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Research Article

Morphological and Genetic Characteristics of Zerdava, A Native Turkish Dog Breed [1][2]

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Abstract

Zerdava dogs are considered as one of the many native animal genetic resources of Turkey. However, the genetic characteristics of these dogs and detailed phenotypic studies related to them have not been reported yet. The aim of this study was to determine the morphological and genetic characteristics of Zerdava dogs. Blood samples (n = 100) were collected from Zerdava dogs. The morphological characteristics of these dogs were also taken. The mean live weights of Zerdava dogs were found to be 16.02±0.35 kg. The mean withers height, rump height and body length were measured as 48.20±0.21, 47.08±0.24 and 51.24±0.23 cm, respectively. According to the results of microsatellite markers, the mean F_{IS} (inbreeding coefficient) value was documented as 0.0361±0.0003. Observed (Ho) and expected heterozygosity (He) values were found to be 0.708±0.091 and 0.694±0.077, respectively. In addition, the frequency of A018 (72%) and B001 (16%) haplotypes were high in Zerdava dogs. The mitochondrial DNA sequence results show that the majority of Zerdava dogs originate from two different maternal lines. According to the results, the phenotypic and genotypic variations of Zerdava dogs were low. Therefore, these results may suggest that Zerdava dogs may have been protected by local breeders and can be considered a separate breed.

Keywords: *Genetic characteristics, Dog, Morphological characteristics, Turkey, Zerdava*

Türkiye'nin Yerli Bir Köpek Irkı Olan Zerdava'nın Morfolojik ve Genetik Özellikleri

Öz

Zerdava köpekleri Türkiye'de birçok yerli hayvan gen kaynaklarından biridir. Ancak, bu köpeklerin genetik ve ayrıntılı fenotipik özellikleri hakkında çalışmaya rastlanılmamaktadır. Bu araştırmanın amacı, Zerdava köpeklerinin morfolojik ve genetik özelliklerini belirlemektir. Zerdava köpeklerinden kan örnekleri (n = 100) alınmıştır. Köpeklere ait morfolojik özellikler de incelenmiştir. Zerdava köpeklerinin ortalama canlı ağırlığı 16.02±0.35 kg olarak bulunmuştur. Ortalama cidago, sağrı yüksekliği ve vücut uzunluğu sırasıyla 48.20±0.21, 47.08±0.24 ve 51.24±0.23 cm'dir. Mikrosatellit işaretleyicilerinin sonuçlarına göre, ortalama F_{IS} (nispi katsayı) değeri 0.0361±0.0003. Gözlenen (Ho) ve beklenen heterozigot (He) değerleri sırasıyla 0.708±0.091 ve 0.694±0.077 olarak tespit edilmiştir. Buna ilave, Zerdava köpeklerinde A018 (%72) ve B001 (%16) haplotiplerinin görülme sıklığı yüksek bulunmuştur. Mitokondriyal DNA sekans sonuçları çoğu Zerdava köpeğinin iki farklı anne soyundan köken aldığını göstermektedir. Mevcut sonuçlara göre, Zerdava köpeklerinin fenotipik ve genotipik varyasyonları düşük olduğu belirlenmiş olup bu köpeklerin yerel yetiştiriciler tarafından korunmuş olabileceği düşünülmektedir.

Anahtar sözcükler: *Genetik özellikler, Köpek, Morfolojik özellikler, Türkiye, Zerdava*

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INTRODUCTION

Zerdava dogs have been grown and raised in the Eastern Black Sea Region, especially in Trabzon and Giresun provinces of Turkey for more than at least a hundred years. This genotype is well known as the gate dog or Eastern Black Sea Spitz. Nowadays, these dogs are owned for hunting (e.g. fox, jackal, badger and pigs), guarding, search and rescue aims. In terms of temperament, they are known to have brave, fearless, aggressive and stubborn characteristics [1]. Hence, Zerdava dogs can be shown as a candidate dog to meet the military and police dog needs of our country.

