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Assessment of genetic diversity and conservation priorities among five White Leghorn Lines based on SSR markers*

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Genetic diversity in livestock is required in breeding studies, to meet requirements of current production as well as adapt to rapidly changing environmental conditions. Identification of genetic diversity within species, breeds or lines is important for the development of conservation strategies. This study was aimed at identifying genetic diversity, population structure and conservation priorities in five white layer pure lines (Blue, Brown, D-229, Black, Maroon). In this study a total number of 150 samples (30 samples from each line) were genotyped using 19 microsatellite loci to identify genetic diversity. All loci were found to be polymorphic, with the mean number of alleles per locus ranging from 3.95 (Blue) to 4.84 (D-229). The highest average observed heterozygosity was recorded in the D-229 line (0.52), while it was lowest in the Blue line (0.41). The inbreeding coefficient ranged from 0.26 (Black) to 0.39 (Maroon). In the analyses conducted to identify conservation priorities, the chicken line with the highest contribution to total genetic diversity was found to be D-229 (1.34). Genetic differentiation coefficients (pairwise F_{ST}) ranged between 0.07 (Brown and D-229) and 0.20 (Blue and Black) among the chicken lines used in the study. Research findings indicate that pure chicken lines have low levels of genetic diversity, but high levels of inbreeding and genetic differentiation. Selection applied for various purposes over extensive periods of time to the chicken lines with the same genetic origin has resulted in genetic differentiation. For the sustainable use of these populations, inbreeding in lines should be reduced and genetic diversity, particularly in the D-229 line, should be conserved.

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Genetic diversity in livestock is required in breeding studies, to meet current production levels and to promote adaptation to environmental conditions that will possibly change in the future. Genetic diversity is also an important part of conservation genetics. Genetic diversity of livestock species or breeds has significantly been reduced in the last century due to intensive selection processes applied to increase some economically important yield traits, producers' preference of some high yielding breeds over local breeds and geographical barriers being overcome by easier intercontinental transportation. Today, the decrease of genetic diversity in livestock leads to significant concerns [Mahmoudi *et al.* 2010, FAO 2011, Ramadan *et al.* 2012].

The reduction in genetic diversity of livestock has become more obvious in commercial chicken production. Because of breeding systems employed in commercial chicken production, the numbers of chicken breeds in use have significantly decreased. Currently, layer chickens have three and broiler chickens four dominant genotypes worldwide. Today White Leghorn (WL) is the dominant breed used to produce white layer chickens [Hillel *et al.* 2003].

In the case of the Turkish commercial poultry sector, studies on breeding material production are performed only by the Ankara Poultry Research Institute. There are six brown layer pure chicken lines, i.e. Rhode Island Red-(RIRI and RIRII), Barred Rock – (BARI and BARII), Colombian Rock-(COL) and Line 54-(L-54), as well as five white layer pure chicken lines (Black Line, Brown Line, Blue Line, Maroon Line and D-229) tested at the above-mentioned Institute. Brown layers and Black, Brown, Blue, Maroon lines were imported from Canada in 1995 and the D-229 line was imported in 2010 from the Czech Republic. Studies undertaken on pure lines resulted in the development of three hybrid materials, i.e. two brown lines (ATAK, ATAK-S) and one white line (ATABEY) of layers [Göger *et al.* 2017].

The Black, Brown, Maroon and Blue lines are used as sire lines, while D-229 is used as a dam line to obtain the white layer hybrid. At the Institute studies are carried out in order to obtain higher levels of egg production traits in pure lines, which constitute the basis for breeding studies by selection. Hens are selected according to individual phenotypic values, while cocks are selected according to their family values. Firstly experiments are carried out in order to increase egg production traits of pure lines with inline selection. In the second step, grandparents from pure lines and parents from grandparents are obtained. In the last step, the sire and dam parent lines are crossed to produce hybrid materials. Between 30 and 50 families (consisting of 9 hens and 1 cocks) are established on each pure line and chicks are produced each year to form a next generation [Göger *et al.* 2017].

Microsatellite or Simple Sequence Repeat (SSR) markers are used in indicating genetic diversity, because they demonstrate co-dominant inheritance, are distributed throughout the genome and feature a high rate of polymorphism and repeatability [Hillel *et al.* 2003, Tadano *et al.* 2007, Rajmukar *et al.* 2007]. SSR markers are used

to identify genetic variation in livestock as well as determine conservation priorities in breeds, eco-types or lines [Ramadan *et al.* 2012, Tadano *et al.* 2013].

