2013 International Conference on Food and Agricultural Sciences
IPCBEE vol.55 (2013) © (2013) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2013. V55. 3

Genetic Variation Analysis of Sinai Chicken and Japanese Quail Populations Using Microsatellite DNA Markers

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Abstract. Tow avian species; Japanese quail and Sinai chickens were examined genetically using 3 microsatellite markers to detect genetic variation. The studied loci on average produced 5.666 alleles per locus (range: 4-8). The mean observed heterozygosity (H_o) was 0.568 and ranged across loci from 0.125 to 0.900, whereas the mean expected heterozygosity (H_e) was 0.697 and ranged between 0.611 and 0.843. The polymorphic information content (PIC) values varied among loci and ranged between 0.519 for locus GUJ0063 and 0.806 for locus GUJ0087 with overall mean 0.637. Differentiation among populations was high (F_{ST} = 0.311; R_{ST} = - 0.042). Sinai chickens showed no departure from Hardy-Weinberg equilibrium, while Japanese quails were not in Hardy-Weinberg equilibrium. These results reflect that, the set of studied markers can be used effectively to capture the magnitude of genetic variability in both of Sinai chicken and Japanese quail populations.

Keywords: Japanese quail, Sinai chickens, microsatellite, genetic variation.

1. Introduction

Knowledge-based phenotypic and genotypic data is essential for the characterization of indigenous animal genetic resources providing needed information for effective conservation of useful gene pool against future uncertainties in the face of current global challenges such as agricultural, socio-economic, environmental, emerging diseases, population growth, and rising consumer demands challenges. Recently, several studies were conducted to characterize the genetic diversity in chickens and quails [1]-[7]. Genetic variation is the basic material for animal breeding. Molecular studies bring complementary information to social surveys and phenotypic data, and allow to setting up an integrated program of characterization and conservation of indigenous populations [5], [8]. There is a growing interest in suitable genetic markers for assessing population differences in qualitative and quantitative traits.

As DNA-based genetic markers, microsatellites (Short Tandem Repeats (STR)) are widely used in gene marker studies due to their co-dominant, highly polymorphic nature, dense distribution in the genome and easy genotyping, so the microsatellites are identified as reliable and effective markers in poultry species [4], [6], [8]-[10]. In Egypt, microsatellites marker analyses were involved in some recent studies to assess genetic diversity within and between local chicken strains [11]-[13].

Chickens play very significant socio-cultural and economic roles in most African societies. Despite the importance, little is known about its genetic diversity regarding the different types and local population sizes [5], [10]. Japanese quails are likely to be well-adapted to the hard conditions and resistance to diseases as it has attained economic importance as an agricultural species [7]. The objective of the current study is to assess genetic variation of two common poultry species in Egypt (Sinai chickens and Japanese quails) using nuclear microsatellite markers.

2. Materials and Methods

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2.1. Blood Sampling and DNA Extraction

A total of 30 individual blood samples representing two poultry species (Sinai chicken which were collected from Sinai desert at 1985 and improved in poultry farm at the Faculty of Agriculture, Menofia University and Japanese quail) were used. Approximately 1 ml of blood was drawn from the jugular vein for each individual 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%.

2.2. Microsatellite Genotyping

Each bird was genotyped for 3 nuclear microsatellite loci that were chosen based on their degree of polymorphism (Table 1). Polymerase chain reaction (PCR) amplifications were performed in a 20 µl reaction volume containing 30 ng genomic DNA, 130 µM of dNTP mix, 2.5 µl 10x reaction buffer (with 15 mM $MgCl_2$), 0.3 µM of forward and reverse primer, and one unit of AmpliTaq DNA polymerase (BioReady rTaq, BioFlux). For the 3 microsatellites used for genotyping, amplifications were performed using Techne® TC-3000 thermal cycler (Bibby Scientific Limited, UK) with the following temperature profile: i) initial denaturation at 95 ℃ for 9 min, ii) 30 cycles of 95 ℃ for 30 s, 55 ℃ for 1 min, and 72 ℃ for 1 min, and iii) 5 min final extension at 72 ℃. Fragments were separated on 8% polyacrylamide gel on vertical plates (with 5 V/cm) [14]. Allelic sizes were estimated using the GelAnalyzer (v. 2010a) software [15].

Table 1: Description of Microsatellite Primers Used in the Current Study

Primer	GenBank AN[a]	Repeat array		Primer (5´-3´)	TA[b] (℃)
GUJ063	AB063131	(CA)7CT(CA) 2CT(CA)7	F	GCTCAGGTTCTCAGCTGATG	55
			R	GGGAGAGATCAAGGGAACAG	
GUJ085	AB063153	(GT)14	F	ACAACCACTTCTCCAGCTAC	55
			R	GCTTGTGCTGCTGTTGCTAA	
GUJ087	AB063155	(CT)12AA(CA)11	F	CATGCCGGCTGCTATGACAG	55
			R	AAGTGCAGGGAGCGAGGAAG	

[a] *GenBank AN*: GenBank accession number [b] *TA*: temperature of annealing.

