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Molecular Comparison of Egyptian and Saudi Local Chickens using RAPD Markers

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Abstract

This study aimed at comparing the genetic variation levels of two Egyptian (Fayoumi and Dandrawi) and two Saudi chicken lines (Hajar1 and Hajar2), with commercial broiler and layer chicken breeds. RAPD-PCR analysis, using 15 random primers, was used. 10 primers only were subjected to data analysis due to their productivity. The total number of amplified bands was 3967 on 123 loci. The percentage of polymorphic loci was averaged 34.56% and was the highest in Fayoumi, Dandrawi and layer (37.4%) and the lowest in broilers (26%). The average number of detected alleles was 1.173. However, the effective number of alleles averaged 1.238. The average of within-breed genetic variability estimates ranged between 0.135 in broilers to 0.229 in Fayoumi chickens. The Analysis of Molecular Variance (AMOVA) indicated that the six breeds were significantly different from each other. The phylogenic analysis revealed that the two Saudi breeds were too close to each other, although they are not in the same cluster. Also, all local breeds were close to the meat-type than the egg-type commercial breed.

Introduction

Overall the world, poultry production is depending mainly on commercial strains. However, local poultry breeds are acting as a reservoir for genetic variation, such variation may be exploited in breeding programs to improve the productivity of local breeds. Furthermore, all sustainable production trends depend on the use of local breeds which were mostly neglected for a long time due to their poor productivity. Moreover, developing countries should give more concern to the improvement of their local breeds, which may help in the reduction of poverty [1]. The accurate characterization of genetic resources of local breeds is a prerequisite for the successful conservation and breeding programs [2].

Molecular genetics offered different techniques for addressing the genetic variation and characterizing different populations. Such techniques depending PCR amplification of different DNA regions [3]. A wide range of DNA markers was extensively used to estimate genetic diversity of different poultry populations, including AFLP, RAPD, SSR, and SNP. RAPD markers are depending on the use of random primers to amplify different DNA fragments [4]. RAPD technique is cheap, easy and does not require prior knowledge of the target DNA sequence. On the other hand, the lake of reproducibility and low power of resolution compared to microsatellites and SNPs are the main disadvantages of RAPD. Nevertheless, it can be used for preliminary estimation of within- and among- breeds variation as well as genetic relationships among different breeds and populations. Van De Zande and Bijlsma [5] reported that RAPD is a useful technique to study population divergence. Besides, the strategy is profoundly helpful for strain and species recognition. Few attempts have been made to genetically characterize local chicken breeds in the middle-east region [6-10]. But most of these studies were not focused on the levels of diversity as much as the resulted banding patterns. Hence, the aim of this study was to compare the genetic parameters of local Egyptian and Saudi chicken breeds with commercial broilers and layers, as a primary step for setting conservation priorities and designing breeding programs to improve their productivity.

Materials and Methods

Populations

A total of forty-eight individuals from six chicken populations were used in this study.

Populations were two local Egyptian breeds (Fayoumi and Dandrawi), two local Saudi chicken lines (Hajar1 and Hajar2), commercial meat-type chicken breed (broiler), and commercial egg-type chicken breed (layer).

Blood sampling and PCR amplification procedures

Approximately three ml of blood were collected from the wing vein in a sterile 5-ml tube containing EDTA as an anticoagulant agent. Total genomic DNA was then extracted using

blood genomic DNA extraction kit (Bioflux, China) with slight modifications. DNA samples were amplified using 15 random primers (Vivantis, Malaysia), all primers were 10-mer primers (Table 1). PCR reactions were performed in a total volume of 20 µl composed of 10 µl PCR-master mix, 2 µl of each primer, 2 µl of template DNA (30-50 ng), 1 µl MgCl2, and 5 µl ddH2O. The steps of PCR program were initial denaturation for 10 min at 94°C, followed by 40 cycles of denaturation (2 min at 94°C), annealing (1 min at 34°C) and extension (2 min at 72°C), final extension for 10 min at 72°C, and then hold at 4°C. PCR products were electrophoresed on 1.7% agarose gels stained with ethidium bromide and then visualized under UV light.

Data Analysis

Amplified bands were scored and then analyzed using Popgene [11], Within-breed genetic variability was calculated according to [12]. Analysis of molecular variance (AMOVA) was carried out using GENALEX V6.5 [13]. The hierarchical cluster plot of the evolutionary relationships, using UPGMA method, was constructed using MEGA6 software [14].

