

International Journal of DAGENE

Danubian Animal Genetic Resources

Volume 7, Issue 2 (2022)

DAGENE
International Association for the Conservation
of Animal Breeds in the Danube Region
1078 Budapest, István street 2.
Hungary



FEDO.HU – Online COI calculator of planned litters for mate selection in Kuvasz dog breed

FÜRLINGER, Dóra¹ – BÁN, Beáta² – PALLOS, Réka³ – PISCHOF, Ferenc⁴ – ZSÓRI, Zsolt⁴
– KISS, Daniella Panna¹ – GÁSPÁRDY, András¹ – MARÓTI-AGÓTS, Ákos^{1*}

¹Institute of Animal Breeding, Nutrition and Laboratory Animal Science, University of Veterinary Medicine Budapest, István u. 2, 1078 Budapest, Hungary

²Veterinary Diagnostic Directorate, National Food Chain Safety Office, Kitaibel Pál u. 4, 1024 Budapest, Hungary

³Institute of Biology, Faculty of Sciences, University of Pécs, Ifjúság u. 6, 7624 Pécs, Hungary

⁴Hungarian Kuvasz Breeder Association, Gervay u. 117/a, 1147 Budapest, Hungary

*corresponding author: maroti-agots.akos@univet.hu

Abstract

Nowadays, more than ever before, the breeding of purebred dogs is based on their appearance. However, using mating of close relatives in pure-bred breeding to maintain "aesthetic" traits drastically reduces the genetic diversity of the breed and increases the occurrence of genetic diseases. Inbreeding value of Kuvasz dogs registered in the pedigree book of Hungarian Kuvasz Breeder Association (MKFE) was determined using two different methods (pedigree calculations and molecular genetic identification). Molecular genetic analyses used for parentage control in NÉBIH-ÁDI matched the inbreeding values calculated from pedigree data. The inbreeding values (COI) of the indigenous Kuvasz dog breed are low. FEDO.HU is a transparent, public, attitude-shaping, scientifically reliable professional decision support system, which is available to all breeders of our indigenous dog breed.

Keywords: COI, molecular inbreeding coefficient, Kuvasz breeding, pure-bred dog breeds, online calculator

Introduction

Most dog breeds were created in the mid-1900s, which is when we can date the development of purebred breeding methods and the establishment of breed standards (WILCOX et al. 1995, PARKER et al., 2004). After the foundation, due to the closed registers, further gene flow was forbidden, the aim was to maintain the characteristics of the founding individuals of the breed. Strict selection for appearance traits can have serious consequences. It may involve the complete loss of some genes, thereby reducing genetic diversity, or an enrichment of another allele variant and so resulting unwanted health consequences (PEDERSEN et al., 2015; KEIJSER et al., 2018; LAMPI et al., 2020).

Thorough pedigrees are an important source of information on genetic diversity and population structure (GOLEMAN et al., 2021). The calculation of the inbreeding coefficient (COI) based

on pedigree data dates back nearly a hundred years (WRIGHT, 1922). Determination of inbreeding value by molecular genetic methods is for some decades in practice, using short tandem repeats (STRs). From the results of the molecular tests, in addition to the number of alleles, values used to characterise genetic balance can also be calculated: E.g., the fixation index (which is in fact equivalent to the COI) expresses the probability that two alleles of a locus are derived from a common ancestor (PÉNZES, 2011). Inbreeding can be calculated based on both expected (H_e) and observed heterozygosity (H_o) of alleles on a certain locus. A low H_o value indicates a decrease in the number of heterozygous individuals for a given locus, which can be seen, for example, in the French bulldog ($H_o = 0.376$). In an ideal population, in Hardy-Weinberg equilibrium, the frequency of allelic variants of a gene is constant, does not change from generation to generation, so that, among other things, the observed heterozygosity remains close to the expected value (DZITSIUK et al., 2017).

