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Genetic diversity of village chickens in Central Black Sea Region and commercial chickens in Turkey by using microsatellite markers

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Abstract: Local chicken populations in the Central Black Sea Region of Turkey have been intensively affected by governmental poultry culling due to avian influenza outbreak risks. The aim of this study was to investigate the genetic diversity of indigenous chicken populations raised in the Central Black Sea Region in order to assess genetic structures of these populations and to determine genetic relationships between the study populations and certain commercial chicken genotypes. Genotypic diversity of 45 Turkish village chicken populations located in 5 provinces in the Central Black Sea Region of Turkey and 2 commercial hybrid populations were compared using 28 autosomal microsatellite loci. In total, 363 alleles were observed within 47 populations in 28 microsatellite loci. These loci showed 12.96 ± 4.97 alleles per locus and the mean number of alleles per population was 2.33 ± 0.19. The most polymorphic locus was LEI0234 with 28 alleles and 0.944 polymorphism information content (PIC) value. The least polymorphic locus was LEI0192 with 6 alleles and 0.720 PIC value. The results suggested that despite the extensive culling the studied local chicken populations showed a high genetic diversity compared to commercial hybrid populations.

Key words: Village chickens, genetic diversity, microsatellite markers

1. Introduction

Gallus gallus gallus as the Red Jungle Fowl is accepted to be the maternal ancestor of the domesticated chicken. Currently, the diversity of chicken populations in Europe is based on the crossing of Red Jungle Fowl genotypes, Mediterranean-type populations, local breeds and lines, and Chinese and Malay types of chicken genotypes (1). Crossing between these genotypes resulted in the commercial chicken breeds in the early 20th century. However, expansion of commercial hybrids in the chicken sector caused a loss of genetic diversity in local chicken populations, which saves gene resources for future breeding and production purposes in in vivo conditions.

Chicken production is an important part of the poultry sector, contributing to a high proportion of the meat and egg supply for human consumption in Turkey. For chicken production small-holder systems are widely preferred by farmers because of the low capital investments and sufficient cost-efficiency in Turkey. Although there is large phenotypic variation in these local village populations compared to commercial ones, limited data on morphological characteristics and genetic structures of only two indigenous breeds have been published to date (2).

Microsatellite markers are widely used for establishing genetic diversity of chicken genotypes (3,4). The easy scoring and establishment of heterozygosity levels, measurement of genetic parameters, and number of effective alleles make the microsatellites useful tools (5–8).

Consciousness about conserving genetic reservoirs is of great importance because of the irreversible structure of these resources (9). Nevertheless, prioritization is crucial for conservation programs due to limited economic funds. Thus, determinations of allelic richness levels of the populations by molecular markers gives robust information on prioritization for conservation programs.

The Central Black Sea Region of Turkey was affected by the H5N1 avian influenza pandemic in 2005. A high proportion of avian influenza was seen in this region, because this region is on the migration routes of migratory birds and is one of the main stopover and wintering grounds for the birds that come via the Black Sea (http://www.kusgribi.gov.tr). Consequences of such extreme interventions as seen in poultry culling on genetic resources cannot be assessed if there is a lack of former genetic data on populations. Because of these concerns, the aims of the study were measurement of the genetic diversity of local chickens in order to get familiar with

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the status of commercial and local chicken genotypes' genetic relationships in the area, and determination of the populations' genetic structure for future evaluations and comparisons.

2. Materials and methods

Forty-five populations were visited to cover a wide range of populations from 5 provinces including 3 counties with 3 villages or districts located in the Central Black Sea Region of Turkey (Figure 1). Acreage of the study area was 36.919 km² from 41°43′44″N to 40°24′34″N and from 34°52′27″E to 37°24′27″E. Its altitude ranged from 3 to 1234 m above sea level. The number of local chickens sampled from all of the populations was 364 in total. Sample sizes picked from each flock ranged from 5 to 14. Blood samples from commercial broilers (25 individuals from one line) and layer hybrids (50 individuals from 10 lines from the Ankara Poultry Research Institute in Turkey) were included in the study as a reference.

