IJAS Iranian Journal of Applied Animal Science

Assessing Genetic Diversity in Two Local Chicken Breeds in Egypt Using Microsatellite Markers

Research Article

M.I. El-Hefnawy[1*], E.A. El-Gendy[1], A.M. El-Kaiaty[1] and M. Helal[1]

[1] Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt

Received on: 30 Mar 2023
Revised on: 28 May 2023
Accepted on: 24 Jun 2023
Online Published on: Sep 2023

*Correspondence E-mail: mohammed.is.kamel@agr.cu.edu.eg

Online version is available on: www.ijas.ir

ABSTRACT

Local chicken breeds play vital roles in rural development and sustainability; therefore, the preservation of local breeds' genetic diversity is crucial, also, assessing and advancing local breeds is crucial considering the impact of climate change on chicken production. The current study evaluated the differences between a fully feathered local chicken line (CE2) and a naked-neck local chicken line (CE4) at the microsatellite loci level. For this purpose, nine microsatellite primers were used to scan the genomes of both lines. The results generated by different loci between the two lines showed that the lowest allele numbers were 0 and 4 alleles detected at loci LEI0094 and MCW217, respectively, and the highest allele number was 10 detected at MCW328 and ADL299 loci, with an overall mean of 6.69 alleles/locus. The levels of heterozygosity were relatively high and were higher for line CE4 than for line CE2. The genetic distance between males and females of line CE2 was shorter (0.018) than that between males and females of line CE4 (0.104), and the opposite was observed for genetic identity. Also, ΦPT values were 0.088 ($P<0.001$), and 0.080 ($P<0.01$) for line CE2 and line CE4, respectively. The association between alleles and body weights revealed different alleles of microsatellites MCW217, MCW328, MCW193, ADL299, and MCW064 contributed significantly to body weight variance at different ages. The study provided an outlook on the genetic diversity of two chicken lines and indicated substantial room for improvement within Egyptian native breeds.

KEY WORDS diversity, genomic information, local chickens, microsatellites.

INTRODUCTION

Indigenous chicken breeds are very important from poultry breeder point of view. This is mainly because they have high levels of genetic diversity and can survive persistent exposure to severe environmental circumstances (Mpenda *et al*. 2019). Local chicken breeds contribute to biodiversity conservation by maintaining genetic diversity within the chicken population. They are important source of nutrition and income for rural communities. Also, they are often raised under low-input free-range or organic production systems, which are environment friendly and can help to reduce the use of synthetic inputs in agriculture (Padhi, 2016; Verma, 2021).

Moreover, local chicken breeds are also important for sustainable production, the conservation of local breeds gives us the opportunity to produce animals that fit better into the environment for the production of good quality products for present and future generations in an acceptable manner. However, with the complete dependency of the industry on commercial strains, the loss of genetic diversity in local breeds can be substantial. Additionally, there is an influence for climate change on farm animal genetic resources, adaptation, and conservation (Verma, 2021).

In Egypt, local chicken had a high genetic variation and is yet unexploited, extensive diversity, adaptation to local environments, genetic resources for survival in local environments.

The genetic diversity of poultry genetic resources allows for the sustainable ability of populations to respond to selection for increased productivity as well as to adapt to changing environmental conditions, including not only those related to climate, but also to changes in the market, management, breeding practices, and disease challenges (Malvika *et al*. 2019), and conservation of genetic diversity makeup of local poultry breeds are very essential in developing countries (Besbes *et al*. 2007). It is well known that the reduction in genetic variation and genetic diversity can lead to reductions in fitness and adaptability to the change in environmental conditions (Allentoft and O'Brien, 2010). Microsatellites are short tandem repeats which exist in both coding and non-coding regions of the genome and are co-dominant and highly reproducible (Okumu *et al*. 2017). Also, it has a very rapid rate of evolution making them particularly useful in working out the relationships among very closely related species. Despite the developing of single nucleotide polymorphism markers, microsatellites remain the favorite tool in the genetic diversity studies (Canales Vergara *et al*. 2020). Also, Microsatellite alleles were also associated with loci affecting antibody responses (Yonash *et al*. 2001), and Immunocompetence in chickens (Chatterjee *et al*. 2008). Therefore, microsatellite markers are one of the most significant genetic tools to accurate characterization of local chicken breeds and can be used as a tool for marker-assisted selection (El-Gendy and Helal, 2011). In this concern, Helal and El-Gendy (2023) estimated the increase in 6-week body weight in local chickens by 16.6 to 25.1% if microsatellite markers have been considered in the selection program.

Moreover, it is essential to consider the sex effect when estimating genetic diversity parameters. Sex may influence genetic diversity through different ways, including differences in gene expression, which can lead to differences in physical traits and behaviors between males and females. These differences can contribute to genetic diversity in a population by increasing the number of possible genetic combinations. Additionally, Abasht *et al*. (2006) documented that several QTL displayed sex interaction on both the autosomal and Z chromosomes in chickens. The sex effect leads to differences in allele frequencies between males and females, this can be due to a variety of factors, such as differences in selection pressures, mating patterns, or mutations.

