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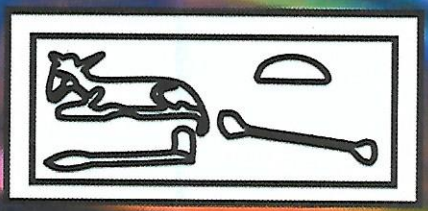

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ASSESSING GENETIC VARIATION, STRUCTURE AND RELATIONSHIP OF EGYPTIAN SIX LOCAL CHICKEN STRAINS WITH SPECIAL REFERENCE TO CROSSBREEDING INFLUENCE

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Poultry especially chicken is more efficient feed converter and has a shorter production cycle than red meat animals. Thus, in the past, government policies directed much toward increasing its meat and egg production. The current chicken genetic resources in Egypt are the result of a long history of human activities and trade dispersal process as early as 1840 BC. (Clotherd, 1966). So far later, some chicken strains were well established in Egypt mainly for food production about 600 B.C during Greek and Persian influence where they remained until now (Clotherd, 1966). Based on molecular data, El-Tanany (2011) proposed that Fayoumi standard breed is the most original chicken strain in Egypt. Furthermore, Dandarawi is an old pure local chicken breed. However, they both are bred in very restricted geographical regions (Upper-Egypt mostly) and as experimental populations in institutional research farms as well.

In particular Fayoumi, the ancient best known chicken breed for high survival, resistance and wild aggressive life (Tixier-Boichard *et al.*, 2009), was not only utilized intensively as ancestor in crossbreeding and creation of newly synthetic breeds but also suffered from intensive selection (Hosny, 2006). Subsequently, the genetic diversity and uniqueness of Fayoumi could be at risk to be lost (through intensive selection, subdivision and inbreeding) or diluted (via sever crossbreeding). In addition, there are in Egypt local mongrel chickens with no specific characteristics such as village chickens (Baladi) and Sinai. The former is well known and distributed allover Egyptian villages and commercial farms (Hosny, 2006) to serve as dynamic genetic entities of chicken reservoir in Egypt. There are lots of synthetic improved breeds originated from crosses between local and exotic standard breeds to possess both high economic and adaptive values. Nonetheless, they are inbred in agricultural research farms and poorly known and utilized in commercial, small holder and rural sections. On contrary, commercial chicken industries (broilers and layers) in Egypt are increasingly growing since 1964 up till now especially in last decades on expense of native chicken resources improvement and maintenance (Hosny, 2006). Therefore, this wide gap between commercial and local chicken industries and production may lead to loss of Egyptian indigenous chicken varieties.

In this respect, the genetic diversity of these local chicken strains is in an urgent appeal to be monitored and prioritized for conservation and developed sustainably. Conservation of genetic diversity of Egyptian chicken resources helps ensure long-term food security. In addition, conservation of specific chicken breeds of particular cultural and historical values such as Fayoumi may be necessary to sustain the bequest value of livestock, and to fulfill the rights of an existing genetic resource to continue to exist (Hanotte *et al.*, 2005). The Global Plan of Action for Animal Genetic Resources (FAO, 2007) promotes the sustainable use and conservation of animal genetic resources. It includes four Priority Areas: 1. Characterization, inventory and monitoring of genetic diversity and associated risks; 2. Sustainable use and development; 3. Conservation; and 4. Policies, institutions and capacity-building. Therefore, prioritization methods of breeds for conservation mainly those are based on molecular genetic information are highly needed (Boettcher *et al.*, 2010) such as: 1. the Weitzman method designed for decision making across species and measures diversity as distinctiveness, 2. alternatives that measure diversity as co-ancestry (i.e. also within-breed variability) have been proposed. The availability of molecular markers can provide useful information on relatedness between populations, breed identity and within-breed diversity (Tixier-Boichard *et al.*, 2009).

There were some recent diversity studies based on biochemical and molecular characterization for several Egyptian chickens mainly using RAPD-PCR and microsatellite markers (Mohmed *et al.*, 2001; El-Gendy, 2005; El-Tanany, 2005; Roushdy *et al.*, 2008 a&b; El-Tanany, 2011). Major advantages of microsatellites are: they are unique, abundant and evenly distributed loci over the whole genome and can be detected by PCR. In addition, they are considered as highly polymorphic genomic markers. They reflect influences of the genetic diversity due to their high information content and function in population identification and assignment. Furthermore, considering neutrality of microsatellites makes them a maker of choice to yield a reliable picture of the diversity status away from different selection stresses. Therefore, there are many studies that have been conducted to evaluate genetic diversity within and between distant chicken populations including wild, domestic and commercial types using microsatellite markers (Wimmers *et al.*, 2000; Granevitze *et al.*, 2009).

The first purpose of the present study is to monitor and further characterize genetic variation, uniqueness, structure and relatedness for six Egyptian chicken strains, selected on basis of genetic overlap from previous study of Eltanany (2011), using 29 genome-wide microsatellite markers and further different statistics and analytic criteria. In addition to monitor how great crossbreeding and selection influenced their genetic diversity that can help to establish a promising trend of sustainable improvement. Secondly, optimum contribution to genetic diversity

components and prioritization of strains studied for conservation are to be measured as well.

MATERIALS AND METHODS

1. Chicken populations

The strains used in the present study were the native Fayoumi (two selected lines: PP & GG) and four synthetic breeds including Doki-4, Golden-Montazah, Gimmizah and Bandara. They are bred as genetic pools in Al-Azzab station-4, belonging to the Poultry Integrated Project at Fayoum governorate. In which all strains bred with flock size of 5000 birds, sex ratio of one male to 10 females and under selection program based on individual performance since 1983. Some features of strains under study were described in Table (1).

2. Blood sampling

Approximately 2-5 ml venous blood samples were taken from the ulnar vein and collected in vacuum plastic tubes containing EDTA and then stored at 4°C. The number of birds sampled per population was as the followings: 26 (Fayoumi PP line), 26 (Fayoumi GG line), 25 (Doki-4), 25 (Golden-Montazah), 25 (Gimmizah) and 23 (Bandara).

3. DNA extraction

DNA samples were extracted by modification of the traditional salting-out method (Miller *et al.*, 1988). 50µl of blood was resuspended in 1 ml lysis buffer (10.95 g Sucrose, 1 ml Tris-HCL, 0.5 ml $MgCl_2$, TritonX 1 ml, pH 7.5) and after centrifugation and washing with distilled water, the pellets were incubated with 50 ml Proteinase-K digestion buffer (1 ml Nacl (5 M), 0.5 ml Tris-Hcl pH 8 (1 M), 2.5 ml EDTA pH 8 (0.5 M), 1.25 ml SDS (20%) and 50 ml distilled water) and 2 µl of Proteinase-K (20 ng/ml) over night, then washing the samples from protein traces using 5 M NaCl. DNA was precipitated from the transferred supernatant with 1.1 ml absolute ethanol. After drying, DNA was allowed to be dissolved overnight at 37°C in 50-500 µl 1x T E buffer. DNA was stored at 4°C until genotyping.

