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GENETIC CHARACTERIZATION AMONG FAYOUMI BREED USING MICROSATELLITE MARKERS

By

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ABSTRACT: *This study represents the first results on assessing the genetic characterization of three populations generated from Egyptian Fayoumi native breed (P, G & R). On the basis of molecular genetic characterization six highly polymorphic microsatellite markers were utilized. The discriminating power of such microsatellite was estimated. A total of 106 alleles was detected averaged 17.67 with allele frequency ranged 0.02 (P) to 0.75 (R). The lowest heterozygousity was found in P (20%) and the highest (27%) in G population. A total of 13 unique alleles (12%) was detected indicating its validity in diagnostic and fingerprint tool for breed identification. The genetic distance (D) among the three populations clustered P and G as more closely related than R population. It was concluded that this particular set of microsatellite markers (especially, ADL136. ADL172 and ADL176) can be of use a measure of genetic variability in native chicken breeds and/or strains in Egypt.*

INTRODUCTION

Fayoumi is an active and hardy type of fowl, the origin of this breed is not definitely known. Concerning this situation, different theories are attended; the first is that it was introduced to Egypt from a village called "Biga" in Turkey during the period of Mohamed Ali Pasha. The second one is it originates from the Silver Campine breed and was introduced at the time of Napoleon's Occupation of Egypt (Hossari, 1958). While, Abdel-Warith (1993) mentioned that Fayoumi looks much more phenotically like the Silver Campine than any other breed but its genomic constituents might be different. Since, barring is a sex linked trait in Fayoumi breed, while, it is autosomal in the Silver Campine. Furthermore, Lamont (1997), as listed in Iowa State University web site, found that the Fayoumi genetics is very different from other chicken. She confirmed that Fayoumi birds are much

more resistant to viral diseases than others. (URL:http://www. iastate.edu/general/Iastater/ ....chicken. html).

Genetic characterization contributes to breed definition, especially populations which are not well defined and provide an indication of their genetic diversity. It also has potential to identify unique alleles in the breeds or lines studied. Conservation of farm animals including poultry will be important for future designing of sustainable breeding programs (Toro et al., 2006). Microsatellites are widely implemented in exploring genetic variation and phylogeny between populations of same species (Buchanan et al., 1994; MacHugh et al., 1994). The usefulness of microsatellite markers in estimating genetic relatedness and diversity in chickens have been demonstrated in a number of indigenous breeds, inbred strains and in commercial chicken lines (Crooijmans et al., 1996; Zhou & Lamont, 1999; Romanov & Weigend, 2001; Zhang et al., 2002; Tadelle, 2003; Qsman et al., 2006; Tadano et al., 2007 & more recently, Roushdy et al., 2008). These markers are co-dominant and highly reproducible. The microsatellite marker is extensively used for assessing genetic structure, diversity, and relationships because of many advantages such as being numerous and ubiquitous throughout the genome, showing a higher degree of polymorphisms, and codominant inheritance (Tautz, 1989). Especially, high degree of polymorphisms is considered to be greatly useful for assessing genetic diversity and relationships among closely related livestock breeds (FAO, 1998). As for conservation of livestock genetic resources, if there are limitations of costs or breeding places, the population may be maintained as a core collection, which possesses as much genetic variability as possible with appropriate population size. To develop the core collection, exact genetic evaluation for the population would be required by molecular markers. It would lead to a correct management of the population as a genetic resource.

Two strains were originated from a random-bred control (R) by convenient selection from a Fayoumi flock; G and P. Individual selection was carried out in the growth strain (G) for seven generations to increase body weight at eight weeks of age. While, a full-sibs family selection was practiced in production strain (P) for five generations to increase egg number (Hossari, 1970). Up to date, a few information is available on the genetic diversity of Egyptian indigenous chickens, which are becoming important to design effective selection and conservation strategies. Therefore, the current study was carried out to estimate genetic

characterization of three Fayoumi chicken populations (G, P and R) based on 6 microsatellite markers. Consequently, the obtained results would contribute to the appropriate managements avoiding loss of genetic variability in these lines and to future improvements.

