

GENETIC EVIDENCE FOR THE DISTINCTNESS OF KANGAL DOGS

VAHDETTİN ALTUNOK, EVREN KOBAN¹, LOUNÈS CHIKHI², ALISON SCHAFFER³,
NIELS C. PEDERSEN³, MEHMET NİZAMLIOĞLU AND İNCİ TOGAN¹

Department of Biochemistry, Faculty of Veterinary Medicine, Selçuk University, 42031 Konya, Turkey

¹Department of Biological Sciences, Middle East Technical University, 06531 Ankara, Turkey

²UMR 5174 Evolution et Diversité Biologique, Université Paul Sabatier, 31062 Toulouse, France

³Veterinary Genetics Laboratory, University of California, Davis 95616, California, USA

e-mail: togan@metu.edu.tr

valtunok@selcuk.edu.tr

Received for publication December 09, 2004.

Abstract

The genetic diversity of Kangal dogs (n=23) was analysed using 100 canine microsatellites, and the results were compared to Central Anatolian feral dogs (n=51), Akbash dogs (n=6), and Turkish greyhounds (TG, n=3). The Kangal, Akbash, Turkish greyhound and feral dogs were found to be significantly different from each other by F_{ST} measure. Factorial Correspondence Analysis (FCA), which evaluated the span of genotypic variation between individual dogs, yielded 4 distinct groups of the animals. Group I was composed of 12 pure Kangal dogs (Kangal I) without the Kangal looking hybrids of Kangals and feral dogs. Group II contained the remaining 11 Kangal dogs (Kangal II), 1 Turkish greyhound, and all feral dogs except for one. Group III was comprised of the remaining 2 Turkish greyhounds, while Group IV consisted of all of the Akbash dogs. Kangal I, Akbash and Turkish greyhound groups were scattered in different parts of the three-dimensional FCA plot. We conclude that Kangal dogs are genetically distinct and hence they deserve to be identified as a breed. Furthermore, it has been observed that microsatellites can be employed in the conservation efforts of Kangals.

Key words: dogs, microsatellites, genetic variability, population genetics.

Kangal dogs are the most popular dogs in Turkey and are often used as guard dogs to watch over livestock, factories and houses. They are known throughout Turkey and abroad for their strength, intelligence, loyalty, endurance to extreme temperatures, and lack of predatory behaviour towards livestock. Moreover, these characteristics have made Kangal dogs appealing to pet owners around the world; since the 1950s, breed clubs have been established in U.S.A, England, Germany, Holland, France and Belgium (5).

Kangal dogs are the dogs of central Anatolia and originally they are shepherd dogs. In Anatolia there is another well known shepherd dog, namely Akbash

dog. This is pure white dog of western Anatolia. Unfortunately, there are no formal written records or pedigrees for these dogs in Turkey. It should be emphasised that although they are recognised as separate breeds by Turkey and several western kennel clubs, the Fédération Cynologique Internationale (FCI) groups Kangal and Akbash dogs, along with other Turkish guardian types, into one breed known as the Anatolian Shepherd Dog with the breed number 331. Thus, the criteria under the breed number 331 span a broad range (6).

An effort is being made to preserve and breed the regional breeds of Turkey separately on farms such as the Military School of Veterinary and Training Center in Gemlik and the University of Selçuk Research and Application Unit (RA unit) in Konya. The Kangal dog in particular has been the object of government breeding and conservation efforts for decades. It is important, however, that foundation Kangal Dogs chosen to be used in such institutions truly represents the Kangal breed as it is known in Turkey. Furthermore, an effort to have FCI official recognition of these two separate breeds by their true breed names: Kangal and Akbash and to reconsider their distinct standards is underway.

The Kangal dogs of the RA unit were collected for the present study from local people of different parts of Central Anatolia in 1992-1993, basing on their phenotypic traits. Phenotypic traits may not be adequate, however, because many progeny of Kangal and Turkish feral dogs display the Kangal dog phenotype (10, 12). Accordingly both, phenotypic as well as genotypic measures of breed identity are required.

Genetic studies of Kangal dogs are scarce and limited to "classical" polymorphism (1). Therefore, more in depth genetic studies of Kangal dogs are needed to assure proper genetic management of the breed in the future. The present study is concerned with the genetic diversity and distinctness currently found in Kangal

dogs and is a pioneering study of their distinctness, based on molecular markers.

