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The microsatellite allele diversity on chromosomes 3, 4, and 5 in native Egyptian chickens

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ABSTRACT

This study aimed at the evaluation of genetic variation in 5 different local chicken populations in Egypt. The populations were Fayoumi, Sinai Bedouin, White Baladi, normally-feathered Baladi (CE2) and naked-neck Baladi (CE4). Four microsatellite markers were used to screen the genomes of the chromosomes 3, 4 and 5 in the chicken populations. A total of 35 alleles were detected at the microsatellite loci, and averaged 8.75 alleles per locus. Sinai Bedouin fowl had a total of 19 alleles overall loci with an average of 4.75 alleles/locus, whereas Fayoumi, White Baladi, CE2 and CE4 had less total numbers of alleles with averages of 2.33, 2.75, 2.25 and 2.50 alleles/locus, respectively. Sinai Bedouin showed more allelic diversity than the other breeds. In addition, many alleles at many loci were population-specific, and Sinai Bedouin had the highest number of them, with a total of 10 alleles overall loci. White Baladi, CE2 and CE4 had a total of 2, 3 and 3 specific alleles, while Fayoumi did not have any. The genomic variability within populations was in general lowly moderate to moderate, with averages of 0.519, 0.447, 0.286, 0.417 and 0.542 in Fayoumi, Sinai Bedouin, White Baladi, CE2 and CE4, respectively. Sinai Bedouin showed the least percentage of allele similarity with all other populations with an average of 2.21%. Also, White Baladi and Fayoumi showed low allele similarity with other populations with percentages averaging 2.34 and 2.43%, respectively. The populations CE2 and CE4 showed the highest allele similarity with other populations with averages of 3.75 and 3.69%, respectively. The populations CE2 and CE4 shared an average 6.90% of the same alleles.

Key words: Allele diversity, allele similarity, genomic variability, native chickens.

INTRODUCTION

Local chicken breeds possess unique genetic compositions enabling them to adapt harsh environmental conditions, such as hot climate and poor management (El-Gendy, 2009; Islam and Nishibori, 2009; Niknafs *et al.*, 2012). The interaction between the chicken genotype and the housing systems has been reported by N'Dri *et al.* (2007); and significant effects for breed by management system interactions on the productivity of Rhode Island Red and Fayoumi were observed by Bekele *et al.* (2008). El-Kashef *et al.* (2017) showed that the growth of warm-region chickens under natural prolonged severe heating conditions was due to the genetic composition for growth and the genetic composition of heat tolerance. In this concern, Higazy *et al.* (2017) detected QTL contributing to growth performance of warm-region chickens under natural prolonged heating conditions. The productivity of native chicken breeds could be greatly improved by breeding (El-Gendy, 2009; Padhi *et al.*, 2016), in which the genetic variation plays a central role.

Rosário *et al.* (2009) genotyped two F_1 reciprocal crosses and their parental lines (two Brazilian reference populations). The observed heterozygosity was higher (0.68–0.71) in both crosses compared to their parental populations, and this was attributed to linkage disequilibrium. Mitileni *et al.* (2010)

compared the level and distribution of genetic variation among conserved and village chicken populations in South African. The results indicated that mean number of alleles per locus ranged from 3.52 to 6.62. The conserved flocks displayed considerable between-breed genetic variability which was different from that of the respective village chicken populations. The conserved flocks were at most a partial representation of the genetic diversity found in the village chicken populations in South Africa. Esfahan *et al*. (2012) used 10 microsatellites to evaluate the genetic diversity of Isfahan chicken population in Iran. A total 31 alleles was detected with average allele number of 3.1allele/locus. El-Gendy and Helal (2014) used 27 microsatellites to evaluate the consequences of practicing selection for 8 generations on the genomic variability in two lines (normally-feathered selected line, CE1, and naked-neck selected line, CE3) in comparison with their genetic control lines (CE2 and CE4, respectively). The average number of alleles was 3.15 per locus. Low estimates of variability were found in both selected lines and were attributed to selection. El-Gendy *et al*. (2013) used 7 microsatellite markers to scan the genomes of lines CE3 and CE4. The total number of alleles detected among line and sex ranged from 6 to 11 alleles, with an average of 2.14 alleles per locus. The average variability was 0.54 in line CE3 and 0.46 in line CE4.