MATERIALS & METHODS

Chicken populations

A total of 74 blood samples represented three Fayoumi chicken populations (P,25; G,31 and R,18) were collected from Poultry Breeding Station at Fayoum Governorate , Animal Production Research Institute.

Blood sampling & DNA Isolation:

A half ml of blood sample was withdrawn from Jugular vein on EDTA tube as anticoagulant (0.2 ml of 0.5 M EDTA). DNA was freshly extracted from whole collected EDTA-blood . Two and half ml of lysis buffer TSTM (20 mM Tris-HCl pH 7.6, 640 mM sucrose, 2% Triton X-100, 10 mM MgCl2) was added to the aliquot. The mixture was centrifuged and the pellet suspended in 150 µl Proteinase K, 1.5 ml nuclei lysis buffer and 110 µl SDS 20%. After overnight incubation at 37° C, the proteins were removed by NaCl 6M and the DNA was precipitated by ice cold absolute ethanol.

Microsatellite loci:

Six microsatellite loci (Table 1) were selected based on the degree of polymorphism and genome coverage have been recommended for the Measurement of Domestic Animals Diversity (MoDAD) (FAO, 2004), for application in diversity studies. Detailed informations about used microsatellites are available at the FAO website (www.dad.fao.org/en/refer/library/guidelin/marker.pdf).

PCR & Amplification Conditions:

The.PCR was carried out in a volume of 20 µl comprising 50 ng of template DNA, 10 pmol of each primer and 5 µl of Taq master (LAROVA GmbH.Rheinstr.17a.14513 Telow. Germany). The amplification conditions were: 2 min denaturation at 95° C followed by 35 cycles of denaturation at 94°C for 30 s, annealing at (47-52°C, accordingly) for (30-60 s) and elongation at 72° C for 30s. The PCR products were separated in 8% TBE polyacrylamide gel.

Microsatellite & Genetic Analysis:

All resulted gels were visualized and scored with Alphaimages2200 software Version 4.0.1. All scored microsatellite data was firstly corrected to estimate each allele size according to its number of repeats for each marker. A Tandem Repeat Analyzer software package was adopted for this purpose. Then, a spread sheet program (Microsoft Excel) was used to arrange the included data for each breed regarding each locus. All possible extracted population figures were carried out employing a Arlequin 3.11 software package after data conversion using CONVERT program. It is common in such cases no amplicon is produced in certain samples for such primer rather than other. Thus, the absence of PCR product in these samples is manipulated as missing data. As a consequent, the analysis program account them as null (unknown) alleles not exceeded 0.1 of data as our default analysis.

RESULTS AND DISCUSSIONS

The present investigation utilized six microsatellites highly polymorphic markers. Two out of them, are located on chromosome 8 linkage group and cover 26-35 cM for ADL0171 and 70:105 cM for ADL0172 locus. The remaining four loci are located on chromosome2 (ADL0176), chromosome9 (ADL0136), chromosome10 (ADL0102) and chromosome11 (ADL0210) covering 116 cM, 107 cM, 88 cM and 54 cM, respectively.

Allelic frequencies were calculated on the basis on all six microsatellite loci. The genetic diversity within the three populations (P, G & R) analyzed herein was described by the number of alleles per locus, the mean expected and observed heterozygosity and total gene diversity. Genetic differentiation between populations was assessed by an analysis of molecular variance (F-index) employing a new version of most widely used for molecular population genetics analysis Arlequin3.11 software package.

Table (1) summarizes all information of six microsatellites markers used and shows locus name, genome location, flanking sequences, reported number of alleles, gene bank NCBI accession number, microsatellite repeat type and sequence tagged site (STS) size in base pairs.