Approximately 1 mL of blood was collected from the brachial vein of each sampled chicken into vacuum tubes with anticoagulant (K_3EDTA) using an obtainer needle. Blood samples were frozen at –20 °C. DNA from blood samples was extracted using a BILATEC commercial kit. Concentrations of the individual DNA samples were measured by spectrophotometer and standardized to 10 ng/µL. Equal amounts of 5–14 DNA samples were pooled as a bulk sample to reduce the amount of genotyping.

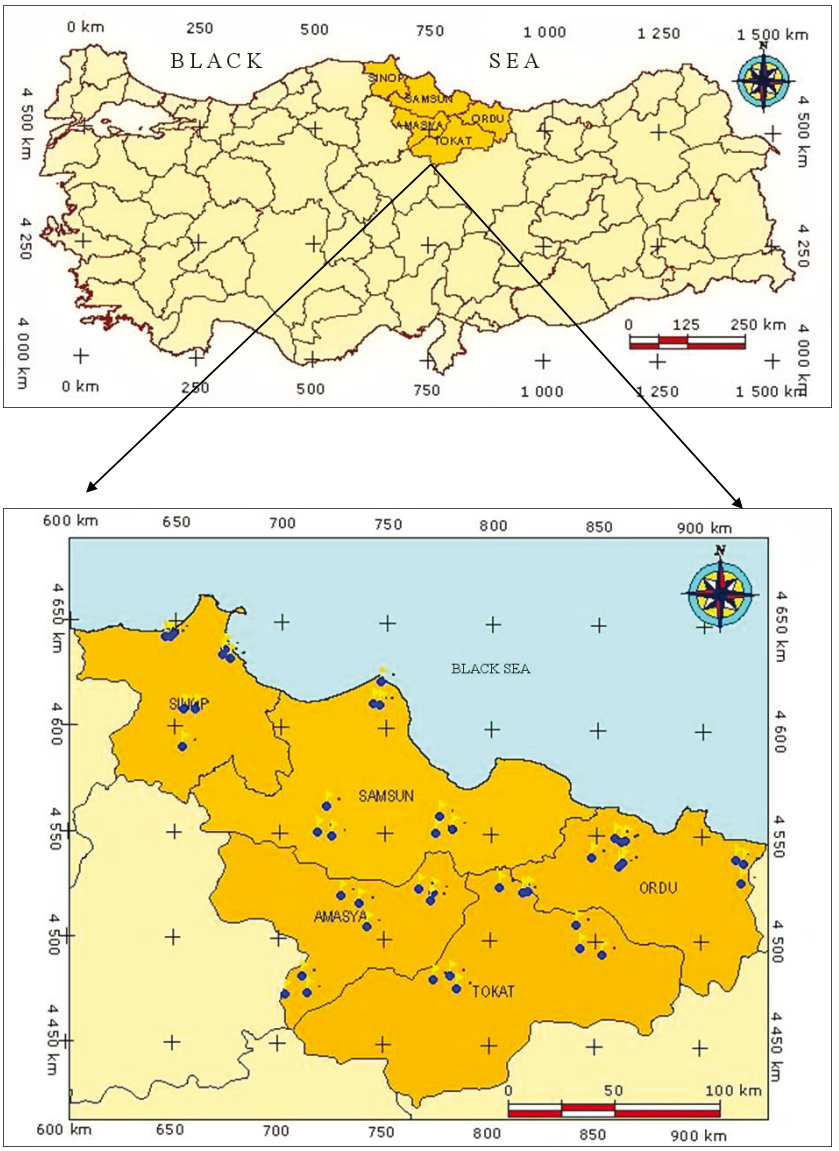

Figure 1. Sampling locations of village chicken populations.