Material and methods

Pedigree data

FEDO.HU was inspired by the English Kennel Club - Mate Select open access system (JANES et al., 2020). The database of the Hungarian Kuvasz Breeders Association (MKFE) being looked after by the Hungarian Kennel Club (MEOESZ), currently contains the pedigree data of 7713 Kuvasz individuals. An algorithm written in C++ performs graph theoretical COI computations, thereby after selecting two individuals from the database, degree of relatedness of these two individuals can be analysed taking into account 7 generations of data. The algorithm only counts 7 generations because including additional ancestors has no substantial influence on the results, for the present purpose.

Molecular genetic testing

STR-test results of parentage control carried out by the Animal Genetics Laboratory in NÉBIH-ÁDI were used. NÉBIH has created a database containing 768 dogs from the test results of dogs registered by MKFE, including the following data: pedigree name, breed, sex, chip number, pedigree number, date of birth, type and laboratory number of the sample, as well as the mother's and father's pedigree name. The database also includes the 21 canine-STRs investigated and recommended by ISAG and the amelogenin marker for sex determination.

For genetic calculations Genetic Analyses in Excel (GenAIEx) 6.5 macro was used (PEAKALL et al., 2012), which was applied to perform allele frequency analyses (F-statistics, heterozygosity, fixation index (F_{is}) and deviations from Hardy-Weinberg equilibrium). The number of alleles for the whole population and their frequency at each locus and the relative genetic entropy were determined (GÁSPÁRDY et al., 2018).

For statistical comparison of the inbreeding coefficients obtained, a differential test of the ratios as a function of percentage and number of samples.

Results and discussion

FEDO.HU

From the database, we have created an online inbreeding calculator freely available on the internet. Kuvasz dogs born after 01.10.2011. are registered on FEDO.HU as breeding animals and the website lists a total of 7713 individuals. Based on the data, the average inbreeding coefficient (COI) of the population is 5.36%. The few higher COI values are typical of

individuals born in the last century, with a maximum of 25%. However, there are also some of the recently born individuals with COI around 20%.

The number of founding individuals is 517. Among the founder individuals, in the case of 232 animals we found a recorded date of birth. According to these data, the first founder was born on 04.08.1987 and the last one on 29.09.2000.

On the website, the COI of the planned progeny of two breeding animals can be calculated. Based on the traffic data so far, users tend to retrieve the data of several hundreds of possible litters per month. The calculator is available in English and Hungarian and the COI values are automatically categorised: below 3.125% low, between 3.125-6.25% mild, above 6.25% we would get very highly inbred pups from the given parents. These categories were defined by the Association's management in the previous year in accordance with the average COI of the breeding population, and are going to be updated every year on the basis of new data. In addition to the COI values, the pedigree data of the breeding animals are freely available (Figure).

