

Preliminary studies of the genetic structure of "Cimarron uruguayo" dog using microsatellite markers

Estudios preliminares de la estructura genética del perro cimarrón uruguayo usando microsatélites

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ABSTRACT

Objective. To analyze the population structure, using microsatellite markers in a sample of "Cimarron Uruguayo" dogs. **Materials and methods.** Thirty dogs were analyzed in different areas of Uruguay with a set of nine molecular microsatellite markers using PCR. The population structure was analyzed using the free distribution software "Structure". **Results.** According to our data, the preliminary results show that it is not possible to establish a subdivision among the animals in the sample. **Conclusions.** The study supports the hypothesis that the currently existing canines derive from a founding nucleus that took refuge in the Northeastern region of the country. The distribution of the breed among the different areas of Uruguay continues nowadays, so there is no isolation among the different groups of animals, and the exchange is constant

Key words: Population structure; DNA markers; Cimarron Uruguayo.

RESUMEN

Objetivo. Analizar la estructura poblacional en una muestra de perros "Cimarrón Uruguayo" usando marcadores moleculares tipo microsatélites. **Materiales y métodos.** Se analizaron treinta caninos de diferentes zonas de Uruguay con un set de nueve marcadores moleculares microsatélites empleando PCR. La estructura poblacional se analizó con el software de distribución libre "Structure". **Resultados.** Según nuestros datos, los resultados preliminares

muestran que no es posible establecer una subdivisión entre los animales de la muestra. **Conclusiones.** El estudio realizado apoya la hipótesis de que los perros que existen en la actualidad derivan del núcleo fundador que se refugió en la región noreste del país. La distribución de la raza entre las distintas áreas de Uruguay continúa hoy en día, no existe aislamiento entre los diferentes grupos de animales y el intercambio es constante.

Palabras clave: Estructura poblacional, marcadores de ADN, Cimarrón Uruguayo.

INTRODUCTION

The "Cimarron Uruguayo" is the only dog breed which is originary from Uruguay. On 21 February 2006, the "Fédération Cynologique Internationale" recognizes the breed in primary form, which means that it is in a proof period of five years before its final recognition (1). This has led to an increase in the interest in the breed on the part of the breeders and the exhibitors, which is why strict controls are being made to ensure the standardization and prevent the occurrence of undesirable hereditary characteristics. At different times in history, these animals have represented a serious danger to man and livestock. Some historians, in the XVIth century, talked about their multiplication and the damage they produce, even putting a price on their heads.

In 1788, the extermination of these dogs was approved by Buenos Aires Council. During the XIXth century, the persecution of these dogs still continued. The second time when these dogs represented a problem was during the "Guerra Grande". At this time, there was another pursuit of Cimarron dogs. However, a few individuals still remained in rural places of the provinces of Cerro Largo and Treinta y Tres (2-3). Characterization of the population structure of this dog breed is useful in a variety of contexts. Genetic ascertainment of within-species population structure has been widely applied for classifying subspecies, for defining intraspecific conservation units, for understanding events in the history of a species, for identifying ongoing speciation events and for testing hypothesis about evolutionary processes (4). "Structure" is one of the freeware used to analyze population structure. It uses multi-locus

genotypic data, so it can be applied to the majority of the commonly-used genetic markers (5). Microsatellites are among the plausible genetic markers to be used for these analyses. It is assumed that there is no linkage among the chosen loci.

Having in consideration its history and the interest this breed has arisen, the objective of this investigation was to analyze the population structure, using microsatellite markers in a sample of "Cimarron Uruguayo" dogs.

MATERIALS AND METHODS

Animals and study area. Thirty "Cimarron Uruguayo" dogs were analyzed: 13 animals from the northeastern (NE) area of Uruguay and 17 from the southern (S) area. These areas were selected taking into account: the background of the founding nucleus in the origins of the breed (NE area: provinces of Cerro Largo and Treinta y Tres) and animal density (S area: provinces of Montevideo and Canelones).