4. Microsatellite markers

In this study 29 microsatellite markers distributed on 15 chromosomes of the chicken genome with a minimum distance of 17 cM were exploited (Table 2). Twenty-eight of microsatellites (Table 2) were recommended by FAO (http://dad.fao.org/). The other one, MCW80, was used in AVIANDIV project. Additionally, they have been widely used in previous biodiversity studies in chickens

(Hillel *et al.*, 2007; Granevitze *et al.*, 2009). The four markers (*ADL0268, MCW0034, MCW0183* and *MCW0295*) are highly polymorphic and were reported as more effective markers for cluster analysis and individual assignment in chickens (Rosenberg *et al.*, 2001).

5. PCR procedures and genotyping

DNA was amplified in seven multiplex reactions as given at the AVIANDIV website (http://aviandiv.tzv.fal.de/primer_table.html, Weigend *et al.*, 1998). Each PCR tube contained 17 µl of 2 µl genomic DNA and 15 µl master mix including 10 pmol of each forward primer labelled with either IRD700 or IRD800 (Eurofins MWG Operon, Ebersberg, Germany), 10 pmol of each unlabeled reverse primer, 5-6% DMSO (dimethyl sulfxide) and 10% 10 x $MgCl_2$-containing PCR buffer (Q-Biogene, Heidelberg, Germany).

The amplification protocol comprised of an initial denaturation and enzyme activation phase at 94°C (4 min), followed by 35 cycles of denaturation at 94°C (30 sec), primer annealing at 55°C (1 min), and extension at 72°C (30 sec) and finally cooling at 4°C for 10 min. DNA fragments were scored on 6% polyacrylamide gel using an LI-COR automated DNA analyzer (LI-COR Biotechnology Division, Lincoln, NE). Fragment size was estimated by plotting produced bands versus 50-350 bp standard (LI-COR, Lincoln NE, USA) and reference samples genotyped as given on the AVIANDIV website (http://aviandiv.tzv.fal.de).

6. Data analysis

6.1. Microsatellites variation

The data was analyzed across strains by calculating observed number of alleles (NA); expected number of alleles (Ne) using POPGENE software package version 1.3 (http://www.ualberta.ca/~fyeh/fyeh/); expected heterozygosity (H_E) and polymorphic information content (PIC) via using CERVUS software version3.0.3 (http://www.fieldgenetics.com). In addition, markers Wright's fixation indices (1969), F_{IS}, F_{IT} and F_{ST}, across populations were measured by Weir and Cockerham's (1984) method implemented in FSTAT software version 2.9.3 (Goudet, 2002).

6.2. Genetic variation and uniqueness in each strain

This parameter was evaluated across markers per strain by estimating total number of alleles (TNA); mean number of alleles per locus (MNA); expected number of alleles; expected heterozygosity (H_E) and inbreeding coefficient (F_{IS}) in POPGENE software package version 1.3 (http://www.ualberta.ca/~fyeh/fyeh/). Strain uniqueness

was evaluated through number and frequencies of unique alleles executed via GENALEX 6 in Excel (Peakall and Smouse, 2006). The genetic similarity (marker estimated kinship, MEK) and relatedness (Coancestry coefficient, Co) between individuals in each strain were measured as probabilities of alike alleles in state (AIS) according to Eding and Meuwissen (2001) and identical alleles by descend (IBD) according to Wang (2011), respectively.

6.3. Relationship between studied strains

The Analysis of Molecular Variance (AMOVA) was tested first for all six strains studied and secondly for the three synthetic breeds using GENALEX 6 in Excel (Peakall and Smouse, 2006) that allows the hierarchical partitioning of genetic variance among populations.

The genetic similarity (MEK) and relatedness (Co) between strains under study were estimated according to Eding and Meuwissen (2001) and Wang (2011), respectively. Moreover, Nei standard genetic distance (DA, Nei *et al.*, 1983) matrix to discriminate between studied Egyptian chicken strains was calculated in POPULAION software package (Ollivier and Foulley, 2005; http://bioinformatics.org/project/?group_id=84).

6.4. Cluster and structure analysis of studied strains

This was based on the following criteria: 1. Phylogenetic relationships between studied strains derived from MEK-based distances as well as Nei standard genetic distance (DA) using Neighbour-Net method (Bryant and Moulton, 2004) to construct a phylogenetic network utilizing SPLITSTREE4 software (Hudson and Bryant, 2006). 2. Multivariate analysis was carried out through principle component analysis (PCA) implemented in PCAGEN 1.2.1 (Goudet, 2010; http://www.soft82.com/download/windows/pcagen/). This nonparametric method identifies the primary axes of variation in data and projects the samples onto these axes in a graphically appealing and intuitive manner so it can uncover the underlying genealogical history and demographic process of the studied strains. 3. STRUCTURE software (Pritchard *et al.*, 2000) was used to study population structure and stratifications using genotype data. An admixture model was applied with correlated allele frequencies with $2 \leq K \leq 6$ (for all six strains) and $2 \leq K \leq 3$ for sub-clustering three synthetic strains. There were 20 runs for each K value executed. Iteration number used in each run was 10.000 in Burn-in followed by 50.000 of Markov Chain Method length (MCMC). True number of populations was identified according to Pritchard and Wen (2003) and Evanno *et al.* (2005). Pair-wise comparisons of the 20 solutions of each K value were run along with 100 permutations using CLUMPP software (Jakobsson and Rosenberg, 2007). Therefore, the most frequent solution,

which got the highest similarity index (H), was considered the most probable one. Finally the clustering pattern was graphically displayed using DISTRUCT software (Rosenberg, 2004).

6.5. Contribution of each strain studied to aggregate genetic diversity

The measurement of breed contribution to aggregate or total genetic diversity (due to the within-breeds diversity and the between-breeds genetic distance) was done as follows:

1. According to Ollivier and Foulley (2005) who implied defining for each breed its contributions to the between-breed and to the within-breed diversity. The contribution to between-breed diversity (CB) was computed by estimation of Weitzman values based on the DA genetic distance (Nei *et al.* 1983) with WEITZPRO (Derban *et al.*, 2002; http:// www-sgqa.jouy.inra.fr/ article.php3?id_article=3). Within-breed (CW) contributions to diversity were calculated using the average values of within-breed expected heterozygosity. The aggregate diversity (D1) was obtained after weighting CB by F_{ST} and CW by 1-F_{ST}. Since the F_{ST} estimate represents the proportion of the total genetic variation which is due to differences in allelic frequencies between populations.

2. According to Eding *et al.* (2002) who defined the quantitative assessment of genetic diversity within and between populations as the maximum genetic variance that can be obtained in a random mating population that is bred from the set of populations S. The relative contribution of populations C (i) to a core set of populations (S) which was computed as the overlap of genetic diversity was minimized. Therefore, this method conserves the founder population (and thus minimizes the loss of alleles). The minimum kinship in the core set, $f(S)$ min is proportional to the genetic diversity Div(S) in set S defined as Div$(S) = 1 - f(S)$min.

3. According to Caballero and Toro (2002) who proposed setting priorities for conservation using a criterion of maintenance of the maximum overall Nei's (1987) gene diversity (GD) in the preserved set of breeds. Note that this is equivalent to minimize the overall molecular coancestry (f) because $GD = 1 - f$. The positive contributions to diversity from a given population using the Caballero and Toro's (2002) method mean that the remaining dataset increases the overall diversity; consequently, the assessed population would not be preferred for conservation. This was computed using MolKin version 2.0 software package (Gutiérrez *et al.*, 2005).

