ASSESSING THE GENETIC VARIABILITY IN EGG PRODUCTION AND EGG QUALITY TRAITS OF FOUR EGYPTIAN LOCAL STRAINS OF CHICKENS USING RAPD AND SSR TECHNIQUES

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SUMMARY

   Local chicken strains are important, especially to the smallholder systems in developing countries. In this study we attempt to estimate the genetic characterization of some Egyptian local chicken strain/populations using SSR and RAPD markers. In four populations of Fayoumi, Gimmizah, Bandarah and Mandarah strains, egg production and quality parameters (egg number, weight, mass, eggshell strength, albumen weight, albumen percentage, Haugh units and yolk index) were recorded and evaluated for the first 3 months of the production cycle. Fayoumi eggs recorded a significant increase in the eggshell strength and yolk percentage when compared to other strains. On the other hand, Fayoumi hens recorded a significant decrease in egg production, egg weight and egg mass compared to Gimmizah, Bandarah and Mandarah ones. Yolk index showed a significant increase for Fayoumi eggs compared to all studied eggs strains. Moreover, albumen weight and albumen percentage recorded a significant increase for eggs of Bandarah and Mandarah compared to those of Fayoumi and Gimmizah strains. Six SSR and five RAPD primers were used to estimate genetic heterogeneity. Molecular marker analysis indicated that Bandarah and Mandarah showed a similarity of 60% in one group, while Gimiezah's similarity only reached 53%. On the other hand, Fayoumi was less similar to the other tested strains (about 40%). Heterozygozity within each studied strain -based on allele frequency- was also studied. Fayoumi breed showed lower heterozygozity (0.23) than Gimieizah, Mandara and Bandara which showed 0.26, 0.30 and 0.31 heterozygote values respectively. So, we recommend that SSR technique is useful in establishing genetic relationship among the populations and in indicating the purity of the breeds.

Keyword: RAPD, SSR, genetics, Egyptian strains, production parameters

INTRODUCTION

   Poultry production is an important livestock sector for supplying humans with animal protein. It is especially favorable to the smallholder systems in developing countries due to low capital investment and high cost efficiency. Egyptian local chicken strains are important, but the information on their genetics with respect to performance, adaptability, resistance, genetic variability and genetic relationships are little. Chicken are rich in genetic diversity and have a high recombination rates (Siegel *et al*., 2006). Molecular markers such as RAPD and SSR have so far proved to be useful in establishing genetic relationship among the livestock populations including poultry (Hillel *et al*., 1992). Different genetic marker systems have successfully been used in poultry for estimation of genetic characterization of chicken breeds and populations including RAPD (random amplified polymorphic DNA) (Singh and Sharma, 2002) and microsatellites (Kaiser *et al*., 2000 and Osman *et al*., 2006). Information on genetic relationship, both within and between the populations has several potential applications in breeding programs, such as selection of parents for crossing predicting the genetic gains and testing population genetic hypotheses. Most of the genetic studies demonstrated that epistatic effects have been increasingly recognized as important genetic factors in the inheritance of quantitative traits (Sheridan and Randall, 1977; Sheridan, 1980; Fairfull *et al*., 1985 and 1987 and Abou El-Ghar and Abdou, 2004).

   Abou El-Ghar *et al*. (2011) had a framework of crossing Matrouh as a common chicken parent with different strains of chickens that was done to investigate the effect of single crossing on some productive traits; as a consequence of crossing Matrouh as a sire parent with Silver Montazah, Mandarah and Inshas developed strains of chicken. Abou El-Ghar *et al*. (2007) ; Ghanem *et al*. (2008) reported that egg production can be benefited by reducing age and increasing body weight at sexual maturity while increasing egg weight at different ages of production. Moreover, crossing Matrouh as a female parent with Silver Montazah, Mandarah and Inshas improved egg number and egg mass at different periods of laying (Ghanem *et al*., 2008). Genetic selection procedures for important economic traits may negatively impact the subsequent productive efficiency of breeds, for both meat and egg production (McGary *et al*., 2002). Estimation of genetic diversity within and between chicken populations as well as association between

microsatellites and economic traits has been undertaken using such markers (Pandey *et al*., 2005; Chatterjee *et al*., 2008&2010; Rajkumar *et al*., 2008 and Radwan *et al*., 2014). In this research, the role of the genetic diversity in improving some egg production and egg quality traits were studied for fours Egyptian local strains using six microsatellites (SSR) and five RAPD primers.