The Naked-neck (Na) gene is thought to have originated in tropical regions, where the loss of feathers on the neck may help the chicken to dissipate heat more effectively. In addition, the gene has been associated with increased resistance to some diseases. By breeding chickens with the naked-neck gene, farmers and breeders can potentially improve their ability to adapt to different environmental conditions and disease pressures. The Na gene can affect diversity in chickens in several ways. Helal and El-Gendy, (2023). El-Gendy *et al*. (2013) evaluated the genomic changes related to genetic selection of 4 populations using 7 microsatellite markers, the populations were broiler dam (line D) with its genetic control (line CD) and the local naked-neck line (CE3) that has been selected for six weeks body weight for six generations with naked-neck chicken (line CE4), and they reported reduction in the diversity due to the existence of NA gene. Therefore, the current study aimed to evaluate the genomic differences between a fully feathered local chicken line and a naked-neck local chicken line.

MATERIALS AND METHODS

The study was conducted during the winter season at Cairo University's Department of Animal Production, where chickens were raised at the Poultry Research Farm and their genomes were analyzed at Poultry Genetics Laboratory (El-Gendy's Lab). All procedures of the current study were approved from the institutional animal care and use committee at Cairo University (CU-IACUC).

Populations, breeding history, and phenotypic performance

For the current study, two local chicken lines were used, normally feathered Baladi (CE2) and naked-neck Baladi (CE4). The two lines were derived from a breeding program that was conducted by El-Gendy (2009) to improve local meat type chickens. The two lines were considered randombred genetic control for their corresponding selected lines. A total of 130 chickens were used for the current analysis, 76 (31 ♂♂ and 45 ♀♀) belong to line CE2, and 54 (22 ♂♂ and 32 ♀♀) belong to line CE4. Chicks were housed in floor chambers in a conventional house, and subjected to an ambient temperature of 32 ˚C during the first three days, and lowered by 2-3 ˚C every week until reached to 22-24 ˚C. Water was provided *ad libitum* during all ages. Commercial diets were provided *ad libitum* from hatch until 8 weeks of age, and 110 g/bird/d during 9-18 weeks of age. Individual day-old and biweekly body weights (BW) until 18 weeks of age were recorded.

Genotyping

Blood collection

At six week of age, blood samples were individually collected from both sexes within each line to sterile tubes con

taining ethylene diamine tetra acetic acid (EDTA) as an anticoagulant. Following that, samples were kept at -20°C until further analysis.

DNA extraction

The genomic DNA from all the gathered samples was obtained by utilizing the phenol/chloroform extraction method explained by Sambrook *et al*. (1989). The whole DNA was then electrophoresed on a 1% agarose gel to verify that the DNA extraction process was successful.

Microsatellite-PCR amplification and allele separation

Nine microsatellite primers distributed across nine autosomal chromosomes were used in a microsatellite-PCR analysis (Table 1). The PCR was carried out in a thermal cycler (Techne, TC 3000, Barlworld scientific Ltd., UK) with a total volume of 12.5 µL of the reaction component (1.5 µL DNA, 1 µL of both forward and reverse primers, 5 µL Master mix, and 4 µL ddh_2o). Amplification was carried out with an initial denaturation and enzyme activation of 95 ˚C for 5 minutes, involved 35 cycles of denaturation at 94 ˚C for 45 seconds, primer annealing at 50-60 ˚C for 45 seconds, and extension at 72 ˚C for 45 seconds. The final extension step involved 72 ˚C for 10 minutes, and the final hold temperature was adjusted to 10 ˚C. The PCR fragments were separated using 8% non-denaturing polyacrylamide gel electrophoresis.

Data analysis

Analysis of variance was carried out on the body weight and number of amplified bands using the general linear model (GLM) procedure of the statistical analysis system (SAS, 1999). The model included line, sex, and their interaction. Popgene (Yeh *et al*. 1999), and Genalex software packages (Peakall and Smouse, 2006) were used to calculate number of alleles, genetic distance, heterozygosity, and F-statistics. Analysis of molecular variance (AMOVA) was performed with 999 permutations tests using Genalex as well.

RESULTS AND DISCUSSION

All the markers were polymorphic and had successfully amplified the selected loci of the genomes. All the previous studies reported that microsatellite or SSR markers are multi-allelic highly polymorphic markers, and widely accepted tools for estimating genetic diversity and measuring divergence within and between populations (Sheriff and Alemayehu, 2018; Canales Vergara *et al*. 2020).

All the nine microsatellites amplified different alleles of the two lines except LEI0094 locus that, did not amplify any alleles in the naked-neck chickens (line CE4), althou-gh the same markers have successfully amplified up to six alleles in the individuals of the fully feathered chicken (line CE2). Moreover, the polymorphic nature of the bands amplified by the different primers is also obvious within line and sex.