4. According to Petit *et al.* (1998) who measured rarefacted number of alleles per locus (k) to assess the contribution of the i^{th} population to the total allelic richness (D2) The positive contributions to diversity from a given population using the Petit *et al.*'s (1998) method mean that the remaining dataset has a lower number of alleles than the original one; consequently, the assessed population would be preferred for conservation. This was computed using MolKin version 2.0 software package (Gutiérrez *et al.*, 2005).

RESULTS AND DISCUSSION

1. Microsatellites variation and within-strain genetic variation and uniqueness

Genotyping of 29 microsatellite loci used in the present study produced 190 alleles totally with a range of 2-16 alleles per locus. The mean number of observed alleles per locus was 6.55±0.69 and mean effective (expected) number of alleles (Ne) was 3.15±0.27 (Table 3). Thus, both observed and expected number of alleles referred to enough sample size and reliable polymorphism information supplied by highly distributed common loci across studied strains. Moreover, microsatellite loci analyzed showed high genetic polymorphism indicated by high mean values of polymorphism information content (PIC= 0.58±0.03) and expected heterozygosity (H_E= 0.62±0.03) according to Botstein *et al.* (1980) and Ott (2001). (Table 3)

The lowest polymorphic loci were *MCW103* and *MCW98*, while the greatest polymorphic ones were *LEI234* and *LEI94* (Table 3). Comparably, the marker variation assessed here was slightly lower than that obtained in the previous study of Eltanany (2011) who used the same marker set to characterize genetic diversity for ten Egyptian chicken strains (NA = 209; PIC = 0.59 and H_E = 0.64). However, that previous study involved the same six breeds investigated here in addition to Dandarawi, Sinai, Inshas and Silver-Montazah. This could refer to the slight contribution of crossbreeding to introduce more genetic variation to whole Egyptian local strains. Moreover, such low comparable difference indicated repetitive exploiting of limited number of local standard breeds to create new improved synthetic breeds. Hence, in that previous study it was found that Dandarawi had no genetic uniqueness and supposed to have Fayoumi as an old ancestor; Sinai having both exotic and local chickens as founders showed close relatedness to both Fayoumi and synthetic types; Inshas had Sinai as sire ancestors; as well as Silver-Montazah had founders (Rhode Island Red sires and Doki-4 dams) as same as Golden-Montazah. In addition the observed variation here was less than that observed by the same marker genotyping across local Chinese and Zimbabwean chickens (Bao *et al.*, 2008; Muchadeyi *et al.*, 2007). However, the found microsatellite variation across Egyptian

six chicken strains in current study was greater than that found using the same marker set across 65 distant chicken breeds (Granevitze *et al.*, 2007).

It was found global highly significant heterogeneity deficiency of microsatellite loci across strains (F_{IT} =0.095***) contributed to highly significant heterogeneity differentiation between them (F_{ST} =0.074***) rather than to low significant within-strain heterogeneity deficiency (F_{IS}= 0.023*). It was deserved to notify that the Wrights' fixation indices estimated in this study were slightly higher than those observed in the previous study (Eltanany, 2011; F_{IT}= 0.089*** and F_{ST}= 0.068***). This indicated the influence of crossbreeding in diluting the total genetic diversity specially between-strain genetic diversity components. However, the fixation index (F_{IS}) remained unchanged in both studies which is attributed to the same breeding and management strategies undertaken in Al-Azab poultry Integrated Project in El-Fayoum for all bred strains.

Locus *MCW123* demonstrated highly significant differentiation potentiality between the six strains (F_{ST} =0.20***), while *MCW103* did not show significant differentiation in between. However, *MCW103* had the highest significant potentiality to discriminate between ten strains in the previous study in addition to *MCW123*. This may due to the involvement of more hybrids sharing great genetic entities leading to the distribution of common alleles' frequencies in between to be normal (increased in pure strains and decreased in hybrids).

The parameters measuring genetic diversity and uniqueness within each strain are postulated in Table (4). The studied strains had mean values of mean number of alleles (MNA) =4.86±0.40; Ne = 2.85±0.18; PA = 15.3%; H_E =0.58±0.04; F_{IS} = 0.023±0.03; MEK= 0.22±0.07 and Co = 0.18±0.02. It can be seen that value of Ne was higher than fifty percent of value of MNA indicating enough and efficient sampling process in this study. Moreover, value of Co was lower than MEK which inferred from the probability of IBD originated from inbreeding using same ancestors was lower than probability of AIS derived from genetically similar individuals not having same ancestors. Mean values of intra-population genetic diversity were almost equal to those observed in the study of Eltanany (2011). As inferred before, this could be due to the effect of crossbreeding which produces closely related, admixed and variable synthetic hybrids of wider genetic base.

Hence, the narrow spectrum of Egyptian native chicken strains (only two pure breeds, Fayoumi and Dandarawi and two mongrel chickens, Baladi and Sinai) was used intensively in crossbreeding as early as 1950 (Kosba and Abd El-Halim, 2008). This can explain the risk amount laid on their genetic uniqueness in addition to the

risk predicted on aggregate genetic diversity through production of closely related new hybrids sharing the genetic entities of indigenous resources.

As seen in Table (4), Fayoumi lines had the least within-population genetic diversity and most within-population genetic overlapping and relatedness. This is supposed to be attributed to their narrow genetic base as they are ancient native chickens bred as closed populations or subpopulations in restricted geographical areas. This was in agreement with the earlier study of Eltanany (2011). It was observed that Fayoumi GG, selected line for higher growth rate (El-Hossary, 1970), had complete fixation of allele (178 bp) of marker *MCW14* proposed to be attributed to the presence of such an allele in hitchhiking with a cryptic additive allele controlling the trait under selection. However, this line displayed very low non-significant heterozygosity deficits (0.003), but the other Fayoumi selected line PP for higher egg production exhibited significantly high heterozygotes deficiency (0.059*). Both lines had the same value of within-population Co indicating the same degree of inbreeding. These results could indicate occurrence of subdivision of Fayoumi PP population as a result of bottle neck, or that the full-sib family selection undertaken to blossom such a line could be accompanied with decreasing heterozygosity within. Furthermore, the least allelic uniqueness was seen for Fayoumi (lines 1 (2%)) indicating the excessive usage of Fayoumi's genetic pool in synthesis of new hybrids which in turn led to substantial violation and loss of its genetic uniqueness. However in study of Granevitze *et al.* (2007), who used the same marker set used herein to study genetic diversity of 64 distant chicken populations including a Fayoumi population inbred in France since 1978 and reported that Fyoumi population had no allelic privacy.