Allele size in base pair, their frequencies for each locus and population and average of allele frequencies for each locus for each population as observed in the present study are given in Table (2). The

highest allele frequency overall loci was 0.75 of allele 108 at locus ADL171 in the case of R population. Whereas, the lowest one was 0.02 associated with P population at four loci, ADL102 (for allele 162), ADL136 (for alleles, 132 and 154), ADL172 (for allele 144) and ADL176 (for allele 156). The highest average of allele frequency estimated was for P population at locus ADL171 (0.5). On the other hand, the lowest one was 0.09 in the case of locus ADL136 for two populations (P &G).

The variations for each microsatellite are summarized in Table (3). A total of 106 distinct alleles were detected at the 6 microsatellite loci in 74 birds. Across all populations, the average number of alleles per locus was 17.67, with the range from 9 (ADL172) to 30 (ADL136). The total number of alleles detected in each population overall loci were almost equal 36, 35 and 35 for P, R and G, respectively. The lowest number of alleles was 2 (P, ADL171) while the highest one was 11 (P, ADL136). Similar results with regard to the number of alleles were reported by Wimmers *et al.* (2000), where, a range of 2 to 11 alleles per locus was detected for the local chickens from Africa, Asia and South America. Generally, microsatellite markers are found to be medium to highly polymorphic when tested in various chicken lines (Vanhala *et al.*, 1998; Romanov & Weigend, 2001; Osman *et al.*, 2006). The mean number of alleles for each locus overall populations ranged from 3 (ADL171) to 10 (ADL136). The observed variability of average number of alleles seemed to reflect different potentialities of genetic markers to detect genetic variability among such breeds. Roushdy et al. (2008) reported the same conclusion in comparison of three breeds (Fayoumi, Dandarawi & Hyline). Regarding this situation the results presented in Table (3) support utilizing ADL0136 microsatellite marker for population genetic differentiation among chicken breeds. Regarding unique alleles (Table 3), a total of 13 unique alleles (0.12) were detected overall loci and populations. Referring unique allele for each locus versus population, a total of 2, 2, 1, 4, 2 and 2 were found for ADL102, ADL136, ADL171, ADL172, ADL176 and ADL210, respectively. While, for P, R and G populations a total of unique ones were 4, 5 an 4, respectively. As given in Table (3), at least each population was characterized by unique allele for such locus. Consequently, these unique alleles would be utilized as population fingerprint even one allele for one locus.

As shown in Table (4), the H_E per locus ranged from 0.48 (ADL171) to 0.87 (ADL136). In terms of the H_E, higher values were observed at

ADL136 (0.87), ADL176 (0.82) and ADL172 (0.74). As for number of alleles, ADL136 (30), ADL176(25) and ADL172(18) were more highly polymorphic. In regard to the FIS value, which indicates component of genetic variation within population, ADL176 (0.76), ADL136 (0.62) and ADL172 (0.61) had higher values than others, Table (4). These observations evidence a correlation between showed high expected heterozygosity and many alleles at the same time for these three markers. As a consequent, these markers (ADL136, ADL176 and ADL172) proved to be more effective for our aim in the present study and would be utilized in the same kind of study. The concluded remarks herein are in agreement with Tadano et al. (2007). All 3 populations revealed unbiased deviation of HWE (Hardy-Weinberg equilibrium) at high significant levels (P value ranged 0.000 – 0.013). This indicates a such degree of inbreeding level and strong selection, this results are in accordance with Marelli et al., 2005, who used eight microsatellite markers for genetic characterization of some Italian chicken breed, fortunately, five out of these markers are used in the present work.

Table (5) represents analysis of molecular variance (AMOVA) estimated by Arlequin3.11 software package as standard genetic population input data. Variance components proved that the majority of genetic diversity obtained in the current study is represented by among individuals within populations (61.77%) rather than others. Population fixation indices traced a 0.68 of variation referring to differences among individuals versus total variance (FIT). While, among populations differences versus total variance was the lowest fixation indices (FST= 0.06) indicating low level of population differentiation. This observations might be explained as approximate equality of average total number of alleles detected for each population overall loci as shown in Table (3), where it was as, P (6), R (5.83) and G (5.83).

