

Genetic Diversity Among Turkish Native Chickens, Denizli and Gerze, Estimated by Microsatellite Markers

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Abstract The genetic diversity of the Turkish native chicken breeds Denizli and Gerze was evaluated with 10 microsatellite markers. We genotyped a total of 125 individuals from five subpopulations. Among loci, the mean number of alleles was 7.5, expected heterozygosity (H_e) was 0.665, PIC value was 0.610, and Wright's fixation index was 0.301. H_e was higher in the Denizli breed (0.656) than in the Gerze breed (0.475). The PIC values were 0.599 and 0.426 for Denizli and Gerze, respectively. A phylogenetic tree was constructed using genetic distance and the neighbor-joining method. Its topology reflects the general pattern of genetic differentiation among the Denizli and Gerze breeds. The present study suggests that Denizli and Gerze subpopulations have a rich genetic diversity. The information about Denizli and Gerze breeds estimated by microsatellite analysis may also be useful as an initial guide in defining objectives for designing future investigations of genetic variation and developing conservation strategies.

Keywords Turkish native chickens · Denizli · Gerze · Genetic diversity · Microsatellites

Introduction

Native chickens are known to be good foragers and efficient mothers, and they require minimal care to grow. They are, therefore, most suited for raising under village conditions. These birds do, however, need special attention with respect to

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their conservation and improvement. Turkish native chicken breeds exist as names in the literature, but there is no information on their characteristics, such as the extent of genetic diversity. Furthermore, native chicken breeds are becoming extinct because of their poor commercial performance. Consequently, there is a need to define existing chicken populations and to develop improvement and conservation programs so as to benefit people living in rural areas.

It can be assumed that local breeds contain the genes and alleles pertinent to their adaptation to particular environments and local breeding goals. Such local breeds are needed to maintain genetic resources permitting adaptation to unforeseen breeding requirements in the future and can serve as a source of research material (Romanov and Weigend 2001).

Düzgüneş (1990) claimed that the Denizli and Gerze breeds are two of the Turkish native chickens. These breeds are primarily located to the western (Denizli) and northern (Gerze) parts of Turkey. Denizli hens are reared for eggs and as a hobby, and Denizli cocks are famous for their long crowing (app. 15–16 s). Gerze chickens, reared in the province of Sinop in northern Turkey, are primarily reared for eggs and as a hobby. Additional reports on Denizli and Gerze phenotypes include feathering characteristics (Aksoy et al. 2002), adult body weight, egg number, reproduction performance (Özdoğan et al. 2007), and blood group alleles (Aksoy et al. 2000).

Recent advances in molecular technology have provided new opportunities to assess genetic variability at the DNA level. Microsatellites are tandem repeats of one to six bases. They are widely used since they are numerous, randomly distributed in the genome, and highly polymorphic, and they show codominant inheritance (Cheng and Crittenden 1994). A number of publications have revealed that microsatellite markers are useful in determining many descriptive statistics such as heterozygosity, genetic distance, number of effective alleles, and polymorphic information content among closely related populations. Relatively few publications have addressed the genetic diversity of local chickens (Wimmers et al. 2000; Zhang et al. 2002; Hillel et al. 2003; Kong et al. 2006; Shahbazi et al. 2007).

Microsatellite analysis is regarded as the most convenient tool in the determination of heterozygosity and genetic distance, and many microsatellite loci are available for use in chickens. For a more general view on the importance of the exotic populations as genetic resources, it would be interesting to look at genetic distance to other commercial strains. Furthermore, the presence of unique alleles or allelic combinations coding for specific (production) traits and characteristics related to adaptability is of interest. An example of this is the Indian Kadaknath breed as a source for the valuable dark meat genes (Wimmers et al. 2000). In addition, with the increased focus on genetic conservation, unique alleles may be of use in decisions to maintain such birds. These decisions will be important, especially if the alleles are associated with economically important traits (Emara et al. 2002).

