GENETIC DIVERSITY AND POPULATION STRUCTURE OF THE SORRAIA HORSE ASSESSED BY MICROSATELLITE ANALYSIS

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Introduction

Genetic variability studies are one of the first steps in conservation management programs [1] of endangered populations, as they are essential for evaluating genetic health and survival capacity (e.g. 2).There are two major subpopulations of the Sorraia horse breed, one in Portugal and the other in Germany. The latter was established in 1976 with six founders imported from Portugal, followed by some more recent acquisitions of animals born in Portugal [3]. The genetic isolation promoted by the closed management of this breed, combined with the reduced number of founders (only 12, since 1937) and the reduced effective population size, led to high inbreeding values (F=0.38). Therefore, the Sorraia is one Portuguese autochthonous equine breed recognized as extremely endangered and is considered in critical-maintained risk status according to FAO criteria [4,5]. Microsatellites are mainly located in non-coding regions, highly polymorphic, co-dominantly inherited and still an useful tool to describe and analyze livestock genetic variability [e.g. 6,7]. The genetic variability of the Sorraia horse has been analyzed in the past [e.g. 8,9,10] and here we update that information.

Objectives

- Evaluate genetic variability by microsatellite (mSat) analysis (50 autosomal loci - ABGe099, ABGe121, ABGe151, ABGe241, AHT004, AHT005, AHT107, AHT58, ASB002, ASB009, COR003, COR007, COR012, COR058, COR062, COR065, COR073, COR089, COR105, HMS003, HMS006, HMS007, HTG004, HTG006, HTG010, LEX020, LEX023, LEX036, NVHEQ100, NVHEQ43, SGCV24, TKY034, TKY1001, TKY315, TKY321, TKY384, TKY412, TKY448, TKY477, TKY478, TKY523, TKY568, TKY623, TKY741, TKY806, UCDEQ005, UCDEQ405, UM011, UMNE158 and VHL020; 5 X-linked loci - LEX003, LEX024, LEX027, UCDEQ502 and TKY038);
- Compare genetic parameters on the whole (TOTAL), Portuguese (PT) and German (GER) populations and look for signs of population structure.

Materials and Methods

DNA was extracted (N=190, 13 different breeders) from whole blood and hair samples following standard protocols. Microsatellite genotyping was carried out in a Li-Cor 4200S (Li-Cor, Lincoln, NE) or an ABI 3730 sequencer. Alleles were scored according to PCR product size with RFLPscan 3.1 software (Scanalytics CPS Inc., Rockville, MD) and GeneMapper® v4.1 (Applied Biosystems). The mean number of alleles (MNA), observed heterozygosity (H_O), unbiased expected heterozygosity (H_E), polymorphic information content (PIC), probability of paternity exclusion (PE), individual heterozygosity, mean d^2 distance, heterozygote deficiency coefficient (F_{IS}) and F_{ST} coefficient were calculated using MS Office Excel, CERVUS [11] and Genepop [12]. Population structure was assessed using STRUCTURE [13] and by FCA analysis in GENETIX [14]. The total population was considered as well as the two subpopulations separately (Portugal and Germany).

Results and Discussion

X-linked mSats

- MNA was 2.8 in the TOTAL, 2.6 in PT and 2.8 in GER. H_O was similar in PT (0.333) and in GER (0.325), although H_E was higher in GER (0.484 *vs* 0.382) (Table 1). F_{IS} was almost half in PT and F_{ST} was 0.1152.

Autosomal mSats

- MNA was 3.7 in the TOTAL, 3.7 in PT and 3.5 in GER (Table 1). This set of mSats allows a high probability of paternity exclusion (PE=1) being appropriate for parentage testing. H_O and H_E were slightly higher in GER (0.580 and 0.572, respectively). F_{IS} was lowest in GER and F_{ST} between subpopulations was 0.0672.

- Average inbreeding, d2 and individual heterozygosity (Figure 1) in the total population were 0.3816, 60.6449 (in bp^2) and 0.5708, respectively. Inbreeding was higher in PT. GER had better values of mean d2 and individual heterozygosity. These values are direct results of the different breeding strategies: in PT, one stallion is chosen per herd, per year; in GER, one stallion is chosen per mare, per year, resulting in a higher number of stallions used yearly and increasing genetic variation and decreasing inbreeding.