In the same context, no monomorphic bands were detected within sex or line, where the percentage of polymorphic bands reached 100% for bands detected by all microsatellite markers. The number of amplified bands is considered the criterion of calculating the similarity, variability, genetic distances, differentiation, and parentage analysis (Bhattacharya *et al*. 2003; Figueira *et al*. 2010).

The results of the current study indicated that the number of amplified bands, overall individuals and markers was 730, where 415 and 315 bands were amplified for lines CE2 and CE4, respectively. Although these numbers imply the richness of the genome of the CE2 line compared to the CE4 line, these absolute numbers are misleading due to the differences in the number of individuals per line. To avoid biased consideration of the absolute number of bands, the relative number of bands was used to compare the amplified bands in males and females of the two lines (Table 2). The lowest relative number of the detected bands was 0.00 at the LEI0094 locus in the males and females of line CE4, as mentioned before there were no bands detected at that locus in the naked-neck chicken line. Also, ADL240 and MCW193 loci showed a low relative number of the detected bands in males of line CE2 with values of 0.60 and 0.70 alleles/individual, respectively. On the other hand, the highest relative number was detected for females of line CE4 at MCW328 and ADL299 loci with values of 7.43 and 6.14 alleles/individual, respectively. It can be noticed that the relative number of bands was higher in females compared to males in both lines.

The effects of line, sex, and line × sex interaction on the relative number of detected alleles is presented in Table 2. The effect of the line was significant at MCW328, MCW193, ADL299, ADL158, and LEI0094 loci, and the effect of sex was significant at LEI0079, MCW193, and MCW64 loci. However, the interaction effect was absent except for the MCW193 locus.

Table 3 presents the information on alleles detected by different microsatellite primers. The results indicate that the microsatellite loci were multi-allelic. Overall loci, the total allele number was higher in line CE2 compared with line CE4 in both males and females. The lowest allele numbers were 0 and 4 alleles detected at loci LEI0094 and MCW217, respectively, and the highest allele number was 10 detected at MCW328 and ADL299 loci, with an overall mean of 6.69 alleles/locus in line CE2, the number of alleles was higher in females (62 alleles) compared to males (61 alleles).

Table 1 List of microsatellite primers used in the current study

Primer	Chromosome	Base sequence	
		Forward	Reverse
LEI0079	1	TCATTATCCTTGTGTGAAACTG	AGGCTCCTGAATGAATGCATC
LEI0094	4	TCTCACACTGTAACACAGTGC	GATCTCACCAGTATGAGCTGC
MCW0193	5	ATTACGTCTGCACCAGTACAG	TATTCAATAGAGTTACGCTGTC
MCW0064	8	TCTCAGCACTACAAAATACACAGG	CTTCAAGAGCCATAGGTGGTCT
ADL0158	10	TAGGTGCTGCACTGGAAATC	TGGCATGGTTGAGGAATACA
ADL0240	12	CGTCCCGTCCTGANTGTTTG	ACCTGGGAGATTGGATTCAA
MCW0217	18	CTGCACTTGGTTCAGGTTCTG	GATCTTTCTGGAACAGATTTC
MCW0328	27	CTCCAATCCCAGGCTCCAAC	ATGGAAACAGATGGAGCTGGC
ADL0299	28	CCACCCCCATGTTCAGGTCA	GTCTAGGCCCCTTGCCAAAC

Table 2 Effects of line, sex, and their interaction on the relative number of detected bands/individual

Microsatellite locus	Line CE2		Line CE4		P-value		
	♂♂	♀♀	♂♂	♀♀	Line	Sex	Interaction
MCW217	2.90	2.46	1.40	1.86	0.145	0.989	0.528
MCW328	4.17	3.15	3.60	7.43	0.001	0.059	0.931
LEI0079	1.60	2.15	1.60	2.86	0.166	0.103	0.463
MCW193	0.70	1.54	4.80	1.00	0.019	0.032	0.001
ADL299	4.20	4.85	5.00	6.14	0.049	0.618	0.156
ADL240	0.60	1.69	1.60	1.00	0.586	0.387	0.650
ADL158	1.40	1.00	2.00	2.14	0.045	0.498	0.831
MCW64	2.70	3.08	2.60	4.43	0.627	0.048	0.330
LEI0094	2.90	1.77	0.00	0.00	0.001	0.055	0.055
Mean	2.35	2.41	2.5	2.98	0.048	0.065	0.074
SE	0.45	0.39	0.55	0.84			

SE: standard error.

Table 3 The allele information at the different microsatellite loci

Microsatellite locus	Number of alleles			
	Line CE2		Line CE4	
	♂♂	♀♀	♂♂	♀♀
MCW217	6.00	5.00	4.00	4.00
MCW328	8.00	10.00	10.00	10.00
LEI0079	5.00	4.00	7.00	7.00
MCW193	7.00	7.00	9.00	8.00
ADL299	10.00	9.00	9.00	9.00
ADL240	6.00	6.00	5.00	5.00
ADL158	5.00	6.00	6.00	6.00
MCW64	8.00	9.00	7.00	9.00
LEI0094	6.00	6.00	0.00	0.00
Total	61.00	62.00	57.00	54.00
Mean	6.10	6.20	5.70	6.40
SD	1.64	2.03	3.08	3.13
	Number of effective alleles			
Mean	1.482	1.529	1.365	1.435
SD	0.096	0.093	0.102	0.098

SD: standard deviation.

