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GENETIC DIVERSITY OF CRESTED AND NON CRESTED DANDARAWI EGYPTIAN NATIVE CHICKEN BASED ON MICROSATELLITE MARKERS

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ABSTRACT: The present study was conducted to evaluate the genetic diversity and relationships of Dandarawi Egyptian native chicken phenotypes (crested (Cr) and non-crested (cr), using four microsatellite markers. According to morphological appearance this study was estimated various in reproductive traits of Dandarawi Egyptian native chicken phenotypes (crested (Cr) and non-crested (cr)). Seventy-two female from each of Cr and cr layer Dandarawi hens were chosen when egg production (EP) reached 50 %. Crested phenotype had higher significantly egg weight and egg number than cr during 2nd month and overall period. Four microsatellite markers were chosen based on the degree of polymorphism reported in the previous literature. The results revealed that all studied markers exhibited a varied percentage of polymorphism among native chicken populations. Based on cluster analysis two main clusters of chicken populations were identified. Two lines comprised of Dandarawi Egyptian native chicken phenotypes (crested (Cr) and non-crested (cr). Higher similarity was found between either (S10, S11), (S19, S29), (S2, S3), (S25, S26), or (S6, S7) populations. Genetic distance between each couple was 89.6%, 86.9%,78%,77.4% and 69.5% respectively. The genetic diversity we found among populations may be of interest for improving productive performance and adaptability.

Key words: Dandarawi-Crest gene-Microsatellite Markers-Simple Sequence Repeats Assay

INTRODUCTION

The Crest phenotype is characterized by a bunch of outspread feathers upper the head (Wang *et al.,* 2012) and found in many bird species (Price, 2002). The appearance of the Crest depends on the length and shape of the cranial feathers (Wang *et al.,* 2012). Crest is controlled by autosomal incompletely dominant gene (Fathi and Galal, 2001). The genetics experiments observed the head crest segregates as a simple Mendelian recessive trait (Wang *et al.,* 2012). The chicken's phenotype crest has feather mass more about 12% of non-crest chicken (Galal, 2003).Dandarawi chicken was developed in Upper Egypt and could be simply sexed by feather color and considered good layer (Thomas *et al*., 2010). Fathi *et al*. (2000) reported that crest gene in Dandarawi chicken remarkably improved the abnormality and viability of spermatozoa. Genetic diversity of chicken genetic resources provides the basis for genetic improvement not only to increase productivity but also to adapt domestic populations to changes in production environments as well as in markets, management practices and disease challenges (Boettcher *et al.,* 2010). Microsatellite markers have been shown to be an appropriate tool to estimate the genetic diversity among chicken populations (Abebe *et al*.,2015) The usefulness of microsatellite markers in estimating genetic similarity and diversity in chicken have been demonstrated in a number of indigenous breeds, inbred strains and in commercial chicken lines (Tadele, 2003).These markers are co-dominant and highly reproducible. Additionally microsatellites have been reported to be a very efficient tool for diversity analysis because of their high degree of polymorphism, random distribution across the genome, co-dominance, possibility of automated scoring of genotypes and neutrality with respect to selection (Dixit *et al.,* 2012).Our research has adopted a series of experiments to study their genetic and phenotypic characteristics. Therefore, the current study was designed to evaluate the diversity level and genetic relationship between 30 populations of chicken using 4 microsatellite markers.

MATERIALS AND METHODS

This experiment was conducted at Fayoum poultry research station and Molecular genetic lab at faculty of agriculture, Ain Shams University.

Experimental design and productive performance:

The female Dandarawi laying hens from each of (Cr) and (cr) were chosen when they reached 50% egg production percent (EP %). In addition, the chickens were fed control basal diet that satisfied recommendations of NRC (1994).Each bird was housed separately in single cage for three months. Eggs from each replicate were collected, numbered and weighed daily. Egg production percent, average egg weight calculated according to Murugesan and Persia (2013). Ten eggs from each replicate were chosen to evaluate egg quality during the third month of egg production.