A set of 30 microsatellite marker loci, developed in the European Research Project AVIANDIV (EC Contract No. BIO4-CT98-0342 (1998–2000)) and distributed throughout the genome, was used to examine genetic variability (Table 1). These loci were also recommended by the FAO MoDAD project (http://dad.fao.org/en/refer/library/guidelin/marker.pdf) for assessing chicken genetic diversity. However, two loci (MCW0020 and MCW0165) displayed difficulty for amplification in all populations. Therefore, these two loci were not included in the further analyses. The PCR products were handled in a total volume of 20 µL using a QPlus Thermal Cycler. Each reaction consisted of 40 ng of genomic DNA, 5 pmol of reverse and forward primers, 6 µL of master mix (Promega), and ultrapure water. The PCR amplification was performed according to Romanov and Weigend (3) with a touchdown PCR procedure to reduce stuttering. DNA fragments were visualized by 29:1 acrylamide/bis-acrylamide (10%) using a manual polyacrylamide gel system (15 × 15 cm double gel system, 1 mm gel thickness, 2 h and 30 min running time, 250 V voltage). The gel pictures were taken using the SYNGENE Gel Documentation System after staining by ethidium bromide solution. Gel scorings of allele peaks and intensity were made using SYNGENE GeneTools image analysis software.

We used Nei's equation (1987), assuming Hardy–Weinberg equilibrium, to determine pooled populations' allele frequencies and heterozygosities per population (gene diversity) per locus (polymorphism information content, PIC), according to Crooijmans et al. (10). Classification was completed as cluster and principal coordinates analysis (PCoA) to see groups in two and

Table 1. Locus names, number of total/private alleles, allele size range (in base pairs), and PIC values of loci.

Locus name[a]	Allele numbers		Allele range (bp)	PIC values
	Total	Private		
ADL0112	13	3	120–144	0.885
ADL0268	9	1	98–116	0.865
ADL0278	15	2	102–130	0.916
LEI0094	15	4	235–269	0.901
LEI0166	16	3	360–394	0.903
LEI0192	6	1	342–362	0.720
LEI0234	28	2	244–380	0.944
MCW0014	9	2	178–200	0.809
MCW0016	18	1	134–178	0.916
MCW0034	16	2	212–242	0.916
MCW0037	16	7	144–178	0.903
MCW0067	10	1	172–190	0.847
MCW0069	16	3	144–182	0.911
MCW0078	9	3	133–149	0.734
MCW0080	16	3	280–320	0.917
MCW0081	17	-	110–143	0.927
MCW0098	10	1	285–303	0.873
MCW0103	9	2	268–286	0.838
MCW0104	20	5	186–232	0.919
MCW0111	10	-	96–114	0.795
MCW0123	9	2	76–94	0.807
MCW0183	10	2	290–320	0.779
MCW0206	11	1	225–245	0.884
MCW0216	7	1	143–157	0.796
MCW0222	8	1	218–234	0.811
MCW0248	9	2	201–221	0.841
MCW0295	11	1	86–108	0.869
MCW0330	20	4	260–302	0.932
Average	12.96 ± 4.97	2.31 ± 1.46	76–394	0.863 ± 0.060

[a]http://w3.tzt.fal.de and Hillel et al. (1).

three dimensions using NTSYSpc version 2.11 (11). A phylogenetic tree dendrogram was obtained by means of the unweighted pair-group method using arithmetic averages (UPGMA) after genetic similarity was calculated using Nei's coefficient.

3. Results

Locus names, number of total and private alleles, allele size range, and PIC values of the loci are shown in Table 1. Gene diversity and total number of alleles for all loci within each population are shown in Table 2. All of the 28

Table 2. Gene diversity and number of alleles observed in the study populations.