On the contrary, in line CE4, the number of alleles was higher in males (57 alleles) than in females (54 alleles). Ramadan *et al*. (2012) evaluated the genetic diversity in Egyptian chickens using twenty-one microsatellite markers and detected 162 alleles with an average of 7.7.

The same tendency was observed for the number of effective alleles, where it was higher in line CE2 (1.505) compared with line CE4 (1.400). Also, it can be noted that the number of effective alleles was higher in females (1.482) than in males (1.424), which can be attributed to the

low number of males in the random bred populations compared with the number of females, where the sexual ratio was set to one male: 10 females. The differences between the two lines are mainly attributed to the effect of the Na gene.

The observed heterozygosity (Ho) levels are presented in Table 4. The levels of heterozygosity were relatively high and were higher for the naked-neck line than for the fully feathered line. The values ranged between 0.71 to 0.87 in line CE2, and from 0.7 to 0.90 in line CE4. Pandey *et al.* (2002) reported high levels of observed heterozygosity in chickens that ranged between 0.23 and 1.00 and attributed that to the low inbreeding levels. The existence of moderate to high heterozygosity indicates that there is still much within-line variation (El-Gendy *et al.* 2013). Nei (1973) suggested that the frequency of heterozygous to be the most important parameter to measure genetic diversity. The current estimates of heterozygosity are also higher than that estimated by Sabry *et al.* (2021) where they evaluated the heterozygosity levels of different chicken ecotypes from Saudi Arabia and Egypt using fourteen microsatellite markers. They reported that the observed and expected heterozygosity values were 0.46 and 0.75, respectively. Do Rosário *et al.* (2009) reported a direct relationship between the number of alleles per locus and both of polymorphism and heterozygosity. Hence, the increase in the number of alleles per locus from 2 to 8 alleles, increased polymorphism information content from 0.40 to 0.80, and the heterozygosity from 0.45 to 0.80.

The results of F-statistics are presented in Table 5. The inbreeding index (F_{IS}) was higher in females compared to males, where the values were 0.038 *vs.* 0.018 and 0.082 *vs.* 0.027 in females and of lines CE2 and CE4, respectively. Also, the F_{IS} values were higher in line CE4 compared to that in line CE2. The inbreeding index (F_{IS}) indicated a possible incidence of inbreeding in population. These values of this study in general indicated the low inbreeding within each line. The same trend was observed for the fixation index (F_{IT}), where the values were negative but higher in females compared to males (-0.043 *vs.* -0.034 in males and females of line CE2, and -0.044 *vs.* -0.043 in males and females in line CE4). The values were also higher in line CE4 compared to line CE2.

The negative F_{IT} in most of the loci indicated the excess in heterozygosity and the decrease in homozygosity. The F_{ST} which denoted obvious breed differentiation had the same trend as F_{IS} and F_{IT}. Hartl and Clark (1997) stated that F_{ST} is the result of the diversity within population and the differentiation among different populations. Accordingly, low F_{ST} values may be obtained in larger populations rather than in smaller populations. The results found in this study coincide with the results obtained on the native breeds, where low F_{ST} values were reported for the Egyptian indigenous breeds. Sabry *et al.* (2021) reported an average population differentiation index F_{ST} of 0.143, overall heterozygosity deficiency F_{IT} of 0.156, and global inbreeding of individuals within breeds F_{IS} was 0.319. Also, Ramadan *et al.* (2012) evaluated the genetic diversity in Egyptian chickens and reported low pairwise F_{ST} value (0.006) between Sinai and Baladi chickens.

Different diversity approaches were used to estimate the diversity for the two lines and for sex within line (Figure 1). The within-line diversity values were almost the same when estimated by Shannon's information index, diversity index, or unbiased diversity index. No specific relationship tendency for values was observed using the three diversity approaches, where a higher value of Shannon's information index was obtained for females of line CE2 and for males of line CE4, and the same was found for unbiased diversity. Pairwise genetic distance (GD) and genetic identity (GI) were estimated between males and females of each line (Table 6). The genetic distance between males and females of line CE2 was shorter (0.018) than that between males and females of line CE4 (0.104), and the opposite was observed for genetic identity. Within-line diversity is an important parameter that reflects the genetic makeup of populations. El-Gendy and Helal (2014) indicated that the genetic distance incidence was 0.8 between CE2 and CE4 lines in a previous generation. Ezzulddin *et al.* (2020) reported a genetic of 0.128 with local Iraqi chickens and attributed that to the high within-population similarity. Sabry *et al.* (2021) also reported short genetic distance for local Egyptian chickens.

