the present study are in consistent with the finding of [1]. In their study, they found that *GUJ*0028, *GUJ*0029, *UBC*0004, *UBC*0005, and *UBC*0006 sites are possessing high polymorphism and concluded that these markers can also be used for finding the quantitative trait loci.

However, the average Nei genetic distance for wild quail was 0.256 (Table 4.2). However, in case of Japanese quail the mean Nei genetic distance was 0.214.

4. CONCLUSION

On the basis of our data it can be concluded that Japanese and wild quail have substantial genetic diversity in term of numbers of alleles, Polymorphic information contents, heterozygosity, and Nei genetic distance. But maximum value of PIC content was 0.38 showing that genomic sites tested in this study were having polymorphism up to medium extent. The present study shows that even with the limited number of samples and marker considerable level of genetic diversity between the breeds, under study, was observed.

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