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Microsatellite Genetic Differentiation Analysis of Two Local Chicken Breeds Compared with Foreign Hy-Line Strain

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Abstract: The present study was conducted on two Egyptian native breeds (Dandarawi and Fayoumi) and commercial laying hens (brown Hy-line) to estimate genetic differentiation using microsatellites and their association with egg production traits. Five microsatellite markers, four located on Z chromosome and one located on chromosome 1 were used in this study. The present results indicated that the Hy-line strain had significantly better egg production parameters and feed conversion ratio compared to two native breeds. Inversely, the two native breeds had better eggshell quality measurements compared to Hy-line hens. The five microsatellite genetic markers applied in the present study success to reveal high degree of polymorphism among the three breeds used here. Also, a clear discriminating power was achieved in differentiation among studied chicken populations. The genetic distance revealed that Fayoumi breed is mostly related to Hy-line strain more than Dandarawi breed.

Key words: Dandarawi, Fayoumi, brown Hy-line, microsatellites, egg production

INTRODUCTION

The use of DNA marker technology in poultry as a strains identification has progressed rapidly during the last decade. Molecular markers appear particularly useful for:

- 1) measuring local gene flow and migration,
- 2) assigning individuals to their most likely population of origin,
- 3) measuring effective population size through the between generation comparison of allele frequencies and
- 4) detecting past demographic bottlenecks through allele frequency distortions (Jehle and Arntzen, 2002).

Most of the widely used and applicable genetic markers for genome dissection and breeds differentiation is so called microsatellites (simple sequence repeats; SSRs, or short tandem repeats; STRs). The effectiveness of microsatellite in detecting polymorphism between chicken populations and their applicability in population studies and establishing genetic relationships among chicken populations has been reported by Zhang *et al.* (2002a,b). Microsatellites are useful for a number of analyses. They were originally utilized for genetic mapping (Tuiskula *et al.*, 2002) and have been extensively used for linkage analyses in the association with disease susceptibility genes (McElroy *et al.*, 2005). In addition, they have proven to be useful in the analysis of paternity and kinship (Queller *et al.*, 1993) and in the probability of sample identity at both the individual (Edwards *et al.*, 1992) and population levels (Ya-Bo *et al.*, 2006). In the study of entire populations microsatellites are also very useful.

Many investigators were studied egg production traits for our local and foreign breeds (Abdel Galil, 1993; Fathi, and El- Sahar, 1996; El-Full *et al.*, 2005; Zaky, 2006). El-Full *et al.* (2005) showed that Dandarawi hens had significantly higher egg number during the first 90 days of production than Golden Montazah or Fayoumi hens. Regarding Haugh units (as a measure to evaluate albumin quality of eggs), Abd El-Galil (1993) found that Haugh units values were higher in LSL strain than those of local strains. Similarly, Zaky (2006) reported that Fayoumi breed had lower Haugh units compared to White Leghorn. El-Full *et al.* (2005) indicated that Fayoumi breed was significantly increased in Haugh units than Dandarawi breed. Fathi and El-Sahar (1996) realized that the force of breakage the eggshells were significantly differed among strains and genotypes. Also, they found that mean of breaking strength of the brown shells was approximately 13% higher than those of white ones.

Therefore, the study herein is conducted to:

- 1) estimate productive performance as comparative study among different three breeds, two local Fayoumi and Dandarawi and foreign one Hy-line strain.
- 2) investigate genetic differences among the three breeds used herein based on available microsatellite loci reported as in linkage with studied quantitative traits (Tuiskula-Haavisto, *et al.*, 2002).

MATERIALS AND METHODS

Birds and management: Hy-line strain and two Egyptian local breeds of chicken (Fayoumi and Dandarawi) were raised at the Poultry Breeding Farm, Ain Shams

University under the same environmental, managerial and hygienic conditions. At 16 weeks of age, a total of 300 females (100 Hy-line, 100 Fayoumi and 100 Dandarawi) were randomly assigned to the current experiment. They were housed in individual cages placed in an open-sided house. They were fed a laying diet containing 18.3% CP and 2752.9kcalME/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 50 weeks of age. The lighting schedule was maintained at 17 hours of daylight and 7 hours of darkness throughout the experiment.