In the present study, in five different white layer pure chicken lines produced from the White Leghorn were analysed in terms of their genetic diversity, population structure and conservation priorities using 19 microsatellite loci. With the findings of this research it is aimed to make contributions to the sustainable use and conservation of these lines that have been reared as closed flocks for a long period of time and have been subjected to selection.

Material and methods

Sample collection and DNA extraction

Blood samples (approximately 1 ml) were collected into tubes with K3 EDTA from a total number of 150 samples, including 30 samples from each white layer pure chicken line at the Ankara Poultry Research Institute. Genomic DNA extraction was conducted in line with the protocol reported by Miller *et al.* [1988] applying minor optimisation at our laboratory conditions.

PCR and microsatellite genotyping

The present study used 19 microsatellite loci (ADL0112, ADL0268, LEI0094, LEI0166, LEI0192, LEI0234, MCW0020, MCW0034, MCW0037, MCW0067, MCW0069, MCW0078, MCW0081, MCW0111, MCW0123, MCW0183, MCW0248, MCW0301, MCW0330) recommended by FAO [2011] for the identification of the genetic structure among white layer pure chicken lines. The PCR reaction mixture consisted of 3 µl genomic DNA (50 ng/µl), 3 µl 10X PCR buffer (75 mM Tris-HCl, pH 9.0), 0.3 µl of each primer (10 pmol/µl), 0.1 µl Taq DNA polymerase (Thermo, Cat. No. EP0402) (5U/µl), 2.5 µl $MgCl_2$ (2.5 mM/µl) and 3 µl dNTPs (2.5mM/µl), with the volume of the mixtures made up to 25µL with distilled deionized water. PCR amplification was performed as follows: initial denaturation at 94°C for 10 minutes, followed by 30 cycles of denaturation at 94°C for 45 seconds, annealing (at 50-60°C) for 30 seconds, extension at 72°C for 45 seconds and final extension at 72°C for 10 min.

In the present study, 96 automated capillary electrophoresis systems (Advanced Analytical Technologies-AATI, Ames, Iowa, USA) were used to determine the size of PCR products. The gel, inlet buffer, capillary conditioning solution and 35-500 bp marker were prepared according to the manufacturer's instructions using a dsDNA 900 Reagent Kit (35bp/500 bp). After capillary electrophoresis separation, the data was recorded and band sizes calculated using PROSize® 2.0 version 1.3.1.1 (Advanced Analytical Technologies, Inc., Ames, IA, USA).

Data analyses

The Convert version 1.31 [Glaubitz 2004] programme was used in order to determine private allele, allele range and frequency. The presence of null alleles in

all lines for each locus was tested using the Ml-Nullfreq programme [Kalinowski and Taper 2006]. Genetic variation parameters (observed heterozygosity, expected heterozygosity, number of allele and number of effective alleles), genetic identity and genetic distance were calculated using the Popgene version 1.31 [Yeh *et al.* 1997]. PIC values were established with a Microsatellite Toolkit [Park 2001], while the inbreeding coefficient (F_{IS}) per line was calculated using Fstat v.1.2 [Goudet 1995]. Genetic differentiation values (pairwise F_{ST}) between each pair of pure lines was calculated using the Arlequin software [Excoffier *et al.* 2006].

Conservation priorities of chicken lines were evaluated according to the method described by Petit *et al.* [1998] and Caballero and Toro [2002] using the MolKin program [Gutierrez *et al.* 2005]. According to Caballero and Toro [2002], the contribution of each subpopulation to total genetic diversity may be determined when each of the subpopulations is removed from the overall data set. When each subpopulation is removed from the data set, the negative value (-) that is found in the overall data set is the contribution of that population to total genetic diversity. In summary, the subpopulation with the highest negative value contributes the most to the total genetic diversity. In contrast, Petit *et al.* [1998] reported that the highest positive value contributes the most to the total genetic diversity.