On the other hand, the synthetic breeds under study displayed high intra-population genetic diversity and most allelic uniqueness, however, least within-population genetic overlapping and relatedness. This might be inferred to their wide genetic base consisted of indigenous and exogenous chicken genetic pools. Noticeably, the Co values were greater than MEK values for synthetic strains in contrast to Fayoumi lines and Doki-4. This could be inferred to that the later types are pure strains have not experienced sever inbreeding; hence IBD originated from inbreeding were less than AIS originated from genetically similar founders and ancestors within their populations (i.e. narrow genetic base). On contrast, the former types are hybrids originated from genetically non similar founders (i.e. wide genetic base), however, their populations involved same ancestors due to inbreeding. These hybrids MEK values (AIS%) less than Co values (IBD%). Golden-Montazah was the greatest variable strain, while Gimmizah was the most unique strain in the present study. Although, Golden-Montazah had largest number of private alleles, they were in lower frequencies (9 (2-6%)) than of Gimmizah 8 (2-10%). Moreover, it was found

that Doki-4 and Golden-Montazah showed excess heterozygosity might be as a result of non-random mating. Gimmizah population showed highly significant heterozygotes deficiency and highest Co value across synthetic types attributed to high inbreeding encountered in it posing its potentially endangerment according to Simon and Buchenauer (1993). (Table 4)

2. Relationship between studied strains

AMOVA test across six strains revealed partitioning of genetic variance to 7% attributed to between-strain genetic differentiation and 93% due to within-strain genetic variation. This value was the same as that obtained for ten Egyptian strains in the study of Eltanany (2011). As mentioned before, producing more hybrids sharing indigenous genetic entities could dilute the between-strain genetic variance component. This was in agreement with the obtained result from application of the AMOVA test for only three synthetic breeds in the current study (Golden-Montazah, Gimmizah and Bandara); revealing only 3% of genetic variation which was attributed to genetic differentiation between them. Hence, both Golden-montazah and Gimmizah had Doki-4 as indigenous founder, while Bandara had Gimmizah as indigenous founder. Note, all these indigenous founders had Fayoumi as the base population. These findings were higher than those among Zimbabwe, Malawi and Sudan chickens (Muchadeyi *et al.*, 2007). Some local and conserved chicken breeds exhibited greater between-breed microsatellite differentiation than that detected in the present study like those from Hungary (22%), France (19%), China (16%) and South-Africa (0.13%) (Bodzsar *et al.*, 2009; Bao *et al.*, 2008; Berthouly *et al.*, 2008; Marle-Köster *et al.*, 2008).

The values of genetic overlapping and relatedness between studied strains are given in Table (5). It was observed that the mean value of between-strain MEK (0.13) was less than that of within-strain MEK (0.22). It is interesting to mention that the current mean value of between-strain MEK is less than that in the study of Eltanany (2011). This observation confirmed the conception mentioned before that crossbreeding with excessive usage of indigenous chicken genetic pools increase genetic overlapping between Egyptian chickens in total. The most genetic overlapping in this study was seen between Fayoumi GG and Doki-4 which had Fayoumi sire ancestors (MEK= 0.34). Agreeably to study of Eltanany (2011), it is suggested that Fayoumi GG is closer to original Fayoumi. Furthermore, this suggestion is in consistent with the observation that Fayoumi GG showed higher genetic similarity (overlapping) to all studied strains than Fayoumi PP. However, the closest genetic relatedness was detected between Fayoumi lines PP&GG (C0 = 0.24) having non-selected Fayoumi chicken as common ancestor.

Golden-Montazah and Gimmizah showed the least genetic overlapping (MEK= 0.01) proposed owing to very low or ever gene flow in between. Moreover, this might be due to low genetic similarity between exotic ancestors of Golden-Montazah (Rohde Island Red) and Gimmizah (White Plymouth Rock). However, there was a higher genetic relatedness detected between Golden-Montazah and Gimmizah (Co = 0.09), as they had Doki-4 as the indigenous ancestor. It was interesting to find that Doki-4 exhibited higher genetic similarity and relatedness to Gimmizah (having ½ Doki-4 in its genetic composition, (Taha *et al.,* 1982a) than to Golden-Montazah (having ¼ Doki-4 in its genetic composition, Taha *et al.*, 1974). Furthermore, Fayoumi lines displayed greater genetic overlapping and relatedness to Gimmizah than to Golden-Montazah. This implied higher genetic overlapping and closer relatedness between Fayoumi and White Plymouth Rock (dam ancestor of Gimmizah) than Rohde Island Red (sire ancestor of Golden-Montazah).

This was consistent with the study of Granevitze *et al.* (2009) in which Fayoumi was clustered by STRUCTURE with European chickens and broiler sire lines (derived from White Cornish (Crowford, 1990)) and dam lines (derived from White Plymouth Rock (Crowford, 1990)) till K=4. On the other hand, the same study showed early genetic discrimination between Fayoumi and Rohde Island Red at K=2. The presumed ancient relationship between Fayoumi and exotic standard breeds was in agreement with the history of establishment of chickens in Egypt about 600 B.C during Greek and Persian influence while they were remaining until now (Coltherd, 1966). In addition, this complied with clustering Fayoumi with some European chicken breeds (Berthouly *et al.*, 2008).

Regarding genetic discrimination between studied strains owing to mutational influences and harboring new alleles, pair-wise Nei's standard genetic distances, DA, were calculated (Table 6). The mean value of DA in current study was less than in the previous study of Eltanany (2011) who involved more Egyptian breeds harboring their own new alleles originated from exotic components of their genetic composition. The closest genetic relationship was detected between Fayoumi lines, while the widest genetic distance was between Fayoumi lines and Gimmizah, the most unique breed.

3. Cluster and structure analysis of studied strains

The phylogeny based-cluster analyses using both MKK-based distance (Dk) (Fig. 1A) and Nei's standard genetic distance, DA (Fig. 1B) were in good agreement. There were two major clusters identified: one cluster with Fayoumi lines and Doki-4 and another with three synthetic breeds, Gimmizah, Bandara and Golden-Montazah. The most distant strain in both cases was Golden-Montzah. Note achievement of the previously mentioned concept that crossbreeding produced closely related synthetic

breeds which were clustered together. It was worth to notice that Gimmizah had closer phylogenetic relationship to Fayoumi and Doki-4, in contrast to Golden-Montazah. This might be attributed that Fayoumi had ancient more genetic admixture with White Plymouth Rock (dam ancestor of Gimmizah) than Rohde Island Red (sire ancestor of Golden-Montazah). This presumption was in agreement with clustering pattern produced by the multivariate analysis using PCA (Fig. 2). In which Gimmizah was positioned on the horizontal axis while Fayoumi lines were located on perpendicular axis revealing presence of an ancient genetic admixture in between (Gil, 2009). However they seemed to be distantly apparent which might be due to genetic drifts and mutational events influenced the presumed anciently admixed genetic entities between Fayoumi (common ancestor of Fayoumi lines) and White Plymouth Rock (dam ancestor of Gimmizah). This should be confirmed through further mitochondrial analysis. PCA revealed that Doki-4 having Fayoumi sire ancestor showed the closest position and so genealogy to Fayoumi lines. Gimmizah closely inbred population had its own structure. Golden-Montazah and Bandara were allocated in the same synthetic space displaying some genealogy which might go back to a phylogenetic relationship between their exotic ancestral components (Rohde Island Red and White Cornish), or due to unexpected migration in between.