Measurements and observations:

Productive performance: Age at sexual maturity was recorded from hatching time to the first egg by days. Egg production (number and weight) was recorded for the first 3 months of production cycle to calculate egg mass. To assess egg quality parameters (internal and external), a total of 90 eggs were randomly collected from each breed at 26 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 0.1g 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 towel to remove any adhering albumen and weighed. Albumen yield was determined by subtraction of the yolk and shell with shell membranes 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). The percentage of dry eggshell was calculated. The thickness (mm) of the shell with intact membranes was measured at three different points in the middle part of the egg using a dial gauge micrometer. The shell breaking strength (kg/cm^2) was determined according to Fathi and El-Sahar (1996). Specific gravity was determined by the flotation method using salt solution with specific gravity ranging from 1.060-1.100 at increments of 0.005. Egg volume was estimated by measuring the quantity of water dislodged in cm^3 after immersing the egg into known water volume.

Blood samples: Thirty eight blood samples were collected from 3 different breeds as follows: 13 (Fayoumi), 11 (Hyline) and 14 (Dandarawi). A half ml of blood sample was withdrawn from Jugular vein on EDTA tube as anticoagulant (0.2ml of 0.5M EDTA) and stored at -20°C .

DNA Isolation: DNA was extracted from 0.5mL of whole EDTA-blood. Two and half ml of lysis buffer (20mM Tris-HCl pH 7.6, 640mM saccharose, 2% Triton X-100, 10mM MgCl_2) was added to the aliquot. The mixture was centrifuged and the pellet suspended in 150 μl Proteinase K, 1.5ml nuclei lysis buffer and 110 μl SDS20%. After overnight incubation at 37°C , the proteins were removed by NaCl 6M and the DNA was precipitated by ethanol.

Microsatellite loci: Five microsatellite loci (Table 1) were selected based on published chicken genome database. All markers used are linked to egg production traits, four markers; ADL-273, MCW-241, MCW-246 and MCW-258 are located on Z chromosome linkage group, whilst, the remainder marker (ADL-188) is located on chromosome one linkage group.

The polymerase chain reaction: The PCR was carried out in a volume of 20 μl comprising 50ng of template DNA, 10 pmol of each primer and 5 μl of Taq master* (Thermostable DNA polymorphism, dATP, dCTP, dGTP, dTTP, reaction buffer with $(\text{NH}_4)_2\text{SO}_4$, MgCl_2 and Triton X-100, stabilizers. Two pairs of primers were amplified in the same reaction. To these reactions 10 pmol of each primer was applied. The amplification conditions were: 5 min denaturation at 94°C followed by 35 cycles of denaturation at 94°C for 30 s, annealing ($47-55^\circ\text{C}$) for (45-60 s) and elongation at 72°C for 60 s. The PCR products were separated in 8% polyacrylamide gels. Results were visualized and the genotyping done with the Alphasoft 2200 software Version 4.0.1.

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Statistical analysis:

Quantitative traits: Data were subjected to a one-way ANOVA using GLM (SAS Institute, 2001) with strain and breeds as fixed effect. Data of egg production traits are presented as means and the standard errors SE.

Microsatellite and genetic analysis: 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 GENPOP software package after data conversion using CONVERT program.

RESULTS AND DISCUSSION

Productive performance: Effect of Hy-line strain and local breeds on maturation measurements is presented in Table 2 the Hy-line strain had significantly heavier body weight measured at sexual maturity compared to

Table 1: Microsatellite loci used herein, their accessions, flanking primers, annealing temperature (AT), location on genome and linked traits

| No | Trait | Locus | location | Primer Sequence | AT | Reference |
|----|-------|--------|----------|---|----|---------------------------------|
| 1 | AFE | MCW241 | Z-72cM | AACCAGTTTGTAAACATCAGC ATTGGAGTTGGTACCACTC | 55 | Crooijmans <i>et al.</i> , 1996 |
| 2 | EN | ADL273 | Z-65cM | GCCATACATGACAATAGAGG TGGTAGATGCTGAGAGGTGT | 47 | Cheng <i>et al.</i> , 1995 |
| 3 | ESS | MCW246 | Z-104cM | TCATAAGGCAGAGAATTCATC TTTCCATTCCAGACAACAAGGC | 55 | Crooijmans <i>et al.</i> , 1996 |
| 4 | EW | MCW258 | Z-63cM | TTCTTAGTCCTTGCCAGAGGC CTGCAGGAGGATGTGTCCTAG | 55 | Crooijmans <i>et al.</i> , 1996 |
| 5 | HU | ADL188 | 1-107cM | CACTTCCAGTATTAACGTGA GTGGACACAATGAGTTCCTC | 47 | Cheng <i>et al.</i> , 1995 |