Factorial Correspondence Analysis (FCA), the Unweighted Pair Group Method with Arithmetic Mean Analysis (UPGMA) dendrogram and STRUCTURE clustering analysis were conducted to identify the relationship between lines and individuals. FCA analysis using Genetix v. 4.05 [Belkhir *et al.* 2004] and Bayesian model-based clustering using Structure software [Pritchard *et al.* 2000] were constructed. First the programme was run to assume the number of distinct populations defined as K. The analysis involved an admixture model with correlated allele frequencies. One hundred independent runs with 500,000 Markov Chain Monte Carlo iterations and a burn-in of 100,000 steps were performed for $2 \leq K \leq 5$ (where K is the number of clusters to be tested) to estimate the most likely number of clusters present in the data set. The most probable K was determined using Structure Harvester [Earl and van Hold 2012] by calculating the distribution of the ΔK statistic as described by Evanno *et al.* [2005]. The Structure Plot [Ramasamy *et al.* 2014] was used to visualise the Structure output.

Results and discussion

Genetic diversity parameters within lines

Genetic diversity parameters obtained in each chicken line, PIC values and inbreeding coefficients are summarised in Table 1. The number of alleles per locus ranged between 3.95 (Blue) and 4.84 (D-229), while the number of effective alleles was between 3.02 (Blue) and 3.73 (D-229). The lowest average observed heterozygosity was observed in the Blue and Maroon lines (0.41), while the highest Ho value was recorded in the D-229 line (0.52). The lowest PIC value (0.56) was found in the Blue population, whereas the highest PIC value (0.65) was determined in the D-229

population. Inbreeding coefficients in the Blue, Brown, D-229, Black and Maroon populations were identified as 0.36, 0.33, 0.28, 0.26 and 0.39, respectively.

The mean numbers of Na (3.95-4.84) and Ne (3.02-3.73) per locus obtained in this study were higher than the values reported by Hillel *et al.* [2003] (2.70-3.70 in two commercial WL populations), by Granevitze *et al.* [2007] (2.96 in a white layer pure line), and by Tadano *et al.* [2007] (2.47, 2.90 and 3.05 in the WL-A, WL-B and WL-C lines). In contrast, they are lower than the Na value (5.33) given by Rajkumar *et al.* [2007] in one WL pure line (WLH-IWD).

The observed heterozygosity values (between 0.41 and 0.52) calculated for five different white layer pure chicken lines are higher than the Ho values (0.33 and 0.31) reported by Muchadeyi *et al.* [2007] in the white layer LS-S and WL-A pure lines, while they were lower than the observed Ho values (0.61, 0.92) reported by Rajkumar *et al.* [2007] in the WL pure lines (WLH-IWD and WLH-IWF). Contrary to those studies, Ho values in this study are similar to Ho values (0.48, 0.43 and 0.49) reported by Tadano *et al.* [2007] and the Ho value (0.42) reported by Ramadan *et al.* [2012] in the WL lines.

Inbreeding coefficients obtained in pure chicken lines (between 0.26 and 0.39) were higher than the Fis values presented by Tadano *et al*. [2007] (-0.050, -0032, -0.020 in WR-A, WR-B and WR-C lines, respectively), those reported by Muchadeyi et al. [2007] (0.067 and 0.086 in the white layer LS-S and WL-A lines), or Rajkumar *et al*. [2007] (-0.053 and -0.11 in the WLH-IWD and WLH-IWF lines) and Granevitze *et al*. [2007] in the white layer pure line (0.086).

Table 1. Genetic diversity parameters within five white layer pure lines

Item	n	MNa		MNe		Ho		He		PIC		PA	Fis
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD		
Blue	30	3.95	1.13	3.02	1.17	0.41	0.20	0.61	0.21	0.56	0.19	10	0.36**
Brown	30	4.53	1.54	3.41	1.37	0.46	0.22	0.61	0.20	0.61	0.17	10	0.33**
D-229	30	4.84	1.54	3.73	1.52	0.52	0.23	0.71	0.13	0.65	0.14	11	0.28*
Black	30	4.21	1.23	3.11	1.16	0.48	0.21	0.64	0.15	0.58	0.15	4	0.26*
Maroon	30	4.26	1.45	3.31	1.39	0.41	0.18	0.65	0.20	0.58	0.21	6	0.39**

*p<0.05; **p<0.01.
MNa – mean number of alleles per locus. MNe – mean number of effective alleles per locus. Ho – average observed heterozygosity per locus. He – average expected heterozygosity per locus. PIC – *Polymorphism Information Content*. PA – number of private alleles (frequency ≥0.01). Fis – inbreeding coefficient.