2.3. Microsatellite DNA Polymorphism and Deviation from Hardy-Weinberg Equilibrium

The number of alleles per locus, observed heterozygosity (H_o), and Nei's unbiased estimates of expected heterozygosity (H_e) were calculated using program Cervus 3.0.3 [16]. The information content of each locus was calculated by the polymorphism information content (PIC) using program Cervus 3.0.3. Departures from Hardy-Weinberg equilibrium were assessed across populations and for each locus based on exact tests as implemented in Cervus 3.0.3. Also, deviation from HWE at each locus in each population was obtained using program Genealex 6.4 [17].

2.4. Analysis of Molecular Variance (AMOVA)

To quantify the extent of molecular variation, locus-by-locus analysis of molecular variance (AMOVA) was performed using Genealex 6.4. 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 Genealex 6.4 [17].

3. Results

3.1. Microsatellite DNA Polymorphism and Deviation from Hardy-Weinberg Equilibrium

A total of 17 alleles were detected across all populations for the 3 microsatellites examined. Number of alleles per locus overall populations ranged between 4 (GUJ0063) and 8 (GUJ0087) with mean of 5.666 alleles per locus as presented in Tables 2 and 3. The mean number of alleles per locus overall 3 investigated loci were 2.333 and 4.000 in chickens and quails, respectively (Table 3) revealed a reduction of genetic variation in chickens. The difference in allele size varied among loci and ranged from 31 bp (145-176 bp) for locus GUJ0087 to 59 bp (190-249 bp) for locus GUJ0085 (Table 2). The mean effective number of alleles

per locus was 3.660 across all investigated loci in all populations (Table 3). The mean effective number of alleles overall investigated loci was 1.774 and 3.279 in chicken and quail populations respectively (Table 3).

Data presented in Tables 2 and 3 showed that, overall populations the mean observed heterozygosity (H_o) was 0.568 and ranged across loci from 0.125 to 0.900, whereas the mean expected heterozygosity (H_e) was 0.697 and ranged between 0.611 and 0.843. The observed heterozygosities were 0.533 and 0.572 for chickens and quails respectively. Among the investigated populations H_e was 0.415 and 0.606 for chickens and quails, respectively. The polymorphic information content (PIC) among loci ranged between 0.519 for locus GUJ0063 and 0.806 for locus GUJ0087 with general mean of 0.637 (Table 3). The majority of the studied microsatellite loci used in this study was highly revealing. All of markers used in the current study had highly informative PIC values. In the current study chickens showed no departure from HWE while, quails showed significant deviations from HW expectation for both combined and individual loci.

3.2. Analysis of Molecular Variance (AMOVA)

AMOVA results based on the Stepwise Mutation Model (SMM) indicated that approximately ~100% of the microsatellite variation resided within populations (Table 4). Differentiation among populations was moderate but highly significant (F_{ST} = 0.311; R_{ST} = -0.042).

Table 2: Genetic Variation Measures Obtained from the Analysis of Microsatellite Markers Used in the Current Study by Locus by Population

Pop	locus	N_a	N_e	Size range (bp)	H_o	UH_e	PIC	HWE
Quail	GUJ0063	2	1.972	227-251	0.880	0.503	0.371	**
	GUJ0085	3	1.819	242-249	0.053	0.462	0.404	***
	GUJ0087	7	6.046	145-176	0.783	0.853	0.814	***
Chicken	GUJ0063	3	2.632	219-251	1.000	0.689	0.548	NS
	GUJ0085	2	1.471	190-197	0.400	0.356	0.269	NS
	GUJ0087	2	1.220	165-173	0.200	0.200	0.164	NS
Over pops.	GUJ0063	4	2.503	219-251	0.900	0.611	0.519	***
	GUJ0085	5	2.672	190-249	0.125	0.639	0.587	***
	GUJ0087	8	5.807	145-176	0.679	0.843	0.806	***

N_a = No. of different alleles, N_e = No. of effective alleles, H_o = Observed heterozygosity, UH_e = Unbiased Expected heterozygosity, PIC = Polymorphism information content, HWE = Hardy-Weinberg equilibrium

Table 3: Genetic Diversity Measures for the Studied Populations Overall Loci

Population	N_a	N_e	H_o	UH_e	PIC	HWE
Quail	4.000	3.279	0.572	0.606	0.529	**
Chicken	2.333	1.774	0.533	0.415	0.327	NS
Over pops.	5.666	3.660	0.568	0.697	0.637	**

N_a = No. of different alleles, N_e = No. of effective alleles, H_o = Observed heterozygosity, UH_e = Unbiased Expected heterozygosity, PIC = Polymorphism information content, HWE =Hardy-Weinberg equilibrium