Table 2: Means±SE of egg production characteristics for Fayoumi, Dandarawi breeds and Hy-line strain

| Trait | Breed | | | Prob |
|---------------------------|-----------------------------|-----------------------------|-----------------------------|--------|
| | Fayoumi | Dandarawi | Hy-line | |
| Body weight, g | 1276.4 ^b ±24.89 | 1173.8 ^c ±21.5 | 1486.5 ^a ±24.65 | 0.001 |
| Age at sexual maturity, d | 151.22 ^b ±1.89 | 152.44 ^b ±2.89 | 137.08 ^a ±1.55 | 0.0001 |
| Egg number | 63.93 ^b ±1.15 | 59.9 ^c ±1.09 | 84.2 ^a ±2.24 | 0.0001 |
| Egg production% | 71.03 ^b ±1.02 | 66.56 ^c ±1.09 | 93.56 ^a ±0.95 | 0.005 |
| Egg weight, gm | 42.6 ^b ±0.30 | 40.22 ^c ±0.46 | 59.61 ^a ±0.59 | 0.003 |
| Egg mass, gm | 2723.42 ^b ±39.65 | 2409.18 ^c ±44.59 | 5019.16 ^a ±48.69 | 0.0002 |

^{abc}values with different superscripts are statistically different within the same row.

local breeds. Also, the Fayoumi breed was significantly increased of body weight compared to Dandarawi breed. With respect to age at sexual maturity, it could be noticed that the Hy-line strain reached sexual maturity earlier than that of local breeds. The mean values were 137.08, 151.22 and 152.44 for Hy-line strain, Fayoumi breed and Dandarawi breed. The Hy-line chicken produced significantly highest egg number, egg production%, egg weight and egg mass compared to Fayoumi and Dandarawi breeds. The same trend Fayoumi breed significantly higher of these traits compared to Dandarawi breed. Egg production depends on many characters such as age at sexual maturity, egg number, body weight, egg weight, shell thickness, egg specific gravity and others (El Full *et al.*, 2001) which influence egg production system independently and/or associated with each other. The breed or strain variation in the associations among these traits to perform egg production system was reported by Fairfull and Gowe (1993) and El Gendy *et al.* (1997).

Data presented in Table 3 showed that the effects of Hy-line strain and local breeds on egg weight and internal egg quality of chicken. The Hy-line strain significantly increased egg weight of chicken and strata the Fayoumi breed increase egg weight. The Hy-line strain significantly increase Haugh units compared to local breeds and showed that slightly increase in yolk index to Hy-line strain compared to native strain. Hy-line strain significantly increased Albumen, yolk weight compared to local breeds. Also, the Fayoumi breed significantly increase this traits compared to Dandarawi breed. There was no significant difference between Hy-line strain and native breeds of chicken for Albumen and yolk

percentage. One of the most important egg quality traits to be considered in a poultry breeding program is shell strength. According to Grunder *et al.* (1989), specific gravity (SG) is eggshell strength as it relates to resistance to breakage and is relatively simple to measure (Gowe and Fairfull, 1995). Albumen height (AH) and Haugh units (HU) are traits used to evaluate albumen quality, which also deteriorates with age (Liljedahl *et al.*, 1984).

Table 4 showed significant different between the three strains studied at 30 wks of age. The specific gravity of Fayoumi and Dandarawi hens had higher value than the standard strain Hy-line. There was no significant difference among of three strains for shape index. It could be noticed that the Fayoumi and Dandarawi breeds were significantly higher for (shell strength, shell thickness and shell percentage) traits compared to Hy-line strain. But Hy-line strain had higher of egg volume than local breeds. Egg production is the yield of overall performance of a bird concerning many variable such as egg number, rate of lay, sexual maturity age, egg weight, shell thickness and external and internal egg quality characteristics (Eitan and Soller, 1993) these variable are correlated with egg production and with each other in positive or negative trends (Fairfull and Gowe, 1993). Similar findings were found by Kul and Seker (2004). They explained that there were significant positive correlations among egg weight, shell weight, shell thickness and shell strength. However, these correlations could be used to predict shell strength (force required to break egg). Regarding egg shape index, there was slightly increased associated with