Rectal temperature and respiration rate:

At the end of 3rd month of egg production two hens from each replicate were chosen randomly while, respiration

rate and rectal temperature were recorded. Rectal temperature (°C) was measured by inserting thermometers approximately 5 cm into rectal. Respiration rate was recorded by counting the wave cycles of breast up and down per minute. The ambient temperature was 15°C when these items were measured.

Statistical analysis:

One-way ANOVA using SPSS Ver.20 was carried out to compare means among breeds for the production traits assessed. Significant scales ($P \leq 0.05$) were tested using Duncan's Multiple Range Test (Duncan, 1955).

Microsatellite Markers

A total of 4 microsatellite markers were chosen based on the degree of polymorphism reported in the literature to identify the genetic diversity among Dandarawi Egyptian native chicken phenotypes (crested (Cr(S1- S15) and non-crested (cr (S16-S30). Four markers were chosen from the list of microsatellite markers recommended by the Standing Committee (MoDAD project, FAO, 2004). Four markers correlated with body weight, body temperature and egg production traits were assigned according to Roushdy *et al.* (2008). Additionally, all used marker information presented in table (1).

Blood Samples and DNA Extraction

Nucleic acid extraction a total of blood samples representing Dandarawi Egyptian native chicken phenotypes (crested (Cr) and non-crested (cr) were used to extract DNA. Approximately one mL blood/bird from a wing vein was collected in EDTA tubes and stored at –20◦C. genomic DNA was extracted a whole blood (Thermo Fisher Scientific) for automated specimen processing by use of a Maxwell blood purification kit, according to the manufacturer's instructions (Thermo Fisher Scientific), with elution into 200 µl of 10 mM Tris-Cl–0.5 mM EDTA buffer (pH 9.0). PCR assays were evaluated on calibrated DNAs (2 to 10 ng/µl), except when indicated. The concentration of DNA was determined spectrophotometrically by measurement of the absorbance at 260/280 nm using a Nanodrop 1000 apparatus (Thermo Fisher Scientific).

Simple Sequence Repeats Assay (SSR)

Amplification was carried out in 25 µl reaction volumes, containing 1X Taq polymerase buffer (50 mM KCl, 10 mM Tris, pH 7.5, 1.5 mM MgCl2) and one unit of Taq polymerase (Pharmacia Biotech, Freiburg, Germany) supplemented with 0.01% gelatin, 0.2mM of each deoxynucleotide triphosphates (dNTPs) (Pharmacia Biotech, Freiburg, Germany), 50 bp of SSR primers, and 50 ng of total genomic DNA. Amplification was performed in a thermal cycler (Thermolyne Amplitron, Barnstead Thermolyne Corporation, Dubuque, IA) programmed for one cycle of 2 min at 94˚C; and 35 cycles of 30 s at 94˚C, 45 s at either 42 or 50˚C, depending on the melting temperature (Tm) value of the primer pair, and 1.3 min at 72˚C; followed by 20 min at 72˚C. After completion of PCR, samples were cooled immediately to 10˚C and stored at 4˚C until gel separation. A gel-loading solution (5 µl) was added, and 10 µl of the total product volume was resolved in 1.5% agarose in 1X Tris-Boric-EDTA (TBE) buffer for 1 h aside, with a 100-bp ladder (Pharmacia, Germany) as the size standard. Gels were stained in ethidium bromide and images were recorded.

Data Analysis

Data of SSR analyses were scored based on the presence or absence of the amplified products for each primer. If a product was present in a cultivar, it was designated "1," if absent it was designated "0." Pair-wise comparisons among the Dandarawi Egyptian native chicken phenotypes (crested (Cr (S1-S15) and non-crested (cr (S16-S30) based on SSR markers with 25 to 75% heterozygosity, were used to generate Dice coefficients of similarity (Nei and Li, 1979). The similarity coefficients were then used to construct a dendrogram by Unweighted Pair-Group Method with Arithmetical Average (UPGMA) using NTSYS-PC software version 2.0 (Rohlf, 2000).