Table 1 – Summary statistics for the 50 autosomal mSats (N=190) and 5 X-linked microsatellites (N=98): mean number of alleles (NA), observed heterozygosity (H_O), unbiased expected heterozygosity (H_E), polymorphic information content (PIC), probability of paternity exclusion (PE), heterozygote deficiency coefficient (F_{IS}).

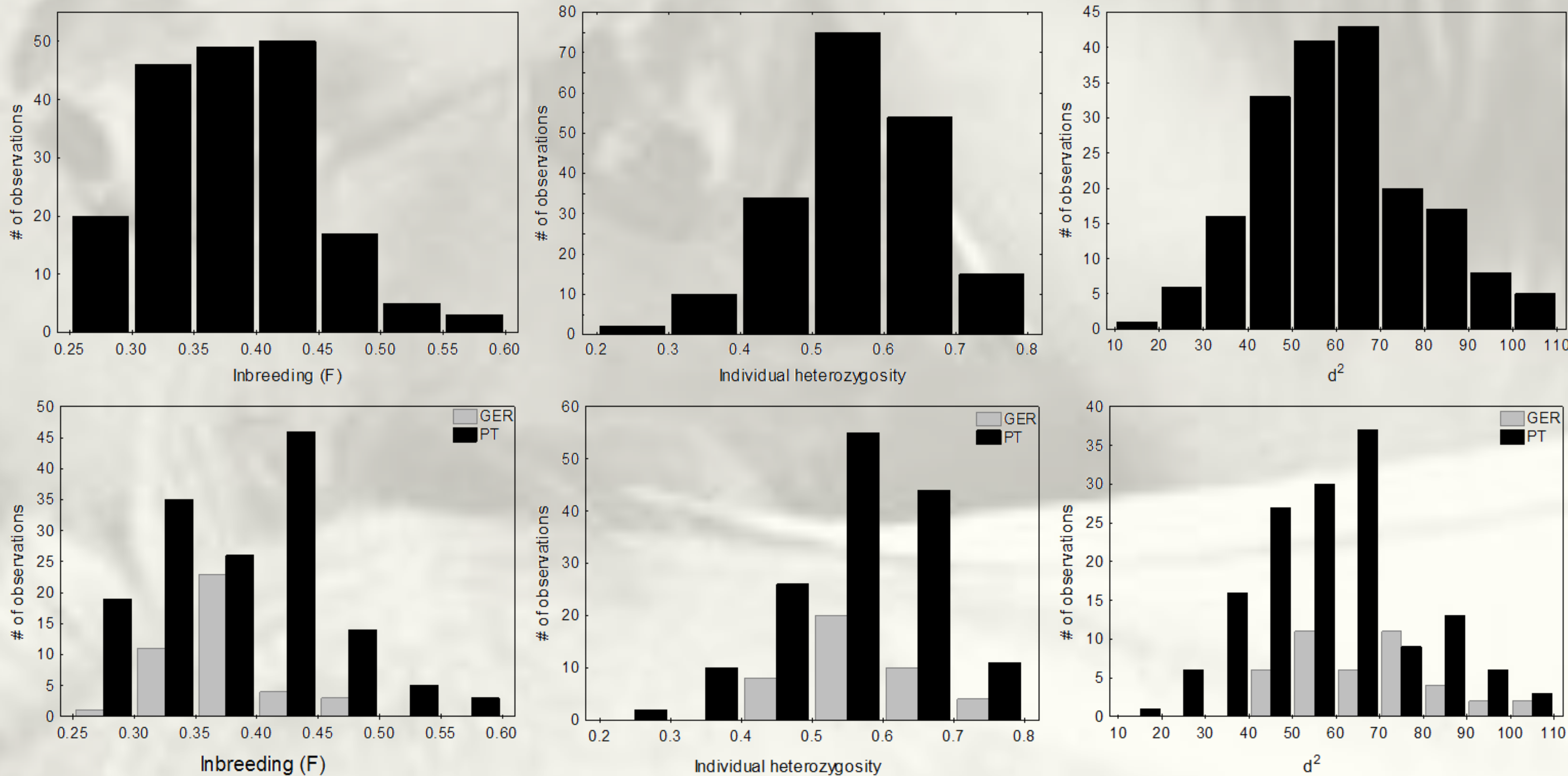|	TOTAL				PORTUGAL				GERMANY			
	NA	H_O	H_E	PIC	PE	NA	H_O	H_E	F_{IS}	NA	H_O	H_E	F_{IS}
X-linked	2.8	0.332	0.423	0.374	0.88	2.6	0.333	0.382	0.132	2.8	0.325	0.484	0.386
Autosomal	3.7	0.573	0.586	0.526	1	3.7	0.570	0.571	0.003	3.5	0.580	0.572	-0.009