The objectives of this study were to look at the genetic variation within five native Egyptian chicken populations that have been originally arisen in different ecological zones, and to assess the genetic relationships between them.

MATERIALS AND METHODS

a. Genetic populations and sampling

Five native Egyptian chicken breeds from different zones in Egypt were used. They were Fayoumi (Fay), Sinai Bedouin (SB), White Baladi (WB), normally-feathered Baladi (CE2) and naked–neck Baladi (CE4). Each population was represented by 15 randomly chosen birds. Blood samples of about 2ml were collected from the brachial veins in sterilized 3-ml tubes containing EDTA (ethylene-di-amine-tetra-acetic acid) as anticoagulant. The collected blood samples were stored at -20˚C until use.

b. Genome Banding

Upon use, the blood samples were thawed and the genomic DNA was extracted by Promega genomic DNA purification kit (Promega Corporation, Madison, WI, USA), and the extraction procedures were according to the manufacturer's guide. Extracted DNA samples were first visualized on 1% agarose gel. The individual DNA sample was diluted in TE buffer (5µl DNA/495µl TE), and DNA concentration was determined using spectrophotometer (PG instruments, Alma park, wibtoft, Lutter worth LE 175BH, UK), and according to Sambrook *et al*. (1989). Pooled DNA samples were prepared by mixing 5 individual DNA samples of same population, with equal concentrations to bring the total concentration of 25ng/µl in each pooled sample. Three pooled DNA samples were prepared for each population. The pooled samples of different populations were screened by the microsatellite markers, and the PCR products were electrophoresed on 2% agarose gel and also on 8% non-denaturating polyacrylamide gel electrophoresis (PAGE).

Microsatellite-PCR procedure was performed to screen genomic DNA, using four microsatellite markers targeting three autosomal chromosomes (Table 1). PCR was performed in the thermal cycler (Techne, TC3000, Barloworld Scientific Ltd, Beacon Road Stone, UK), using a total volume of 25µl of the reaction components (Table 2). The PCR program included initial denaturation at 95°C/5

min., followed by 35 cycles (denaturation at 94°C/45 sec,annealing at 50-54°C/45 sec and extension at 72°C/45 sec), final extension at 72°C/10 min, and final hold at 10°C. Theannealing temperature differed for different microsatellite markers.

The PCR products were first separated on 2% agarose gel containing 20µl ethidium bromide, EtBr(0.5mg/ml EtBr in 100ml dH_2O). A DNA marker of 100 bp (GeneDireX, 100-bp DNA ladder RTU) was used to determine the lengths (bp) of the amplified bands. The electrophoresis was performed at 75v/60min by a power supply (Cleaver scientific Ltd., UK). The DNA bands were visualized by an ultraviolet trans-illuminator in a dark chamber and photographed. Upon recognition of the genomic bands, the PCR products were separated on 8% non-denaturating PAGE (Table 3). A 50-bp DNA marker (GeneDireX, 50-bp DNA ladder RTU) was loaded. Electrophoresis was run at 100v for 240 minutes or until the lower dye was escaped from the gel. The gel was gently submerged in EtBrstaining solution (20µl EtBr added to the buffer 1X-TBE) for 20 minutes at room temperature. The electrophoretic genomic bands were visualized and photographed.The PCR products were visualized and photographed using the WGD-30 WiseDoc gel documentation (Daihan Scientific, Co., ltd, Seoul, Korea).

c. Genomic measurements and Statistical Analysis:

The DNA images were analyzed for band detection and segmental length (bp) of the genes using TotalLab software (TotalLab ltd, Keel House, Garth Heads, NewCastle, UK). The generated DNA patterns were used to recognize the alleles in each microsatellite locus. The allele frequencies were estimated within populations and were used to estimate the genomic variability according to the formula of Kuhnlein *et al*. (1989), and similarity index using the formula of Haymer and McInnis (1994).