Turkey has undertaken a national project (TAGEM-97/17/01/0003) to genetically improve indigenous native chickens. The Lalahan Livestock Central Animal Research Institute has operated a national program of genetic preservation of native chickens, titled “The conservation of Turkish native chickens, Denizli and Gerze,” since 1997. This project specifically aimed to identify, characterize, and protect the Denizli and Gerze breeds for quantitative traits. It has also tried to determine and maintain genetic

diversity in these breeds. There is, however, no comprehensive published genetic description of current Turkish native chickens. According to the Turkish Ministry of Agricultural and Rural Affairs, there are two indigenous chickens, Denizli and Gerze, and these breeds are now in serious danger of extinction. The present analysis is of great importance because it is probably the first genetic study of Turkish native chicken biodiversity using microsatellite markers. The objective of this research was to determine genetic diversity within the breeds and to compare the Denizli and Gerze Turkish native chicken breeds. To achieve this goal, we individually genotyped 10 microsatellite loci in 125 chickens from five subpopulations.

Materials and Methods

Experimental Populations

Turkish native chicken subpopulations and the number of individuals used in this study are as follows: Denizli Cock Rearing Farm (DHUC, $N = 25$), Denizli Lalahan Livestock Central Animal Research Institute (DLHMAE, $N = 25$), Denizli Private Farms (DOI, $N = 25$), Gerze Lalahan Livestock Central Animal Research Institute (GLHMAE, $N = 25$), and Gerze Private Farms (GOI, $N = 25$). The DHUC, DLHMAE, and DOI subpopulations contain only the Denizli breed. The DLHMAE subpopulation was derived from the DHUC subpopulation for genetic conservation purposes in 1997, and they have been reared closely. The GLHMAE and GOI subpopulations contain only the Gerze breed. The GLMHAЕ was derived from the GOI subpopulation for genetic conservation in 1997, and they have the same genetic background. In total, 125 chickens from the five subpopulations were genotyped.

DNA Isolation

Blood samples were collected from the wing vein with syringes into a tube containing EDTA as an anticoagulating agent. DNA of individuals was isolated from 100 μ l of blood in EDTA using a Wizard Genomic DNA Purification Kit (Promega).

Microsatellite Loci

Ten microsatellite primers (ADL0102, ADL0136, ADL0158, ADL0171, ADL0172, ADL0176, ADL0181, ADL0210, ADL0267, and ADL0268) have been recommended by the FAO/MoDAD (2004) advisory group and were provided by the Coordinators of the U.S. National Poultry Genome Research Program.

PCR Procedure

PCR reactions were carried out in a total volume of 25 μ l containing 50–100 ng genomic DNA, 2.5 mM $MgCl_2$, 200 μ M dNTP, 0.5 U *Taq* Polymerase, 50 nM each primer (one of which was labeled with a fluorescent dye). The cycling conditions consisted of 5 min at 95°C followed by 30 cycles of 30 s at 94°C, 45 s at 50°C, and

90 s at 72°C, and a final extension step of 10 min at 72°C (Cheng et al. 1995). A mixture of 1 µl PCR product and 80% formamide was made, denatured by heating to 94°C for 5 min, and analyzed by an ABI Prism 310 sequencer (Applied Biosystems, USA). The size of each fragment was determined relative to the TAMRA 350 size standards (Applied Biosystems) using GeneScan.

Statistical Analysis

Based on microsatellite genotyping and allele frequencies, the number of alleles, allele size range (in base pairs), observed heterozygosity, Nei's (1987) expected heterozygosity, and Wright's (1978) fixation index were estimated using the computer software package PopGene version 1.31 (Yeh et al. 1997). Allele frequencies obtained from the microsatellite genotypes were used to calculate PIC (polymorphism information content) values (Botstein et al. 1980) using the computer software package Cervus 3.0 (Marshall et al. 1998; Kalinowski et al. 2007) in order to measure the degree of information obtained by a microsatellite. Based on microsatellite genotyping, Nei's (1978) unbiased genetic distance between subpopulations was estimated. These results were used to construct phylogenetic trees by neighbor-joining cluster analysis with the appropriate options of computer software package PopGene version 1.31.

Results

Microsatellite Allele Distribution

All microsatellite primers gave PCR products that were polymorphic in the five subpopulations (Table 1). Allele size range differences between the alleles observed within the loci ranged from 18 bp (ADL0181) to 40 bp (ADL0171), with an average of 25.4 bp per locus. The number of alleles per locus varied from 3 (ADL0210) to 12 (ADL0136) alleles detected. The total number of alleles was 75 across all populations. The mean number of alleles across all microsatellite loci was 7.5 ± 0.76 (Table 1).