Morphological characteristics are important in distinguishing and explaining the genetic relationship among breeds [2-7]. The body of Zerdava dogs is of medium size and has a colour, varying from light chestnut to dark chestnut. However, white hairs can be seen in some parts of the body. The whiteness at tail is one of the most important morphological features of these dogs. In general, they have small round spots on the white on their legs. The colours of the spots are the same with the body colours. These dogs, which have adapted to living in mountainous regions, are small and have an athletic structure. The physical characteristics of these dogs include upright ears, deep and wide chest and inward-curved tail, which is covered with longer hairs relative to those in the body [1,8].

Because of their features, Zerdava dogs have been grown locally in the Black Sea region of Turkey for many years [1,8]. In recent years, an increasing interest has been developed for these dogs in many regions in Turkey. This situation may cause uncontrolled genotype crossings and disruption of genotype characteristics. Thus, these dogs should be identified morphologically and genetically and should be protected. However, the genetic characteristics of these dogs and detailed or enough phenotypic studies related to them have not been reported. The aim of this study is to determine the morphological and genetic characteristics of Zerdava dogs.

MATERIAL and METHODS

Samples

In this study, 100 (50 females and 50 males) blood samples were collected from twelve month and older (Mean mature live weight: fourteen months) Zerdava dogs from Trabzon (Latitude-longitude: 41′ 0145-39′ 7178) and Giresun (Latitude-longitude: 40′ 2811-38′ 8953) provinces in the Eastern Sea region of Turkey *(Fig. 1)*. Trabzon and Giresun are neighbouring provinces and have a rainy climate that is unique to the Black Sea region. The study was conducted by the consent of Ethics Committee of Ankara University (protocol no: 2013-18-135). All animals that had been included in this study had an owner. The animals were hosted individually in kennels. In general, mature dogs were fed with home-made meals containing meat once a day, although the diets differ according to their owners. These animals were the offspring of the parents who had the all characters of the breed. During mating, the females in estrus were kept in the male's kennel for three days. All dogs were selected based on morphological standards and on information about their pedigree. The data about the animals were collected from Zerdava dog clubs and associations (Trabzon Zerdava Dog Association). The samples were collected from non-relative individuals that are thought to be best representatives of their breed characteristics. The blood samples collected from the animals were placed into 10 cc tubes containing anti-coagulants (EDTA) from *Vena cephalica antebrachii* of the dogs. The samples were stored at -20°C until DNA isolation.

Morphological Studies

For morphological studies, the live weight and head and body measurements were taken as indicated in the literature [2]. The significances of differences among gender groups were identified by a t-test, whereas those among age groups were discerned by a one-way analysis of variance. Duncan's test was performed for multiple comparisons of age groups, SPSS software was used for the statistical analysis of data [9].

Genetic Studies

DNA Isolation: A DNA isolation kit (Thermo Scientific Co., California, USA) was used for the DNA isolation of the blood samples. The procedure was carried out in accordance with the manufacturer's recommendations. The absorbance values of the DNAs were measured with a spectrophotometer at 260 and 280 nm. All samples were stored at -30°C until polymerase chain reaction (PCR) analysis.

Amplification and Analysis of Microsatellite Markers: Thermo Scientific Canine Genotypes Panel 1.1 kit (Thermo Scientific Co., California, USA) was used for the amplification of microsatellite markers from the DNA samples. PCR amplification and imaging were performed in accordance with a previous study [10].

The mean heterozygosity values of the dog population were carried out according to the method of Nei [11] whereas the F_{IS} values and deviations in the Hardy-Weinberg equilibrium were estimated using the Genetix 4.05 program [12].

A factorial similarity analysis (FSA) was performed to show the relationship between breeds, where the Genetix 4.05 computer package program was used [13].

Mitochondrial DNA d-loop Analysis: The amplification of the mitochondrial DNA (mtDNA) control region (d-loop) was performed by a PCR analysis [14]. The DNA sequences were edited using Sequencher 5.4.5 (Gene Codes Corporation, Ann Arbor, MI, USA), such that the sequences

were arranged with a length of 582 base pairs (bps). The sequence was then aligned with ClustalX 1.81 and BioEdit 7.0.9 sequence alignment program. The sequences of five samples were submitted to GenBank (Zerdava-TRZ.1-3 and Zerdava-GRS.1-2). Nucleotide differences (π) among the dog populations, population mutation rate (Θ) and Tajima's D values were identified using MEGA 4 computer package program [15].