RESULTS AND DISCUSSION

Egg weight (EW)

The data presented in Table 2 showed that EW of *Cr* gene was significantly higher than *cr* gene for second month and for the overall period. These results agree with those of Galal and Fathi (2002) who reported that presence of *Cr* allele increased egg mass by about 4.2% compared with *cr* genotype. In addition to that Youssef *et al*. (2017) recorded average egg weight of *Cr* was significantly ($P \leq 0.05$) compared to *cr*.

Egg number (EN)

The egg number was observed in all genotypes so the second month of experiment the *Cr* gene had higher significantly ($P \leq 0.05$) of egg number than cr gene see Table 3. Furthermore, since many reports have shown that light strains of birds are good layers (Adedeji *et al.,* 2006) so the crested-head genotype may also be a good layer.

Egg quality

Concerning Haugh unit, Table 4 collates from crest gene and non-crest gene Dandarawi chicken. The results show not significantly in Haugh unit and shape index. Youssef et al. (2017) found not significantly in Haugh unit and shape index between *Cr* gene and *cr* gene.

Body temperature

Body temperature of *Cr* phenotype during cold stress was lower by about 1°C than *cr* phenotype Table 5. This result in agreement with Galal and Fathi (2002) who reported that *Cr* recorded lower rectal temperature by about 0.26°C than *cr*. Addition Youssef *et al.,* (2017) observed body temperature of *Cr* gene during cold stress was lower by about 0.38°C than *cr* gene. This may be due to *Cr* phenotype had higher feather mass by about 11.8% compared with *cr* (Fathi and Galal, 2001).Feather mass associated positively with body temperature under high ambient temperature (Deeb and Cahaner, 1999) and reduction in feather mass was associated with an increase in body temperature (Booth *et al.,*1993).

Genetic Diversity and Similarity Among Chicken Populations

The genetic distance matrices among chicken populations are shown It could be noticed that the genetic distances among the 30 chicken populations ranged from 13.7% to 89.6% as shown in figure 1. Higher similarity was found between (S10 & S11) and (S19 & S29) respectively. They had a closer relationship to each other. The genetic distance between each couple were 0.896 and 0.869, respectively. This high similarity suggested that the 2 populations of chicken may be genetically derived from the same genetic

origin. The applicability and effectiveness of microsatellite panels in assessing genetic variation and setting the conservation priorities for Egyptian local chicken strains were affirmed by Ramadan *et al.,* (2012).

Dendrogram based on Genetic Diversity and Similarity Among Chicken Populations

The dendrogram figure 1 was divided into 2 main clusters at 27% similarity with 7 chicken populations falling one cluster and the other 23 chicken populations in the second cluster. The first cluster was further sub divided at similarity into 2 sub-groups discriminating S1 from the rest of the group. The second main cluster included the rest of the 23 chicken populations. This shows that molecular information of genetic diversity is playing an important role in conservation chicken resources. It is suggested that increasing morphological and productive uniformity within each breed will aid in the prevention of both losing genetic diversity and increasing inbreeding. Even though genetic analyses can reveal the extent of biodiversity in chicken breeds (Dorji *et al.,* 2012), additional data on exact environmental adaptability and efficient performance is needed to adequately evaluate each breed for breeding and conservation programs. (Osman *et al.,* 2016).

In conclusion, evaluation of genetic diversity among chicken populations based on 4 microsatellite markers studied in the current study was efficient and gained reliable results. Knowledge of the levels and distribution of genetic diversity at the molecular level are important to protect genetic resources from extinction and contributes to the general genetic pool of the chicken

Table (1): Nucleotide sequences of the primers used to amplify locus

Locus	Direction	Sequence	Chromosomes (GGA)
MCW0248	Forward	AGAAGATGCATGGTTGTTCAAA	1
	Reverse	TTGCATTAACTGGGCACTTTC	
MCW0206	Forward	ACATCTAGAATTGACTGTTCAC	2
	Reverse	CTTGACAGTGATGCATTAAATG	
MCW0183	Forward	ATCCCAGTGTCGAGTATCCGA	7
	Reverse	TGAGATTTACTGGAGCCTGCC	
MCW0165	Forward	CAGACATGCATGCCCAGATGA	23
	Reverse	CGTCCTGCAGGCTGCGATCCA	