Table 4: Analysis of Molecular Variance (AMOVA) in Studied Populations, Input as Microsatellite Distance Matrix for Calculation of Rst (Within Individual Analysis Suppressed)

Source of variance	df	SS	MS	Est. Var.	%	Rst value	Fst value (S.E.)
Among populations	1	3772.54	3772.54	0.000	0	-0.042	0.311 (0.068)
Within populations	58	664370.52	11454.66	11454.66	100		
Total	59	668143.06		11454.66	100		

4. Discussion

4.1. Microsatellite DNA Polymorphism and Deviation from Hardy-Weinberg Equilibrium

The mean number of alleles per locus overall 3 investigated loci could be informative and revealed a reduction of genetic variation in chickens. Higher mean number of alleles per breed were previously reported, may be due to the higher number of populations and markers used in such studies [1], [8], [10], [12] in chickens and 7 in quails]. For Japanese quail lower values of size range (mean 12.6 bp) recorded compared

with our findings [18]. Lower mean number of alleles per locus as 3.7 (range 1-6 alleles) in a set of 100 microsatellite marker in Japanese quail randombred population was reported [18]. Higher values for average number of alleles per locus were reported, 11.4 in 64 population of chickens from different continents [19] and 7.5 for five subpopulations of Turkish native chicken breeds [20] and 4.9 for Egyptian native breeds (Fayoumi and Dandarawi) and commercial laying hens (brown Hy-line) [11]. Finally, average number of alleles per locus as 11.0 was previously recorded [2]. Results showed that, the genetic variation was higher in quails as compared to chicken population and reflected the efficiency of used set of microsatellite markers in studying the genetic variation within and between poultry populations. 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. Our results were in a good agreement with those obtained in Japanese quail [18], and in Egyptian chickens [13]. The genetic diversity of the Turkish native chicken breeds Denizli and Gerze was evaluated with 10 microsatellite markers [20] and H_e was 0.665. Furthermore, in Iran, higher values of expected heterozygosity were recorded and varied between 0.708 and 0.849 for four strains of Japanese quail [21]. Higher values of heterozygosity were recorded in Guangxi Three-yellow chickens from China [1], in Pakistani chickens [4], while, mean values of H_o= 0.49 and H_e= 0.52 in Italian chicken breeds were reported [8]. In addition higher values of heterozygosity in Japanese quail [7] than these obtained in the current study were recorded. On the other hand lower values of heterozygosity were recorded in British chicken breeds [3]. Thus, variation in heterozygosity may be adduced to differences in location, different sample sizes, different experimental birds and number/sources of microsatellite markers.

The majority of the studied microsatellite loci used in this study was highly revealing. According to classification of Botstein et al. [22], 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. All of markers used in the current study had highly informative PIC values. Overall high PIC value indicates that the particular locus is highly informative which may be used to resolve queries of forensic nature and help to evaluate the genetic diversity of different breeds of poultry [4]. Lower values than those obtained in our experiment ranged between 0.00 and 0.729 with mean value of 0.4769 were obtained previously [18], while, PIC values ranged between 0.427 in panda strain (lowest) and 0.815 in Golden strain (highest) for four strains of Japanese quail [21]. In chickens, values of PIC varied in many previous studies on, Turkish native chicken breeds 0.426-0.599 [20]. Our results also were similar to these obtained in previous studies [1], [4]. On the other hand, higher PIC values were reported in Nigerian chicken populations [10], and in Japanese quail [7].

In the current study chickens showed no departure from HWE, while quails showed significant deviations from HW expectation for both combined and individual loci. These results reflected that, quails were generally not in HWE, according to the history of the studied population, they were under artificial selection, and thus we concluded that, the set of studied markers can be used to determine the genetic variation in different populations of poultry species effectively. Our findings are in agreement with Amirmia et al. [21] in Japanese quail, in addition, no departure from Hardy–Weinberg equilibrium was observed for 64 chicken populations from different continents [19].

4.2. Analysis of Molecular Variance (AMOVA)

Our results were no much differed from those obtained in British chicken breeds (F_{ST} = 0.25) as previously reported [3], while in Pakistani Aseel chickens F_{ST} = 0.1264 [4]. Furthermore, 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 [2]. On the opposite trend, Wright's *F*-statistics revealed lower F_{ST} = 0.07 and 0.082 in Egyptian local chicken breeds [12], [13] respectively, or negligible genetic differentiation (F_{ST}) in local Ghanaian chicken populations [23]. 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.

In conclusion, the three nuclear microsatellite markers used were sufficient to differentiate among two domesticated poultry species (Sinai chicken and Japanese quail). Within-population variation was very high compared to among-population variation. The studied chicken population followed HW expectation, while deviation from HWE was observed at studied quail population. 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 populations was high and measurable. Results from the current study are useful in determine genetic variability in chicken and quail populations as well as designing prospective breeding programs for different traits.

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