The genetic distinctness of Kangal dogs was measured against two phenotypically different Turkish breeds (Akbash and Turkish Greyhound), and against feral dogs from Konya, Central Anatolia. One hundred highly polymorphic canine microsatellite loci were examined in the study.

Material and Methods

Three Turkish regional dogs, Kangal, Akbash and TG, are found in Central, Western and North-Eastern Anatolia and are morphologically very distinct. The Research and Application Unit of the Veterinary Faculty of Selçuk regularly collects individuals of Turkish guarding dogs to form representative populations of the breeds in the Research Unit. Yet, the sizes of the populations are kept modest. The criterion of choice is based on morphological characteristics and the individuals that are thought to be "good representatives" of these breeds are selected. Overall, 23 Kangal, 6 Akbash, and 3 TG individuals were sampled for the present study. Pure breed dogs selection was based on criteria by FCI (6). These represented all the dogs available at the Research and Application Unit. The feral dog samples ($n=51$) were obtained from a semi-restricted region of 7000 m² owned by the Konya municipality. Buccal swab samples (epithelial tissue samples from the mouth) were taken from each dog using a small cytological brush (Medical Packaging Corporation, Camarillo, USA).

The procedures for the DNA isolation, DNA amplification and the panels of microsatellite loci were developed by the Veterinary Genetics Laboratory, University of California in Davis. Details of the procedures can be found in Koban's study (8) which can be sent upon request. List of the microsatellite loci used is as follows: AHT136, C06.636, C08.618, C09.173, CPH02, CXX763, FH2001, FH2004, FH2054, FH2079,

FH2161, AHT130, AHT133, AHT139, AHTk292, C01.424, C05.771, CXX002, CXX391, FH2145, FH2289, Wilms-TF, AHT111, AHT121, C07.620, C22.123, CXX147, CXX365, CXX758, FH2274, LEI004, PEZ08, PEZ12, AHT137, C03.877, C20.253, CXX140, FH2199, FH2247, FH2313, FH2361, LEI006, PEZ13, AHT103, AHT132, AHTk211, CPH14, CXX608, FH2175, LEI002, PEZ22, C08.410, CFMSAT, CPH03, CPH16, CXX279, FH2164, FH2293, PEZ02, C20.446, CPH08, LEI003, PEZ11, PEZ18, RVC1, VIASD10, C15.402, CXX161, CXX263, CXX750, FH2328, LEI007, CXX176, CXX213, FH2130, FH2140, FH2356, CXX130, CXX646, FH2137, FH2326, INRA21, LEI005, PEZ03, PEZ05, AHTk253, C09.250, FH2138, FH2148, FH2165, FH2201, FH2283, FH2324, C10.404, C16.671, CXX866, FH2200, FH2202, FH2233, FH2305.

For all the samples the number of alleles (n_A) and the mean number of alleles (MNA) across loci, observed heterozygosity (H_o), and unbiased expected heterozygosity (H_e) were estimated. Using Weir and Cockerham's (14) approach two of the Wright's F-statistics (F_{IS} and F_{ST}) (16) were calculated and their significance tests were performed by permuting the data 1000 times. Factorial Correspondence Analysis (FCA) was also performed. These computations were performed using GENETIX 4.0 (2)

Results

The results of genetic variability for all the samples are given in Table 1. Basing on n_A , MNA and H_e measures, the highest genetic variability was observed in feral dogs. The next highest variability was in Kangals. The highest H_o was observed in Akbash dogs. However, it is important to note that the number of Akbash and TG samples was small (6 and 3, respectively). As a consequence, some measures (n_A , MNA, H_o) are influenced by the sample number and must be interpreted appropriately for these samples.

Table 1
Unbiased expected heterozygosity (H_e), observed heterozygosity (H_o), total number of alleles (n_A) and mean number of alleles (MNA) seen in each dog group

	Kangal $n=23$	Akbash $n=6$	Turkish greyhounds $n=3$	Feral $n=51$
H_e	0.743	0.620	0.705	0.789
H_o	0.701	0.715	0.710	0.709
n_A	764	312	318	1074
MNA	7.64	3.12	3.18	10.74

Departures from Hardy-Weinberg (HW) expectations, evaluated within sample F_{IS} values, are given in Table 2. These values indicate significant ($P < 0.001$) departures from HW expectations in all samples, except that in TG.

The pairwise F_{ST} values in Table 2 show that the largest value was observed between the TG and the Akbash. The second highest value was then observed between Kangal and Akbash and this value was higher than that between greyhounds and Kangal, indicating strong differentiation between them.