Table (2): Egg weight for cr and Cr phenotypes of Dandarawi laying hens

Phenotype / **Traits Main Effects**		**cr**	**Cr**
Average egg weight (gm)	1st month	41.7	41.6
	SE	0.24	0.24
	Probability	NS	NS
	2nd month	41.2b	42.2a
	SE	0.3	0.25
	Probability	0.01	
	3rd month	42.2	42.4
	SE	0.27	0.24
	Probability	NS	NS
	Overall period	41.6b	42.1a
	SE	0.16	0.14
	Probability	0.027	

Cr= crest. cr = non-crest.

*a, b, = Means in the same column within each main effect with different superscripts, differ significantly (P<0.05); N.S. = Not Significant (P>0.05).

Table (3): influence of crest gene on egg number

Phenotype / **Traits Main Effects**		**cr**	**Cr**
Egg number	1st month	20.2	19.6
	SE	0.31	0.34
	Probability	NS	NS
	2nd month	17.1^{b}	18.7^{a}
	SE	0.4	0.39
	Probability	**0.007**	
	3rd month	17.3	18.3
	SE	0.43	0.34
	Probability	NS	NS
	Overall period	18.3	18.8
	SE	0.24	0.21
	Probability	NS	NS

Cr= crest. cr = non-crest.

*a, b, = Means in the same column within each main effect with different superscripts, differ significantly (P<0.05); N.S. = Not Significant (P>0.05).

Table (4): Egg quality for cr and Cr phenotypes of Dandarawi laying hens

Phenotype **Traits Main Effects**	**cr**	**Cr**
HU **SE**	94.7 0.63	95 0.6
Probability	NS	NS
Shape index **SE**	77.2 0.66	77.3 0.32
Probability	NS	NS

Cr= crest. cr = non-crest.

*a, b, = Means in the same column within each main effect with different superscripts, differ significantly (P<0.05); N.S. = Not Significant (P>0.05).

Table (5): impact of crest gene Dandarawi laying hens on body temperature during cold stress.

Phenotype **Traits Main Effects**	**cr**	**Cr**
Body temperature SE Probability	42.8 0.09 NS	41.8 0.2 NS

Cr= crest. cr = non-crest.

*a, b, = Means in the same column within each main effect with different superscripts, differ significantly (P<0.05); N.S. = Not Significant (P>0.05).