Table 3: Means ± SE of internal egg quality measurement for Fayoumi, Dandarawi breeds and Hy-line strain

| Trait | Breed | | Strain | Prob. |
|--------------------|--------------------------|--------------------------|--------------------------|--------|
| | Fayoumi | Dandarawi | Hy-line | |
| Egg weight, gm | 41.88 ^b ±0.55 | 38.24 ^c ±0.54 | 62.2 ^a ±0.66 | 0.001 |
| Haugh unit | 85.13 ^b ±0.63 | 84.16 ^b ±0.75 | 88.41 ^a ±0.81 | 0.001 |
| Yolk index | 49.07 ^b ±0.06 | 47.5 ^c ±0.09 | 49.84 ^b ±0.11 | 0.002 |
| Albumin weight, gm | 23.91 ^b ±0.23 | 22.05 ^c ±0.37 | 34.92 ^a ±0.41 | 0.005 |
| Albumin % | 56.97±1.39 | 57.61±1.46 | 58.95±1.59 | NS |
| Yolk weight, gm | 12.81 ^b ±0.22 | 11.48 ^c ±0.24 | 18.41 ^a ±0.26 | 0.0001 |
| Yolk % | 30.58±0.39 | 29.88±0.41 | 30.81±0.38 | NS |

^{a,b,c} values with different superscripts are statistically different within the same row.

Table 4: Means ± SE of external egg quality measurement for Fayoumi, Dandarawi breeds and Hy-line strain

| Trait | Breed | | Strain | Prob. |
|------------------------------------|---------------------------|---------------------------|---------------------------|--------|
| | Fayoumi | Dandarawi | Hy-line | |
| Specific gravity | 1.089 ^a ±0.001 | 1.085 ^b ±0.001 | 1.081 ^c ±0.001 | 0.01 |
| Egg shape index | 75.43±.38 | 75.14±0.49 | 75.46±0.48 | NS |
| Shell strength, kg/cm ² | 4.75 ^a ±0.18 | 4.12 ^b ±0.23 | 3.91 ^c ±0.19 | 0.0001 |
| Shell thickness, mm | 0.39 ^a ±0.01 | 0.38 ^b ±0.01 | 0.35 ^c ±0.01 | 0.05 |
| Shell % | 12.45 ^a ±0.19 | 12.44 ^a ±0.22 | 10.24 ^b ±0.18 | 0.003 |
| Egg volume, ml | 38.48 ^b ±0.54 | 35.41 ^c ±0.52 | 56.51 ^a ±0.61 | 0.0002 |

^{a, b, c} values with different superscripts are statistically different within the same row

Dandarawi eggs compared to Fayoumi one. This result was supported by Kul and Seker (2004), where the shape index was not referred to be a good estimator for the shell thickness and the shell ratio. Conversely, both eggshell area and egg volume were increased in Fayoumi breed compared to Dandarawi one.

Microsatellite analysis: Five microsatellites highly polymorphic markers were used in the present investigation. Four out of them, are located on Z-chromosome linkage group and cover 41 cM (centi Morgan) of Z chromosome map, approximately. The Z-chromosome four marker are associated with four egg traits; MCW-241 for AFE (age at first egg; 72cM), ADL-273 for EN (egg number, 65cM), MCW-246 for ESS (egg shell strength, 104cM) and MCW-258 for EW (egg weight; 63cM). The remaining marker (ADL-188 for HU) is located on 1st chromosome linkage group at 107cM site. Table 1 summarizes all information of five microsatellites used and shows associated traits, locus name, genome location, flanking sequences, annealing temperatures and corresponding references. Fig. 1 illustrates apart of data analyzed herein showing polymorphic microsatellites bands of different locus for each breed.

Allelic frequencies, expected heterozygosities and genetic differentiations were calculated on the basis on all 5 microsatellite loci. The genetic diversity within the three populations analyzed herein was described by the mean number of alleles per locus and the mean expected and observed heterozygosity or total gene diversity (Nei, 1978). Genetic differentiation between populations was assessed by an analysis of molecular variance (F-index).

For all 3 used populations, the overall mean number of alleles detected per locus was 4.9 (Table 5), although the actual number of observable alleles at each locus ranged from 2 at locus ADL-188-8 at locus MCW-258 (Table 5). Average number of alleles per locus versus three breeds varied from 3.3-6.7 at locus ADL-188 and locus MCW-241, respectively. It might be concluded that average number of alleles per locus divided into three groups. The first is that of highest estimate (6.7 at locus MCW-241 and 6.3 at locus MCW-258). The second group showed moderate average (4.3 at locus MCW-246 and 4 at locus ADL-273), whereas, the third group is associated to locus ADL-188 (3.3).