Although the genetic diversity parameters in five different pure chicken lines derived from the WL breed were generally low, they are found to be at medium levels compared to the values reported in literature for the WL lines. The F_{IS} values obtained in the populations indicate a fairly high level of inbreeding. Deviation from the Hardy-Weinberg equilibrium is caused by the lack of heterozygotes due to increased inbreeding in all lines. These results are not surprising, given the methods, with which

the populations are raised. When populations have been raised as closed flocks and subjected to selection, it is expected to reduce genetic diversity and lead to an increase of inbreeding. In addition, it should not be forgotten that in small populations, random chance factors may cause a significant change in the gene pool. Simon and Buchenauer [1993] reported that if the F_{IS} value is above 0.40 conservation studies have to be started in the population. The F_{IS} values we calculated for five different chicken lines are between 0.26 and 0.39. These values indicate that actions should be taken to reduce the level of inbreeding in populations. It may be recommended to increase the population size and to use molecular methods for mating plans in order to reduce the level of inbreeding.

Another reason for excessive homozygosity in populations may be related with the null alleles. In this study null allele frequencies in the LEI0192 (Blue, Brown, D-229, Maroon), MCW0034 (Blue, D-229, Maroon), MCW0111 (Blue, Brown, D-229, Black) and MCW0301 (Blue, Black) loci were over 0.20, while they were below 0.20 in the other 15 loci. If the null allele frequencies are below 0.20, the effect of null alleles may be acceptable as non-significant [Mahammi *et al.* 2016]. It is therefore assumed that the high homozygosity in populations is caused by small population sizes rather than null alleles.

The low levels of genetic diversity in this study may be caused by the fact that these populations have been raised as closed flocks and subjected to selection. As mentioned above, Blue, Brown, Black and Maroon pure lines were imported in 1995 from Canada, have been bred as closed flocks since then and subjected to selection in order to improve several yield traits. Also, before being imported to Turkey, these pure lines were known to have been derived from White Leghorns by improvement through selection for a period of 50 years with regard to several yield traits. That is, these lines have been subjected to selection for about 70 years and bred in closed flocks.

When the studied pure lines were compared within their populations, genetic diversity was found to be the highest in the D-229 line. In contrast to the other chicken lines in the study, the D-229 line was imported to Turkey in 2010 from the Czech Republic. Breeding studies in the D-229 line started later than in the four other lines. Consequently, the D-229 line is considered to have higher genetic diversity parameters and a lower level of inbreeding than the other lines.

Genetic differentiation among populations

Genetic differentiation (pairwise F_{ST}) values among populations are given in Table 2. In the study, the lowest pairwise F_{ST} value (0.07) was recorded between the D-229 and Brown populations, while the highest pairwise F_{ST} value (0.20) between the Black and Blue populations. All pairwise F_{ST} values identified in the study were found to be significantly different from 0 ($p<0.05$). These values indicate that all lines have been genetically differentiated.

Pairwise F_{ST} values between pure lines were similar to values previously reported by Tadano *et al.* [2011] in seven WL lines (between 0.071-0.259). However, pairwise

Table 2. Genetic differentiation (pairwise F_{ST}) values among white layer pure lines

Item	Blue	Brown	D-229	Black	Maroon
Blue	0.000				
Brown	0.13*	0.000			
D-229	0.15*	0.07*	0.000		
Black	0.20*	0.14*	0.13*	0.000	
Maroon	0.17*	0.13*	0.12*	0.13*	0.000

All pairwise F_{ST} are significantly different from 0; *p<0.05; mean = 0.14.