Across breeds, the mean number of alleles in Denizli was 6.1 ± 0.6 , and in Gerze it was 5.0 ± 0.7 (Table 2).

Genetic Variability

The estimates of expected heterozygosity (H_e) and PIC were obtained using the allele frequency data for each locus in each subpopulation and across breeds. Expected heterozygosities were quite high, ranging from 0.498 (ADL0181) to 0.852 (ADL0136), and the mean H_e was 0.665 ± 0.04 among loci (Table 1). The estimates of H_e at different loci between subpopulations showed a large variation. Among breeds given in Table 2, the mean H_e was 0.656 ± 0.045 in Denizli and 0.475 ± 0.074 in Gerze. This result showed that genetic diversity is higher in the Denizli breed than in the Gerze breed.

Table 1 Ten microsatellite markers used in Denizli and Gerze subpopulations of Turkish native chickens

Locus	Allele size range (bp)	Number of alleles	H_e	PIC	F_{is}
ADL0102	90–112	8	0.524	0.420	0.402
ADL0136	125–159	12	0.852	0.830	0.415
ADL0158	162–192	5	0.607	0.546	0.431
ADL0171	85–125	9	0.681	0.631	0.540
ADL0172	131–157	7	0.785	0.749	0.161
ADL0176	181–201	9	0.831	0.835	0.487
ADL0181	174–192	7	0.498	0.457	0.322
ADL0210	102–126	3	0.506	0.381	−0.017
ADL0267	98–118	8	0.733	0.687	0.189
ADL0268	93–113	7	0.632	0.590	0.073
Mean \pm SE	–	7.5 ± 0.76	0.665 ± 0.04	0.610 ± 0.05	0.301 ± 0.05

Note: H_e , expected heterozygosity; PIC, polymorphism information content; F_{is} , Wright's fixation index

The PIC among loci was highest for ADL0136 (0.830) and lowest for ADL0210 (0.381). Among breeds, the mean PIC value was 0.599 ± 0.049 in Denizli and 0.426 ± 0.068 in Gerze (Table 2).

Wright's fixation index (F_{is}) values among loci ranged from -0.017 (for ADL0210) to 0.540 (ADL0171). The mean F_{is} for 10 microsatellite loci was 0.301 ± 0.05 (Table 1). The mean of observed heterozygosity (H_o) was 0.508 ± 0.037 in the Denizli breed and 0.380 ± 0.065 in the Gerze breed (Table 2).

Genetic Distance and Phylogenetic Analysis

Using Nei's (1978) unbiased genetic distance (Table 3) and the neighbor-joining method, a phylogenetic tree was constructed for the Denizli and Gerze subpopulations. The smallest genetic distance, between DLHMAE and DHUC, was quite low (0.0652). A similar result was obtained for the GLMHAE and GOI subpopulations, with a very low genetic distance (0.0783). The larger genetic distances (greater than 0.4193) were found between the Denizli and Gerze subpopulations. The neighbor-joining dendrogram in Fig. 1 was drawn using the genetic distances given in Table 3. The Denizli (DHUC, DLHMAE, and DOI) and Gerze (GLHMAE and GOI) breeds were clearly clustered as different groups according to their origin, supporting the reliability of this analysis.

Discussion

Microsatellite Allele Distribution

All microsatellite loci recommended by the FAO/MoDAD (2004) Advisory Group were extremely proficient at obtaining highly polymorphic PCR products within and between Turkish native chickens. They also demonstrated their utility as

Table 2 Genetic diversity within Denizli and Gerze subpopulations of Turkish native chickens