Table 2
Pairwise F_{ST} values and within-sample F_{IS} values (diagonal) for the dog groups

	Kangal	Akbash	Turkish greyhound	Feral
Kangal	0.057***	0.115***	0.067**	0.026***
Akbash		-0.172***	0.173**	0.084***
Feral			0.101***	0.033**
Turkish greyhound				-0.008

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

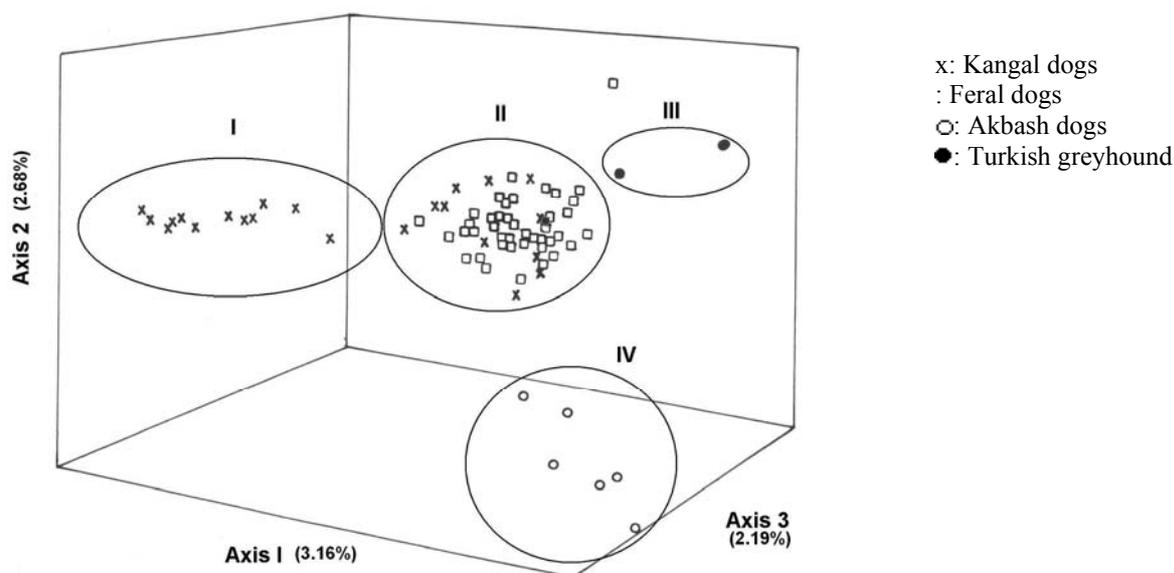


Fig. 1. FCA. Groups indicated as I, II, III and IV are explained in the text.

The FCA was performed to visualise and explore the relationships between the individuals. The FCA plot for the first 3 axes is given in Fig. 1. It shows a clear set of 4 clusters. While there is not a perfect match between breeds and clusters, a rather clear-cut pattern emerges. All the members of group I are Kangal dogs and are called Kangal I. The rest of the Kangals (Kangal II, $n=11$) as well as all but 1 of the feral

individuals (only non-circled individual in Fig. 1) and 1 TG constitute group II. Group III is composed of other 2 TGs. Finally, all the Akbash individuals constitute Group IV. In this figure feral dogs and Kangal I (group I) occupy a central position, Akbash (group IV) and Greyhounds (group III) occupy quite compact and distinct places.

Discussion

In the following, we compare our results with those obtained in other breeds of dogs, in order to interpret the extent of genetic variability observed in the Kangal dogs. Since Akbash and TG dogs were represented by small sample number, these comparisons were limited to H_e values.

A number of studies report the amount of genetic diversity in different breeds of dogs, but in general the number of loci used is more limited than that described in our study. Wilton *et al.* (15) studied the microsatellite variation in a group of Australian dingo ($n=15$) and in a group of mixed-breed dogs ($n=16$) using 14 microsatellites. Only two of the loci used by Wilton *et al.* (15) (AHT103 and CXX263) were also used in the present study. They found that the average H_e for these two loci were 0.78 in the mixed-breed dogs and 0.51 in dingos. For the same loci, we found 0.75 in Kangal, 0.61 in Akbash, 0.43 in TG, and 0.76 in feral dogs. In another study, Koskinen and Bredbacka (9) used 10 microsatellite loci to assess the genetic variability and differentiation among Finnish populations of 5 domestic dog breeds (Golden Retriever, German shepherd, Wirehaired Dachshund, Pembroke Welsh Corgis and Bedlington terrier). Five of these loci (FH2001, FH2004, FH2054, FH2137 and FH2175) were also used in our study. In that study ranges of average H_e values were between 0.51 and 0.75 in the 5 breeds. In our samples H_e values were 0.84 in Kangal, 0.76 in Akbash 0.80 in TG and 0.84 in feral dogs.