F_{ST} values were lower than those reported by Tadano *et al.* [2013] in seven Plymouth Rock lines (0.201-0.422) and by Karslı and Balcıoğlu [2019] in six brown pure layer lines (0.115-0.352). Although all pure lines in the study came from the same genetic origin, it is considered that the selection applied to these lines may have resulted in the genetic differentiation of all lines. Contrary to other evolutionary forces such as mutation, selection may very rapidly change gene and genotype frequencies depending on its intensity. This condition is thought to be the main source of genetic differentiation among populations. It is thought that selection started later in the D-229 and Brown populations than the other lines, because of the lowest pairwise F_{ST} values obtained D-229 and Brown populations. The high genetic differentiation obtained by Tadano *et al.* [2013] in seven Plymouth Rock lines may have resulted from the selection performed in different directions (PR1, PR4 and PR6 for meat production traits; PR5 for egg production traits). It is natural that in a study by Karslı and Balcıoğlu [2019] genetic differentiation was higher in brown layers than in white layers, because while white layers originated from a single breed (WL), brown layers originated from three different breeds (Rhode Island Red, Colombian Rock, Barred Plymouth Rock).

A total of 41 private alleles were identified for 19 microsatellite loci in five chicken lines. The population with the lowest number of private alleles is Black (4), while the one with the highest number is D-229 (11). The private allele percentage calculated in this study (26.7) is higher than the percentage (10%) obtained by Granevitze *et al.* [2007] in 64 different populations. Individuals migrating between populations reduce genetic differences and consequently the number of private alleles. If there is no transition between populations, that is if populations are bred in closed flocks as it is in this study, genetic differentiation and the number of private alleles increase. In that sense, it is natural for the number of private alleles in this study to be high.

The pairwise F_{ST} values obtained in the study and the number of private alleles all support each other. The chicken lines, despite having the same genetic origin, have been genetically differentiated because of breeding systems and the selection applied in order to obtain different yield traits.

Clustering analyses

The values of Nei's genetic distance and similarity obtained between the populations are shownin Table 3. In the study, the lowest genetic distance values

Table 3. Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Item	Blue	Brown	D-229	Black	Maroon
Blue	****	0.75	0.67	0.59	0.61
Brown	0.28	****	0.80	0.68	0.63
D-229	0.40	0.22	****	0.67	0.69
Black	0.53	0.39	0.40	****	0.74
Maroon	0.49	0.46	0.40	0.31	****

were obtained between the D-229 and Brown populations (0.22), whereas the highest genetic distance values were found between the Black and Blue populations (0.53).

Rajkumar *et al.* [2007] reported the genetic distance between two WL populations as 0.06. In turn, Tadano *et al.* [2011] reported that genetic distance values in seven different WL lines ranged between 0.07 (WL4-WL5) and 0.26 (WL1-WL7). Ramadan *et al.* [2012] reported that the genetic distance was 0.33 between the RIR and WL populations. The genetic distance values calculated in our study were within the range of 0.22-0.53. In turn, Karslı and Balcıoğlu [2019] reported that the genetic distance values in six different brown layer pure chicken lines ranged between 0.28 (BARI and BARII) and 1.44 (L-54-COL). It was found in the study that the genetic distance values between five populations are higher than the populations having the same origin. This may be caused by the fact that selection procedure started earlier in the pure chicken lines used in our study.

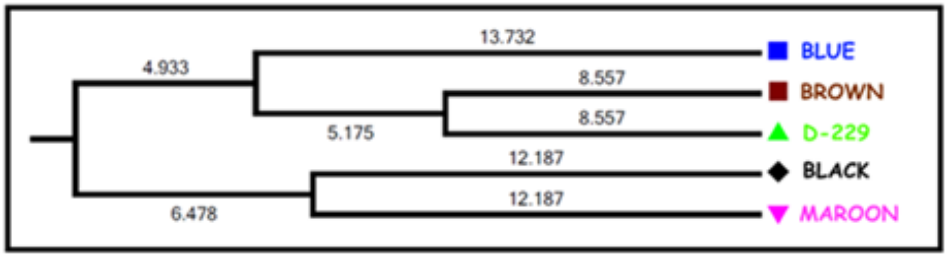

Fig. 1. UPGMA dendrogram among the white layer pure lines based on genetic distance.

The UPGMA dendrogram based on Nei's genetic distance to identify the phylogenic relation between the pure chicken lines is shown in Figure 1. The results of the FCA analysis, where the phylogenic relation was presented on a three dimensional plane, are given in Figure 2. Similarly to the UPGMA dendrogram, the D-229, Brown and Blue populations were clustered closer to each other, whereas the Black and Maroon populations were located closer to each other in a different region. According to the FCA results based on individuals, although there are differences between the populations, they are not yet separated with clear boundaries and there are transitions between populations. This seems rather likely, given that the five pure chicken lines have the same genetic origin (White Leghorn).