MATERIALS AND METHODS

This experiment was designed to study the genetic variability in some egg production and egg quality traits of three Egyptian developed strains (Gimmizah, bandara and mandara) and one pure local breed (Fayoumi). The farm work was carried out at El Takamoly Poultry Project, Fayoum, Egypt, while the lab work was done at the Dept. of Poultry Production, Faculty of Agriculture, Ain Shams University and the Dept. of Genetics, Faculty of Agriculture, Tanta University. The flocks after hatching used in this experiment were reared under the same environmental, managerial and hygienic conditions. They were housed in individual cages placed in an open-sided house. They were fed a laying diet containing 16.3% CP and 2924 kcal ME/kg. Feed and drinking water were offered to birds *ad libitum*, whereas conventional breeding and management procedures were applied throughout the experimental period which lasted up to ٥٠ weeks of age. The lighting schedule was maintained at 16 hours of light and 8 hours of darkness throughout the experiment.

Production performance:

Egg production (number and weight) was recorded for the first 3 months of the production cycle to calculate egg mass. To assess internal egg quality parameters, a total of 240 eggs were randomly collected from each strain at 35 weeks of age. The dimensions of eggs (width and length) were measured using a digital caliper to calculate shape index. Each egg was first weighed to the nearest 0.1 g and broken open. The height of albumen and yolk was measured using a micrometer mounted on a stand with a platform on which the liquid content was placed. Each egg yolk was separated from the albumen using a plastic egg separator, rolled on a tissue paper to a well to remove any adhering albumen and was then weighed. Albumen yield was determined by subtraction of the yolk and shell weight intact from the whole egg weight. The percentage of egg components (yolk, albumen and shell) was calculated as the ratio of egg component to egg weight multiplied by 100. Yolk index (yolk height/yolk diameter) was also calculated. Haugh units were calculated according to Stadelman *et al.* (1988). Eggshell was weighed to the nearest 0.01g; then its percentage was calculated.

Genomic DNA isolation:

Twelve blood samples were randomly collected from each strain into vacuum tubes containing EDTA and stored at -20°C. Genomic DNA was isolated from blood samples using AXYGEN kit (Axyprep TM) from Axygen Scientific, inc. USA Cat. No. AP-MN-BL-GDNA-50. DNA concentration was determined using spectrophotometer and the final concentration was adjusted up to 50 ng/μl for PCR analysis.

Molecular marker analysis (SSR and RAPD):

Genomic DNA of twelve individuals from each strain was used as a template for PCR which were carried out against six SSR and five RAPD primers. The codes, sequences and melting temperatures of these primers are shown in Table (1). PCR was performed in 25 μl volumes containing 12 μl of PCR Master mix 2x (CinnaGen/ Iran), 2 μl of each primer (10 pmol/ μl), 1 μl genomic DNA (50 ng/ μl) and 10 μl sterile deionized water were added.

PCR analysis:

PCR reactions were achieved in thermal cycles (LongGene - MG96G / china) that were differently programmed for each of the primers. The initial denaturation temperature of the first cycle was 94 C° for 4 min for all primers. The next 30 cycles varied in temperature settings (for 1 min) according to each primer. Final extension was 72 C° for 5 min for all primers.

PCR products (15 μl of each reaction) and 100 bp DNA ladder (Larova GmbH- Germany) as DNA size marker were resolved by electrophoresis on 2 % agarose gel containing ethidium bromide for 90 min. at 70 volt, visualized via UV transilluminator and then photographed. Molecular size of the amplified fragments separated on gels was measured by analyzing gel images with Gel Analyzer software package version 2010a.