Locus ^a	Denizli breed				Gerze breed			
	DHUC	DLHMAE	DOI	Total	GLHMAE	GOI	Total	Total
ADL0102	bp	92–110	90–110	92–110	92–112	92–110	92–110	92–112
	<i>N</i>	3	4	2	4	3	5	5
	<i>H_o</i>	0.280	0.320	0.320	0.400	0.240	0.320	0.400
	<i>H_e</i>	0.308	0.460	0.274	0.440	0.431	0.432	0.440
ADL0136	PIC	0.267	0.392	0.233	0.379	0.361	0.372	0.379
	bp	127–157	125–155	127–155	127–157	129–159	127–159	127–159
	<i>N</i>	7	7	6	5	7	9	9
	<i>H_o</i>	0.520	0.480	0.640	0.360	0.480	0.420	0.360
ADL0158	<i>H_e</i>	0.761	0.856	0.679	0.610	0.560	0.658	0.610
	PIC	0.708	0.818	0.629	0.540	0.514	0.591	0.540
	bp	184–192	184–192	184–192	186	162–192	162–192	186
	<i>N</i>	4	4	4	1	3	3	1
ADL0171	<i>H_o</i>	0.400	0.520	0.560	0.000	0.240	0.120	0.000
	<i>H_e</i>	0.549	0.670	0.566	0.000	0.222	0.116	0.000
	PIC	0.476	0.591	0.456	0.000	0.205	0.111	0.000
	bp	85–105	93–105	85–105	95–125	99–125	95–125	95–125
ADL0171	<i>N</i>	4	5	5	4	5	6	4
	<i>H_o</i>	0.280	0.280	0.360	0.240	0.400	0.320	0.240
	<i>H_e</i>	0.349	0.576	0.626	0.350	0.530	0.449	0.350
	PIC	0.318	0.524	0.578	0.322	0.460	0.407	0.322

Table 2 continued

Locus ^a	Denizli breed				Gerze breed			
	DHUC	DLHMAE	DOI	Total	GLHMAE	GOI	Total	Total
ADL0172	bp	131–155	131–157	131–157	131–157	131–157	131–157	131–157
	<i>N</i>	6	5	7	4	4	4	4
	<i>Ho</i>	0.640	0.800	0.560	0.600	0.680	0.640	0.640
	<i>He</i>	0.701	0.734	0.750	0.734	0.617	0.585	0.585
ADL0176	PIC	0.630	0.668	0.695	0.686	0.550	0.525	0.525
	bp	183–201	183–201	183–201	181–201	181–201	181–201	181–201
	<i>N</i>	5	6	6	5	7	7	7
	<i>Ho</i>	0.600	0.640	0.320	0.520	0.240	0.280	0.280
ADL0181	<i>He</i>	0.739	0.727	0.698	0.774	0.712	0.738	0.738
	PIC	0.679	0.664	0.629	0.729	0.662	0.691	0.691
	bp	176–182	174–192	176–182	174–192	174–180	174–180	174–180
	<i>N</i>	4	6	4	7	2	2	2
ADL0210	<i>Ho</i>	0.640	0.640	0.360	0.547	0.040	0.020	0.020
	<i>He</i>	0.657	0.650	0.663	0.653	0.115	0.059	0.059
	PIC	0.581	0.584	0.582	0.590	0.106	0.057	0.057
	bp	118–126	118–126	118–126	118–126	102–126	102–126	102–126
ADL0210	<i>N</i>	2	2	2	2	3	3	3
	<i>Ho</i>	0.440	0.600	0.480	0.507	0.560	0.520	0.520
	<i>He</i>	0.429	0.507	0.470	0.483	0.444	0.446	0.446
	PIC	0.332	0.373	0.355	0.365	0.341	0.352	0.352