Results and Discussion

Banding patterns

The resulted RAPD banding patterns of five primers (OPA-09, OPA-10, OPB-08, OPA-10, and OPC-11), out of fifteen, were not informative enough (Table 1), and did not generate any polymorphism. Accordingly, they were excluded, and only the 10 polymorphic markers were subjected to data analysis. The percentage of polymorphism ranged between 50 to 100%. Ott [15] considered primers with more than 70% polymorphism are highly polymorphic markers. Accordingly, 6 markers, out of the ten polymorphic markers, were highly polymorphic markers. The total number of bands amplified by the ten primers, overall breeds and primers were 3967 on 123 loci (Table 2). However, the total number of amplified bands per breed, overall individuals, ranged between 31 in broiler chickens primed by primer OPB-07 to 93 bands in Hajar2 individuals primed by primer OPB-14. Overall primers, the average number of detected bands ranged between 7.57 bands/individual in broiler to 8.53 bands/individual in Hajar 2 chickens. Nevertheless, no significant differences were found between the averages of detected bands in different breeds. Previous studies on using RAPD to characterize chicken breeds reported that the number of amplified bands was varied from 5 to 12 [16,17].

Table 1: Primer sequence, melting temperature, GC contents, and percentage of Polymorphic bands.

Primer	Sequence	Tm (c)	GC%	% Polymorphic bands
OPA-01	CAGGCCCTTC	41	70	72.3
OPA-03	AGTCAGCCAC	41	70	75
OPA-05	TGCGCCCTTC	41	70	100
OPA-06	GGTCCCTGAC	41	70	64.3
OPA-08	GTGACGTAGG	36.9	60	78.6
OPA-09	GGGTAACGCC	41	70	0
OPA-10	GTGATCGCAG	36.9	60	0
OPA-18	AGGTGACCGT	36.9	60	69.2
OPB-03	CATCCCCCTG	41	70	85.7
OPB-07	GGTGACGCAG	41	70	83.3
OPB-08	GTCCACACGG	41	70	0
OPB-10	CTGCTGGGAC	41	70	0
OPB-14	TCCGCTCTGG	41	70	53.8
OPC-06	GAACGGACTC	36.9	60	50
OPC-11	AAAGCTGCGG	36.9	60	0

The average number of alleles ranged between 1.008 in broilers to 1.228 in Hajar2 line (Table 3). However, the effective number of alleles ranged between 1.19 in broiler to 1.28 in layer chickens. Higher values were estimated for local Jordanian chickens where the effective number of alleles ranged from 1.47 to 1.7 with an average of 1.65 [8]. Furthermore, the results is in accordance with Pandey [18] who reported that the effective number of alleles maintained in local chicken breeds is always less than the actual number of alleles.

Genetic variability

Within-population genetic variability is very important for the adaptation to different environmental conditions and, hence, for maintains a good level of production [19]. In the current study, within-breed genetic variability estimates were, in general, low (Table 4). Primer OPA-01 did not generate any variability between broiler individual with variability estimate of 0.000. However, the maximum variability was found within the individuals of Fayoumi (using primer OPA-03) and Dandrawi chickens (using primer OPA-01) with a value of 0.375. The average of within-breed genetic variability estimates ranged between 0.135 in broiler to 0.229 in Fayoumi chickens. The low levels of variability within both broilers and layers were expected as the individuals of commercial breeds are expected to be more similar to each other than the native breeds. El-Gendy et al., [6] reported that genetic variability was higher in Fayoumi chickens (0.50) compared to commercial broiler chicken (0.38). Also, high levels of similarity (0.74 - 0.87) were found between indigenous chicken strains in Egypt [20]. The analysis also revealed that the two Saudi chicken lines were genetically variable compared to the two Egyptian breeds. This difference may be ascribed to the differences in population structure.

Table 2: Total number of amplified bands, overall individuals, by primer and breed.

Primer	Fayoumi	Dandrawi	Hajar1	Hajar2	Broiler	Layer
OPA-01	64	55	61	61	64	76
OPA-03	45	50	53	51	47	77
OPA-05	55	54	62	69	56	68
OPA-06	84	78	79	83	85	85
OPA-08	74	64	75	60	55	67
OPA-18	69	62	73	82	78	53
OPB-03	72	83	82	68	62	78
OPB-07	35	45	38	38	31	33
OPB-14	71	83	85	95	82	78
OPC-06	78	73	66	76	78	63
Average±SE	64.7±4.8	64.7±4.4	67.4±4.6	68.3±5.3	63.8±5.5	67.8±4.8

Table 3: Average (Mean ± SE) number of different alleles (Na), effective number of alleles, Shannon's information index (I) and expected heterozygosity (He).