Statistical analysis:

Data were subjected to a one-way ANOVA using GLM (SAS Institute, 2001) with strains as fixed effect.
The statistical model used in this study was as follows;
$Y_{ijk} = \mu + B_i + e_{ij}$
Where; μ= Overall mean, B_i = strain effect and e_{ij} = Experimental error.

Microsatellite and RAPD data analysis:

The amplified bands were scored for each microsatellite and RAPD marker based on the presence or absence of bands, generating a binary data matrix of 1 and 0 for each marker system. The data matrix was then analyzed using the PAST, ver. 1.90 (Hammer *et al*., 2001), Then was used to calculate the genetic similarity based on Jaccard's similarity coefficients.

Table 1. Codes, sequences and annealing temperatures of the used primers

Primer Name /	Primer sequence	Annealing temp.	No of allele expected	Allele size (bp)
ADL 115	F (5⁻ GGATGAGAAGAAAGGCA 3⁻)	50° C	5	85-500
	R (5⁻ CAATGGTGGTTCAGGTAATC 3⁻)	56° C		
ADL 209	F (5⁻ GGTTAGCTCCCTCCTTCCAG 3⁻)	63° C	4	150 -300
	R (5⁻ AAGGAAACAAAGAGAAATCC 3⁻)	52° C		
ADL 231	F (5⁻ ACTATTAGCCTGGGGAGAGC 3⁻)	60° C	5	100 – 670
	R (5⁻ TCACTCCAGCTTGAGACAGG 3⁻)	60° C		
APH 23	F (5⁻ GGATGAGAAGAAGAAAGGCA 3⁻)	56° C	6	95- 270
	R (5⁻ AAGGAAACAAAGAGAAATCC 3⁻)	52° C		
APH 24	F (5⁻ GGATGAGAAGAAGAAAGGCA 3⁻)	56° C	5	100- 490
	R (5⁻ CAATGGTGGTTCAGGTAATC 3⁻)	56° C		
APH-09	F (5⁻ GGATGTTGCCCCCACATATTT 3⁻)	56° C	5	95- 370
	R (5⁻ TTGCCTTGTTTATGAGCCATTA 3⁻)	52° C		
OPA-01	5\ CAGGCCCTTC 3\	34° C	9	220 - 1700
OPB-03	5\CATCCCCCTG 3\	34° C	11	200 – 2800
OPB-14	5\ TCCGCTCTGG 3\	34° C	8	350 – 1700
OPG-01	5\ CTACGGAGGA 3\	34° C	6	290 – 1500
OPZ-01	5\ TCTGTGCCAC 3\	34° C	7	290 – 1650

Table 2. Means ±SE of egg production characteristics for different Egyptian strains

Traits	Strain				P-Value
	Fayoumi	**Gimmizah**	**Bandarah**	**Mandarah**	
Percent egg production	79.25± 1.89[b]	82.50±1.48[ab]	85.45±1.90[a]	80.48±1.76[ab]	0.03
Egg mass (gm)	3256.48±78.76[b]	3749.88±86.27[a]	3599.81±93.92[a]	3608.56±105.60[a]	0.002
Egg weight (gm)	42.33±0.56[b]	50.47±0.65[a]	50.92±0.57[a]	49.56±0.46[a]	0.0001

a,b values in the same row for each parameter with different superscripts are significantly different (P<0.05)

RESULTS AND DISCUSSION

Table (2) shows egg production characteristics for the four different strains studied. The Fayoumi hens had a significant) P<0.05) decrease in egg production, egg weight and egg mass when compared with the other strains (Gimmizah, Bandarah and Mandarah).

Table (3) shows the means ±SE of egg quality characteristics for the Egyptian strains studied. The eggshell strength recorded a significant (P<0.05) increase for Fayumi hens (4.88) when compared with Gimmizah (4.10), Bandarah (4.37) and Mandarah (4.01) strains. Mandarah eggs recorded a significant decrease in Haugh units compared to Fayoumi, Gimmizah and Bandarah eggs. Yolk index showed a significant increase for Fayoumi (49.02) than Gimmizah (46.76), Bandarah (42.56) and Mandarah (41.66). Moreover, albumen weight and albumen percentage showed a significant increase for Bandarah (31.70g, 61.35%) and Mandarah eggs (32.47g, 59.72%) when compared with Fayoumi (26.54g, 57.45%), Gimmizah (25.55g, 52.84%) ones.