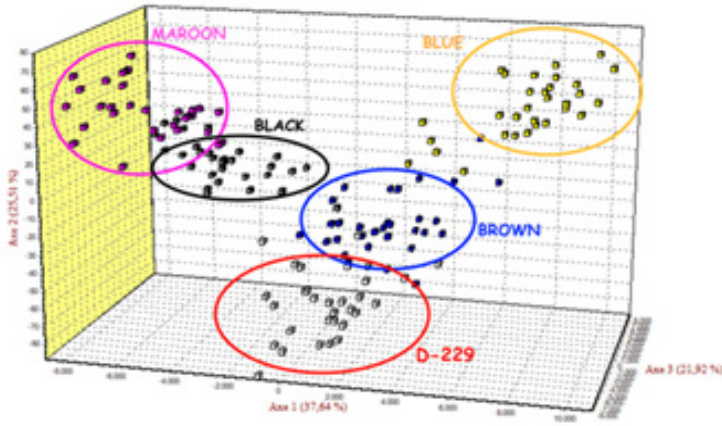

Fig. 2. Factorial Correspondence Analysis of the studied white layer pure lines for 19 microsatellite loci.

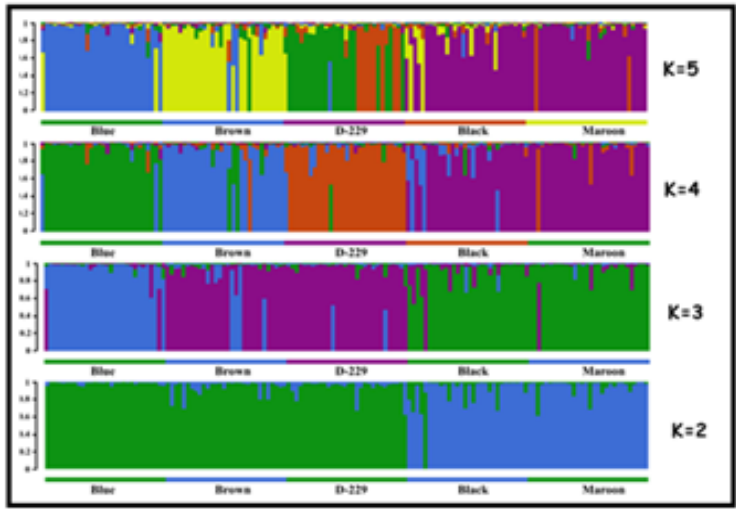

Fig. 3. Bayesian cluster analyses of the studied individuals from five white layer pure lines Each individual was represented by a vertical bar. The highest ΔK value was obtained for K = 3 of the studied layer pure chicken individuals.

According to the Structure analysis results, when the K value is 2, meaning the five pure chicken populations are divided into two clusters, the first cluster includes the Blue, Brown and D-229 populations, while the other includes the Black and Maroon populations, similarly to the UPGMA dendrogram. In the case with the best K value (K=3), the first cluster comprises the Blue, the second consists of the Brown and D-229 populations, while the third -the Black and Maroon populations, respectively. There are transitions especially between the Blue population in the first cluster and the Brown and D-229 in the second, and between the Brown and D-229 populations

in the second cluster and the Black and Maroon in the third. This condition once again reflects the fact that populations that are bred as closed flocks are genetically differentiated by selection and that the populations have the same genetic origins.

Conservation priority

Table 4 shows the contribution of each line to the genetic diversity based on the methods defined by Caballero and Toro [2002] and Petit *et al.* [1998].

Table 4. Contributions of the chicken lines to genetic diversity according to Caballero and Toro [2002] and Petit *et al*. [1998]

Item	Caballero and Toro [2002]			Petit *et al*. [1998]		
	total (%)	within line (%)	between line (%)	total (%)	within line (%)	between line (%)
Blue	-0.42	0.72	-1.14	3.73	0.25	3.48
Brown	-0.21	-0.23	0.02	0.51	0.41	0.10
D-229	-1.34	-1.43	0.09	4.10	2.95	1.15
Black	-0.38	0.60	-0.98	-1.32	-1.97	0.65
Maroon	-0.50	0.34	-0.84	0.49	-1.65	2.14

In the study, according to the method by Caballero and Toro [2002], the Black line provided the lowest contribution to the genetic diversity between populations. The greatest contribution to the total genetic diversity comes from the D-229 line. Similarly, according to the method developed by Petit *et al.* [1998], the highest contribution to the total genetic diversity was obtained from the D-229 line. The D-229 line is followed by the Blue line with 3.739. According to both methods, the D-229 line makes the highest contribution to genetic diversity and this population should be the starting point for conservation studies. Also among the studied five pure chicken lines, the highest Na (4.842), Ne (3.732), Ho (0.515) and He (0.706) values per locus were obtained in the D-229 line. These values support the above given results.