The observed variability of average number of alleles seemed to reflect different potentialities of genetic markers to detect genetic variability among such breeds. As postulated in Table 5, allelic frequencies showed wide range for each locus among all breeds. The highest allele frequency overall loci was 0.818 of 143 allele at locus ADL-273. Whereas, the lowest one was 0.036 associated with Dandarawi breed at two loci, MCW-241 (for allele 350) and MC258 (for alleles, 99, 110, 165 and 176). The obtained results demonstrate highly observed heterozygosity among three populations.

Among 5 microsatellite markers applied in the present investigation, it might be concluded a such association between specific allele(s) on the basis of their frequencies and such quantitative traits. In this respect, the highest frequency of allele 270 (0.455) at locus MCW-241 in Hy-line population might be associated to AFE trait. With respect to EN trait, the highly significant performance of egg number of Hy-line strain (84.2 eggs, Table 2) might be correlated to high allele frequency (0.818) of allele 143 at locus ADL-273. Since the ESS

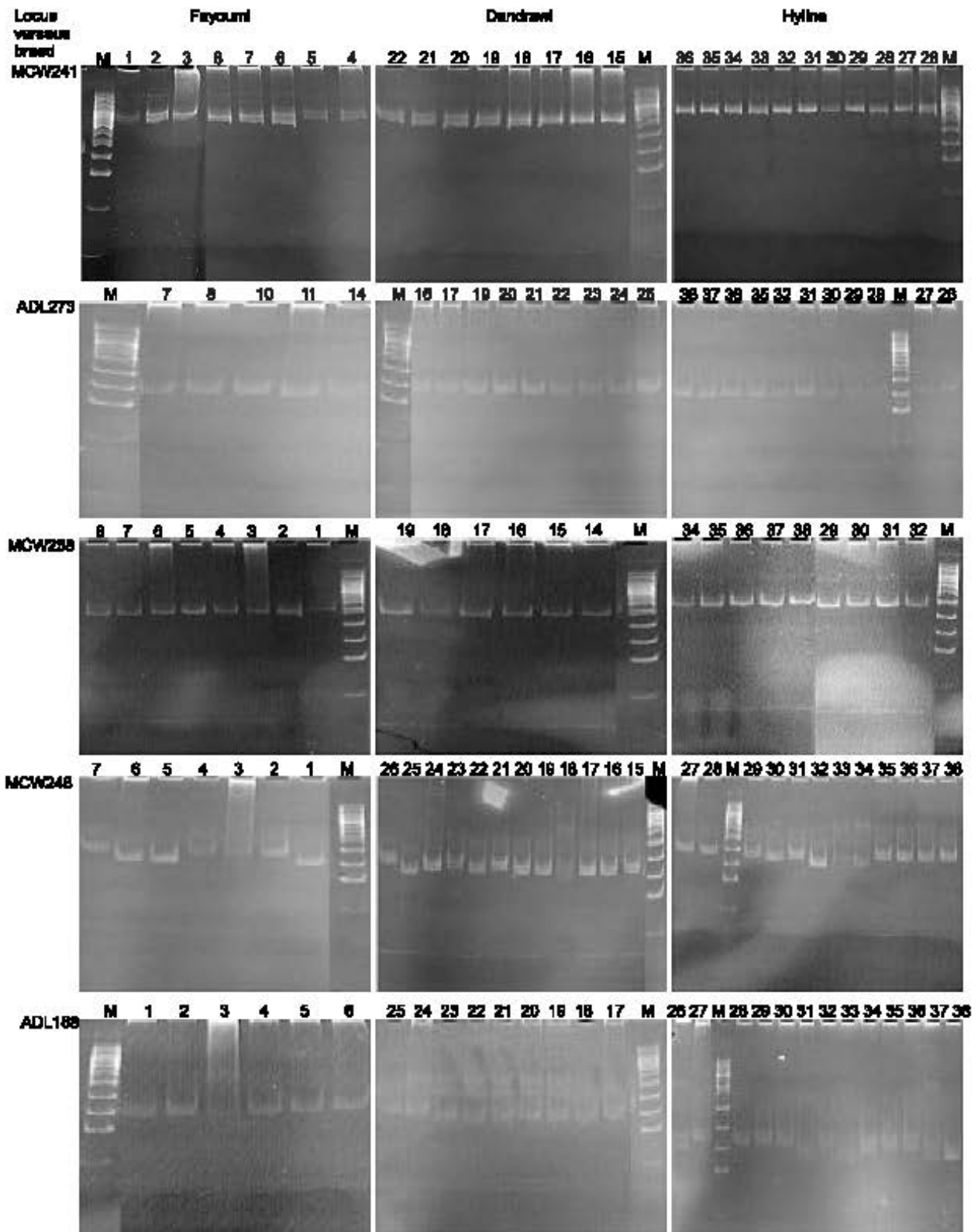


Fig. 1: Examples of some individual microsatellite profile showing marker polymorphism at each locus for each breed

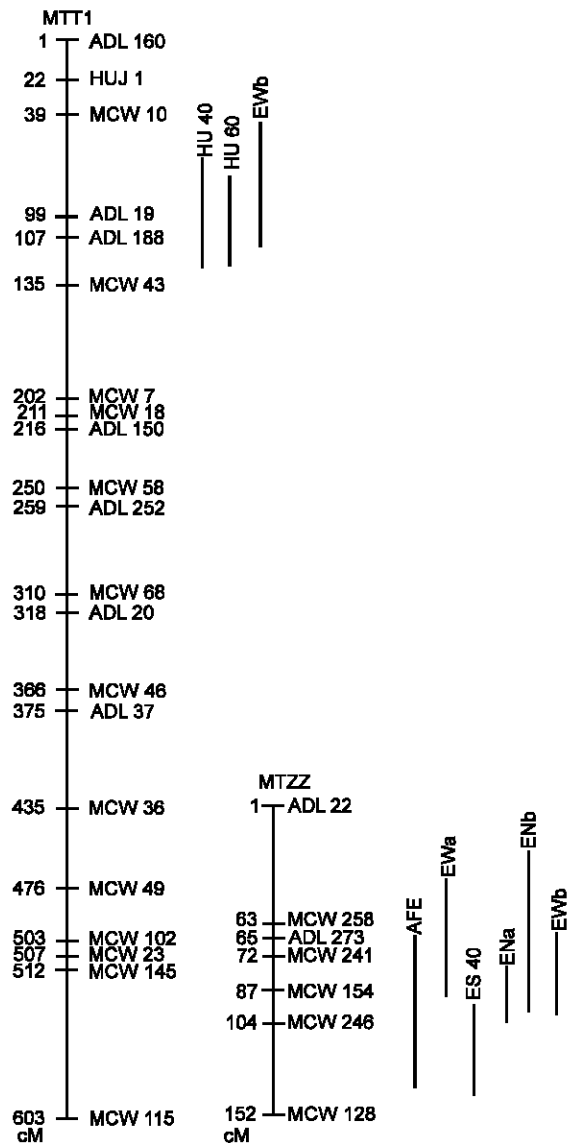