Analysis of molecular variance was performed in two times (Figure 2), the first one considered all individuals to evaluate the variance component within and among lines, and the second was used to evaluate variance components within and among sexes within each line. The analysis revealed a higher percentage of molecular variance among males and females of line CE4 (8%, $P<0.001$) compared to that of line CE2 (2%, $P<0.05$). When all individuals were considered, a higher among-lines variance was obtained (11%, $P< 0.001$). Like AMOVA, PhiPT (Φ_{PT}) values were calculated. The values were 0.162 ($P<0.001$), 0.088 ($P<0.001$), and 0.080 ($P<0.01$) for all individuals of line CE2, and line CE4, respectively. PhiPT value represents the genetic differentiation between pairs of populations (Muñoz *et al.* 2017).

However, it suppresses the variance between individuals to allow the estimation of genetic differences. The obtained PhiPT value indicates that the divergence of the two lines is higher than that between the two sexes within each line, and the divergence between males and females of the naked-neck population is higher than the fully feathered one.

Table 4 Observed heterozygosity levels at the different microsatellite loci

Microsatellite locus	Line CE2		Line CE4	
	♂♂	♀♀	♂♂	♀♀
MCW217	0.78	0.76	0.78	0.77
MCW328	0.87	0.87	0.90	0.89
LEI0079	0.80	0.78	0.70	0.78
MCW193	0.73	0.74	0.85	0.82
ADL299	0.82	0.89	0.89	0.89
ADL240	0.83	0.81	0.78	0.78
ADL158	0.71	0.72	0.80	0.76
MCW64	0.84	0.84	0.84	0.86
LEI0094	0.81	0.80	-	-
Mean	0.80	0.80	0.82	0.82
SD	0.05	0.51	0.28	0.27

SD: standard deviation.

Table 5 The average of fixation indices (F_{IT}, F_{ST} and F_{IS})[1] within line and within sex/line

Fixation indices		Line CE2		Line CE4		Line CE2	Line CE4
		♂♂	♀♀	♂♂	♀♀		
F_{IS}	Mean	0.018	0.038	0.027	0.082	0.033	0.045
	SD	0.021	0.023	0.014	0.023	0.015	0.043
F_{IT}	Mean	-0.043	-0.034	-0.044	-0.043	-0.043	-0.040
	SD	0.012	0.023	0.021	0.032	0.023	0.032
F_{ST}	Mean	0.026	0.051	0.031	0.036	0.034	0.032
	SD	0.032	0.002	0.022	0.014	0.023	0.012

[1] F_{IS}: inbreeding index; F_{IT}: fixation index and F_{ST}: population differentiation index.
SD: standard deviation.

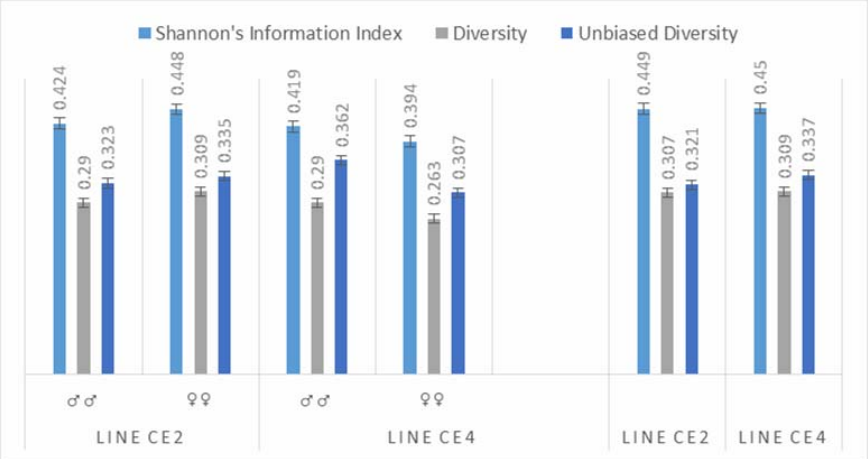

Figure 1 Diversity indices within lines and within sex/line

Table 6 Pairwise Nei genetic distance and Nei genetic identity between males and females of the two lines

Distance/identity	Line CE2	Line CE4
Nei genetic distance	0.018	0.104
Nei genetic identity	0.982	0.901

Moreover, the higher value of PhiPT in line CE2 compared to line CE4 may be associated with the number of alleles detected in each line.

Also, it indicates the purity or identity of those two lines, which is confirmed by the percentage of with-line variance inferred by AMOVA analysis.