In conclusion, in this study where 19 microsatellite loci were used in five different white layer pure lines, they determined low levels of genetic diversity and high levels of inbreeding in chicken lines. It was observed that the breeding system and selection cause a significant genetic differentiation even in populations of the same genetic background (White Leghorn). The analysis of microsatellite data revealed that the D-229 line among all lines provided the highest contribution to genetic diversity and therefore this population should be given priority in conservation of existing genetic diversity. For a sustainable use of these populations, inbreeding in lines should be reduced and genetic diversity, particularly in the D-229 line, should be conserved.

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REFERENCES

1. BELKHIR K., BORSA P., CHİKHI L., RAUFASTE N., BONHOMME F., 2004 – GENETIX 4.05, logiciel sous Windows pour la ge´ne´tique des populations. Universite´ de Montpellier II, Montpellier, France. In French, with English summary; available from: http://www.genetix.univ-montp2.fr/genetix/genetix.htm

2. CABALLERO A., TORO M.A., 2002 – Analysis of genetic diversity for the management of conserved subdivided populations. ***Conservation Genetics***, 3, 289-299.
3. CHATTERJEE R.N., BHATTACHARYA T.K., DANGE M., RAJKUMAR U., 2010 – Assessment of genetic relatedness of crossbred chicken populations using microsatellite markers. ***Biochemical Genetics,*** 48, 727-736.
4. EARL D.A., VONHOLDT B.M., 2012 – Structure harvester: a web-site and program for visualizing structure output and implementing the Evanno method. ***Conservation Genetics Resources***, 4, 359-361.
5. EVANNO G., REGNAUT S., GOUDET J., 2005 – Detecting the number of cluster of individuals using the software structure: a simulation study. ***Molecular Ecology***, 14, 2611-2620.
6. EXCOFFİER L., LAVAL G., SCHNEIDER S., 2006 – ARLEQUIN version 3.01: an integrated software package for population genetics data analysis. University of Bern, Institute of Zoology, Switzerland.
7. FAO., 2011 – Molecular genetic characterization of animal genetic resources. Animal Production and Health Guidelines; 9, pp:84-85. Available at http://www.fao.org/docrep/014/i2413e/i2413e00.pdf (accessed date: August 30, 2018).
8. FLOCK D., PREISINGER R., 2002 – Breeding plan for poultry with emphasis on sustainability. 7th World Congress on Genetics Applied to Livestock Production, 19-23 August, Montpellier, France.
9. GLAUBİTZ J.C., 2004 – CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. ***Molecular Ecology Notes***, 4, 309-310.
10. GOUDET J., 1995 – FSTAT (Version 1.2), A computer program to calculate F-Statistics. ***Journal of Heredity***, 86, 485-486.
11. GÖGER H., DEMİRTAŞ Ş.E., YURTOĞULLARI Ş., TAŞDEMİR A.N., ŞENKAL U.E., BOYALI B., 2017 – Breeding Studies on Pure Lines at Poultry Research Institute. ***Tavukçuluk Araştırma Dergisi***, 14, 30-38.
12. GRANEVİTZE Z., HİLLEL J., CHEN G.H., CUC N.T.K., FELDMAN M., EDİNG H., WEIGEND S., 2007 – Genetic diversity within chicken populations from different continents and management histories. ***Animal Genetics***, 38, 576-583.
13. GUTIERREZ J.P., ROYO L.J., ALVAREZ I., GOYACHE F., 2005 – MolKin (Version 2.0): a computer program for genetic analysis of populations using molecular coancestry information. ***Journal of Heredity***, 96, 718-721.
14. HİLLEL J, GROENEN MAM, TİXİER-BOİCHARD M, KOROL A.B., DAVİD L., KİRZHNER V.M., BURKE T., BARRE-DIRIE A., CROOİJMANS R.P.M.A., ELO K., FELDMAN M.W., FREİDLIN P.J., MAKİ-TANILA A., OORTWİJN M., THOMPSON P., VİGNAL A., WİMMERS, K., WEIGEND, S., 2003 – Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools. ***Genetics Selection Evolution***, 35, 533–57.
15. KALINOWSKİ S.T., TAPER M.L., 2006 – Maximum likelihood estimation of the frequency of null alleles at microsatellite loci. ***Conservation Genetics***, 7, 991-995.
16. KARSLI T., BALCIOGLU M.S., 2019 – Genetic characterization and population structure of six brown layer pure lines by using microsatellite markers. ***Asian-Australasian Journal of Animal Science***, 32, 49-57.
17. MAHAMMI F.S., GAOUAR S.B.S., LALOE D., FAUGERAS R., TABET-AOUI N., RAGNON X., TIXIER-BIOCHARD M., SAIDI MEHTAR N., 2016 – A molecular analysis of patterns of genetic diversity in local chickens from western Algeria in comparison with commercial lines and wild jungle fowls. ***Journal of Animal Breeding and Genetics,*** 133, 59-70.
18. MAHMOUDI B, BAYAT M, SADEGHİ R, BABAYEV MS, ABDOLLAHI H., 2010 – Genetic diversity among three goat populations assessed by microsatellite DNA markers in Iran. ***Global Veterinaria***, 4, 118-124.