Genetic distance (D):

Based on genetic diversity estimate (FST) a genetic distance among used population was computed as pair-wise differences. Table (6) shows a genetic distance matrix produced by analysis software (Arlequin3.11). Genetic distance (D) revealed that both two populations; P & G, are closed related genetically to each other than the third unselected population, R. Since the lowest (D) value is 0.052 in the case of P and G populations while the highest (D) value is between R and G populations (0.104) followed by

(0.083) between P and R. The obtained (D) values reflect genetic histories of the three populations. since, G population was attended for seven generation of individual section. On the other hand, a P population was introduced to family selection for five generations.

Generally, Any study to identify the molecular genetics underlying important traits in livestock consists of different stages. From these stages, the molecular genotypic data, such as candidate genes, microsatellites, or microarray expression profiles, must by generated. Then, statistical analysis of the phenotypic and genotypic data must be conducted to identify and interpret the relationships of genetic and phenotypic variation of the resource populations. Ultimately, this approach can lead to the detection of functional genes and DNA markers that can be applied for genetic improvement of populations in marker-assisted selection schemes of poultry breeding (Lamont; 2003). It is not worthy to appoint that several investigators used Fayoumi breed more widely overall the world as it was introduced to different countries since 1940 up to now. It was used in some studies as a partner of crosses program determining such important genes (Zhou et al., 2006). Other studies utilized Fayoumi breed as a reference breed; e.g., Zhou & Lamont (1999) & Tadelle (2003). These studies used different schemes and as well different microsatellite markers revealing a high polymorphic levels, although they severe from the foundation effect.

On the other hand, as for our knowledge, there is no concern to the native breeds of Egypt and the present work is considered the first report conducted to understand Fayoumi breed molecular genetic structure, even for the used three populations. In this regard, a recommended 6 microsatellite primer were utilized to assess molecular genetic structure of two populations (P & G) originated from R unselected population throughout convenient selection programs by Hossari, 1970. The present work revealed a high average number of alleles across all populations in all loci (17.67). This observation is mainly due to high number of alleles detected 30, 25 & 18 alleles for ADL136, ADL176 and ADL172, resp., overall populations. Concerning allele numbers corresponding each locus for such breed identical results were reported by Wimmers et al. (2000), where, a range of 2 to 11 alleles per locus was reported for the local chickens from Africa, Asia and South America. Generally, microsatellite markers are found to be medium to highly polymorphic when tested in various chicken lines (Vanhala et al., 1998; Romanov & Weigend, 2001; Osman et al., 2006).

In conclusion, the present investigation proved the usefulness of used six microsatellite markers herein in discriminating among the three Fayoumi populations assessed, P, R and G. Among 106 alleles overall loci versus all populations a total of 13 unique alleles were obtained (4 alleles for P, 5 alleles for R and finally 4 alleles for G). In this regard, ADL176 produced the highest number (4) of unique alleles rather than other markers. This result could be strongly utilized as a molecular tool in fingerprint analysis of Fayoumi populations. The present work suggests using wide genome scan analysis based on more recommended microsatellites covering chicken genome. This could be utilized in further work concerning MAS (marker assisted selection) and QTL (Quantitative Trait Loci) programs. On the background of Fayoumi breed history a more and more further studies should be done.

Table (1): Characteristics of microsatellite markers used for the genetic analysis.