The best cluster pattern for the six strains under study using STRUCTURE algorism was achieved at K=3 (Fig. 3A) based on the uppermost values of L(K) (posterior probability of data) (Pritchard and Wen, 2003) and of Δ L(K) (Evanno *et al.*, 2005) (Fig. 3C). The highest similarity index of the depicted solutions for each k value was obtained at K=2 (99.9%) and K=3 (94%). At K=2, Fayoumi selected lines PP&GG having Fayoumi non-selected chicken as common ancestor and Doki-4 which had Fayoumi as sire ancestor (El-Itriby and Sayed, 1966) were separated from other synthetic breeds. Hence, Doki-4 constituted ¼ of Golden-Montazah and ½ of Gimmizah genetic compositions, while Gimmizah was dam ancestor for Bandara (Taha *et al.*, 1974; Taha *et al.*, 1982 a&b). Implicitly, more genetic constituents of Golden-Montazah, Gimmizah and Bandara may represent those of exotic chicken breeds, thus they were early separated from Fayoumi and Doki-4. On the other hand, Doki-4 genetic composite could be pertaining more to Fayoumi gene pool or there was uncontrolled migration in between, therefore, it was clustered with Fayoumi till K=6.

Fayoumi lines clustered as one distinct population till K=6 that goes with other studies which pointed that Fayoumi breed was differentiated distinctly from a wide range of chicken breeds (Berthouly *et al.*, 2008; Granevitze *et al.*, 2009). At K=3, Gimmizah was separated as its own clear cluster, while Golden-Montazah and Bandara clustered together showing great admixture till K=6. Sub-cluster of such synthetic breeds (Fig. 3B) exhibited earlier separation of Gimmizah at K=2, whereas

Golden-Montazah and Bandara showed no clear sub-cluster but clustered similarly as mosaic admixed populations. Although these later two breeds had different origins (Table 1), clustering them together might be attributed to gene flows in between or presence of close genetic relationship between their founders.

4. Contribution of each strain studied to aggregate genetic diversity

In current study different prioritization methods were utilized through measuring the breed contribution to aggregate genetic diversity as an important criterion for its conservation (Table 7). All such methods revealed that Fayoumi-GG contributed negatively to aggregate genetic diversity and its component (within-strain genetic diversity), while it was the least to between-strain genetic diversity using Weitzman method (CB = 6.65). The same situation was seen for Fayoumi-PP, however it contributed only the least (C (i) = 0.15) to the core set constituted to provide the most diversity genetic pool according to Eding *et al.* (2002). Therefore, Fayoumi lines according to such a determined criterion may be not prioritized for conservation. This finding complied with aforementioned concept that intensive usage of Fayoumi and its closest descended synthetic breed, Doki-4, in crossbreeding exhausted their allelic privacy and eroded their genetic barriers allowing sever passing of their own alleles to the newly created hybrids. Moreover, this result also confirmed the previously mentioned assumption that Fayoumi is the most original chicken in Egypt (El-Tanany, 2011).

On the contrary, Gimmizah contributed the most to aggregate and between-strain genetic diversity according to Weitzman (1993), Petit *et al.* (1998) and Ollivier & Foulley (2005) (Table 7). Gimmizah was found to have the following criteria: the most unique breed, having its own clear population, showing obvious genetic overlapping and relatedness with Fayoumi (supposed to be due to genetic relatedness between its dam ancestor and Fayoumi), however, highest genetic distance with Fayoumi and Doki-4. Based on the aforesaid findings about Gimmizah, it can be proposed that crossing between originally overlapped and genetically distant founders can rescue the aggregate genetic diversity and adduce clearly structured breed. However, Golden-Montazah, having the highest observed within-strain genetic variation, was found to contribute slightly higher than Gimmizha to within-strain component of genetic diversity (CW = 2.7) in accordance with Ollivier and Foulley (2005). This could be inferred from the wide genetic base of such a synthetic breed originated from crossing between genetically distant and originally less overlapped founder breeds as assumed before. Therefore, Golden-Montaha was reasonably highly admixed population that contributed obviously less to both aggregate and between-strain genetic diversity (CB = 15.77; D1 = 3.61; D2 = 3.24; GD = -1.57) than Gimmizah (CB = 25.88, D1 = 4.03; D2 = 3.98; GD = -1.12).

Regarding coancestry and genetic overlapping (Caballero and Toro, 2002; Eding *et al.*, 2002), the total founder diversity in the Egyptian core set was 0.98. It was observed that the total genetic diversity value provided by Egyptian core set was higher than that provided by Hungarian core set (0.84). Bandara was the highest that contributed to the aggregate genetic diversity (C (i) =0.36) and ranked firstly in the constituted core set, while, the contribution of Fayoumi GG and Doki-4 was negative and had to be set to zero (Eding *et al.*, 2002). The strains that should be prioritized for conservation according to the obtained core set were ranked as: Bandara, Golden-Montazah, Gimmizah and lastly Fayoumi PP.

In conclusion, severely extensive crossbreeding based on the limited indigenous chicken standard strains in Egypt might lead to lose most of their genetic uniqueness threatening maintenance of their genetic diversity. Therefore, these breeds considered as national treasure especially Fayoumi as seen in this study are in urgent appeal to be conserved and sustainably improved. Nevertheless, the synthetic breeds contributed highest to genetic diversity and should be prioritized for conservation (First: Gimmizah; Second: Golden-Montazah; Third: Bandara). In accordance to this study crossing between originally overlapped and genetically distant founders is recommended to save the aggregate genetic diversity of whole species.

SUMMARY

The genetic variation, structure and relationship of six Egyptian chicken strains including two Fayoumi selected-lines and four synthetic breeds (n=150) were evaluated based on genotyping of 29 genome-wide microsatellite loci. Fayoumi lines displayed the lowest within-population genetic diversity (MNA=4.24; H_E=0.48-0.50) and least allelic privacy (PA=1 (2%)) but showed its own distinct structure. Synthetic breeds displayed high genetic variation and uniqueness (MNA=4.59-5.31; H_E=0.55-0.66; PA=3-9 (2%-10%)), whereas unclear-admixed structure except for Gimmizah. This observation indicates sever integration of genetic entities of Fayoumi founders into synthetic breeds loosing the allelic barrier in between. AMOVA revealed greater genetic variation between the six strains (7%) than between three synthetic breeds Gimmizah, Golden-Montazah and Bandara (3%). Therefore, crossbreeding may dilute the aggregate diversity through intensive sharing gene pools of ancestral strains to create different synthetic hybrids. Mean values of Nei's genetic-distances; 0.10, marker-estimated kinship (MEK); 0.13 and coancestry coefficient; 0.09 inferred considerable genetic differentiation, while, high genetic relatedness between strains studied. Nei's genetic distance- and MEK-based phylogenies showed two major clusters: Fayoumi lines and Doki-4 (cluster 1) and other synthetic breeds (cluster2). Multivariate analysis and STRUCTURE demonstrated Fayoumi lines and Gimmizah as clearly separated populations, but other synthetic breeds as mosaic admixed

populations. Regarding genetic variation and uniqueness, Gimmizah contributed the most to between-population and aggregate genetic diversity, Golden-Montazh contributed slightly higher to within-population genetic diversity, whereas Fayoumi lines added negatively to all components. Regarding genetic overlap, Fayoumi-GG and Doki-4 were the most overlapped with other strains; therefore, they were excluded from the core set in which Bandara was ranked firstly. In conclusion, Fayoumi lost its most genetic uniqueness due to intensive crossbreeding and introgression, thus it is in high need to be conserved. It is recommended molecular characterization of founder breeds utilized in crossbreeding and crossing between genetically distant and originally overlapped populations that could rescue the aggregate genetic diversity.