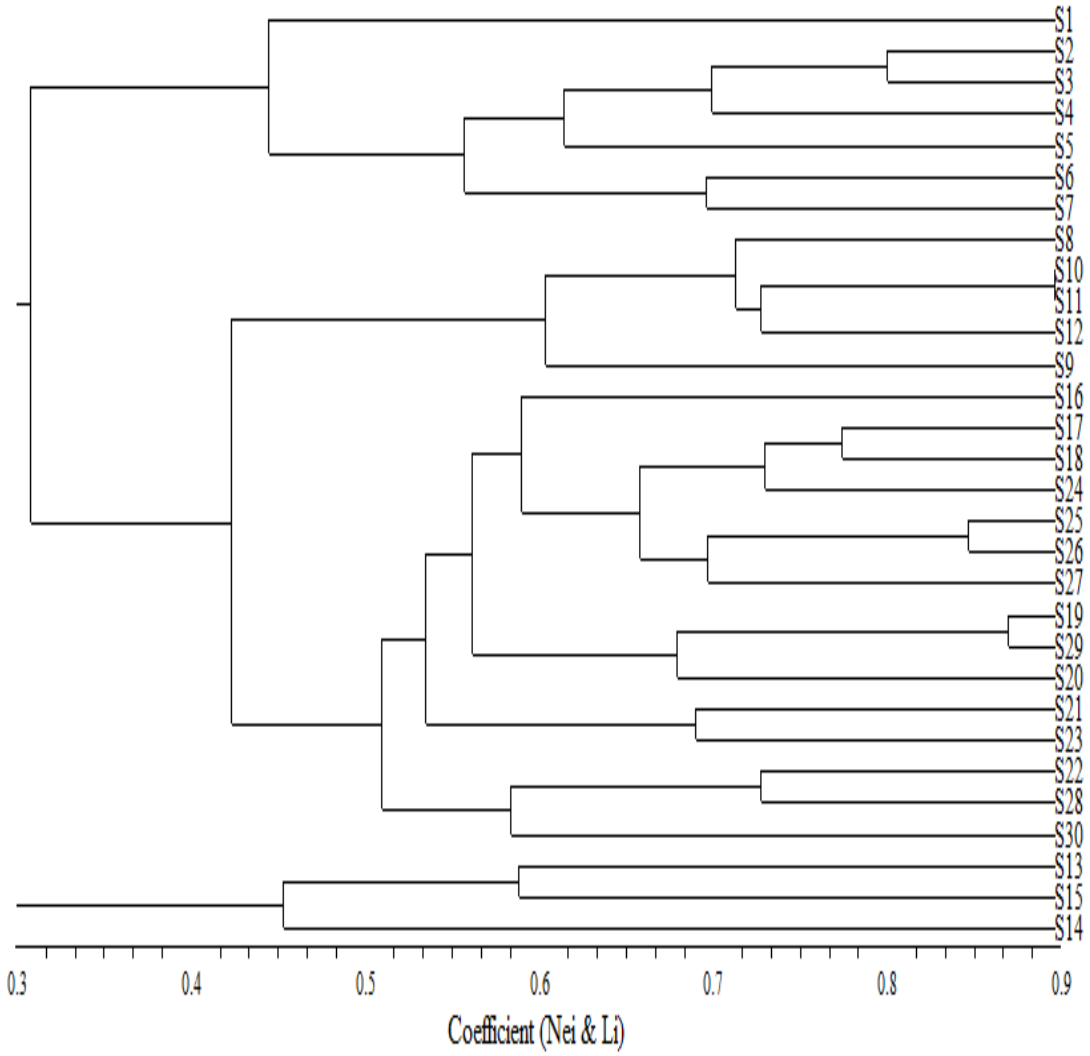

Fig. (1): Phylogenetic relationship of different stocks of chickens based on the locus in chromosomes.

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الملخص العربي

التنوع الوراثي لدجاج الدندراوي ذوالقلنسوة وبدون القلنسوة المصري على اساس الواسمات الوراثيه

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1معهد بحوث الإنتاج الحيواني مركز البحوث الزراعية الدقي الجيزة

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الهدف الاول من هذه التجربه هو الدراسة الاختلاف بين بعض الصفات الإنتاجيه لسلاله دجاج الدندراوي ذوالقلنسوة وبدون القلنسوة. عند وصل مستوى الانتاج الي نسبة 50 % تم اختيار اثنتين وسبعين عشوائيا لسلاله دجاج الدندراوي ذوالقلنسوة وبدون القلنسوة .و من اهم النتائج ان دجاج الدندراوي ذوالقلنسوة كان اعلى معنويه في كلا من وزن البيض وعدد البيض ($p\leq 0.05$) مقابل دجاج الدندراوي بدون القلنسوة بداية من الشهر الثاني و استمر طوال فتره التجربه.

الهدف الثاني من هذه التجربه هو الدراسة لتقييم التنوع الوراثي لسلاله دجاج الدندراوي ذوالقلنسوة وبدون القلنسوة ، وذلك باستخدام أربعة من الواسمات الوراثيه. أظهرت النتائج أن جميع العلامات التي تم دراستها أظهرت نسبة مختلفة من تعدد الأشكال بين دجاج الدندراوي ذوالقلنسوة وبدون القلنسوة.

1. تم العثور على تشابه أعلى بين دجاج الدندراوي ذوالقلنسوة (S6, S7), (S2, S3), (S10, S11) وكانت التنوع الوراثي 89.6 ٪، 78 ٪ ، 69.5 ٪ على التوالي

2. تم العثور على تشابه أعلى بين دجاج الدندراوي بدون القلنسوة (S25, S26), (S19, S29)بينما كان التنوع الوراثي 86.9 ٪ ، 77.4 ٪ على التوالي.