RESULTS

The live weight, body measurements and statistical values of Zerdava dogs in the gender and age groups are presented in *Table 1*. The mean live weight values of Zerdava dogs were 16.02±0.35 kg. The mean withers height, rump height and body length were found to be 48.20±0.21, 47.08±0.24 and 51.24±0.23 cm, respectively. The mean head length was recorded as 20.95±0.09 cm, the face length was 8.49±0.05 and the ear length was 10.04±0.07 cm. The withers height, rump height, chest depth, chest circumference, live weight, shank circumference, distance between the ears, ear length and mouth circumference were statistically significant depending on age. Moreover, the withers height, rump height, body length, chest depth, chest circumference, shank circumference, head length, face length, ear length, tail length and mouth

Fig 1. Zerdava dogs and provinces where the samples are collected from

Table 1. *The statistic values ($X\pm S_x$) for live weight (kg) and some morphological measurements (cm) of Zerdava dogs*

Groups	n	Withers Height	Rump Height	Body Length	Chest Width	Chest Depth	Chest Circumference	Front Cannon Circumference	Back Cannon Circumference	Live Weight
Age		*	**	-	-	***	**	*	**	*
12 month	27	47.03±0.40^{a}	45.64±0.45^{a}	50.52±0.44	16.69±0.33	19.04±0.22^{a}	50.92±0.68^{a}	8.73±0.10^{a}	8.26±0.11^{a}	14.31±0.58^{a}
13-24 month	34	48.45±0.36^{b}	47.52±0.45^{b}	51.85±0.39	17.73±0.29	20.60±0.20^{b}	53.53±0.61^{b}	9.13±0.09^{b}	8.63±0.10^{ab}	15.72±0.53^{ab}
25-36 month	20	48.44±0.48^{b}	47.46±0.53^{b}	51.08±0.52	17.82±0.38	21.16±0.26^{b}	54.44±0.81^{b}	9.14±0.12^{b}	8.84±0.13^{b}	16.74±0.85^{b}
37+ month	19	48.88±0.48^{b}	47.71±0.54^{b}	51.50±0.53	17.77±0.39	21.22±0.02^{b}	54.30±0.82^{b}	9.01±0.12^{ab}	8.54±0.13^{ab}	17.88±0.96^{b}
Gender		***	***	***	-	***	**	***	**	-
Female	50	47.23±0.31	46.12±0.35	50.42±0.34	17.34±0.25	20.13±0.17	52.48±0.54	8.82±0.08	8.43±0.08	15.79±0.51
Male	50	49.17±0.29	48.05±0.33	52.06±0.32	17.66±0.24	20.88±0.16	54.11±0.50	9.18±0.07	8.70±0.08	16.25±0.47
Total	100	48.20±0.21	47.08±0.24	51.24±0.23	17.50±0.17	20.50±0.12	53.30±0.37	9.00±0.05	8.57±0.06	16.02±0.35
Groups	**n**	**Head Length**	**Face Length**	**Ear Length**	**Ear Width**	**Distance Between Ears**	**Distance Between Eyes**	**Mouth Circumference**	**Tail Length**	**Whiteness Length at Point of Tail**
Age		-	-	*	-	*	-	***	-	-
12 month	27	20.57±0.18	8.40±0.09	9.78±0.13^{a}	8.50±0.11	10.49±0.23^{a}	4.06±0.27	17.34±0.19^{a}	28.79±0.58	6.49±0.54
13-24 month	34	21.00±0.16	8.57±0.08	10.36±0.12^{b}	8.84±0.10	10.81±0.20^{ab}	4.86±0.24	18.81±0.17^{c}	28.81±0.51	7.00±0.48
25-36 month	20	21.15±0.21	8.56±0.11	10.00±0.16^{ab}	8.85±0.14	11.37±0.27^{b}	4.32±0.31	18.16±0.22^{bc}	28.85±0.68	6.99±0.64
37+ month	19	21.07±0.22	8.43±0.11	10.01±0.16^{ab}	8.74±0.14	11.48±0.27^{b}	4.69±0.32	18.04±0.23^{b}	28.73±0.69	7.17±0.65
Gender		***	***	**	-	-	-	*	***	-
Female	50	20.56±0.14	8.31±0.07	9.85±0.10	8.67±0.09	11.18±0.18	4.26±0.21	17.78±0.15	27.90±0.45	6.78±0.42
Male	50	21.34±0.13	8.67±0.07	10.22±0.10	8.80±0.08	10.89±0.17	4.70±0.19	18.40±0.14	29.69±0.42	7.04±0.40
Total	100	20.95±0.09	8.49±0.05	10.04±0.07	8.73±0.06	11.04±0.12	4.48±0.14	18.09±0.10	28.79±0.31	6.91±0.29