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Table (1): Some features of Egyptian chicken strains used in the current study according to Fowl Integral Project records, 1999.

Breed	Type	Origin	Female BW (gm)	EW (gm)	Egg No*
FayP	Selected line for egg	Convenient full-sib family selection for five generations to increase egg production from ancient pure Egyptian standard randomly bred Fayoumi population originated in Fayoum in 1970 by Prof El-Hossari at Animal Breeding Research Centre, Agricultural Ministry	At 8 weeks: 470 At 21 weeks: 1150	43	215
FayG	Selected line for meat	The same as FayP but established by convenient individual selection for seven generations to increase BW at 8 weeks of age.	At 8 weeks: 510 At 21 weeks: 1270	45	200
Doki-4	Synthetic breed for egg and meat	From crossing Fayoumi sires and Barred Plymouth Rock dams for 4 self-crossed generations in 1954-1958 by Prof. Abass El-Itribi at Animal Breeding Research Centre, Agricultural Ministry.	At 8 weeks: 540 At 21 weeks: 1340	50	200
G-Mon	Synthetic breed for egg	From crossing Rhode Island Red sires and Doki-4 dams for 5 self-crossed generations in 1968-1973 coupled with selection by Prof. Taha Hussien at Montazah Research Farm and submitted at Animal Breeding Research Centre, Agricultural Ministry.	At 8 weeks: 665 At 21 weeks: 1570	56	205
Gimm	Synthetic breed for meat and egg	From crossing Doki-4 sires and White Plymouth Rock dams for 4 self-crossed generations in 1970-1974 by Prof. Taha Hussien at Gimmizah Poultry Research Farm and submitted at Animal Breeding Research Centre, Agricultural Ministry.	At 8 weeks: 580 At 21 weeks: 1480	53	196
Bandara	Synthetic breed for meat	From crossing White Cornish sires and Gimmizah dams for 4 self-crossed generations in 1973-1977 by Prof Elham Abd El-Gwad at Gimmizah Research Station and submitted at Animal Breeding Research Centre, Agricultural Ministry	At 8 weeks: 750 At 21 weeks: 1960	55	190

FayG = Fayoumi GG line; FayP = Fayoumi PP line; G-Mon = Golden-Montazah; Gimm = Gimmizah; BW= body weight; EW= egg weight; *= egg number in 52 weeks.

Table (2): Some properties of microsatellite loci used in the current study.

Locus*	Chromosome	Motif	Forward primer	Reverse primer
MCW69	26	(CA)11	ATTGCTTCAGCAAGCATGGGA	GCACTCGAGAAAACTTCCTGC
MCW81	5	(TG)17	GTTGCTGAGAGCCTGGTGCAG	CCTGTATGTGGAATTACTTCTC
MCW22	3	(GT)8	GCAGTTACATTGAAATGATTCC	TTCTCAAAACACCTAGAAGAC
MCW34	2	(TG)24	TGTCCTTCCAATTACATTCATG	TGCACGCACTTACATACTTAG
MCW29	4	(CA)10	ATCACTACAGAACACCCTCTC	TATGTATGCACGCAGATATCC
MCW24	1	(CA)9	TTGCATTAACTGGGCACTTTC	GTTGTTCAAAAGAAGATGCAT
ADL278	8	(GT)13	CCAGCAGTCTACCTTCCTAT	TGTCATCCAAGAACAGTGTG
LEI94	4	(CA)16	GATCTCACCAGTATGAGCTGC	TCTCACACTGTAACACAGTGC
ADL268	1	(GT)12	CTCCACCCCTCTCAGAACTA	CAACTTCCCATCTACCTACT
MCW21	13	(GT)9	GGGTTTTACAGGATGGGACG	AGTTTCACTCCCAGGGCTCG
MCW10	3	(CA)8	TTTCCTAACTGGATGCTTCTG	AACTGCGTTGAGAGTGAATGC
MCW20	2	(GT)9	CTTGACAGTGATGCATTAAAT	ACATCTAGAATTGACTGTTC
MCW78	5	(GT)6(AT)4	CCACACGGAGAGGAGAAGGTC	TAGCATATGAGTGTACTGAGC
MCW67	10	(AT)6(GT)11	GAGATGTAGTTGCCACATTCCG	GCACTACTGTGTGCTGCAGTTT
MCW33	17	(CA)n	TGGACCTCATCAGTCTGACAG	AATGTTCTCATAGAGTTCCTGC
MCW98	4	(TG)13	CGATGGTCGTAATTCTCACGT	GGCTGCTTTGTGCTCTTCTCG
MCW10	13	(TG)18	TAGCACAACTCAAGCTGTGAG	AGACTTGCACAGCTGTGTACC
MCW80	15	(TG)10	CCGTGCATTCTTAATTGACAG	GAAATGGTACAGTGCAGTTGG
MCW12	14	(AC)10	CCACTAGAAAAGAACATCCTC	GGCTGATGTAAGAAGGGATGA
MCW20	1	(TG)13	TCTTCTTTGACATGAATTGGCA	GCAAGGAAGATTTTGTACAAA
MCW16	23	(CA)n	CAGACATGCATGCCCAGATGA	GATCCAGTCCTGCAGGCTGC
MCW18	7	(CA)14	ATCCCAGTGTCGAGTATCCGA	TGAGATTTACTGGAGCCTGCC
ADL112	10	(CA)10	GGCTTAAGCTGACCCATTAT	ATCTCAAATGTAATGCGTGC
MCW14	6	(AC)9	ACCGGAAATGAAGGTAAGACT	AAAATATTGGCTCTAGGAACT
MCW37	3	(CA)8	ACCGGTGCCATCAATTACCTAT	GAAAGCTCACATGACACTGCG
MCW16	3	(TG)16	ATGGCGCAGAAGGCAAAGCGA	TGGCTTCTGAAGCAGTTGCTAT
MCW11	1	(AC)8	ATGTCCACTTGTCAATGATG	GCTCCATGTGAAGTGGTTTA
LEI234	2	(CTTT)19	ATGCATCAGATTGGTATTCAA	CGTGGCTGTGAACAAATATG
LEI0166	3		CTCCTGCCCTTAGCTACGCA	TATCCCCTGGCTGGGAGTTT

* Annealing temperature in the used protocol was 55°C for all primers and the source of primer sequences is ArKDB Database web site by the Roslin Bioinformatics Group (http:/www.thearkdb.org/) except the reverse primer of LEI0166 was derived from AVIANDIV web site (http://aviandiv.tzv.fal.de/primer_table.html, Weigend *et al.*, 1998).

Table (3): Marker variations across the studied Egyptian chicken strains.