RESULTS AND DISCUSSION

a. The allelic structure within populations

The number of alleles detected and the population-specific alleles (PSA) at the microsatellite loci are presented in Table (4). A total of 35 alleles were detected at the four microsatellite loci, with an average 8.75 alleles/locus. The microsatellite locus LEI0166 showed nine different alleles with lengths of 105-503 bp. The locus LEI0073 revealed 10 different alleles with lengths of 111-568 bp. The loci ADL0143 and MCW0193, revealed eight alleles for each with lengths of 134-212 and 238-389 bp, respectively. Sinai Bedouin fowl had a total of 19 alleles overall loci with an average of 4.75 alleles/locus, whereas Fayoumi, White Baladi, CE2 and CE4 had less total numbers of alleles with averages of 2.33, 2.75, 2.25 and 2.50 alleles/locus, respectively. The results indicate that locus LEI0073 was relatively the richest in alleles. Also, Sinai Bedouin showed more allelic diversity than the other breeds. In addition, many alleles at many loci were population-specific. Sinai Bedouin had the highest number of the alleles specific to the breed, with a total of 10 alleles overall loci. White Baladi, CE2 and CE4 had a total of 2, 3 and 3 specific alleles, while Fayoumi did not have any. The population-specific alleles contribute to the genetic uniqueness of the breed and indicate whether the populations had been involved in gene exchange. In this concern, Sinai Bedouin seemed to be the breed genetically most-outer to the other breeds. The value of r^2 denotes to the accuracy by which the allelic information has been generated at each microsatellite

locus. The values of r^2 were high at all loci and ranged from 0.9922 at the locus ADL0143 to 0.9960 at the locus MCW0193. Olowofeso*et al.* (2005) reported allele number of 5.73 – 6.00 at 15 microsatellite loci in four Chinese chicken populations. Esfahan *et al*. (2012) used 10 microsatellites to evaluate the genetic diversity of Isfahan chicken population in Iran. A total 31 alleles was detected with average allele number of 3.1alleles/locus. Also, the average number of alleles/locus ranged from 2.14 to 3.15 at 27 microsatellite loci in the native Egyptian chicken breeds (El-Gendy *et al.,* 2013; El-Gendy and Helal, 2014).

b. Genomic variability

The genomic variability within populations was in general lowly moderate to moderate in different populations (Table 5). The genomic variability was absent at locus LEI0166 in White Baladi and at locus ADL0143 in the normally-feathered Baladi, whereas it reached its maximum (0.667) at locus ADL0143 in naked-neck Baladi and at MCW0193 in the normally-feathered Baladi. The average genomic variability overall loci was 0.519, 0.447, 0.286, 0.417 and 0.542 in Fayoumi, Sinai Bedouin, White Baladi, the normally-feathered Baladi (CE2) and the naked-neck Baladi (CE4), respectively. The results indicate the existence of moderate levels of heterozygosity in the chickens, and the inbreeding was noticeable. El-Gendy *et al.* (2013) reported that random mating and keeping populations in small sizes may result in reduction in variability and heterozygosity. Abebe *et al.* (2015) studied the genetic diversity, relationship and population structure of 5 local Swedish chickens. The global heterozygosity was 0.545, the population differentiation index was 0.440, and the global inbreeding of individuals within breed was 0.187. The five local breeds showed low within-breed genetic diversity, but considerable variations were existed between breeds.

c. Similarity index

The similarity indices among the chicken populations are presented in Table (6). The similarity index measures the percentage of microsatellite alleles that are shared by different breeds. In this concern, Sinai Bedouin showed the least percentage of allele similarity with all other populations with an average of 2.21%. Also, White Baladi and Fayoumi showed low allele similarity with other populations with percentages averaging 2.34 and 2.43%, respectively. The populations CE2 and CE4 showed the highest allele similarity with other populations with averages of 3.75 and 3.69%, respectively. The similarity level expresses the rate of gene flow through the breed intercrossing. It also may signal if different breeds have been derived from each other. In this respect, populations CE2 and CE4 shared an average 6.90% of the same alleles. The high similarity between population CE2 and population CE4 apparently reflects that they both have been derived from same base population. Sinai Bedouin fowl showed the least similarity with the other populations because they have been developed in Sinai pensellona and the chance of intercrossing with other breeds was minimal. White Baladi chickens have been raised as a closed population with very little chance to intercross with different ecotype chicken breeds. El-Gendy*et al*. (2006) used 10 RAPD markers to evaluate the gnomic specificity of three native Egyptian chicken breeds (White Baladi, Fayoumi and Sinai Bedouin). The average of genetic distances between White Baladi and each of Fayoumi and Sinai Bedouin was 0.42, and between Fayoumi and Sinai Bedouin was 0.53. Rudresh*et al.* (2015) reported a genetic identity index of 0.802 between two indigenous Indian chicken breeds. Abebe *et al.* (2015) used 24 microsatellites to investigate the genetic relationship between five local Swedish chickens. The