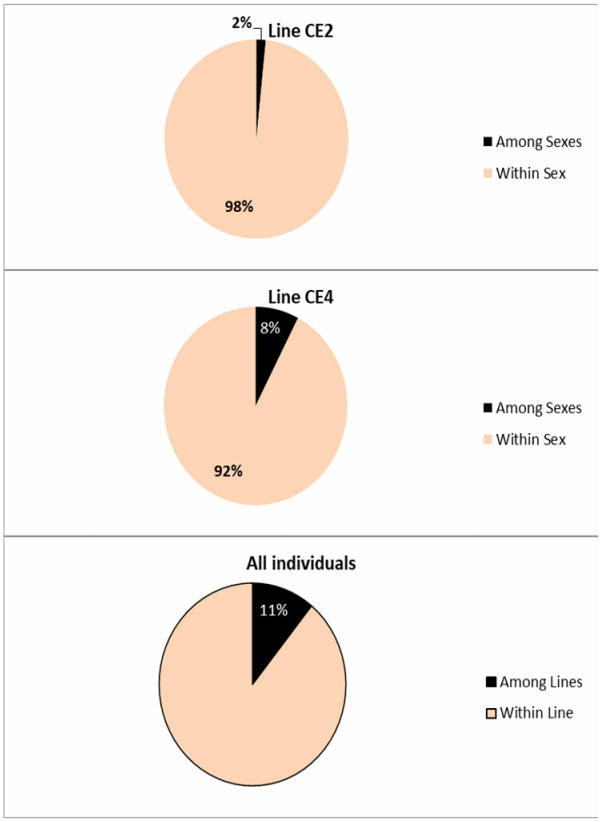

Figure 2 Analysis of molecular variance in the CE2 and CE4 lines

The genetic relationships between the detected microsatellite alleles and loci influencing body weights at different ages of all chickens were estimated. The significant contributions of such loci are presented in Table 7. The results indicated that five microsatellites were linked to loci that contributed to the variations in body weights, where different alleles of microsatellites MCW217, MCW328, MCW193, ADL299, and MCW064 contributed significantly to body weight variance at different ages. In the locus MCW217, there was an allele at 300 bp significantly contributed to the variation in body weights at 2, 4, 6, 10, and 16 weeks of age by 14.22, 12,54, 18.74, 15.43, and 20.22%, respectively. The locus MCW328 had the highest contribution to the variance in body weight at different ages, as there were three alleles contributed to the variance. The first allele was found at 584 bp and significantly contributed to the variation in body weights at the period from 4 to 12 weeks of age by 22.30% (BW4), 15.43% (BW6), 13.34% (BW8), 19.34% (BW10), and 17.54% (BW12), respectively. Another allele at 465 bp contributed to the variance at 12, 14, and 16 weeks of age by 22.23, 24.23, and 14.23%, respectively. The third allele was detected at 214 bp and significantly contributed to the variation in body weights at 6 (10.23%), 8 (13.45%), and 10 (15.34%) weeks of age.

Another allele was detected at 430 bp at MCW193 locus and was found to contribute to the variation in body weights at early ages, where it significantly contributed to the variance at hatch by 32.34%, and during the subsequent ages at 2 (27.54%), 4 (30.21%), and 6 (29.54%) weeks of age. Similarly, the allele detected at loci ADL299 (392 bp) affected the variance in body weights at 2 and 16 weeks of age by 22.43 and 20.21%. Another allele obtained at MCW064 locus at 291 bp significantly contributed to the variance at hatch, 6, 16, and 18 weeks of age by 34.32, 40.23, 16.45, and 13.44%.

Several microsatellites have been recognized for their association with important economic traits like growth and egg production in chickens. In this concern, (El-Gendy *et al*. 2018) reported significant contributions in body weights at 4, and 8 weeks of age in local chickens for alleles detected at LEI0166, LEI0073, ADL0143, and MCW0193 loci. Chatterjee *et al*. (2008) reported microsatellite alleles association with QTLs affecting growth in six crossbred chicken populations, where the alleles at MCW007 locus were significantly associated with body weight at hatch, 8, 12, 20, 28, and 40 weeks of age. A significant association between microsatellite ADL020 locus and body weight at 8, 12, and 40 weeks of age was also reported (Chatterjee *et al*. 2008). Debnath *et al*. (2019) reported a significant contraption of alleles detected at MCW0258 locus to body weight at 8 weeks of age in Rhode Island Red chickens.

Associations between microsatellite markers and traits related to growth and fatness were investigated using resource broiler population (Atzmon *et al.* 2006), where the marker MCW0088 (Chromosome 2 at position 274 cM) was significantly associated with body weight, breast weight and abdominal fat weight. Two other markers (MCW0102 and ADL0255) were significantly associated with body weight, front-half weight, and breast weight, but not associated with abdominal fat weight.

Also, QTLs controlling egg production traits were associated with the polymorphisms of microsatellites. Das *et al*. (2016) studied the association between microsatellites and layer performances in Rhode Island Red selected chickens and reported that alleles detected at locus MCW0075 were significantly associated with age at sexual maturity and egg weight at 28 weeks of age. However, alleles detected at loci MCW0051, MCW0014, and ADL0176 had significant effects on body weight at 40 weeks of age. Similarly, microsatellite loci MCW0041, ADL0210, and MCW0110 were reported to be associated with egg production traits (Chatterjee *et al*. 2010).