Locus Name	Location[1]	Primer Sequence	No. of alleles[2]	Access. No.[3]	Repeat type	STS Size[4]
ADL0102	10 - 88 cM	TTCCACCTTTCTTTTTTATT GCTCCACTCCCTTCTAACCC	10	G01547	$(GT)_{18}$	122bp
ADL0136	9 - 107 cM	TGTCAAGCCCATCGTATCAC CCACCTCCTTCTCCTGTTCA	10	G01561	$(TG)_{10}$	145bp
ADL0171	8 - 26:35 cM	ACAGGATTCTTGAGATTTTT GGTCTTAGCAGTGTTTGTTT	8	G01593	$(TG)_{18}$	104bp
ADL0172	8 - 70:105 cM	CCCTACAACAAAGAGCAGTG CTATGGAATAAAATGGAAAT	7	G01594	$(AC)_{18}$	154bp
ADL0176	2 - 116 cM	TTGTGGATTCTGGTGGTAGC TTCTCCCGTAACACTCGTCA	9	G01598	$(GT)_{12}$	192bp
ADL0210	11 - 54 cM	ACAGGAGGATAGTCACACAT GCCAAAAAGATGAATGAGTA	9	G01630	$(AC)_{15}$	130bp

1,2. Locations & Number of alleles listed as reported by US chicken genome project population tester kit#9. 3. Genebank accession number; www.ncbi.nlm.nih.gov/. 4. STS: sequence tagged site size according to NCBI database.

Table (2): Allele size in base pair, their frequencies for e. ch locus and population & average of allele frequencies as observed in the present study.

Locus	Alleles bp*	Frequencies			Locus	Alleles bp*	Frequencies		
		P	R	G			P	R	G
ADL102	090	0.18	0.64	0.18	ADL172	108	0.18	0.05	----
	108	0.58	0.33	0.52		126	0.10	----	----
	126	0.22	0.03	0.27		144	0.02	0.14	0.19
	144	-----	----	0.03		162	0.36	0.50	0.31
	162	0.02	---	---		180	0.22	0.11	0.24
						198	0.04	0.03	0.13
						216	---	0.11	---
						234	---	0.03	---
						252	---	0.03	---
						null	0.08	----	0.13
Average	---	0.25	0.33	0.25	Average	---	0.17	0.13	0.20
ADL136	066	0.04	---	0.16	ADL176	132	0.18	----	---
	088	0.20	0.06	0.03		144	0.08	----	0.06
	108	----	0.03	---		156	0.02	0.05	0.03
	110	0.04	0.03	----		168	--	0.03	0.08
	132	0.02	0.10	0.06		180	---	0.44	0.11
	154	0.02	0.19	0.13		192	0.08	0.17	0.08
	176	0.20	0.22	0.19		204	0.24	0.22	0.03
	198	0.22	0.17	0.05		216	0.14	0.06	0.06
	220	0.06	0.03	0.13		228	0.20	0.03	0.21
	242	0.04	-----	0.03		240	0.06	---	0.26
	264	0.12	0.17	0.13		252	---	---	0.08
	286	0.04	----	0.03					
	Null	----	----	0.06					
Average	---	0.09	0.11	0.09	Average	---	0.13	0.14	0.10
ADL171	090	----	----	0.18	ADL210	120	0.36	0.22	--
	108	0.60	0.75	0.52		135	0.20	0.44	0.29
	126	0.40	0.22	0.27		150	0.16	0.11	0.48
	144	----	0.03	0.03		165	0.16	0.06	0.19
						180	----	0.06	---
						195	0.04	----	---
						null	0.08	0.11	0.04
Average	---	0.50	0.33	0.25	Average	---	0.17	0.17	0.25

* in such cases some samples produced no amplicon in such primer rather than other, thus they are manipulated as missing data. The program account it as null (unknown) allele not exceeded 0.1 in freq..

Table(3): Number of alleles observed in each locus within each population, total number of alleles, unique alleles and estimated means.

Locus no.	P	R	G	Mean	Total (106)*	Unique alleles
1	4	3	4	3.67	11	2
2	11	9	10	10	30	2
3	2	3	4	3	9	1
4	6	8	4	6	18	4
5	8	7	10	8.33	25	2
6	5	5	3	4.33	13	2
Mean	6	5.83	5.83	5.89	17.67**	2.16
Unique alleles	4	5	4	4.33	---	13 (12%)***

* total number of alleles overall loci between parentheses.
** average number of alleles overall loci.
*** total # of unique alleles & percentage between parentheses.