Breed	Number of different alleles (Na)	Effective number of alleles (Ne)	Shannon's Information Index (I)	Expected Heterozygosity (He)
Fayoumi	1.227±0.062	1.223±0.031	0.197±0.025	0.131±0.017
Dandrawi	1.203±0.064	1.248±0.034	0.206±0.026	0.140±0.018
Hajar1	1.163±0.061	1.220±0.031	0.185±0.025	0.126±0.017
Hajar2	1.228±0.061	1.246±0.034	0.202±0.026	0.138±0.018
Broiler	1.008±0.065	1.192±0.032	0.153±0.024	0.105±0.017
Layer	1.211±0.063	1.282±0.036	0.226±0.027	0.156±0.019

Ne=1/(p^2+q^2), I = -1×(p×Ln (p)+q×Ln(q)), and He = 2×p×q.

Table 4: Within-breed genetic variability estimates.

Primer	Fayoumi	Dandrawi	Hajar1	Hajar2	Broiler	Layer
OPA-01	0.273	0.375	0.307	0.238	0	0.208
OPA-03	0.375	0.306	0.338	0.203	0.266	0.125
OPA-05	0.141	0.156	0.225	0.281	0.3	0.227
OPA-06	0.192	0.25	0.177	0.202	0.183	0.183
OPA-08	0.288	0.273	0.219	0.318	0.141	0.302
OPA-18	0.216	0.139	0.17	0.146	0.025	0.054
OPB-03	0.308	0.259	0.146	0.227	0.031	0.188
OPB-07	0.271	0.063	0.208	0.208	0.225	0.175
OPB-14	0.113	0.057	0.034	0.087	0.068	0.188
OPC-06	0.114	0.088	0.083	0.05	0.114	0.016
Average±SE	0.229±0.028	0.196±0.035	0.191±0.029	0.196±0.026	0.135±0.033	0.166±0.026

The global Analysis Of Molecular Variance (AMOVA) was used to estimate the genotypic variance within and among different breeds (Table 5 and Figure 1) according to Ex coffier et al., [21], and resulted in a significant partitioning of the genetic variation (P<0.001), where 49% of the total genetic variation was occurring within-population and 51% was occurring among populations. Nevertheless, when the levels of variation in the Egyptian and Saudi breeds were analyzed separately, a significant partitioning (P<0.001) was attributable to differences within-breed with percentages of 67 and 63% for Egyptian and Saudi breeds respectively. However, unlike the local breeds, the within-breed variation was found to be significantly (P<0.001) less (35%) than that of among breeds (65%) in the individuals of the two commercial breeds. According to these results, and it became obvious that the individuals of local breeds showed much variation than commercial breeds, which may be attributed to the lack of breeding programs that should be practiced to improve the productivity of such breeds.

Table 5: The analysis of molecular variance (n = 999 permutations) of all breeds, the two Egyptian breeds (Fayoumi and Dandrawi), the two Saudi breeds (Hajar1and Hajar2), and the two commercial breeds (Broiler and Layer).

Source of variation	df	SS	MS	variance component	P-value
All breeds					
Among Pops	5	421.646	84.329	9.405	<0.001
Within Pops	42	381.625	9.086	9.086	<0.001
Egyptian breeds					
Among Pops	1	47.375	47.375	4.705	<0.001
Within Pops	14	136.25	9.732	9.732	<0.001
Saudi breeds					
Among Pops	1	51.938	51.938	5.339	<0.001
Within Pops	14	129.125	9.223	9.223	<0.001
Commercial breeds					
Among Pops	1	130.625	130.625	15.29	<0.001
Within Pops	14	116.25	8.304	8.304	<0.001

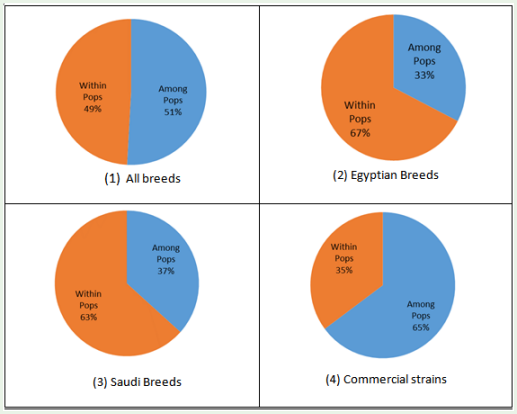

Figure 1: The analysis of molecular variance of (1) All breeds, (2) Egyptian breeds (Fayoumi and Dandrawi), (3) Saudi breeds (Hajar1 and Hajar2), and (4) commercial strains (broiler and layer).