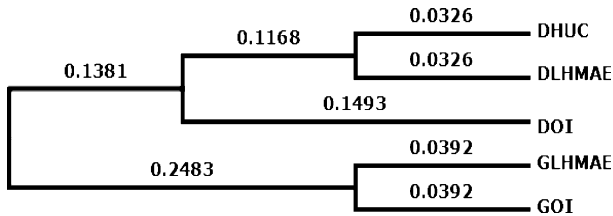
Table 2 continued

Locus ^a	Denizli breed				Gerze breed			
	DHUC	DLHMAE	DOI	Total	GLHMAE	GOI	Total	Total
ADL0267	bp	100–112	98–118	100–110	98–118	98–108	98–106	98–108
	<i>N</i>	6	6	4	8	5	4	5
	<i>Ho</i>	0.640	0.600	0.520	0.587	0.720	0.480	0.600
	<i>He</i>	0.667	0.709	0.676	0.715	0.732	0.725	0.726
ADL0268	PIC	0.610	0.645	0.592	0.670	0.670	0.657	0.670
	bp	93–113	93–113	107–113	93–113	107–113	93–113	93–113
	<i>N</i>	6	5	4	6	3	6	6
	<i>Ho</i>	0.680	0.400	0.720	0.600	0.520	0.600	0.560
Mean ± SE	<i>He</i>	0.622	0.443	0.726	0.673	0.507	0.585	0.545
	PIC	0.572	0.414	0.659	0.627	0.437	0.525	0.489
	<i>N</i> ± SE	4.7	4.4	5.0	6.1 ± 0.6	3.4	4.4	5.0 ± 0.7
	<i>Ho</i> ± SE	0.512	0.484	0.528	0.508 ± 0.037	0.354	0.421	0.380 ± 0.065
	<i>He</i> ± SE	0.578	0.613	0.633	0.656 ± 0.045	0.440	0.487	0.475 ± 0.074
	PIC ± SE	0.517	0.541	0.567	0.599 ± 0.049	0.385	0.440	0.426 ± 0.068

^a bp, allele size range, in base pairs; *N*, number of alleles; *Ho*, observed heterozygosity; *He*, expected heterozygosity; PIC, polymorphism information content

Table 3 Genetic distance among Denizli and Gerze subpopulations of Turkish native chickens

Population	DHUC	DLHMAE	DOI	GLHMAE	GOI
DHUC	–				
DLHMAE	0.0652	–			
DOI	0.3015	0.2959	–		
GLHMAE	0.7603	0.4193	0.4839	–	
GOI	0.7887	0.4318	0.5658	0.0783	–

**Fig. 1** Neighbor-joining dendrogram among five subpopulations of the Denizli and Gerze chicken breeds. Based on Nei's (1978) unbiased genetic distances from Table 3

informative molecular markers in the Denizli and Gerze breeds. The mean number of alleles in this research for overall loci was 7.5 ± 0.76 (Table 1).

Compared with previous studies (Kong et al. 2006; Wimmers et al. 2000), the present research revealed the same microsatellite allele variation in Turkish native chickens. In particular, the mean number of alleles is similar to that of Korean native chickens (Kong et al. 2006). The number of alleles for ADL0158, ADL01171, ADL01176, ADL0210, and ADL0267 was higher than the number reported by Wimmers et al. (2000) in African, Asian, and South American local chickens.

The mean number of alleles for all loci was similar among the five subpopulations. Across breeds, the mean number of alleles in the Denizli and Gerze breeds was 6.1 ± 0.6 and 5.0 ± 0.7 , respectively (Table 2). In chickens, the number of alleles at a single microsatellite locus in any single population has ranged from one (monomorphic) up to several (Emara et al. 2002; Cheng et al. 1995). For instance, Emara et al. (2002) examined 41 microsatellite markers in three commercial broiler pure lines and reported an average number of alleles per marker of 3.5, 2.8, and 3.1 for each of the lines. Hillel et al. (2003) reported that the mean number of alleles was 3.5 within 52 populations. Shahbazi et al. (2007) reported a mean number of alleles of 4.5 per locus in Iranian native chickens. Compared with the data obtained by Crooijmans et al. (1996), who reported an average of 3.6 alleles per marker in broiler lines, and by Kaiser et al. (2000), who reported 2.8 and 2.9 alleles per marker in two broiler populations, we observed higher numbers of alleles (6.1 ± 0.6 and 5.0 ± 0.7) per primer in Turkish native chickens. These values are lower than those reported by Zhang et al. (2002), who estimated a mean of 9.32 alleles for the same primers in Chinese native chicken breeds.