Location			Population Code	Number of samples	Total Number of alleles	Gene diversity (*h*)
Province	County	Village/District				
Ordu	Ünye	Yenikent	ORUY	9	36	0.703
Ordu	Ünye	Beylerce	ORUB	7	39	0.663
Ordu	Ünye	Fatih-Çatak	ORUF	8	37	0.670
Ordu	Kumru	Kıran	ORKK	6	37	0.706
Ordu	Kumru	Ortaçokdeğirmen	ORKO	6	39	0.626
Ordu	Kumru	Yeniergen	ORKY	5	43	0.544
Ordu	Kabadüz	Yokuşdibi	ORKBY	6	37	0.685
Ordu	Kabadüz	Kirazdere	ORKBK	5	37	0.654
Ordu	Kabadüz	Harami	ORKBH	5	34	0.702
Tokat	Erbaa	Karayaka	TKEKY	7	37	0.699
Tokat	Erbaa	Kaleköy	TKEKK	8	35	0.693
Tokat	Erbaa	Salkımören	TKES	7	37	0.675
Tokat	Niksar	Sulugöl	TKNS	7	36	0.664
Tokat	Niksar	Işıklı	TKNI	14	40	0.617
Tokat	Niksar	Hanyeri	TKNH	7	37	0.628
Tokat	Turhal	Asarcık	TKTA	6	35	0.696
Tokat	Turhal	Ortaköy	TKTO	8	35	0.695
Tokat	Turhal	Üçgözen	TKTU	6	36	0.688
Amasya	Göynücek	Karaşar	AMGK	12	37	0.655
Amasya	Göynücek	Pembeli	AMGP	10	33	0.698
Amasya	Göynücek	Damlaçimen	AMGD	8	37	0.676
Amasya	Suluova	Alakadı	AMSA	10	35	0.686
Amasya	Suluova	Saygılı	AMSS	10	36	0.701
Amasya	Suluova	Yüzbeyli	AMSY	6	38	0.653
Amasya	Taşova	Ilıca	AMTI	7	37	0.681
Amasya	Taşova	Akınoğlu	AMTA	8	36	0.652
Amasya	Taşova	Yaylasaray	AMTY	6	35	0.701
Samsun	Asarcık	Aşuru	SMAA	11	36	0.687
Samsun	Asarcık	Uluköy	SMAU	10	38	0.638
Samsun	Asarcık	Gökgöl	SMAG	11	35	0.715
Samsun	Vezirköprü	Bahçekonak	SMVBA	10	36	0.687
Samsun	Vezirköprü	Güder	SMVG	7	37	0.648
Samsun	Vezirköprü	Boğazkoru	SMVBO	8	32	0.756
Samsun	Bafra	Bakırpınar	SMBB	9	30	0.762
Samsun	Bafra	Kaygusuz	SMBK	10	37	0.636
Samsun	Bafra	Tepecik	SMBT	10	36	0.675
Sinop	Gerze	Merkez	SNGM	5	34	0.711
Sinop	Gerze	Yenimahalle	SNGYM	8	37	0.648
Sinop	Gerze	Yaykıl	SNGYK	8	37	0.666
Sinop	Ayancık	Aliköy	SNAA	8	35	0.691
Sinop	Ayancık	Yeşilyurt	SNAY	8	28	0.770
Sinop	Ayancık	Bahçeli	SNAB	6	37	0.626
Sinop	Boyabat	Şıhlı	SNBS	9	35	0.701
Sinop	Boyabat	Bağlıca	SNBB	11	39	0.662
Sinop	Boyabat	Osmanköyü	SNBO	11	40	0.594
Commercial broilers			EP	25	39	0.658
Commercial layers			YP	50	38	0.661

microsatellite loci were found to be polymorphic. A total of 363 alleles were observed with 12.96 ± 4.97 alleles per locus, and the mean number of alleles per population was determined to be 2.33 ± 0.19. Number of alleles per locus ranged from 6 (LEI0192) to 28 (LEI0234). Size differences between the alleles of the smallest and largest fragments within each locus varied from 14 bp (MCW0216) to 136 bp (LEI0234). On the other hand, some loci showed size differences between some alleles in narrow limitations of 2–4 bp. The most polymorphic loci in commercial layer and broiler hybrids were ADL0268 and MCW0216 with 16 alleles, respectively. One to 7 alleles were specific to certain populations.

Mean gene diversity (expected heterozygosity) of all populations was 0.675 ± 0.040. This value was higher than in commercial layer and broiler populations, which were 0.661 and 0.658, respectively. The most polymorphic locus among the 28 tested loci was LEI0234 with 28 alleles across populations and a PIC value of 0.944, while the least polymorphic locus was LEI0192 with 6 alleles and PIC value of 0.720.