Gimmizah and Mandarah eggs had a higher significant heavier yolk weight when compared with Fayoumi and Bandarah. Yolk percentage for Gimmizah eggs (37.08) showed a significant increase than Fayoumi (31.59), Bandarah (29.69) and Mandarah (30.52) ones.

Fathi *et al.* (2010) recorded that the Mandarah strain eggs had better shell strength and ultrastructural formation compared to Bandarah one; but no significant difference between those for internal egg quality properties. On the other hand, Gimmizah strain eggs represented higher significant values for egg weight, yolk index and albumen percentage when compared to Mandarah eggs (Afifi *et al.*, 2010).

In the current study, SSR and RAPD techniques were used to determine the achieved genetic improvement for egg production traits in both developed strains and pure ones; also, similarity and the degree of heterozygousity were measured.

Genetic variability estimates for each strain are summarized in Table (4). A total of 71 alleles were observed at the 11 loci in 48 individuals from 4

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chicken strains. The number of alleles observed across the microsatellite loci studied varied from 4 (ADL209) to 6 (APH23) with an overall mean of 5.00, while the corresponding values recorded when using the RAPD markers varied from 6 (OPG-01) to 11(OPB-03) with an overall mean of 8.20.

To estimate similarity between the four different populations (Fayoumi, Gimmizah, Bandarah and Mandarah); genetic distance between the populations was calculated based on band frequency of all the eleven primers used the PAST program; version 1.90 based on Jaccard's similarity coefficients.

Data in Figure (1) indicate that Bandarah and Mandarah showed similarity of 60% in one group, while Gimiezah had a 53% similarity with them. On the other hand, Fayoumi was less similar to the other tested strains (about 40%) and can be seen as a separate group.
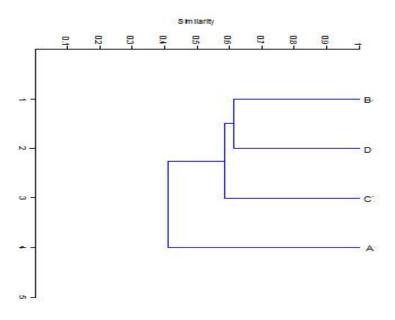
Table 3. Means ±SE of egg quality characteristics for different Egyptian strains

Traits	Strain				P- Value
	Fayoumi	**Gimmizah**	**Bandarah**	**Mandarah**	
Hugh unit	91.01± 1.03[a]	90.70±0.58[a]	90.70±0.58[a]	81.60±0.80[b]	0.0001
Yolk index	49.02±0.65[a]	46.76±0.76[b]	42.56±0.52[c]	41.66±0.39[c]	0.0001
Albumin weight	26.54±0.46[b]	25.55±0.71[b]	31.70±0.49[a]	32.47±0.49[a]	0.0001
Albumin percentage	57.45±0.57[b]	52.84±1.16[c]	61.35±0.36[a]	59.72±0.49[a]	0.0001
Yolk weight	14.59±0.27[c]	17.77±0.49[a]	15.30±0.20[c]	16.52±0.15[b]	0.0001
Yolk percentage	31.59±0.47[a]	37.08±1.19[b]	29.69±0.34[b]	30.52±0.42[b]	0.0001
Shell weight	4.55±0.25[b]	4.87±0.11[ab]	4.63±0.14[b]	5.32±0.18[a]	0.01
Shell percentage	9.92±0.49[ab]	10.21±0.13[a]	8.97±0.25[b]	9.65±0.31[ab]	0.05
Egg weight	45.75±0.70[d]	48.19±0.59[c]	51.64±0.63[b]	54.31 ±0.69[a]	0.0001
Shape index	77.23±0.47	77.91±0.82	75.84±0.38	76.52±0.50	NS
Shell strength	4.88±0.07[a]	4.10±0.07[c]	4.37±0.10[b]	4.01±0.12[c]	0.0001

a,b,c values in the same row for each parameter with different superscripts are significantly different (P<0.05)