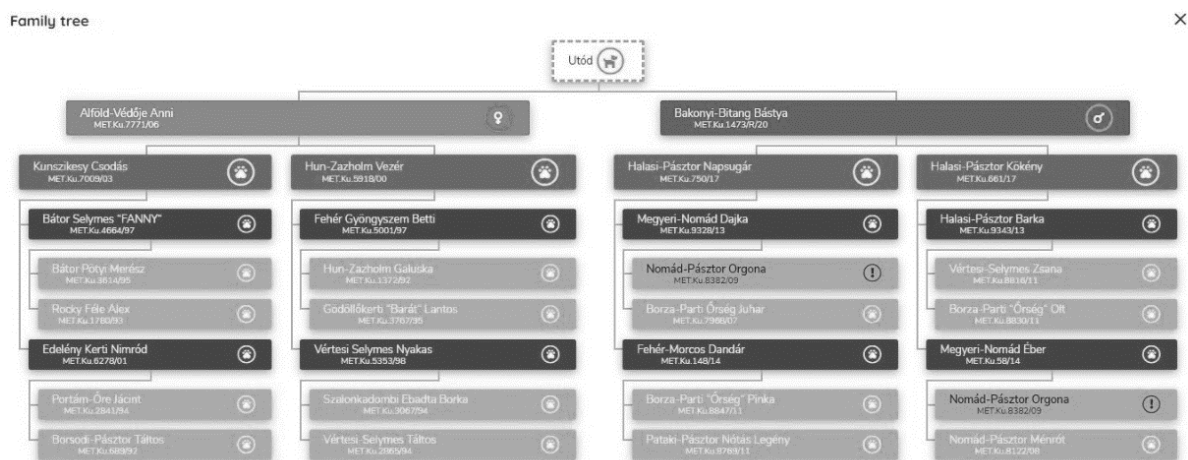


Figure 1. Family tree of the planned litter

Molecular genetic testing

The total number of alleles observed ranges from 4 (REN247M23, AHTk253) to 11 (AHT121) with an average of 6.76 (SD = 0.4). The lowest number of effective alleles (1.47) was found at the REN247M23 locus, and the highest (5.28) at the AHT121 locus. The mean number of effective alleles was 3.14 (SD = 0.21). Also, the REN247M23 locus had the lowest expected heterozygosity (0.32), and the AHT121 had the highest (0.81).

The mean expected heterozygosity (H_e) of the population was 0.65 (SD = 0.03). The mean observed heterozygosity (H_o) of the population was 0.65 (SD = 0.03). The lowest value of H_o was also associated with the REN247M23 locus ($H_o = 0.33$), the highest was associated with AHT121 ($H_o = 0.82$).

The lowest fixation index was observed at locus AHTk211 ($F_{is} = -0.079$). Seven STRs showed positive values, of which $F_{is} = 0.00$ occurred at the REN5P11 locus, and the highest value ($F_{is} = 0.07$) at the INU030 locus.

The average value of F_{is} for all loci 0.07 (SD = 0.08).

The two coefficients of inbreeding (pedigree 5.36%, $n = 7713$ and 6.76%, $n = 768$) showed no statistically proven difference (two-sided differential test, $p = 0,104$).

The 21 STRs showed no significant deviation from the Hardy-Weinberg equilibrium in 11 cases. Weaker significance ($p < 0.01$) for 4 loci (CXX279, REN169O18, INRA21, AHT137), while

stronger significance ($p < 0.001$) for 6 loci (REN162C04, FH2054, AHT121, AHTh260, AHTh130, REN5P11) was observed (Table). The Table also presents the values of the Shannon information index. The genetic diversity of each microsatellite is more directly reflected in its relative indicator, the relative entropy, which can be called the effective genetic diversity. It can be seen that the microsatellite AHTh130, despite having 10 allelic variants, is characterized by the slightest entropy. In such microsatellites the risk of losing the very rare (low frequency) allelic variants in a short time is increased.

Table. Molecular test results of the loci. N: number of samples, N_a : number of alleles per locus, N_e : number of effective alleles, H_o : observed heterozygosity, H_e : expected heterozygosity, F_{is} : fixation index, I =Shannon's information index, I_{rel} =relative entropy, Signif: statistical analysis of the HWE