Locus	N	NA	Ne	PIC	H_E	Fixation Indices		
						F_{IT}	F_{ST}	F_{IS}
MCW14	150	6	1.5	0.31	0.34	0.146	0.139***	0.006
ADL112	150	5	1.7	0.42	0.45	0.096	0.10***	-0.004
MCW183	150	12	5.3	0.79	0.81	0.072	0.028	0.046
MCW103	150	2	1.3	0.23	0.27	0.319***	0.216	0.132
MCW206	150	7	3.7	0.70	0.74	0.068*	0.053	0.017
MCW295	150	7	1.7	0.40	0.42	0.149***	0.076***	0.079**
MCW69	150	9	3.7	0.70	0.74	0.133***	0.079***	0.059
MCW222	150	4	1.8	0.42	0.45	0.032	0.046	-0.015
MCW81	150	7	4.4	0.74	0.78	0.129***	0.12***	0.011
MCW34	150	8	4.1	0.72	0.76	0.043	0.037***	0.006
MCW78	150	5	2.8	0.58	0.65	0.167***	0.121***	0.051
MCW330	150	5	3.2	0.64	0.70	0.131***	0.064***	0.071
MCW67	150	3	2.7	0.56	0.64	0.024	0.045	-0.022*
MCW98	150	2	1.3	0.23	0.26	0.183	0.105	0.078*
MCW123	150	7	2.1	0.48	0.54	0.202	0.2***	0.005
MCW20	150	4	3.6	0.67	0.73	0.087*	0.029	0.06
MCW80	150	8	4.2	0.73	0.77	0.065	0.03	0.036
MCW165	150	3	2.9	0.59	0.66	0.111	0.026	0.087
MCW104	150	13	2.1	0.51	0.53	0.102	0.113*	-0.014
MCW111	150	5	2.5	0.57	0.62	0.142	0.127*	0.016
MCW16	150	7	3.1	0.63	0.68	-0.078	0.024*	-0.105
LEI234	149	16	7.5	0.85	0.87	0.109***	0.075***	0.037
MCW37	149	4	2.5	0.55	0.61	0.102***	0.079***	0.025
ADL268	149	5	3.5	0.67	0.72	0.067	0.087***	-0.022
MCW216	150	7	2.6	0.56	0.63	0.088	0.069	0.021
LEI94	149	16	6.7	0.84	0.85	0.032	0.039***	-0.007
ADL278	149	7	2.9	0.60	0.66	0.146*	0.138***	0.008
MCW248	149	3	1.8	0.42	0.47	0.153	0.014	0.141
LEI0166	150	33	2.8	0.57	0.65	0.049	0.053*	-0.003
Total/Mean		190/6.5	3.1	0.58	0.62	0.095***	0.074***	0.023*
SE		0.69	0.2	0.03	0.03	0.011	0.008	0.009

N = number of genotyped individuals; NA = observed number of alleles; Ne = effective number of alleles; PIC = polymorphism information content; H_E = expected heterozygosity; F_{IT}, F_{ST} and F_{IS} = inbreeding coefficient overall populations, among populations and within populations, respectively; SE = standard error; *, *** = significant deviation from zero at P value < 0.05 and 0.0001, respectively.

Table (4): Genetic diversity within studied six Egyptian chicken strains.

Population	TNA	MNA (SE)	PA (%)	Ne	H_E (SE)	F_{IS} (SE)	MEK (SE)	Co
Fayoumi PP	123	4.24 (0.36)	1 (2)	2.45 (0.24)	0.50 (0.04)	0.059* (0.03)	0.33	0.26
Fayoumi GG[Π]	123	4.24 (0.35)	1 (2)	2.28 (0.19)	0.48 (0.04)	0.003 (0.03)	0.47	0.26
Doki-4	133	4.59 (0.42)	3 (2-6)	2.64 (0.26)	0.55 (0.04)	-0.012 (0.03)	0.26	0.16
Gold-Montazah	154	5.31 (0.44)	9 (2-6)	3.32 (0.24)	0.66 (0.03)	-0.007(0.03)	0.09	0.13
Gimmizah	149	5.14 (0.42)	8 (2-10)	3.12 (0.22)	0.65 (0.03)	0.067* (0.03)	0.10	0.17
Bandara	145	5.00 (0.40)	7 (2-4)	3.30 (0.27)	0.66 (0.03)	0.027 (0.03)	0.06	0.12
Total / Mean	190	4.86 (0.40)	29 (15.3)	2.85 (0.18)	0.58 (0.04)	0.023 (0.03)	0.22 (0.07)	0.18 (0.02)

TNA = total number of alleles; MNA = mean number of alleles / locus; PA = private alleles number (minimum and maximum private alleles frequency percent); Ne = effective number of alleles; PIC = polymorphic information content; H_E = expected heterozygosity; F_{IS} = inbreeding coefficient ; MEK = marker estimated kinship within populations; Co = coancestry coefficient; SE = standard error; *= significant deviation from zero at P value < 0.05; [Π] = FayoumiGG line has one monomorphic marker (*MCW14*) with one completely fixed allele (178 bp).

Table (5): Marker estimated kinship matrix (below diagonal, with mean value of 0.13±0.02) and pairwise coancestry coefficient (above diagonal, with mean value of 0.085±0.02) Egyptian chicken strains studied.

Population	FayP	FayG	Doki-4	G-Mon	Gimm	Bandara
FayP	----	0.24	0.16	0.04	0.04	0.06
FayG	0.24	----	0.17	0.03	0.04	0.05
Doki-4	0.23	0.34	----	0.05	0.06	0.06
G-Mon	0.03	0.08	0.07	----	0.09	0.10
Gimm	0.09	0.24	0.16	0.01	----	0.09
Bandara	0.09	0.12	0.08	0.04	0.11	----

FayG = Fayoumi GG line; FayP = Fayoumi PP line; G-Mon = Golden-Montazah; Gimm = Gimmizah.

Table (6): Nei's standard genetic distance matrix with mean value of 0.10±0.01, DA (Nei *et al.*, 1983)

Population	FayP	FayG	Doki-4	G-Mon	Gimm
FayG	0.03				
Doki-4	0.05	0.05			
G-Mon	0.15	0.15	0.12		
Gimm	0.16	0.16	0.12	0.09	
Bandara	0.12	0.13	0.10	0.06	0.08

FayG = Fayoumi GG line; FayP = Fayoumi PP line; G-Mon = Golden-Montazah; Gimm = Gimmizah.

Table (7): Quantification of contribution of each strain studied to aggregate genetic diversity.

Population	CW	CB	D1	C (i)	GD	D2
FayoumiPP	-2.9	9.12	-2.06	0.15	0.69	-2.23
FayoumiGG	-3.5	6.65	-2.79	0.0	0.89	-2.23
Doki-4	-1.2	13.50	-0.17	0.0	0.81	-1.15
Gold-Montazah	2.7	15.77	3.61	0.32	-1.57	3.24
Gimmizah	2.4	25.88	4.03	0.17	-1.12	3.98
Bandara	2.6	14.64	3.44	0.36	-0.91	1.28
Coreset diversity				0.98		

CW= contribution to within-population genetic diversity; CB = contribution to between-population genetic diversity (Weitzman, 1993); D1= contribution to aggregate genetic diversity according to Ollivier & Foulley (2005); C (i)*N= contribution of strain (i) to optimal core set according to Eding *et al.* (2002); GD= global diversity contribution according to Caballero & Toro (2002); D2= global diversity contribution according to Petit *et al.* (1998).