Table 7 Significant contributions of loci linked to microsatellite loci and influencing body weights of the local chickens

Microsatellite locus	Allele	Molecular weight	Trait	σ^2_P (Phenotypic variance, %)	P≤0.05
MCW217	*C*	300	BW02	14.22	0.023
			BW04	12.54	0.029
			BW06	18.74	0.032
			BW10	15.34	0.001
			BW16	20.22	0.043
MCW328	*C*	584	BW04	22.30	0.041
			BW06	15.43	0.032
			BW08	13.34	0.023
			BW10	19.34	0.002
			BW12	17.54	0.012
	E	465	BW12	22.23	0.021
			BW14	24.23	0.032
			BW16	14.23	0.072
	G	214	BW06	10.23	0.046
			BW08	13.45	0.035
			BW10	15.34	0.042
MCW193	*D*	430	BW0	32.43	0.032
			BW02	27.54	0.045
			BW04	30.21	0.045
			BW06	29.54	0.034
ADL299	*C*	392	BW02	22.43	0.023
			BW16	20.21	0.001
MCW064	*C*	291	BW0	34.32	0.023
			BW06	40.23	0.031
			BW16	16.45	0.012
			BW18	13.44	0.001

CONCLUSION

This study provided an outlook of the genetic diversity of two chicken lines, and highlighted the efficiency of microsatellite markers genetic characterization and the linkage between microsatllite alleles and body weights in local chcikens. The reuslts denoted that lower diversity and loower allelic reichness in the nack-neck chickens, which requires more studies for identfifying the reseons of this reduction. Moreover, five microsatellites were associated with higher body weights, which support the idea of improving growth by means of marker-assisted selection. Therefore, we recommend that future strategies focus on the accurate characterization of local breeds on both phenotypic and genotypic levels using appropraite techniqes to ensure that the genetic diversity is maintained overtime.

ACKNOWLEDGEMENT

Authors would like to thank the Faculty of Agriculture at Cairo University, Giza, Egypt, for providing farm facilities for collecting the phenotypic data. As well as El-Gendy lab for facilitating animal genotyping. The research has not received an external grant from any funding agency.

REFERENCES

Abasht B., Dekkers J.C.M. and Lamont S.J. (2006). Review of Quantitative Trait Loci Identified in Chicken. *Poult. Sci.* **85,** 2079-2096.

Allentoft M.E. and O'Brien J. (2010). Global amphibian declines, loss of genetic diversity and fitness: A review. *Diversity.* **2,** 47-71.

Atzmon G., Ronin Y.I., Korol A., Yonash N., Cheng H. and Hillel J. (2006). QTLs associated with growth traits and abdominal fat weight and their interactions with gender and hatch in commercial meat-type chickens. *Anim. Genet*. **37,** 352-358.

Besbes B., Tixier-Boichard M., Hoffmann I. and Jain G. (2007). Future trends for poultry genetic resources. Pp. 1-25 in Proc. Int. Poult. Conf. Bangkok, Thailand.

Bhattacharya T.K., Kumar P., Joshi J.D. and Kumar S. (2003). Estimation of inbreeding in cattle using RAPD markers. *J. Dairy Res.* **70,** 127-129.

Canales Vergara A.M., Landi V., Delgado Bermejo J.V., Martínez A.M., Cervantes Acosta P., Pons Barros A., Bigi D., Sponenberg P., Helal M., Banabazi M.H. and Camacho Vallejo M.E. (2020). Design and development of a multiplex microsatellite panel for the genetic characterisation and diversity assessment of domestic turkey (*Meleagris gallopavo*). *Italian J. Anim. Sci*. **19,** 392-398.

Chatterjee R., Sharma R.P., Mishra A., Dange M. and Bhattacharya T.K. (2008). Association of microsatellites with growth

and immunocompetence traits in crossbred layer chicken. *J. Poult. Sci*. **45,** 186-191.

Chatterjee R., Sharma R.P., Bhattacharya T.K., Niranjan M. and Reddy B.L. (2010). Microsatellite variability and its relationship with growth, egg production, and immunocompetence traits in chickens. *Biochem. Genet*. **48,** 71-82.

Das A.K., Kumar S., Rahim A. and Kokate L.S. (2016). Association study between microsatellite genotypes and layer performances in Rhode Island Red chicken. *Indian J. Anim. Sci*. **86,** 1021-1024.

Debnath J., Kumar S., Das A.K. and Rahim A. (2019). Association of microsatellites with pre-housing body weights and age at sexual maturity of Rhode Island Red chicken. *Indian J. Anim. Sci*. **89,** 1118-1122.

Do Rosário M.F., Ledur M.C., Moura A.S.A.M.T., Coutinho L.L. and Garcia A.A.F. (2009). Genotypic characterization of microsatellite markers in broiler and layer selected chicken lines and their reciprocal F1s. *Sci. Agric*. **66,** 150-158.

El-Gendy E.A. (2009). A model for the genetic employment of chickens local to warm climate 1. Crossing with a fast-growing strain and growth patterns of the crossbreds. *Int. J. Poult. Sci*. **8,** 299-306.

El-Gendy E. and Helal M.A. (2011). An approach to marker-assisted selection for increased body weights in local chickens in Egypt. *Poult. Sci*. **90(1),** 1-8.