*- P>0.05; * P<0.05; ** P<0.01; *** P<0.001 ^{a,b,c}Means within columns with different superscripts differ significantly (P<0.05)*

Table 2. *Heterozygote (He and Ho) indexes. Hardy-Weinberg equilibrium and F_{IS} values in Zerdava dog populations*

Lokus	Heterozygote Indexes			Hardy-Weinberg Equilibrium		F_{IS}
	Allele Numbers	He	Ho	P*	Significance	
AHT121	12	0.790	0.781	0.044	*	-0.003
AHT137	10	0.765	0.766	0.000	***	-0.005
AHTh13	10	0.769	0.800	0.000	***	-0.053
AHTh171	10	0.682	0.620	0.000	***	0.133
AHTh260	9	0.765	0.800	0.000	***	0.002
AHTk211	6	0.724	0.713	0.616	-	0.045
AHTk253	5	0.646	0.657	0.243	-	-0.024
CXX279	9	0.679	0.635	0.084	-	0.052
FH2001	9	0.811	0.730	0.333	-	0.149
FH2054	9	0.745	0.701	0.004	**	0.061
FH2328	9	0.812	0.774	0.015	*	0.069
FH2848	10	0.749	0.810	0.000	***	-0.083
INRA21	7	0.718	0.650	0.006	**	0.113
INU005	8	0.650	0.669	0.218	-	-0.037
INU030	7	0.787	0.803	0.087	-	-0.002
INU055	9	0.687	0.672	0.000	***	0.021
LEI004	5	0.735	0.752	0.007	**	-0.071
REN105L0	6	0.802	0.723	0.002	**	0.135
REN162C04	6	0.685	0.699	0.097	-	-0.033
REN169D01	10	0.806	0.672	0.000	***	0.207
REN169O1	8	0.741	0.745	0.764	-	0.007
REN247M2	6	0.498	0.467	0.000	***	0.033
REN54P11	8	0.779	0.737	0.000	***	0.032
REN64E19	6	0.750	0.721	0.304	-	0.081
$h_S \pm S_e$		**0.732±0.071**	**0.712±0.077**			**0.0361 ± 0.0003**

*- P>0.05; * P<0.05; ** P<0.01; *** P<0.001; Mean heterozygosity and Standard error ($h_S \pm S_e$)*

circumference differences were statistically significant depending on gender.

For the genetic analysis, the DNA samples of 100 dogs were analysed using 24 polymorphic microsatellite loci. In the microsatellite loci used in the study, the most alleles were AHT121 loci (12 alleles) and the least alleles were LEI004 and AHTk253 loci (five alleles) *(Table 2)*. Some microsatellite loci are not in the Hardy-Weinberg equilibrium *(Table 2)*. The heterozygosity indices (He and Ho) and mean heterozygosity values of the studied dog populations are presented in *Table 2*. The mean He and Ho values were 0.732±0.071 and 0.712±0.077, respectively. According to the t-test results, the difference between the calculated mean heterozygosity values was not statistically significant (P>0.05).

For the determination of population differentiations, an F_{IS} test was performed for each locus, and the results are shown in *Table 2*. The F_{IS} value of the analysed populations was 3.61%, which is the coefficient of inbreeding and exhibits the average heterozygote deficiency for each population.

In the study, a 582 bp mtDNA d-loop control region was used to determine the present haplotypes in the study group. According to mt-DNA D-loop region, the distributions (%) of A011, A018, A020, A028, A178, B001, B042 and C003 haplotypes were 4%, 72%, 2%, 1%, 1%, 16%, 2% and 2%, respectively. In the dog populations, five haplotypes were identified in haplogroup A, and two haplotypes were found in haplogroup B. The frequency of A018 (72%) and B001 (16%) haplotypes was high.