Table 4. Measures of genetic variation in each of the studied Egyptian chicken strain

chicken strain	Locus											Heterozygosity within the tested population
	ADL 115	ADL 209	ADL 231	APH 23	APH-09	APH 24	OPZ-01	OPB-14	OPG-01	OPB-03	OPA-01	
Fayoumi	Observed No of alleles											0.23
	4	3	4	4	5	5	5	4	4	5	9	
	range size (bp)											
	85-500	150-300	140-670	90-270	95-370	160-490	350-950	350-600	490-1670	320-1400	370-1700	
Gimmizah	Observed No of alleles											0.26
	4	3	4	4	5	5	6	6	3	4	7	
	range size (bp)											
	85-500	150-300	140-670	90-270	95-370	160-490	350-1190	300-1770	490-1670	200-900	220-1100	
Bandarah	Observed No of alleles											0.31
	4	3	4	4	5	5	5	3	4	8	7	
	range size (bp)											
	85-500	150-300	140-670	90-270	95-370	160-490	235-995	350-600	500-1770	200-1100	220-1100	
Mandarah	Observed No of alleles											0.30
	5	3	4	4	5	5	5	3	4	6	7	
	range size (bp)											
	85-500	150-300	140-670	90-270	95-370	160-490	235-995	350-600	500-1770	200-1700	220-1100	

Fig. 1. Rooted neighbor joining (NJ) phylogenetic tree for the four studied Egyptian genotypes based on SSR makers. A, B, C and D are Fayoumi, Bandarah, Gimmizah and Mandarah, respectively

The Fayoumi breed had inserted into selection program to produce different Egyptian development strains, Fayoumi breed is considered as a grandparent to Gimmizah and Mandarah strains; while, Fayoumi breed had considered grand grand parent for Bandarah strain, the similarity between them reflected these relationships.

Table (4) shows the observed heterozygosity within each studied chicken genotype. The high heterozygosity estimate for most of the loci in the present study were probably due to existence of a large number of heterozygous alleles according to Chatterjee *et al.* (2010). Accordingly, the estimated haterozygosity for Fayoumi breed was lower (0.23) than the other three strains (Gimieizah 0.26, Bandara 0.31and Mandara 0.30 stains).

The current chicken genetic map contains at least 1,965 loci within 50 linkage groups, and it covers about 4,000 cM. The remaining loci are anonymous molecular DNA markers, including SSR, AFLP, ISSR, CR1 elements, and others (Emara and Kim, 2003).

The magnitude of additive effect is much greater than the dominance effect in some egg production traits (Szydlowski and Szwaczkowski, 2001 and Abou El-Ghar *et al.,* 2009). However, epistatic effects were detected as a major mechanism of heterosis. Bauman (1959) proposed that epistasis is indicated if the performance of the progeny of a cross between a single cross and a tester deviates significantly from the average performance of the single crosses produced by crossing two inbred lines. Crow and Kimura (1970) reported that physiological epistasis contributes to additive, dominance and interaction genetic values and variances. Evidence supporting epistasis as a major mechanism of heterosis in chickens has been reported (Szydlowski and Szwaczkowski, 2001; Sheridan, 1980 and Fairfull *et al*., 1985 and 1987). The heterotic effects on egg production traits include variability produced by both dominance and epistasis (Fairfull *et al.*, 1985, 1987 and Abou El-Ghar and Abdou, 2004). The objective of the current study was to achieve genetic improvement for egg production in crossbreds by applying crossbred methods between two local developed strains. The high heterozygosity estimate for most of the loci in the present study were probably due to the existence of a large number of heterozygous alleles according to Chatterjee *et al.* (2010). So the estimated haterozygosity for Fayoumi breed was lower (0.23) than the other three strains (Gimmizah 0.26, Bandara 0.31and Mandara 0.30 stains), because the Fayoumi breed is considered as a pure Egyptian local breeds; while, the other strains (Gimmizah , Bandara and Mandara stains) produced cross breeding between a native strain and foreign strain Kosba and Abd El-Halim (2008).Continued genetic improvement of livestock is dependent on the fact that substantial genetic variation exists within individual breeds allowing them to respond to selection for different traits (Slavėnaitė *et al.,* 2004). The RAPD and SSR techniques examining population structure, diversity and gene flow in different (Fayoumi, Gimmizah, Bandara and Mandara) Egyptian local strain.