Locus	N	N_a	N_e	H_o	H_e	F_{is}	I	I_{rel}	Signif
AHTk211	752	5	2.251	0.60	0.56	-0.079	0.925	39.8	ns
CXX279	768	6	2.836	0.66	0.65	-0.026	1.242	48.1	**
REN169O18	768	6	4.487	0.76	0.78	0.023	1.571	60.8	**
INU055	767	6	4.026	0.76	0.75	-0.006	1.472	56.9	ns
REN5P11	767	8	3.817	0.74	0.74	0.000	1.440	48.0	***
AHTh130	764	10	1.850	0.47	0.46	-0.029	0.871	26.2	***
INRA21	767	6	3.509	0.76	0.72	-0.067	1.408	54.5	**
AHT137	768	7	3.373	0.70	0.70	-0.001	1.331	47.4	**
REN169D01	767	7	2.799	0.62	0.64	0.030	1.175	41.9	ns
REN169D01	764	7	3.930	0.72	0.75	0.031	1.395	49.7	***
AHTk253	765	4	2.639	0.63	0.62	-0.006	1.040	52.0	ns
INU005	765	7	3.294	0.68	0.70	0.031	1.389	49.5	ns
INU030	767	8	2.861	0.60	0.65	0.070	1.099	36.6	ns
FH2848	761	6	3.969	0.73	0.75	0.020	1.489	57.6	ns
REN105L03	759	5	2.770	0.66	0.64	-0.037	1.204	51.9	ns
AHT121	767	11	5.278	0.82	0.81	-0.015	1.803	52.1	***
FH2054	764	5	2.022	0.51	0.50	-0.015	1.027	44.2	***
REN162C04	767	7	2.316	0.559	0.57	0.016	0.939	33.4	***
AHTh171	767	10	3.924	0.757	0.75	-0.017	1.511	45.5	ns
REN247M23	767	4	1.474	0.330	0.32	-0.026	0.597	29.8	ns
REN64E19	762	7	2.554	0.636	0.61	-0.046	1.093	38.9	ns

With FEDO.HU, the pedigree data have been placed in a public, freely accessible and organised electronic database. From the parentage and breeding data, the COI calculator provides a professionally verified, accurate method to calculate the inbreeding values of the planned litters. The current COI of 5.36% for the Kuvasz population, based on literature data, is considered as a low inbreeding coefficient (GOLEMAN et al., 2021; DZITSIUK et al., 2017; GOLEMAN et al., 2018). The importance of the calculator is confirmed by the fact that we found individuals with approximately 20% COI value in the current population. By checking the planned mating, the website can efficiently help to change breeders' attitudes, prevent close inbreeding and maintain the genetic diversity and health of the breed.

Based on the 142 alleles observed in STR studies, the Kuvasz is comparable to other local breeds with low inbreeding (RADKO et al., 2017; GOLEMAN et al., 2021; DZITSIUK et al., 2017; BIGI et al., 2018; RADKO et al., 2021). Genetic diversity of the breed is considered to

be conserved, with a mean F_{is} of -0.07 ± 0.08 indicating population stability, although the variance may push this value into the positive range minimally. Only 6 of the 21 loci studied deviate significantly from the Hardy-Weinberg equilibrium. In a previous study including several Hungarian dog breed populations, 90% of the loci deviated from the Hardy-Weinberg equilibrium. The authors used 10 STR loci in their work and drew attention to the fact, that the use of more markers than this is justified for pedigree verification, especially for highly inbred breeds (ZENKE et al., 2007). Our own study uses 21 genetic markers so the results can be considered reliable.

Expected and observed heterozygosity values are in agreement and STR tests did not show significant inbreeding. The coincident values suggest that results based on pedigree data as well as molecular genetic calculations are equally reliable when determining COI.

Relative entropy exceeds 50% only in a minority of microsatellites. This suggests that in a larger proportion of microsatellites genetic diversity is reduced, occasionally critically threatened. This result draws attention to the need for careful breed maintenance.

The two inbreeding coefficients (5.36% and 6.76%) are statistically identical, which means that both methods revealed the same degree of inbreeding and homozygosity. The molecular inbreeding coefficient is usually higher than the other one. Results obtained from pedigree data analysis indicate more diverse population, what is due to the fact that the relatedness between the founding ancestors is ignored.

Conclusion and recommendation

The breeding practice where breeders aim to have dogs that are "healthy, behaving and looking like their breed members, and provide sustainable performance" is now called Dog Breeding 2.0. (BROECKX, 2020).

With the FEDO.HU system, the Hungarian dog breeding has got a transparent, professionally supervised, freely accessible tool, which can provide a basis for a change in approach that can lead to the development of what we call Dog Breeding 2.0.

Acknowledgment

The project is supported by the European Union, the European Regional Development Fund (ERDF) (VEKOP-2.3.2.-16-2016-00012).

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