DNA isolation. DNA was isolated using the AxyPrep Multisource Genomic DNA Miniprep Kit (Axygen biosciences). Nine microsatellite markers tested in previous studies in this breed (6), included in ISAG (International Society for Animal Genetics) panel 2 were analyzed using PCR technique; each of them was amplified separately. The amplifications were performed using standard programmes (denaturation, 95°C, 4 minutes; 35 cycles of 95°C, 1 minute, 44-60°C, 30 seconds, 72 °C, 45 seconds). A mix was made with the amplifications of each animal. In table

1 are shown microsatellite markers used in this work, its respective primers and PCR conditions.

Data analysis. The results were read with the MegaBACE 1000 programme, with the permit of the University of Zaragoza (UNIZAR). The population structure was analyzed with the "Structure 2.3.1" freeware with admixture (7). The "Estimated Ln Prob of Data" obtained from running the software was considered to estimate the number of subpopulations (K). This data corresponds to the estimation of $\ln \Pr(X/K)$, where X are the genotypes. To verify the reliability of the estimations obtained by running the software, three runs were performed for each K.

K	Ln	σ^2	P	
1	-709.7	-706.1	8.3	1
		-710.0	16.5	
		-713.1	17.7	
2	-737.6	-716.7	34.8	0
		-736.7	78.0	
		-759.5	131.1	
3	-744.8	-714.0	26.7	0
		-741.5	87.5	
		-778.9	175.6	
4	-739.7	-773.2	154.7	0
		-721.4	44.1	
		-724.6	47.6	
5	-718.2	-700.6	9.2	0
		-701.0	11.4	
		-753.1	110.7	
6	-721.8	-741.9	82.5	0
		-712.8	24.2	
		-710.8	24.0	
7	-717.3	-720.6	42.7	0
		-718.3	37.5	
		-713.0	24.7	
8	-740.6	-713.8	28.1	0
		-771.1	160.0	
		-737.0	76.1	

Figure 1. Inference of the number of subpopulations of "Cimarron Uruguayo" dogs, in accordance with the date obtained with the "Structure" software.

RESULTS

The results obtained from the simulations performed with the "Structure 2.3.1" software are shown in figures 1 and 2. Obtained alleles, observed and expected heterozygosity for each population are shown in table 2.

The number of subpopulations (K), according to the Bayesian rule [considering K=1, where the highest value of $\ln \Pr(X/K)$ was obtained] was inferred according to Pritchard et al (5).

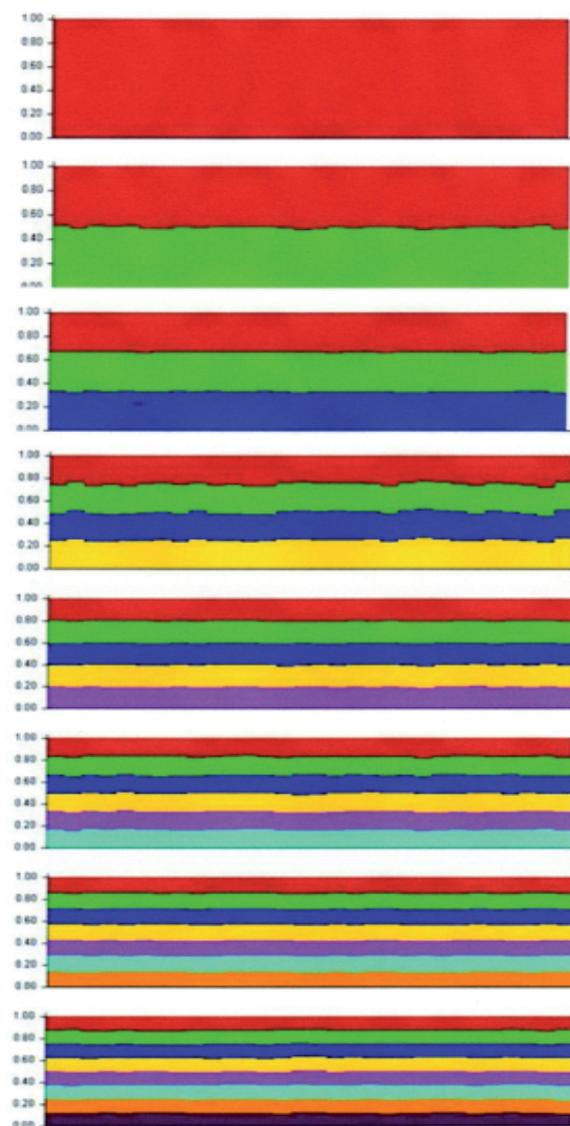


Figure 2. Result obtained from the simulations performed with the "Structure" software. Each simulation was done independently, according to the analysis shown in figure 1.