average within-breed kinship varied from 0.496 to 0.745, showing high co-ancestry.

Conclusion

The native Egyptian chicken breeds showed high allele diversity, with noticeable existence of alleles specifying many breeds. The genomic variability in general was moderate in all breeds. Sinai Bedouin seemed to be the breed genetically most-outer to the other breeds, whereas the normally-feathered Baladi and naked-neck Baladi shared the highest genetic similarity.

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Table 1: The molecular information of the microsatellite primers.

Microsatellite locus	Ch. #	Length, bp	Primer sequence	GC, %
LEI0166	3	F: 21	5′ AAG CAA GTG CTG GCT GTG CTC 3′	57
		R: 20	5′ TCC TGC CCT TAG CTA CGC AC 3′	60
LEI0073	4	F: 21	5′ CCA TAT CAT TTG TCA AGC ACC 3′	43
		R: 21	5′ AAT TCC TGA CCT CCA TGA TAC3′	43
ADL0143	4	F: 20	5′ CCT GTC TCT GGT CTT TAT CC3′	50
		R: 20	5′ AGT TTA CTT CCT TTT CTT GC3′	35
MCW 0193	5	F: 21	5′ ATT ACG TCT GCA CCA GTA CAG3′	48
		R: 22	5′ TAT TCA ATA GAG TTA CGC TGTC3′	36

Table (2): The PCR components.

Component	Amount
Genomic DNA (75 ng)	3µl
Forward primer (25 pmol)	2µl
Reverse primer (25 pmol)	2µl
Master mix	10µl
PCR-grade water	8µl
Total volume	25µl

Table (3): The chemical composition of 8% polyacrylamide gel.

Reagent	Amount
Acrylamide solution (30%)	12.8 ml
ddH_2O	25.6 ml
APS (10%)	800 µl
TEMED	40 µl
TBE (5X)	9.6 ml

Table (4). The number of microsatellite alleles (N) and population-specific alleles (PAS) in the native Egyptian chicken populations.

Microsatellite locus	Ch.	Number of alleles	Length, bp	r^2	Fayoumi		Sinai Bedouin		White Baladi		CE2		CE4	
					N	PSA	N	PSA	N	PSA	N	PSA	N	PSA
LEI0166	3	9	105-503	0.9945	3	0	6	3	1	0	2	1	2	0
LEI0073	4	10	111-568	0.9946	2	0	5	4	2	1	2	1	2	1
ADL0143	4	8	134-212	0.9922	2	0	5	1	5	1	1	0	3	1
MCW0193	5	8	238-389	0.9960	---	---	3	2	3	0	4	1	3	1
Total		35			7	0	19	10	11	2	9	3	10	3
Mean		8.75			2.33	0	4.75	2.50	2.75	0.50	2.25	0.75	2.50	0.75

CE2 and CE4 denote to normally-feathered Baladi and naked-neck Baladi, respectively.

Table (5): The genomic variability within the native Egyptian chicken populations.

Microsatellite locus	Fay	SB	WB	CE2	CE4
LEI0166	0.556	0.300	0.000	0.500	0.556
LEI0073	0.500	0.444	0.500	0.500	0.500
ADL0143	0.500	0.600	0.200	0.000	0.667
MCW0193	---	0.444	0.444	0.667	0.444
Mean	0.519	0.447	0.286	0.417	0.542

Fay, SB, WB, CE2 and CE4 indicate Fayoumi, Sinai Bedouin, White Baladi, normally-featheredBaladi and naked-neck Baladi, respectively.