Another set of 6 loci (LEI002-007) were typed by Zajc *et al.* (17) in 3 different dog breeds (Greyhound, $n=53$, Labrador, $n=52$ and German shepherd, $n=53$). The average H_e values ranged between 0.45 and 0.57, while in our breeds they were 0.58 in Kangal, 0.50 in Akbash, 0.51 in TG and 0.67 in feral dogs.

Comparative studies showed that the amount of genetic variability present in the Kangal, as well as in Akbash and TG, is quite high and comparable or higher than that observed in a number of other domestic breeds.

This can be explained by the fact that many dog breeds are quite inbred. Directional selection aiming at maintaining specific phenotypes, including behavioural patterns, together with founder effects when breeds are developed may lead to the loss of rare alleles at many loci and consequently cause a genetic impoverishment in some breeds. The fact that feral dogs, which are not submitted to any directional selection, exhibit the highest level of genetic diversity is in good agreement with this interpretation. Directional selection for multiple traits (size, behaviour, coat, morphology) has also taken place in Kangal, Akbash, and TG dogs but it has not been so severe.

Another explanation for the diversity observed in Kangal dogs may be the existence of recurrent mating between Kangal and feral dogs. Hybridisation between Kangal and feral dogs is known to produce a crossbred progeny that resembles the Kangal type (10, 12). Therefore, some dogs that appear to be Kangal may actually be. The FCA plot appears to confirm this

analysis since Kangal dogs were divided into two groups, the first (group I) containing about one half of the Kangal dogs, while the second half was within group II, which contained mostly feral dogs. When only Kangal dogs from group I are considered we found a H_e value of 0.68, instead of 0.74 when using all the available Kangal dogs. This shows that a significant amount of diversity observed within the whole Kangal sample could be the result of crossbred between Kangal and feral dogs. Still, we noted that the Kangals from Group I were genetically variable when compared to other dog breeds (recalculating H_e with the loci that were common between our study and those of other authors (9,15,17)): we found values of 0.72, 0.83 and 0.50 that were above or within the range of values observed by these authors.

The within sample departures from HW expectations were highly significant ($P<0.001$) except that for TG. Both Kangal and feral dogs exhibited positive and significant F_{IS} (0.057 and 0.101, respectively), indicating deficits in heterozygotes. These could be caused by a number of factors which include (i) selection, (ii) null alleles, (iii) inbreeding or assortive mating, (iv) population substructure (i.e. Wahlund effect). While selection may generate positive F_{IS} in a limited number of loci it is unlikely to explain the significant departures seen at most loci (only 39 loci in Kangal and 25 loci in feral dogs exhibited negative values, these results are not shown). Null alleles are also likely to explain positive F_{IS} at some loci, particularly when so many loci are analysed using multiplex and co-loading protocols (8). We can use the method of Brookfield (3) to estimate the frequency of null alleles as $(H_e - H_o)/(1 + H_e)$. Using the average H_e and H_o values we found null allele frequencies of 0.02 and 0.07 for the Kangal and feral dogs, respectively. This indicates that the expected frequency of null homozygotes per locus in the two samples would be $4 \cdot 10^{-4}$ for the Kangal and $4.9 \cdot 10^{-3}$ for feral dogs. While such frequencies are too low to be observed when single locus data are used, we expect to see $23 \cdot 100 \cdot 4 \cdot 10^{-4} \sim 1$ and $51 \cdot 100 \cdot 4.9 \cdot 10^{-3} \sim 25$ of such homozygotes in Kangal and feral dogs, respectively. This means that while it is possible to have missed the one null homozygote expected in the Kangal it is unlikely to have missed 25 null homozygotes in the feral dogs. Thus, null alleles may contribute to the total F_{IS} but are unlikely to be the sole cause for it.