Table 6: Pairwise population matrix of Nei genetic distance* (blow diagonal) and pairwise population matrix of Nei genetic identity (above diagonal).

	Fayoumi	Dandrawi	Hajar1	Hajar2	Broiler	Layer
Fayoumi		0.888	0.85	0.822	0.768	0.744
Dandrawi	0.119		0.898	0.861	0.768	0.748
Hajar1	0.162	0.107		0.878	0.762	0.75
Hajar2	0.196	0.15	0.13		0.865	0.749
Broiler	0.264	0.264	0.271	0.145		0.699
Layer	0.296	0.291	0.288	0.29	0.358	

*Nei Genetic Distance = -1 * Ln (Nei Identity).

Genetic relationships

The pairwise matrices of genetic distance and genetic identity are presented in table 6. Moderate to high levels of genetic identity were estimated. The highest identity estimation (0•898) was found between Dandrawi and Hajar2 breeds while the lowest (0•699) was found between the two commercial breeds (broiler and layer). However, the longest genetic distance was found between broiler and layer breeds, and the shortest one was found between Dandrawi and Hajar1 breeds. The Shannon's Information index was generally low and showed greater diversity in Dandrawi compared Fayoumi in the Egyptian breeds, and in Hajar2 compared to Hajar1 in the Saudi breeds. The same trend was found for Jordanian chickens [8], where Shannon's index ranged between 0.42 to 0.60 (mean = 0.58) and genetic distance ranged between 0.04 to 0.37. Tarik et al., [22] reported that the average Shannon index was 1.61 between Egyptian indigenous chicken breeds (Fayoumi, Dokki-4, Golden Montazah, Silver Montazah, and El-Salam). Although Shannon information index is considered a good index of diversity, it considers the diversity is better when the number of breed increases. Moreover, genetic diversity levels maintained in the different population may be attributed to different factors including population size and recurrent gene flow [23]. Although genetic distance among different breeds or species may be calculated by various statistical methods, we used Nei distance [24] which is considered the most common distance used in the phylogenic analysis. The UPGMA phylogenic tree was built in using Mega6 software [14]. The dendrogram (Figure 2) showed that the two Saudi breeds were too close to each other while Dandrawi breed was adjacent to Hajar2 breed, any both share the same ancestral point while Fayoumi was close to Hajar1 breed. The phylogenetic analysis confirmed the results of genetic variability as the two local Saudi breeds were much similar compared to the two local Egyptian breeds. Moreover, all the four local breeds were closer to broiler than layer chickens, which suggest that breeds may be successfully directed to meat production than egg production.

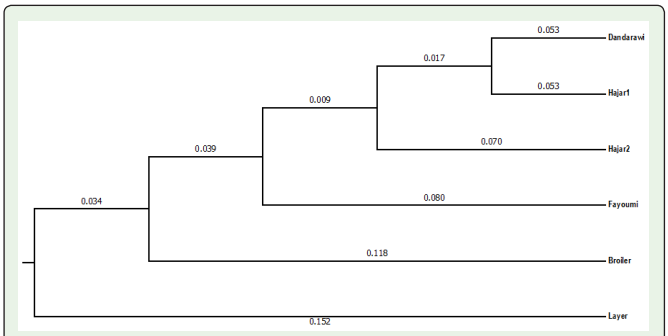

Figure 2: Phylogenetic dendrogram of the six chicken populations, based on Nei genetic distance [24].

Conclusion

RAPD markers are efficient enough when used in estimating variability and diversity levels as well as constructing phylogenic relationship trees among different chicken breeds. However, we could not rely on RAPD markers when used for distinguishing breeds by identifying specific bands, hence, the amplified bands are not universal for each breed and primer. Therefore, this study did no focus on the size of amplified bands and did not mention the different unique bands for each breed. The results of RAPD analysis in this study provided good information in discerning the genetic variability levels within and among Egyptian, Saudi and commercial populations. Such information can be utilized for the designing of breeding programs to improve the productivity of local breeds. In addition, further research on the divergence of the local populations is recommended.

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