Table 1. Primers and PCR conditions used for each microsatellite marker.

Marker	MgCl ₂ (50 µM) (µl)	Annealing temperature (°C)	Primers (5'-3')
AHTk211	1	60	ttagcagccgagaaatcgc attgccccgactttgga
FH 2305	0.5	56	tcattgtctcccttccag aagcaggacattcatagcagtg
FH 2326	0.5	60	gaatcccaatgtacatggc cagccatccaggaaatcg
FH 2328	0.75	60	accaggtagtttcagaaatgc agttatgggacttgaggctg
FH 2361	0.5	60	gcttgaagggtgagactgaatg agcacttagaatgtaccaggcac
LEI2D2	1	65	cgagaccacattgggctcc ccagacgctctccatgctc
PEZ 03	0.5	44	cacttctcataccagactc caatatgtcaactatacttc
PEZ 11	0.5	50	attctctgctctcccttg tgtggataatctcttctgtc
PEZ 12	0.75	56	gtagattagatctcaggcag taggtcctgtagggtgtgg

Table 2. Results obtained for each sub-population. Pop1: sub-population 1, pop2: sub-population 2, H_o: observed heterozygosity, H_e: expected heterozygosity.

LOCUS	Pop 1	Pop 2	Nº of shared alleles	Pop 1		Pop 2	
				H _o	H _e	H _o	H _e
AHTk 211	85, 87, 89, 93	85, 89, 93	3	0.647	0.711	0.462	0.618
FH 2328	185, 189, 205	185, 189, 197, 205	3	0.412	0.392	0.308	0.458
FH 2361	343, 347, 351, 355, 359	343, 347, 351, 355, 359, 363	5	0.688	0.796	0.917	0.786
LEI 2D2	383, 401, 405, 409	379, 383, 401, 405, 409	4	0.647	0.583	0.923	0.791
FH 2326	252, 260, 264, 280	256, 260, 264, 268, 272, 276	2	0.529	0.565	0.769	0.720
FH 2305	131, 195, 207, 215, 227, 239	195, 215, 223, 227, 239	4	0.824	0.791	0.692	0.726
PEZ 03	126, 129, 132, 135, 138, 141, 144	126, 129, 132, 135, 138, 141, 144	7	0.765	0.720	0.692	0.788
PEZ 11	130, 134, 138, 142, 146, 158	134, 138, 142	3	0.706	0.761	0.538	0.649
PEZ 12	264, 268, 272, 276	268, 272, 276	3	0.412	0.401	0.308	0.280

DISCUSSION

According to the data obtained with the software "Structure", it is not possible to establish a subdivision between the animals of the sample, as all of them belong to the same population. The analysis of the population presented in this paper show that the method has power to detect how many populations are presented in the samples studied.

The "Structure" software has been successfully used in different species, with the aim of identifying subpopulations of animals of different breeds or areas (8,9).

The analysis of our populations of "Cimarron Uruguayo" dogs, performed with the "Structure" software, gave a value of K=1 (Figures 1 and 2). This would imply that

the analyzed animals belong to the same population. Previous studies performed with RAPDs showed a high homogeneity and genetic identity among the animals of the NE and S areas (10), which would match the results obtained in this work. However, when making this type of studies, microsatellites have some advantages, including their high variability in populations and their codominant inheritance, which have made them more widely used (11).

In dogs, particularly, this software has been used with the aim of identifying subpopulations corresponding to different breeds (9). In our case, the result coincides with the history of the dog breed "Cimarrón Uruguayo", as it would confirm the hypothesis that current dogs derive from the founding nucleus that managed to take refuge in the

NE region of the country (12). In addition, the distribution of the breed among the different areas of Uruguay continues nowadays, so there is no isolation among the different groups of animals, and the exchange is constant. This would not only not allow the formation of separated subpopulations, but also all the animals would continue being part of the same population. However, we intend to continue with these kinds of studies with other methods as population and quantitative genetics.

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