Table (6). The similarity indices (%) between the native Egyptian chicken populations.

Microsatellite locus	Fay-SB	Fay-WB	Fay-CE2	Fay-CE4	SB-WB	SB-CE2	SB-CE4	WB-CE2	WB-CE4	CE2-CE4	Similarity with all other populations	
LEI0166	1.38	1.80	2.78	1.05	3.02	---	0.59	---	---	1.31	Fay	2.43
LEI0073	2.91	3.70	---	5.93	---	---	---	---	5.93	10.00	SB	2.21
ADL0143	0.65	1.33	---	2.00	2.03	6.21	3.03	2.35	2.71	13.33	WB	2.34
MCW0193	---	---	---	---	1.05	0.46	---	1.60	0.56	2.96	CE2	3.75
Mean	1.65	2.28	2.78	2.99	2.03	3.34	1.81	1.98	3.07	6.90	CE4	3.69

Fay, SB, WB, CE2 and CE4 indicate Fayoumi, Sinai Bedouin, White Baladi, normally-featheredBaladi and naked-neck Baladi, respectively.

The microsatellite allele diversity on chromosomes 3, 4, and 5 in native Egyptian chickens

التنوع الأليلى فى مواقع الواسمات الوراثية الدقيقة على الكروموسومات 3، 4، 5 فى الدجاج المحلى المصرى

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المستخلص

تمت هذه الدراسة بغرض تقييم التباين الوراثى فى خمس سلالات دواجن محلية مصرية، والسلالات هى: الفيومى – السيناوى – البلدى الأبيض، الدجاج البلدى ذو التريبش الطبيعى والدجاج البلدى عارى الرقبة. تمت الدراسة بإستخدام أربع من الواسمات الوراثية الدقيقة التى تتعرف على مواقع وراثية على الكروموسومات 3، 4، 5. أسفرت الدراسة عن التعرف على 35 أليل على كل مواقع الواسمات الوراثية الدقيقة على الكروموسومات، وبمتوسط 8.75 أليل/موقع. أظهر الدجاج السيناوى وجود 19 أليل على كل المواقع، بمتوسط 4.75 أليل/موقع، بينما أظهر كل من الدجاج الفيومى، الدجاج البلدى الأبيض، الدجاج البلدى ذو التريبش الطبيعى والدجاج البلدى عارى الرقبة وجود أعداد أقل من الأيلات وبمتوسطات 2.33، 2.75، 2.25، 2.50 أليل/موقع، على التوالى. أظهر الدجاج السيناوى تنوع أليلى أكثر من السلالات الأخرى. كما أظهرت الدراسة وجود أليلات على بعض المواقع خاصة بسلالة دون غيرها، وكانت هذه الأليلات أعلى عددا فى الدجاج السيناوى بمجموع 10 أليلات فى كل المواقع، بينما تم التعرف على أليلين خاصين بالدجاج البلدى الأبيض وثلاث أليلات خاصة بكل من الدجاج البلدى ذو التريبش الطبيعى والدجاج البلدى عارى الرقبة، ولم تظهر أليلات خاصة بالدجاج الفيومى. كان التباين الجينومى فى السلالات بشكل عام منخفض إلى متوسط، وبمتوسطات 0.519، 0.447، 0.286، 0.417، 0.542 فى كل من الدجاج الفيومى، السيناوى، البلدى الأبيض، البلدى ذو التريبش الطبيعى والبلدى عارى الرقبة، على التوالى. كان الدجاج السيناوى الأقل فى نسبة التشابه الأليلى مع السلالات الأخرى، وبمتوسط 2.21%. أيضا أظهر كل من الدجاج البلدى الأبيض والفيومى نسب منخفضة من التشابه الأليلى مع السلالات الأخرى وبمتوسطى 2.34، 2.43%، على التوالى.وكانت السلالتان الدجاج البلدى ذو التريبش الطبيعى والدجاج البلدى عارى الرقبة هما الأعلي فى نسب التشابه الأليلى مع السلالات الأخرى وبمتوسطى 3.75، 3.69% على التوالى، وقد تشاركت السلالتان فى 6.90% من نفس الأليلات.