Table (4): Observed heterozygosity (H_O), Expected heterozygosities (H_E) and their means estimated for each locus and each population.

Locus#		P	R	G	Mean
1	(observed)	0.16	0.11	0.58	0.28
	(expected)	0.59	0.49	0.63	0.57
	P value	0	0	0.005	---
	FIS*	0.73	0.78	0.09	0.45
2	(observed)	0.32	0.5	0.24	0.35
	(expected)	0.86	0.86	0.88	0.87
	P value	0	0.001	0	---
	FIS	0.63	0.43	0.73	0.62
3	(observed)	0.24	0.11	0.32	0.22
	(expected)	0.49	0.4	0.55	0.48
	P value	0.013	0.001	0.009	---
	FIS	0.52	0.73	0.42	0.51
4	(observed)	0.3	0.33	0.26	0.29
	(expected)	0.75	0.72	0.74	0.74
	P value	0	0	0	---
	FIS	0.6	0.54	0.65	0.61
5	(observed)	0.16	0.28	0.19	0.21
	(expected)	0.85	0.74	0.86	0.82
	P value	0	0	0	---
	FIS	0.82	0.63	0.78	0.76
6	(observed)	0	0	0	0
	(expected)	0.75	0.69	0.63	0.69
	P value	0	0	0	---
	FIS	1	1	1	1
Mean (H_E)		0.72	0.65	0.72	0.69
Mean (H_O)		0.2	0.22	0.27	0.23

* FIS, Population specific FIS indices per polymorphic locus (absolute values).

Table 5: Analysis of molecular variance shows variance components, percentage of variations & overall population fixation indices (FIS, FST & FIT).

S.O.V.	d.f.	S.S.	Variance components	Variations%	Fixation Indices
Among Pops.	2	19.72	0.14	6.34	FIS = 0.659
Within Pops.	71	236.12	1.32	61.77	FST = 0.063
Within individuals	74	50.50	0.68	31.90	FIT = 0.681
Total	147	306.33	2.14	----	----

Table (6): Genetic distances matrix represents genetic relationship among three used Fayoumi populations

Population	P	R
R	0.083	
G	0.052	0.104

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الملخص العربى

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هذه الدراسة تمثل النتائج الأولى لتقييم و قياس التوصيف الوراثى لثلاثة من العشائر من سلالة الفيومى المصرية (P, R & G). اعتمادا على التوصيف الجزيئى الوراثى تم الاستفادة من ستة واسمات من التتابعات الجزيئية و التى تنتج تباينا وراثيا بصورة عالية. و بهذا الصدد تم تقدير قدرة هذه الواسمات فى التمييز بين الأفراد و بين العشائر. و قد تم تحديد ١٠٦ أليلا بمتوسط عام ١٧.٦٧ و بتكرارات ما بين ٢% للعشيرة P إلى ٧٥% للعشيرة R. و كانت أقل قيمة لنسبة الخليط ٢٠ % فى العشيرة P و بلغ أعلاها ٢٧% فى العشيرة G. و بنسبة عامة بلغت ١٢% أمكن تمييز كل عشيرة على حدة بعدد من الأليلات المميزة الفريدة (باجمالى ١٣ أليل) ، مما يوجه الاستفادة من هذه الأليلات فى توصيف و عمل البصمة الوراثية فى حالة تعريف السلالات. و باستخدام مقياس البعد الوراثى اتضح أن كلا من العشيرتين G و P أكثر قرابة من العشيرة R.

و نستخلص من هذه الدراسة أن مجموعة الواسمات الوراثية المستخدمة و خاصة (*ADL136, ADL172 and ADL176*) يمكن استخدامها فى قياس التنوع الوراثى فى سلالات الدواجن فى مصر.