Fig. 2: Genomic regions for production and egg quality traits. HU40 = Haugh units at 40 wk of age, HU60 = Haugh units at 60 wk of age, ES40 = eggshell strength at 40 wk of age, AFE = age at first egg, BW40 = BW at 40 wk of age, EWa = average egg weight from 18 to 40 wk of age, EWb = average egg weight from 41 to 60 wk of age, ENa = total number of eggs from 18 to 40 wk of age, ENb = total number of eggs from 41 to 60 wk of age, FI40 = feed intake per day from 37 to 40 wk of age (after Tuiskula *et al.*, 2002).

traits was highly performed (4.75, Table 4) in Fayoumi breed, it might be attributed to allele 190 (0.385) at locus MCW-246. This illustrates negative correlation of the highest allele frequency (0.607) in Dandrawi breed of allele 210 and Hy-line strain for the same allele (0.409).

In contrary, the two locus of MCW-258 and ADL-188 are inconclusive to extract correlation of such allele for such quantitative traits.

As given in Table 6, the most specific alleles were found in the Dandrawi population (9 versus all loci). The lowest number of specific alleles were traced in Hy-Line population (1 at locus MCW-241). No specific alleles were detected in the case of MCW-246 for all 3 breeds. The highest mean expected heterozygosity (HeA) was recorded in Dandrawi breed (0.65; Table 6). Whilst, the lowest HeA was recorded in Fayoumi breed (0.56) close to Hyline breed (0.59). The analysis of molecular variance (AMOVA) proved that the majority of variations scored herein is referred to within population (FIS) rather than to subpopulation among total variation (FST); 0.61 versus 0.19, respectively.