The phylogenetic tree reconstructed by UPGMA method for the chicken populations is shown in Figure 2.

The Tokat and Ordu populations formed a separate branch with two subgroups. Commercial and other local genotypes also showed two subgroups. However, the Sinop population and commercial genotypes were placed in the same subgroup. Results of PCoA analysis are shown in Figure 3. Results of this analysis showed that the Sinop group was placed in the nearest position to the

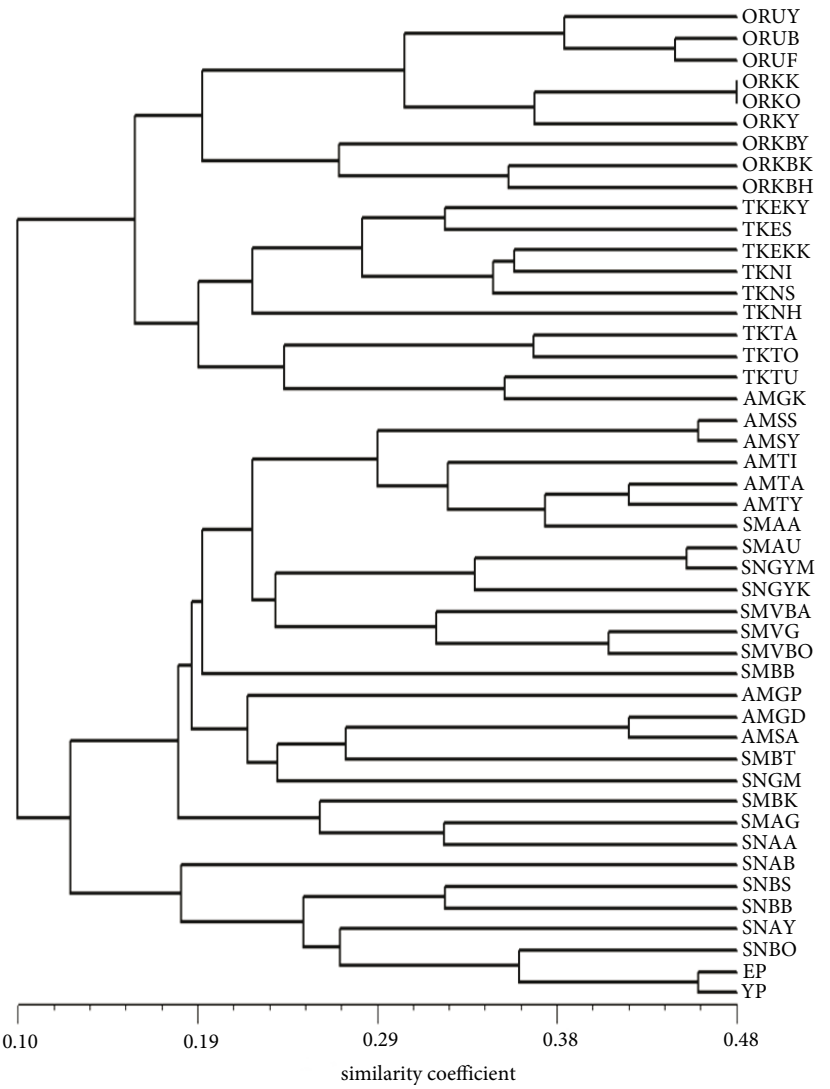

Figure 2. Phylogenetic dendrogram among populations according to UPGMA by NTSYSpc v2.11. SM- Samsun, SN- Sinop, AM- Amasya, TK- Tokat, OR- Ordu. Third and subsequent letters show provinces and villages, while broilers and layers are EP and YP, respectively (see Table 2).