In the present study SSR and RAPD techniques were used to determine the appropriate number of alleles, range size and heterozygosity. This study ended with an important suggestion that chicken specific markers may be useful for examining the

population structure and gene flow in a range of antedate. Although the detected genetic markers may have a known function and marked influence of trait variation. They can be used in combining information from a number of samples to draw a phylogenetic tree which considered as a good characterization of the studied population. The increasing numbers of available markers provides an elementary and powerful tool for better understanding of the genetic variance and population structure related to different Egyptian local strains

CONCLUSION

The Fayoumi breed showed low similarity and heterozygousity than Gimiezah, Bandarah and Mandarah Egyptian strains. SSR technique was used to determine the achieved genetic improvement for egg production traits in both developed strains and pure ones. The genetic findings that were made can be used in selection program for these egg production traits in early ages instead of remaining until they began their production.

ACKNOWLEDGMENTS

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تقييم التنوع الوراثى لصفات انتاج البض وجودة البيض فى اربع سلالات الدجاج المحلى المصرى بإستخدام RAPD وSSR

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تعتبر سلالات الدجاج المحلية مهمة خاصة فى نظم الانتاج الصّغيرة فى الدول النامية. فى هذه الدراسة نحاول تقدير التوصيف الوراثى لبعض سلالات/عشائر الدجاج المصرى المحلى باستخدام واسمات الـ SSR و RAPD . تم تسجيل وتقييم بيانات جودة البيض (عدد ووزن وحجم البيض، صلابة القشرة، وزن الألبيومين ونسبته، ودليل الصفار وHaugh units) فى أربع سلالات محلية هى: فيومى وجميزه و بندره و مندره وذلك فى الثلاث أشهر الأولى من دورة انتاج البيض. أظهر بيض السلالة فيومى زيادة معنوية فى كل من صلابة القشرة ونسبة الصفار مقارنة بالسلالات الأخرى. من جهة أخرى كانت السلالة فيومى أقل بنسب معنوية فى كل من انتاج البيض ووزن وحجم البيض مقارنة بالسلالات الثلاث الأخرى. وقد أظهر دليل الصفار زيادة فى جميع السلالات الأربع. بينما أظهر وزن الألبيومين ونسبته زيادة معنوية فى بيض السلالتين بندرة ومندره مقارنة بالسلالتين جميزه وفيومى. وتم فى هذه الدراسة استخدام ٦ واسمات SSR و خمس واسمات RAPD لتقدير الخلط الوراثى (النقاوة) داخل كل سلالة ودرجة التشابه بين الاربع سلالات. وأظهرت نتائج التحليل الجزيئى أن السلالتين بندره ومندره تقعان فى مجموعه واحدة وبينها تشابه حوالى ٦٠% وتتشابه معها السلالة جميزه بحوالى ٥٣% بينما تقع السلالة فيومى فى مجموعة مستقلة وكانت أقل تشابها معهم بحوالى ٤٠%. أما نسبة الخلط فقد أظهرت النتائج السلالة فيومى كانت أقلهم خلطا بقيمة ٠.٢٣ (أى أكثرهم تماثلا) بينما كانت نسبة الخلط بالسلالات جميزة ومندره وبندره هى ٠.٢٦ و٠.٣٠ و ٠.٣١ على التوالى. ومن خلال هذه الدراسة نستطيع التوصية بأن تكنيك الـ SSR يفيد فى تقدير درجة الخلط داخل السلالات وأيضا درجة التشابه بين السلالات.