The Wahlund effect is a likely factor explaining the deficit in heterozygotes. Indeed, the Kangal dogs could be separated into two discrete groups in the FCA plot, indicating the existence of substructuring in the Kangal breed. Similarly, the feral dogs could be actually made up of two or more cryptic but differentiated groups of dogs. We can test this hypothesis for the Kangal dog by re-analysing the data for the two groups. We would expect the F_{ST} between the two Kangal groups to be approximately equal to the F_{IS} within the whole Kangal bred. Indeed, as shown by the pairwise F_{ST} values in Table 3, F_{ST} between Kangal I and Kangal II was 0.066 as compared to the within-Kangal F_{IS} of 0.057 (Table 2).

Table 3
Pairwise F_{ST} values when Kangal clusters are separated

	Kangal II	Akbash	Turkish greyhound	Feral
Kangal I	0.066***	0.167***	0.121**	0.058***
Kangal II	-	0.095**	0.040*	0.007*

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

The issue of assortive mating has to be interpreted with care in the case of dog breeds, since mating is to some extent directed by humans (but not in feral dogs). Our results suggest that assortive mating could indeed take place when selection of mating pairs is decided on the basis of phenotypic characteristics. However, it is not expected to affect many independent loci.

In conclusion it appears that the two factors that account for most of the deficit in heterozygotes observed in Kangal and feral dogs are cryptic substructuring (Wahlund effect) and some not yet quantifiable degree of non-random mating. The contribution of the two factors may not be the same for the Kangal and feral dogs due to their quite different lifestyles.

The F_{IS} values observed in Akbash (-0.172, $P < 0.001$) and TG (-0.008, not significant) should be interpreted with the understanding that small sample sizes are known to generate negative F_{IS} that have little to do with any biological phenomenon (11, 7).

The genetic differentiation among 4 samples was assessed by using pairwise F_{ST} values and they were all significant ($P < 0.01$ - $P < 0.001$) indicating that the different samples exhibited different allele frequencies. This was reflected in FCA plot (Fig.1) as well. Although the first three axes account for 8% of the genotypic variability, this low percentage is generally seen in the microsatellite-based studies of domestic animals (4). Another interesting observation was the discrete distribution of 3 types of dog samples and central position of the feral dogs on the first three axes of the FCA. Furthermore, 3 regional dog samples were scattered in different parts of the three-dimensional FCA plot. All these indicate the presence of differentiation of Kangals, Akbashes, and TGs from each other in different directions. Yet, 11 Kangals and 1 TG were genetically (based on 100 microsatellite) found in the pool of feral dogs. On the contrary, one feral dog was out of this feral dog group. This result suggests that there are hybrid dogs between the feral dogs and the dogs of the pure-breeds. Based on their appearance the hybrids can potentially be identified incorrectly as Kangal ($n=11$) and TG ($n=1$) in group II (Fig. 1) or the free living ($n=1$) not included in group II. FCA plot proved to be very powerful in identifying substructure in the Kangal breed. We were indeed able to identify individuals that appear to be morphologically like pure Kangal dogs but are likely to have been introgressed, perhaps with feral dogs.

In the original calculations (Table 2), the highest F_{ST} value was observed between TG and Akbash dogs ($F_{ST}=0.17$) and the second highest value was that between Kangal and Akbash ones ($F_{ST}=0.12$). When we accounted for the substructure in Kangal dogs and recalculated F_{ST} values it appeared that F_{ST} increased significantly between Kangal and all others (Table 3). Finally, in relation to the distinctness of Kangal dogs, a recent study carried out by Savolainen *et al.* (13) indicated that a relatively rare mt-DNA haplogroup D is present in Kangal dogs (20%) but not in Akbash ones. The fact that D is also present only in Scandinavian dogs and perhaps in dogs of the Iberian Peninsula raises a new question about the origins of Kangals and dog breeds in general. Regarding all these facts we conclude that Kangal dogs are genetically distinct and they deserve to be identified as a separate breed. Although the numbers examined are very limited the same conclusion may also apply to Akbash and TG dogs.