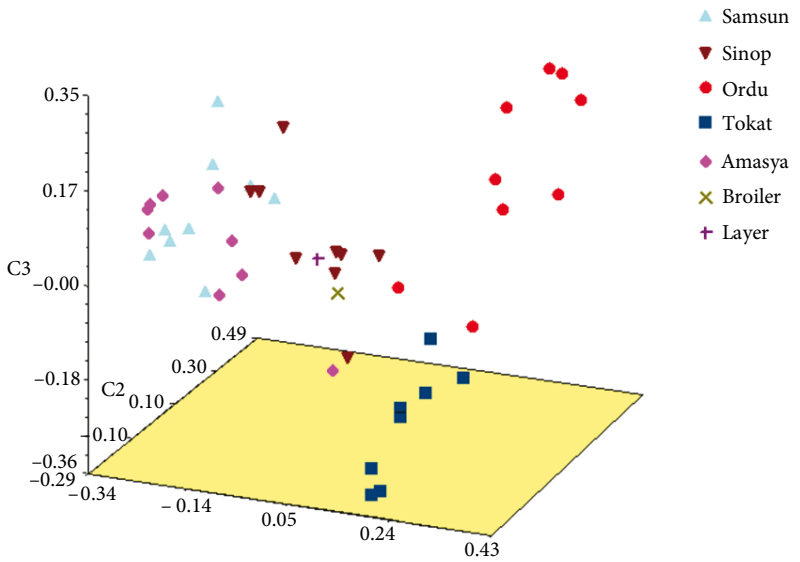

Figure 3. Positioning of populations in three-dimensional space according to PCoA by NTSYSpc v2.11.

commercial genotypes. The Tokat and Ordu groups were placed distantly from the central position, although the Samsun and Amasya groups were placed at the other side of the commercial genotypes with relatively large genetic distances.

4. Discussion

Results of our study are in agreement with those of other researchers reporting that domesticated local populations showed a higher diversity than commercial hybrid genotypes (12). Genetic diversity in terms of expected heterozygosity and number of alleles was higher than that reported by other authors for different chicken populations (1,3,13). Eltanany et al. (14) determined 213 alleles by using almost the same microsatellite loci (29 loci) across Egyptian chicken strains, with an average of 7.3 alleles per locus. In our study the data showed a wide range of genetic diversity in the sampled populations although commercial genotypes shared very limited alleles with the local populations studied. Some chicken flocks from the Sinop, Samsun, and Amasya populations carried certain alleles shared with commercial genotypes. These shared alleles might be introgressed by crossing with commercial lines. However, genotyping of individual samples is necessary in order to confirm the results of the present study. It is known that allele number per locus and size distribution provide useful information for comparing the diversity of populations (10,12). Although we used a common set of microsatellites developed by Hillel et al. (1) for chicken genetic diversity, the results of microsatellite loci analyses were found to be different in comparison to other studies. Scoring the bands was difficult in some cases, for example when length differences of 1–3 bp of the major band occurred. The reason for this might be the creation of stutter bands, insufficient electrophoretic resolution, or point mutations. Stutter bands are amplified products along with the major allele fragment (3). Differences of 1 bp observed in some loci in this study have been accepted as point mutations in the chicken microsatellites (3).

The phylogenetic analysis showed two groups of tree topology. Tokat and Ordu populations formed a cluster together while the Sinop, Samsun, and Amasya populations were clustered with commercial lines. This suggested that the Tokat and Ordu populations were closely related to each other and isolated for many generations without interbreeding. Possibly, in the second group, the Sinop, Samsun, and Amasya populations shared the same alleles from the commercial genotypes. On the other hand, the number of shared alleles between the Sinop population and commercial chicken populations was higher compared to other populations. This suggested that the Samsun, Sinop, and Amasya populations have similar genetic backgrounds or that the local chickens live together with commercial chickens.

These results suggest that despite the extensive culling process local chicken populations in the Central Black Sea Region show high genetic diversity compared to commercial hybrid populations. These results give an optimistic point of view such that there is high genetic diversity in chicken genetic resources, which needs to be conserved in Turkey. The results of microsatellite analysis of other local chicken populations in Turkey will provide further information on utilization and breeding strategies of these genetic resources.

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