The results of the FSA are given in *Fig. 2*. The results detected the presence of two distinct groups in the studied group. There was no relationship between the groups and the provinces where the samples were collected.

The total number of polymorphic regions, polymorphic region ratio, nucleotide differences, population mutation ratio and Tajima's D values were calculated for the nucleotide sequences of the dog populations examined. In the study, eight different polymorphic regions in the mtDNA control region of the populations were determined, and the polymorphism rate was 3.5%. The mutation rate,

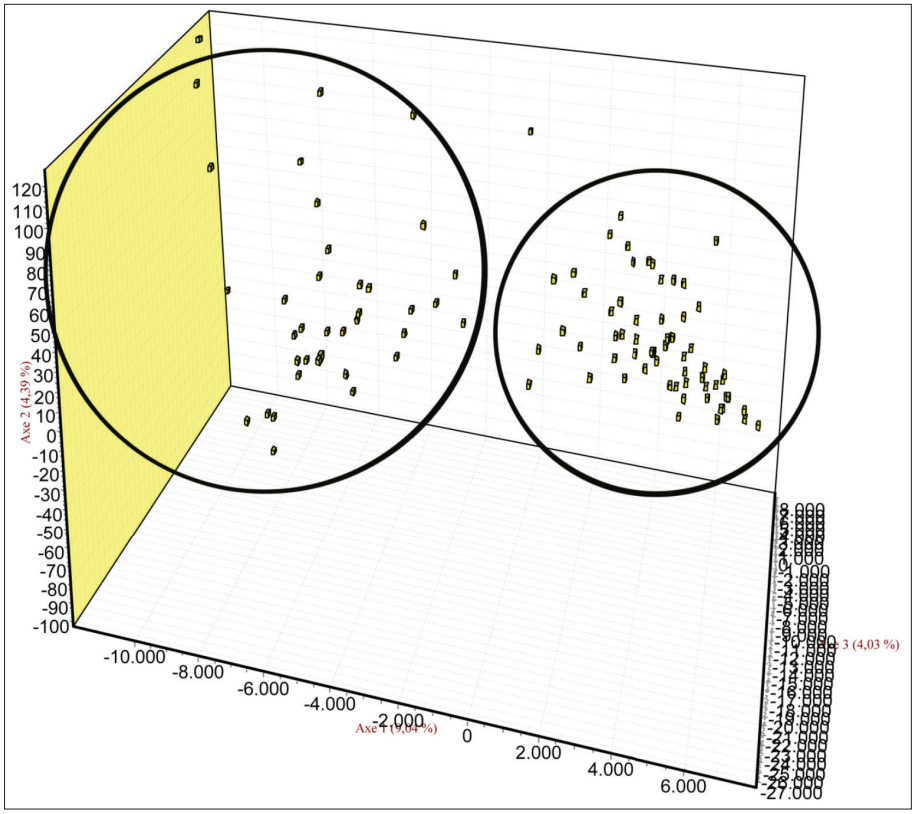

Fig 2. Factorial similarity analysis in the study group (The each circle represents more closely related animals according to genetic similarity)

nucleotide difference and Tajima's D value were 0.006, 0.009 and 1.416, respectively.

DISCUSSION

A previous study was conducted to determine the morphological characteristics of Zerdava dogs [1]. In the study on Zerdava dogs, the withers height, rump height, body length, chest circumference, chest width, shank circumference and head length were 51.2, 51.6, 56.3, 50.1, 25.6, 9.4 and 19.4 cm, respectively. In the present study, the live weight values were 16.02±0.35 kg. The mean withers height, rump height, body length and head length were 48.20±0.21, 47.08±0.24, 51.24±0.23 and 20.95±0.09 cm, respectively. The values obtained from this study for Zerdava dogs are lower than those obtained by Yilmaz and Ertugrul [1]. These differences may be because, the previous study was carried out with a small number (Total 39 dogs) of dogs and conducted with samples in a single province (Trabzon) and a limited group.

The genetic characteristics of some Turkish dog breeds have been reported in previous studies [10,16,17]. However, no studies have been conducted for the genetic characterisation of Zerdava dogs. Therefore, this is the first study performed on the genetic characterisation of Zerdava dogs.