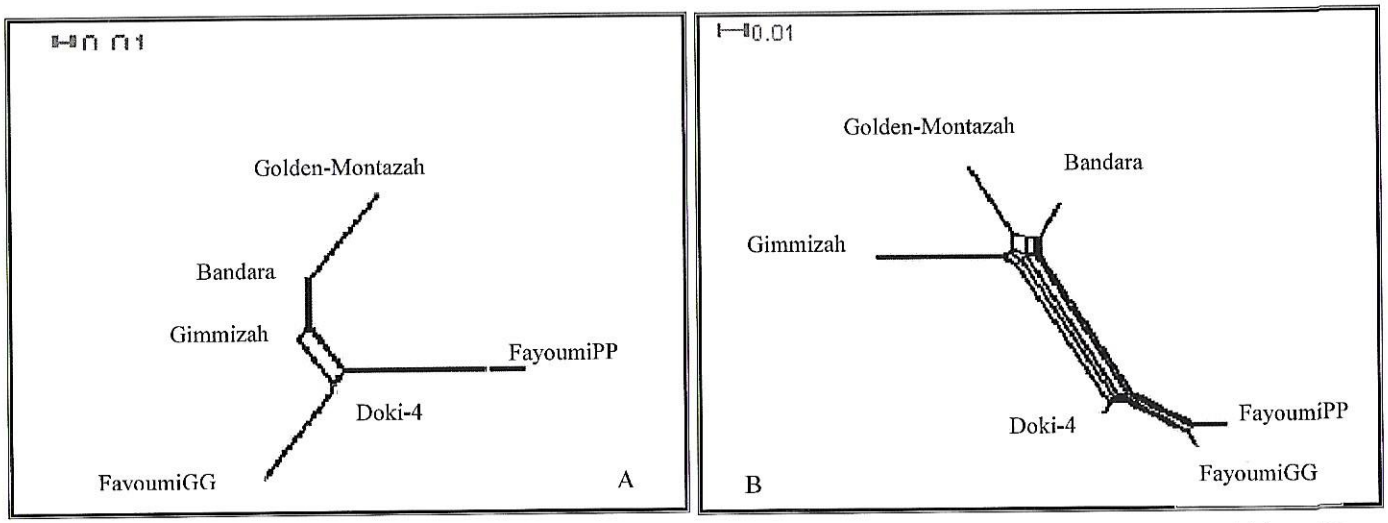

Fig. (1): (A) Neighbour-Net network derived from marker estimated kinship distance (Dk) matrix; (B) Neighbour-Net network derived from Nei's standard genetic distance (DA) matrix; the number refers to bootstraps.

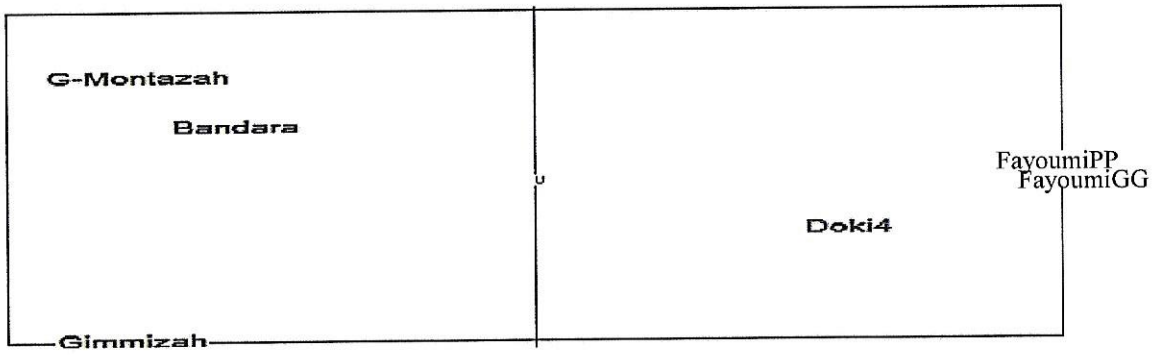

Fig. (2): Principal Components Analysis of six Egyptian chicken strains under current study.

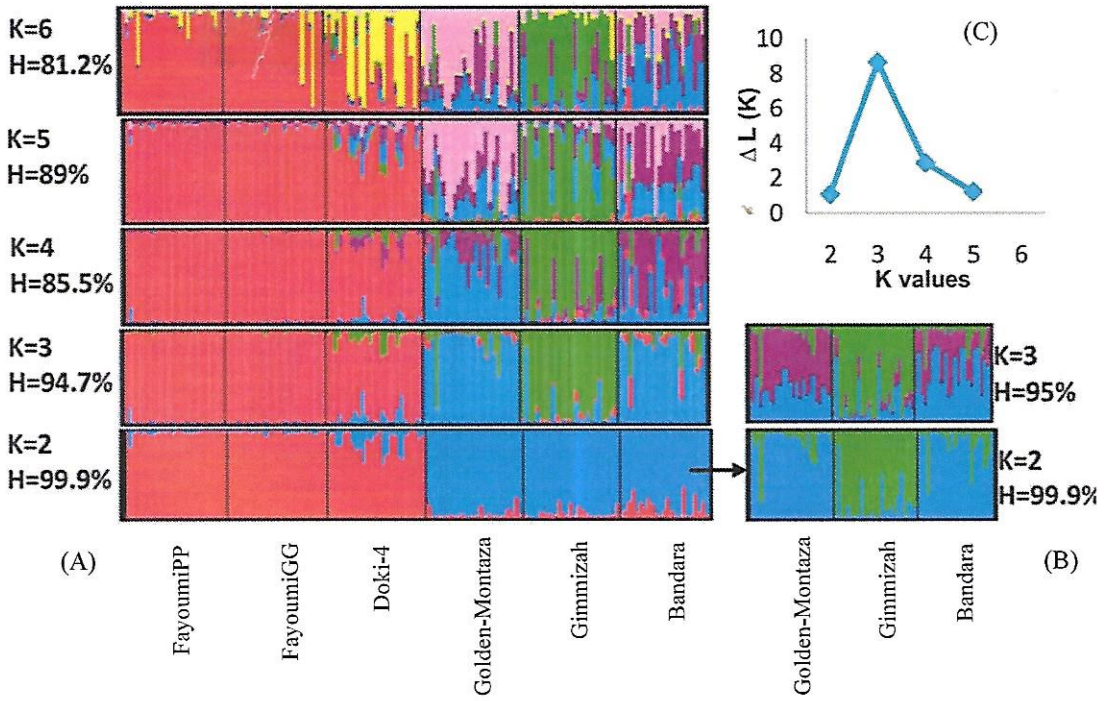

Fig. (3): STRUCTURE cluster pattern of six local Egyptian strains (A); and three synthetic types (B), where K = cluster's number, H = similarity index; K values and their corresponding Δ L(K) values estimated according to (Evanno *et al.*, 2005) were plotted (C), where the true K has the uppermost value of Δ L(K).