El-Gendy E., Abbas E., El-Tantawy S., Mohamed A., Helal M., Zaki El-Deen M. and Wageh A. (2013). A model for the genetic employment of chickens local to warm climate. 2. Genome scanning of two lines selected for growth. *African J. Biol. Sci*. **9,** 95-100.

El-Gendy E. and Helal M. (2014). The genetic variation and polymorphism at microsatellite loci in chickens of warm regions selected for meat production. *Int. J. Biotechnol. Allied Fields*. **2,** 100-116.

El-Gendy E.A., Higazy D.M., El-Kashef A.A.M. and Helal M.M. (2018). The growth pattern of warm-region chickens under prolonged natural severe heating conditions with a reference to association between microsatellite loci on chromosomes 3, 4 and 5 and QTL of growth. Pp. 990-995 in Proc. World Congr. Genet. Appl. Livest. Prod., New Zealand.

Ezzulddin T.A., Jwher D.M. and Dabdoub S.A. (2020). Detection of similarity and genetic distance between Iraqi chicken varieties and different standard strains. *Iraqi J. Vet. Sci*. **34,** 333-337.

Figueira G.M., Risterucci A.M., Zucchi M.I., Cavallari M.M. and Noyer J.L. (2010). Development and characterisation of microsatellite markers for Boraginaceae (*Cordia verbenacea*), an important medicinal species from the Brazilian coast. *Conserv. Genet*. **11,** 1127-1129.

Hartl D.L. and Clark G.C. (1997). Principles of Population Genetics. Sinauer Associates, Sunderland, United Kingdom.

Helal M.M. and El-Gendy E.A. (2023). Marker-assisted selection for improving body weight in local chickens in Egypt. *J. Agric. Sci*. **161,** 135-147.

Malvika S., Ghosh P.R., Dhar B., Devi N.N., Paul R., Halder A., Mazumder A., Choudhury Y., Kumar S. and Ghosh S.K. (2019). Genetic status of indigenous poultry (red jungle fowl) from India. *Gene*. **705,** 77-81.

Mpenda F.N., Schilling M.A., Campbell Z., Mngumi E.B. and Buza J. (2019). The genetic diversity of local african chickens: A potential for selection of chickens resistant to viral infections. *J. Appl. Poult. Res*. **28,** 1-12.

Muñoz L.J., Carballosa-Gautam M.M., Yanowsky K., García-Atarés N. and López D.E. (2017). The genetic audiogenic seizure hamster from Salamanca: The GASH: Sal. *Epilep. Behav*. **71,** 181-192.

Nei M. (1973). Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci*. **70,** 3321-3323.

Okumu O.N., Ngeranwa J.J.N., Binepal Y.S., Kahi A.K., Bramwel W.W., Ateya L.O. and Wekesa F.C. (2017). Genetic diversity of indigenous chickens from selected areas in Kenya using microsatellite markers. *J. Genet. Eng. Biotechnol*. **15,** 489-495.

Padhi M.K. (2016). Importance of indigenous breeds of chicken for rural economy and their improvements for higher production performance. *Scientifica*. **2016,** 1-9.

Pandey A.K., Tantia M.S., Kumar D., Mishra B., Chaudhary P., and Vijh R.K. (2002). Microsatellite analysis of three poultry breeds of India. *Asian-Australasian J. Anim. Sci*. **15,** 1536-1542.

Peakall R. and Smouse P.E. (2006). GENALEX 6: Genetic analysis in excel. Population genetic software for teaching and research. *Mol. Ecol. Notes*. **6,** 288-295.

Ramadan S., Kayang B.B., Inoue E., Nirasawa K., Hayakawa H., Ito S. and Inoue-Murayama M. (2012). Evaluation of genetic diversity and conservation priorities for Egyptian chickens. *Open J. Anim. Sci*. **2,** 183-190.

Sabry A., Mohamed A.A. and Hassen M. (2021). Genetic diversity of indigenous chicken (*Gallus Gallus domesticus*) from ecozones of Egypt and Kingdom of Saudi Arabia. *Open J. Anim. Sci*. **11,** 775-787.

Sambrook J., Fritsch E.F. and Maniatis T. (1989). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Press, Cold Spring Harbor, New York, USA.

SAS Institute. (1999). SAS®/STAT Software, Release 8. Institute, Inc., Cary, NC. USA.

Sheriff O. and Alemayehu K. (2018). Genetic diversity studies using microsatellite markers and their contribution in supporting sustainable sheep breeding programs: A review. *Cogent Food Agric*. **4,** 1-9.

Verma A.K. (2021). Influence of climate change on balanced ecosystem, biodiversity and sustainable development: An overview. *Int. J. Biol. Innov*. **3,** 331-337.

Yeh F.C., Yang R.C. and Boyle T. (1999). POPGENE Version 1.32: Microsoft Window-Based Freeware for Population Genetics Analysis. University of Alberta, Edmonton.

Yonash N., Cheng H.H., Hillel J., Heller D.E. and Cahaner A. (2001). DNA microsatellites linked to quantitative trait loci affecting antibody response and survival rate in meat-type chickens. *Poult. Sci*. **80,** 22-28.