Figure 1 - Distribution of inbreeding, individual heterozygosities and d^2 in the whole population (top row) and by subpopulation (bottom row). Heterozygosities and d^2 calculated with up to 50 autosomal mSats (N=190)

Figure 3 - FCA on autosomal mSats (Portugal - blue; Germany - yellow)(N=190)

Autosomal mSats (cont.)

- STRUCTURE analysis separated our samples in three clusters (Figure 2): cluster **1** (breeder 1, 2, 3, 5, 7, 8 and 11), contains animals from the Andrade family, and studs based on Andrade's animals (2 Portuguese and the most recent German breeder); cluster **2** (4, 6 and 12), all from PT breeders and related to the National Stud Farm; cluster **3** (9, 10 and 13) contains animals from the oldest German breeders and a Portuguese breeder. This analysis clearly demonstrates the historic evolution of this breed and connections between breeders.

- FCA separated analysed animals into two main groups: PT and GER (Figure 3). PC1 clearly separates the Portuguese and "old" German breeders, whereas animals from the recent German breeder are still non-distinguishable from the Portuguese ones due to the newly imported animals. PC2 separates de Portuguese breeders but because of recent efforts by the Breeders Association to promote stallion exchange between stud farms, they do not completely separate from each other, explaining why we could not see 3 clusters as proposed by Structure analysis. This is also in agreement with the low F_{ST} values found between PT and GER.
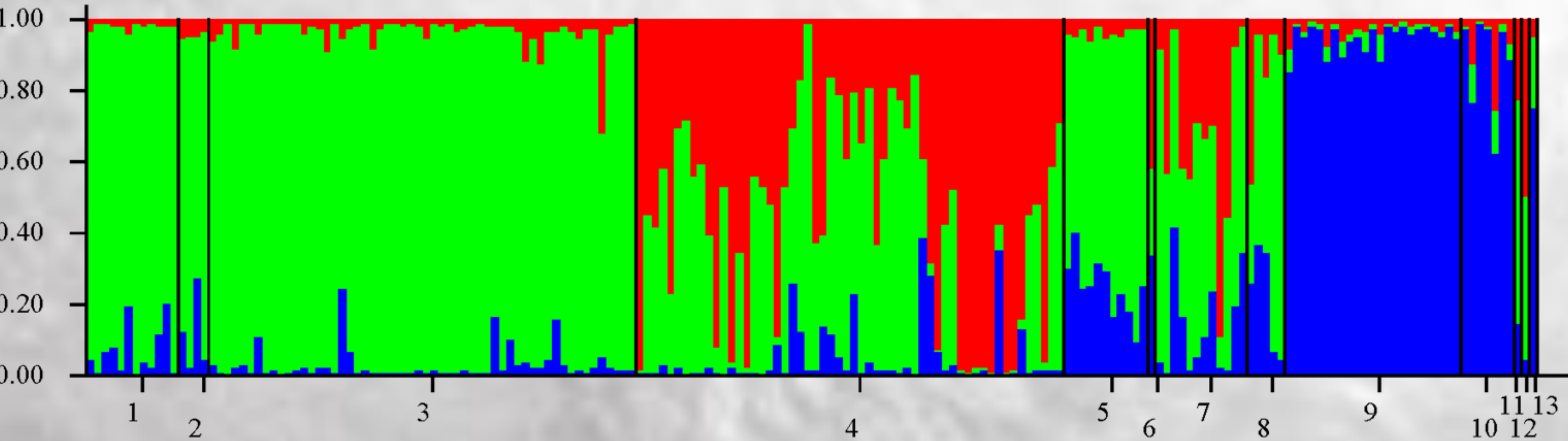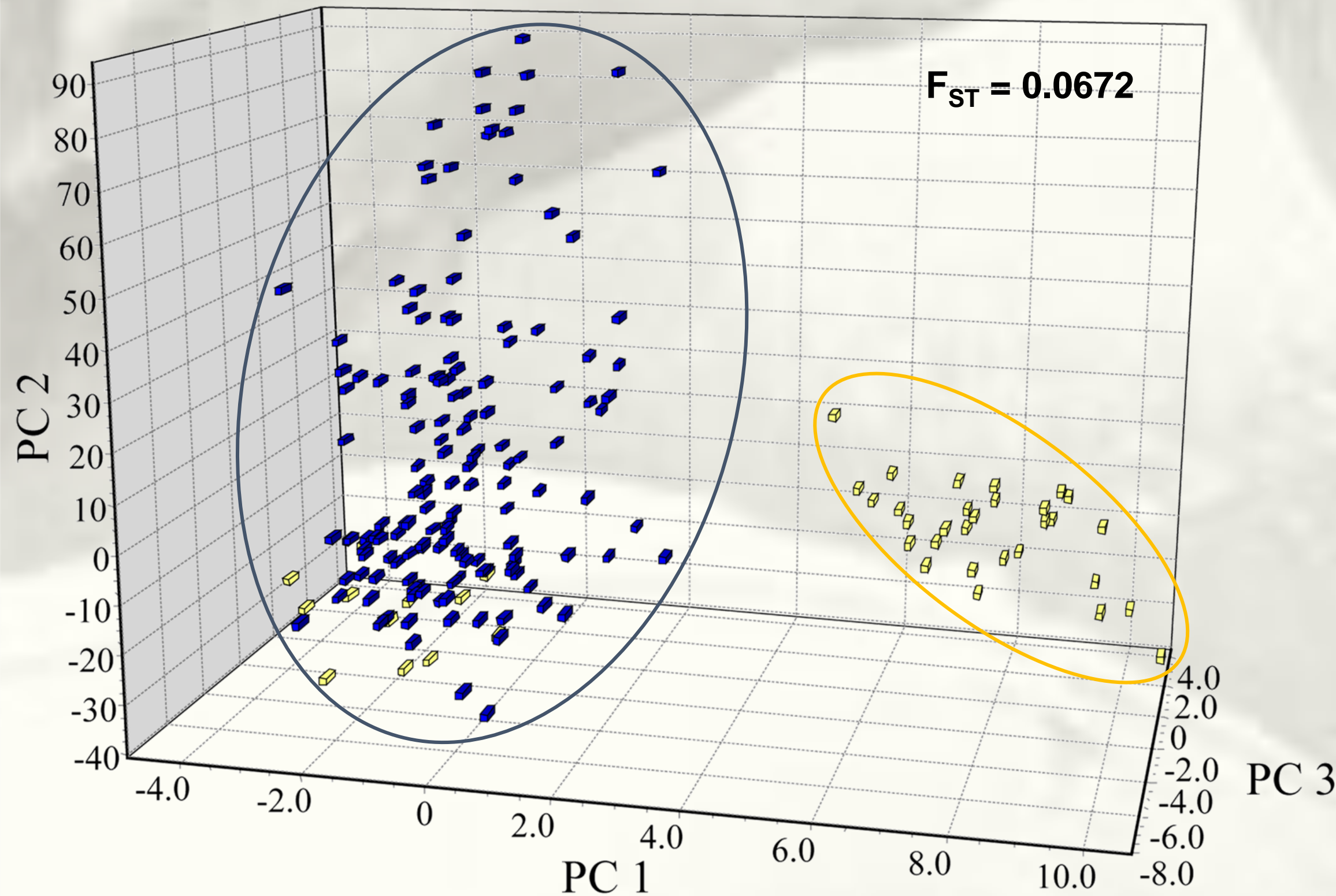

Figure 2 - STRUCTURE assignment of the 13 different breeders into K=3 clusters (N=190, 50 autosomal mSats).

Conclusion

Despite a management breeding plan has been implemented in the last decade in order to retain the existing genetic variability, namely by promoting the population subdivision, stallion routation and maximum avoidance of inbreeding, inbreeding is still very high and genetic variability as measured by different parameters is low. Our results show that the Portuguese and "older" German populations now form two separate clusters with some degree of genetic differentiation between them. It will now be important to promote exchange between PT and GER in future management plans in order to counteract genetic drift effects, further minimize inbreeding and increase genetic variability. This will hopefully improve the breeds' genetic health and prevent the permanent loss of this iconic and important animal genetic resource.

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