Microsatellite markers are one of the standard tools used for the molecular genetic characterisation of breeds [17,18]. Altunok et al.[17] used microsatellite markers to determine the genetic differences among Kangal dogs and other Turkish dog breeds. They reported that the He values were 0.743 in the Kangal breed, 0.620 in the Akbaş breed and 0.705 in the Turkish hounds. Erdogan and Ozbeyaz [16] analysed the polymorphic loci from 276 dogs to determine the genetic relationship among some dog breeds. The mean heterozygosity was estimated between 0.32 and 0.41, and the difference in heterozygosity among dog breeds was insignificant (P<0.05). Although, the heterozygosity determined in the present study (0.732±0.071) was similar to that reported in the study carried out by Altunok et al.[17], but the values are higher than those reported in other studies [16,18,19].

The deviations in the Hardy-Weinberg equilibrium and the genetic differences among the subpopulations provide information about the genetic structure of the population [13]. The F_{IS} (inbreeding coefficient) parameters in Wright's F-statistics model are also commonly referred to as associated with *homozygous index*. In the present study, the microsatellite marker analysis results show that the average F_{IS} was 0.0361±0.0003, and the observed (Ho) and expected heterozygosity (He) indices were 0.732±0.071 and 0.712±0.077, respectively. The t-test results show that the difference between the average heterozygosity calculated in the present study was statistically insignificant. The deviations in the Hardy-Weinberg equilibrium and F_{IS}, Ho and He values suggest that populations can be heterogeneous in terms of the loci examined, closed breeding is less applied and non-relative individuals in the sample were selected successfully. A similar situation is seen more clearly when the FSA *(Fig. 2)* is taken into

consideration. According to this graph, the Zerdava dogs are classified into two groups.

mtDNA has been used as a genetic marker in population genetics for many years [14,18,20,21]. The sequence analysis of the mtDNA d-loop region can detect point mutations in the early stage of replication. Thus, information about maternal evolution can be obtained through an mtDNA sequence analysis in a population. Dog mtDNA haplogroups are divided into six main phylogenetic groups (A, B, C, D, E and F), and the percentage of dogs in these groups are 71.2%, 18.0%, 7.6%, 2.5%, 0.3% and 0.3%, respectively [18,22]. Most of the dogs are in one of the three major haplogroups. Almost all of the old-world dog populations have similar haplogroup rates (A, 55%-85%; B, 10%-35%; and C, 5%-15% [23]. In addition to haplogroups A, B and C, haplogroup D is common in 2.3% of dogs in Southwest Asia. Haplogroups E and F were detected in East Asia [24]. The haplogroups C and D have been detected from Central and West Europe, including France, Germany, Switzerland, and Hungary. On the other hand, haplogroups D, A and C are common in Eastern Europe, Iran and the Middle East [25-27]. The three haplogroups (A, B, and C) can be determined in Kenyan dogs, while Nigerian dogs are in one of four haplogroups (A, B, C, and D) [28]. In this study, the mtDNA sequence analysis found that Zerdava dogs were identified in haplogroups A, B and C. The frequency of A018 (72%) and B001 (16%) haplotypes was found to be high.

The Tajima's test is one of the most commonly used tests to determine mutations and natural selection of populations [29]. In the present study, eight different polymorphic regions were detected in the mtDNA control region, and the polymorphism rate (ps) was determined as 3.5%. The mutation rate *(Θ)* in the population, nucleotide difference and Tajima's D values were 0.006, 0.009 and 1.416, respectively. These results indicate that the population may have a low number of mutations, continuity of breed purity, balanced selection of samples and sampling from limited populations.

This research was conducted to determine the morphological and genetic characteristics of Zerdava dog, which is one of Turkey's native genetic resources. According to the data obtained, the phenotypic and genotypic variations of Zerdava dogs are very low, and thus it can be said that the genotype has been preserved by local breeders. In addition, the findings of this study show that Zerdava dogs may have sub-varieties. For the next stage, necessary steps should be taken for the protection of this breed.

Statement of Author Contributions

FA and FTÖ designed the study and manuscript preparation. The sampling, data collection and laboratory analysis were made by ME, BYÖ, BÖ and FTÖ. All the authors read the manuscript.

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