19. MILLER S., DYKES D., PLESKY H.A., 1988 – Simple salting out procedure for extracting DNA from human cells. ***Nucleic Acids Research***, 16, 1215.
20. MUCHADEYI F.C., EDİNG H., WOLLNY C.B.A., GROENEVELD E., MAKUZAS.M., SHAMSELDİN R., SİMİANER H., WEIGEND S., 2007 – Absence of population structuring in Zimbabwe chicken ecotypes inferred using microsatellite analysis. ***Animal Genetics***, 38, 332-339.
21. PARK S.D.E., 2001 – The Excel microsatellite-toolkit. Animal Genomics Lab., Univ. College Dublin, Dublin, Ireland.
22. PETİT R.J., EL MOUSADI, A, PONS O., 1998 – Identifying populations for conservation on the basis of genetic markers, ***Conservation Genetics***, 12, 844-855.
23. PRITCHARD J.K., MATTHEW S., DONNELLY P. 2000 – Inference of population structure using multilocus genotype data. ***Genetics,*** 155, 945-59.
24. RAJMUKAR U., GUPTA B.R., AHMED N., VENKTRAMAIAH A., REDDY A.R., 2007 – Genetic variation and genetic diversity in chicken populations using microsatellite assay. ***Indian Journal of Animal Science,*** 77, 1194-1198.
25. RAMADAN S., KAYANG B.B., INOUE E., NIRASAWA K.I., HAYAKAWA H., ITO S., INOUE-MURAYAMA M., 2012 – Evaluation of genetic diversity and conservation priorities for Egyptian chickens. ***Jornal of Animal Science***, 2, 83-90.
26. RAMASAMY R.K., RAMASAMY S., BINDROO B.B., NAIK V.G., 2014 – STRUCTURE PLOT: A Program for Drawing Elegant STRUCTURE Bar Plots in User Friendly Interface. Springerplus, 3, 431.
27. SIMON D.L., BUCHENAUER D., 1993 – Genetic diversity of European livestock breeds. European Association for Animal Production Publication No. 66. Wageningen Pers, Wageningen, The Netherlands.
28. TADANO R., GOTO N., TSUDZUKI M., 2011 – Genetic differentiation among White Leghorn lines: Application of individual-based clustering approaches. ***Poultry Science***, 90, 725-730.
29. TADANO R., NAGASAKA N., GOTO N., RIKIMARU K., TSUDZUKI M., 2013 – Genetic characterization and conservation priorities of chicken lines. ***Poultry Science***, 92, 2860-2865.
30. TADANO R., NİSHİBORİ M., NAGASAKA N., TSUDZUKI M., 2007 – Assessing genetic diversity and population structure for commercial chicken lines based on forty microsatellite analyses. ***Poultry Science***, 86, 2301-2308.