Genetic diversity among breeds: Among the three populations analyzed in the current work, the highest variability potential was demonstrated by the Dandrawi breed (5.6), whereas the lowest (4) was demonstrated by the Hyline population (Table 5). Genetic variability among the 3 breeds analyzed was described on the basis of genetic distance (Table 7). The closest genetic similarity was recorded between Fayoumi and Hyline. However, the largest genetic distance was observed between

Hyline and Dandrawi. This implies that the highest heterosis effect could potentially be obtained when crossing birds of those breeds. The breed structure observed in the three populations examined seems to reflect the geographic origin of individual chicken breeds.

The genetic distance, calculated on the basis of the microsatellite analysis, was largest between Hyline and Dandrawi (0.25), whereas the lowest values were observed between Fayoumi and Hyline (0.16). This analysis showed that the populations of Fayoumi were more closely related to the Hyline than to the Dandrawi (0.21). This observation might be attributed to different genetic origin of the three breeds since Fayoumi breed was originated as imported chickens to Egypt long time ago. Another possibility might be referred to the effect of unexpected gene flow between the two breeds (F and H). Finally, this claim requires a further work dealing this point of view based on larger population size as well a comprehensive genome wide span significant analysis. The results showed herein based on microsatellite genetic markers proved the usefulness of this type of marker in chickens genome analysis and differentiation of variable population origin even non-related ones. As reported by Soller *et al.* (2006) the breeding of egg quality traits by traditional methods is difficult because the phenotypic measurements are time consuming. As well, their use in breeding programs is complicated due to unfavorable negative correlations with other relevant

Table 5: Number of detected alleles, range of frequencies, both lowest & highest allele(s) and its frequency corresponding each breed for each locus

| No | Trait | Locus | Breed | No. Alleles | Frequency Range | Alleles | |
|----------------------------|-------|--------|----------------------|-------------|-------------------------------|----------------|---------|
| | | | | | | lowest | Highest |
| 1 | AFE | MCW241 | F | 7 | 0.038-0.346 | 280,290 | 300 |
| | | | H | 7 | 0.045-0.455 | 300,310,350 | 270 |
| | | | D | 6 | 0.036-0.429 | 350 | 310 |
| | | | Average ^a | (6.7) | - | - | - |
| 2 | EN | ADL273 | F | 4 | 0.091-0.500 | 43,176 | 165 |
| | | | H | 3 | 0.091-0.818 | 15,165 | 143 |
| | | | D | 5 | 0.071-0.571 | 13 | 110 |
| | | | Average | (4) | - | - | - |
| 3 | ESS | MCW246 | F | 5 | 0.077-0.385 | 240 | 190 |
| | | | H | 4 | 0.091-0.409 | 190 | 210 |
| | | | D | 4 | 0.071-0.607 | 240 | 210 |
| | | | Average | (4.3) | - | - | - |
| 4 | EW | MCW258 | F | 7 | 0.038-0.269 | 99,176 | 143 |
| | | | H | 4 | 0.136-0.318 | 110 | 121 |
| | | | D | 8 | 0.036-0.429 | 99,110,165,176 | 121 |
| | | | Average | (6.3) | - | - | - |
| 5 | HU | ADL188 | F | 3 | 0.115-0.731 | 96 | 108 |
| | | | H | 2 | 0.364-0.636 | 108 | 96 |
| | | | D | 5 | 0.071-0.571 | 108,144 | 156 |
| | | | Average | (3.3) | - | - | - |
| Total average ^a | | | F | 5.2 | | | |
| | | | H | 4 | | | |
| | | | D | 5.6 | | | |
| | | | | | Overall mean of alleles = 4.9 | | |

a; average no. of alleles for each locus versus breeds. b; average no. of alleles for each breeds versus all loci, Different alleles with the same frequency within each population are listed and separated by comma.