The dramatic decline in the number of Kangal dogs in the last few decades due to decreased agricultural activities, decline in sheep industry and increasing migration of villagers to the cities mean that in order to maintain the genetic variability and genetic distinctiveness of Kangals across Turkey, a careful breeding program should urgently be implemented. Fortunately, in the last few years, a keen great interest in the preservation of the Kangal dogs by the local people of Sivas-Kangal (Central Anatolia) has developed. Every year, the mayor of Sivas-Kangal is organising the "International Kangal Dog Symposium" and arranging Kangal dog competitions. Many dogs from remote village are brought to the competition. Hence, information about these dogs, otherwise unnoticed, was recorded. In a few years, higher number of Kangals will be available as candidates for the new breeding programme. The present study has shown that it is possible to identify clusters of individuals, such as the Kangal dogs from Group I, which could be used to define the genetic standards of the Kangal breed. The typing of 100 loci is of course not feasible to separate the breeds and it would certainly be useful to define smaller sets of loci that could be used.

Acknowledgments: We would like to thank the Veterinary Faculty of Selcuk University Research and Application Unit for giving permission for the collection of swab samples and to Dr Mehmet Maden

for helping us in collection of the samples. In addition, our special thanks go to Carol Karlewicz, Debbie Grossman, Pratibha Daftari, Jennifer Draper and Tamala Gilliland for their technical help, and to Karen Norvell together with all the VGL staff at UC Davis for their kind welcome and support. Finally, we would like to thank Sue Kocher for her valuable suggestions on the manuscript and Sara Banu Akkas for grammar corrections. The study was funded by proceeds from diagnostic services offered at the Veterinary Genetics Laboratory of UC Davis.

References

1. Altunok V., Nizamlioglu M., Ergüven A., Togan I.: Red blood cell enzyme biochemical polymorphism in Anatolian shepherd dog. *Rev Med Vet-Toulouse* 1999, **150**, 625-628.
2. Belkhir K., Borsa P., Chikhi L., Goudet J., Bonhomme F.: GENETIX 4.00 Windows™ software for population genetics. Laboratoire Génome, Populations, Interactions, University of Montpellier, France, 1996-2000 (<http://www.univ-montp2.fr/~genetix/genetix/genetix.htm>).
3. Brookfield J.F.Y.: A simple new method for estimating null allele frequency from heterozygote deficiency. *Mol Biol Evol* 1996, **5**, 453-455.
4. Byrne K., Chikhi L., Townsend S.J., Cruickshank R.H., Alderson G.L.H., Bruford M.W.: Extreme genetic diversity within and among European sheep types and its implications for breed conservation. *Mol Ecol* (in press).
5. Chappell J.: The breeding of Karabaş in Britain. In: *International Symposium on Turkish Shepherd Dogs*, Konya, Turkey 1996, pp. 121-146.
6. F.C.I Anatolian shepherd dog. In: *Federation Cynologique Internationale*. THUIN Belgium, 1989.
7. Goossens B., Chikhi L., Taberlet P., Waits L.P., Allainé D.: Microsatellite analysis of genetic variation among and within Alpine marmot populations in the French Alps. *Mol Ecol* 2001, **10**, 41-52.
8. Koban E.: Genetic identity of Anatolian shepherd dog (ASD) population using microsatellites. MSc.Thesis, Middle East Technical University, Ankara, Turkey, 2001.
9. Koskinen M.T., Bredbacka P.: Assessment of the population structure of five Finnish dog breeds with microsatellites. *Anim Genet* 2000, **31**, 310-317.
10. Nelson D.D.: A general classification of the native dogs of Turkey. In: *International Symposium on Turkish Shepherd Dogs*, Konya, Turkey 1996, pp. 19-96.
11. Pudovkin A.I., Zaykin D.V., Hedgecock D.: On the potential for estimating the effective number of breeders from heterozygote-excess in progeny. *Genetics* 1996, **144**, 383-387.
12. Reed S.: The history of Turkish shepherd dogs. In: *International Symposium on Turkish Shepherd Dogs*, Konya, Turkey 1996, pp. 97-109.
13. Savolainen P., Zhang Y., Luo J., Lundeberg J., Leitner T.: Genetic evidence for an East Asian origin of domestic dogs. *Science* 2002, **298**, 1610-1613.
14. Weir B.S., Cockerham C.C.: Estimating F statistics for the analysis of population structure. *Evolution* 1984, **38**, 1358-1370.
15. Wilton A.N., Steward D.J., Zafiris K.: Microsatellite variation in the Australian dingo. *J Hered* 1999, **90**, 108-111.
16. Wright S.: The genetical structure of populations. *Ann Eugenics* 1951, **15**, 323-354.
17. Zalc I., Mellersh C.S., Sampson J.: Variability of canine microsatellites within and between different dog breeds. *Mamm Genome* 1997, **8**, 182-185.