Genetic Variability

Heterozygosity estimates within the populations were based on a set of markers showing substantial heterogeneity in the number of alleles detected and the polymorphic information content. The use of a mixture of highly variable and less variable microsatellites should reduce the danger of overestimating genetic variability, which might occur if only highly variable loci are used (Wimmers et al. 2000). For all loci, high H_e was observed, and mean H_e was 0.665 ± 0.04 among loci (Table 1). Among breeds in Table 2, the mean H_e was 0.656 ± 0.045 in Denizli and 0.475 ± 0.074 in Gerze. This result showed that genetic diversity in the Denizli breed is higher than in the Gerze breed. This level of mean H_e is quite similar to the value reported for Korean native chickens (0.630) (Kong et al. 2006). Hillel et al. (2003) reported that the average gene diversity within 52 populations across all 22 loci was 0.47. Romanov and Weigend (2001), using microsatellites with chickens, have reported heterozygosity of 0.60 or higher. A similar result (0.45–0.67) was reported by Wimmers et al. (2000) for African, Asian, and South American local chickens. The mean H_e recorded in this research, however, is lower than that reported by Zhang et al. (2002) in Chinese native chickens and by Shahbazi et al. (2007) in Iranian native chickens. Very high heterozygosity values have also been described in Chinese and Iranian native chickens (0.63–0.86 and 0.62–0.74, respectively). The variation of expected heterozygosity may be adduced to differences in location, sample size, population structure, and sources of microsatellite markers.

The mean PIC was an ideal index to measure the polymorphism of allele fragments. According to Botstein et al. (1980), $PIC > 0.50$ indicates a highly informative locus, $0.50 > PIC > 0.25$ indicates a reasonably informative locus, and $0.25 > PIC$ indicates a slightly informative locus. The mean PIC among loci was 0.610 ± 0.05 , and almost all markers (except ADL0210) were highly informative in Turkish native chickens (Table 1). Reasonably informative PIC values for the ADL0158, ADL0171, ADL0176, ADL0210, and ADL0267 loci were reported in African, Asian, and South American local chickens (Wimmers et al. 2000). Among breeds, mean PIC values in the Denizli and Gerze breeds were 0.599 ± 0.049 and 0.426 ± 0.068 , respectively (Table 2). Almost all loci (except ADL0102 and ADL0210) in the Denizli breed are highly informative, whereas 40% of the loci in the Gerze breed were reasonably informative. The ADL0158 and ADL0181 loci in the Gerze breed were slightly informative. The others were highly informative.

The mean F_{is} for 10 microsatellite loci was 0.301 ± 0.05 (Table 1). The results at each single locus revealed that in all cases (except ADL 210) positive F_{is} values were estimated. This means that there may be more heterozygotes than expected for ADL0210 in all five subpopulations (Table 2).

The mean H_o was 0.508 ± 0.037 in the Denizli breed and 0.380 ± 0.065 in the Gerze breed (Table 2). The number of heterozygous genotypes was higher in the Denizli than in the Gerze breed. Thus, the Denizli breed is more variable than the Gerze breed. Therefore, the wide genetic diversity of the Denizli breed allows scientists and farmers to use it in future research and development of quality chicken breeds in Turkey. The high level of variability in the Denizli and Gerze

breeds calls attention to the importance of conserving the Turkish native chicken gene pool.

Genetic Distance and Phylogenetic Analysis

The genetic distance (0.0652) between the DLHMAE and DHUC subpopulations was estimated to be quite low (Table 3), reflecting the fact that these subpopulations are not genetically isolated from each other. The same result can be seen for the GLMMAE and GOI subpopulations, with a very low genetic distance (0.0783). Larger genetic distances (greater than 0.4193) were found between the Denizli and Gerze subpopulations. This result is similar to that of Hillel et al. (2003), who found Nei's mean genetic distance between a given population and all other 51 populations to be 0.44. Hillel et al. (2003) also emphasized that genetic distance measures based on gene frequencies were in good agreement with the genetic diversity of the breeds examined, indicating that these approaches fit the history of the domesticated chickens well.

The genetic differentiation found between the Denizli and Gerze breeds in the neighbor-joining dendrogram (Fig. 1) is confirmed by their breeding origin and evolution.

The present study demonstrates the usefulness of microsatellite primers as molecular markers to identify and compare the Denizli and Gerze subpopulations even with a limited number of loci and samples analyzed. The data also suggest that genetic diversities within and between the Denizli and Gerze breeds are being well preserved by conservation efforts. The information about the Denizli and Gerze breeds estimated by microsatellite analysis may also be useful as an initial guide in defining objectives for designing future investigations of genetic variation and developing conservation strategies.

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