Table 6: Population-Specific allele(s) per locus, heterozygosity (H) regarding each locus, average of heterozygosity (HA) over all loci and average of F-statistics (F-index) over all loci.

| No | Trait | Locus | Breed (N) | Specific Alleles | Frequencies | Heterozygosity (H _j) |
|--|-------|--------|-----------|------------------|-------------------|----------------------------------|
| 1 | AFE | MCW241 | F (1) | 250 | 0.077 | 0.17 ^m |
| | | | H (1) | 250 | 0.182 | 0.73 ^m |
| | | | D (2) | 320 | 0.143 | 0.74 ^s |
| | | | | 340 | 0.071 | - |
| | | | average | - | - | (0.55) ^s |
| 2 | EN | ADL273 | F (1) | 176 | 0.091 | 0.63 ^s |
| | | | H (0) | Nil | Nil | 0.31 ^m |
| | | | D (3) | 110 | 0.571 | 0.62 ^m |
| | | | | 121 | 0.143 | - |
| | | | | 132 | 0.070 | - |
| 3 | ESS | MCW246 | average | - | - | (0.52) ^s |
| | | | F (0) | Nil | Nil | 0.74 ^s |
| | | | H (0) | Nil | Nil | 0.69 ^s |
| | | | D (0) | Nil | Nil | 0.57 ^s |
| | | | average | - | - | (0.67) ^s |
| 4 | EW | MCW258 | F (0) | Nil | Nil | 0.81 ^m |
| | | | H (0) | Nil | Nil | 0.73 ^m |
| | | | D (1) | 165 | 0.036 | 0.69 ^m |
| | | | average | - | - | (0.74) ^m |
| | | | | - | - | - |
| 5 | HU | ADL188 | F (1) | 120 | 0.154 | 0.43 ^s |
| | | | H (0) | Nil | Nil | 0.46 ^m |
| | | | D (3) | 132 | 0.143 | 0.62 ^s |
| | | | | 144 | 0.710 | - |
| | | | | 180 | 0.143 | - |
| Average of expected heterozygosity (H _{e,a}) over all loci | | | average | - | - | (0.5) ^s |
| | | | F | | 0.56 ^s | |
| | | | H | | 0.59 ^s | |
| | | | D | | 0.65 ^s | |
| Average of F-statistics over all loci | | | FIS | | 0.61 | |
| | | | FST | | 0.19 | |

Breed (N); breed name denoted by number of specific alleles between two parentheses. F; Fayoumi, H; Hyline and D; Dandrawi. H_j; Heterozygosity (%) expected and its average of per locus versus breeds. H_{e,a}; Average of heterozygosity (%) expected of each breeds versus all loci. N and S; not significant and significant X² (P value 0.1 X 10⁻⁴). FIS and FST; F-index due to within breeds among individuals and F-index due to among breeds; respectively.

Table 7: Genetic distances (D) matrix among three different breeds estimated as pair-wise differences

| Breeds | Fayoumi | Hyline | Dandrawi |
|----------|---------|--------|----------|
| Fayoumi | - | | |
| Hyline | 0.16 | - | |
| Dandrawi | 0.21 | 0.25 | - |

traits. Genetic diversity measures using microsatellites yield reliable estimations of variability within and genetic relationships among chicken populations, as demonstrated in many studies (for instance; Delany, 2003).

The present work was in focus with, mainly, Z-chromosome (152cM) covering, approximately, 26% of its total length. The QTL region on the Z chromosome was a large area including QTL for sexual maturity, egg weight and number of eggs in laying periods, as well as eggshell strength (Tuiskula *et al.*, 2002).

The highly heterozygosity (expected) presented here demonstrates microsatellite capability to discriminate among the three population used. This result is confirmed by the work of Powell *et al.* (1996). He demonstrated that detection of microsatellite polymorphism results in the greatest expected heterozygosity. As shown by Allen *et al.* (1995), STRs have proven to be useful in the assessment of overall genetic variation estimate, most of populations parameters, as well, to gain insight into the degree of population substructure. As well, Zhang *et al.* (2002a,b) illustrated that microsatellite polymorphisms enable a clearer differentiation, even between closely related breeds, and increase the accuracy of the predicted divergence.

In conclusion, the five microsatellite genetic markers applied in the present study success to reveal high degree of polymorphism among the three breeds used here. Also, a clear discriminating power was achieved in differentiation among studied chicken populations. Genetic diversity based on F-index proved high degree of variability within population level (FIS) more than among population level (FST) in regard to total variations. The genetic distance based on pair-wise differences approach revealed that Fayoumi breed is mostly related to Hy-line strain more than Dandrawi breed. This work presented here support the requirement of further work based on genome wide span included larger population size and applying a set of microsatellite markers covering most of chicken genome of such breeds.

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