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The Sorraia Horse (Portugal): genetic variation and genetic management of an endangered breed

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The Sorraia Horse is a Portuguese native horse breed, believed to be the ancestor of the southern Iberian saddle horse and, consequently, one of the primitive ancestors of the World's light saddle horse breeds. Recovered since 1937 from only 12 founders (5 ; 7), with no further immigration the breed is now represented by less than 160 animals, distributed in two main subpopulations (Portuguese and German). The small number of founders, the reduced effective number and the complete genetic isolation of the breed has led to extremely high levels of inbreeding ($F > 0.40$ for most living animals). Two of the founders are no more represented at the living population and genetic contribution of some under-represented founders are at great risk of loss. Therefore the Sorraia Horse is considered by FAO at a critical risk status and requires a conservation breeding plan in order to establish a long (or even short) term self-sustaining population.

The analysis of genetic markers is a contribution to the evaluation of genetic variation of the breed. We analysed the genetic variability of the Portuguese and German herds using 13 horse microsatellite *loci* (AHT4, AHT5, ASB2, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10, VHL20 and LEX23).

For the analysed *loci* the Sorraia horse showed low levels of variability and with a small number of alleles ranging from 1 to 6.

Recent methods for pedigree analysis can be of great help to guide genetic management of captive populations. We used the GENES software to perform the genetic analysis of the Sorraia Horse studbook data: historical analysis of gene diversity, founder genome equivalents and mean inbreeding coefficient; founder representation analysis, gene drop analysis, inbreeding and kinship calculations for the living population, identifying genetically important individuals for further breeding plans.

THE SORRAIA HORSE (PORTUGAL): GENETIC VARIATION AND GENETIC MANAGEMENT OF AN ENDANGERED BREED

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INTRODUCTION

Most of the breeding programmes used to achieve genetic and demographic management of small populations includes maximal avoidance of inbreeding, minimization of genetic drift and selection through equalization of founder representation and family sizes. The principal objective is to establish long-term self-sustaining populations capable of maintaining high levels of genetic variation.

Genetic diversity is important both for maintaining the evolutionary adaptive potential of the breed and for maintaining the health of the population.

The analysis of genetic markers such as microsatellites may represent a great contribution to the evaluation of genetic variation of a breed.

Pedigree analysis can be used to calculate inbreeding coefficients for existing animals and for the potential offspring of planned matings to identify founders that are under-represented in the current population and produce target representations for future breeding efforts.

Thus, identifying genetically important individuals and how many offspring should be produced per breeder through generations are priorities of any breeding plan for management of genetic variation in pedigree populations.

The Sorraia Horse is a Portuguese native horse breed, believed to be the ancestor of the southern Iberian Peninsula horse, and, consequently, one of the primitive ancestors of the World's light saddle horse breeds. Recovered since 1937 by Dr. Ruy d'Andrade, from only 12 founders (5 and 7), with no further immigration, the breed is now represented by less than 150 animals (101 from Portugal and 47 from Germany). In 1975 the population was subdivided: 5 animals were exported to Germany and the others remained in Portugal. Since then no genetic flux was established between the subpopulations of both countries.

The small number of founders, the reduced effective size (Ne) and the complete genetic isolation of the breed has led to extremely high levels of inbreeding.

Therefore the Sorraia Horse is considered by FAO at a critical risk status and requires a conservative breeding plan.

METHODOLOGY

The AICS*, with the collaboration of the Portuguese National Stud Service and the Sorraia breeders registries, has recovered the pedigree information back up to the founders (N = 496). With this information a dBase file was constructed and analysed using GENES (version 12), a software package for genetic analysis of studbook data developed by R. Lacy (Copyright 1995-1999, Chicago Zoological Society).

Genetic metrics as defined by Lacy (1989,1995), Lacy *et al.*, 1995 and Ballou *et al.*, 1995 were calculated by the "kinship additive matrix" (Ballou, 1983) and "gene drop simulation" (10 000 iterations) (MacCluer *et al.*, 1986).

We analysed the genetic variability of the Portuguese (N = 49) and German (N = 28) subpopulations using 13 horse microsatellite loci (AHT4, AHT5, ASB2, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10, VHL20 and LEX23)

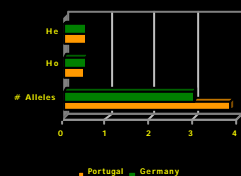
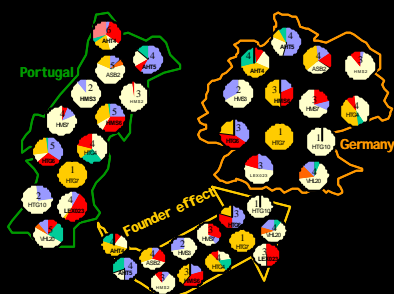
RESULTS

Genetic summary of descent population

Number of founders	10
Number of living descendants	148
Descendant population Mean Kinship	0.3621
Mean Ne over past 7.58 generations	9.774
Current Ne estimated from 12 male and 42 female breeders	37.333
Ne / N ratio	0.252

	LIVING POPULATION	POTENTIAL
Number of Founders	10	10
Mean retention	0.2.60	0.2.60
Founder genomes surviving	2.600	2.600
Founder Genome Equivalents (FGE)	1.407	2.600
Fraction source gene diversity retained	0.645	0.808
Fraction wild source gene diversity lost	0.355	0.192
Mean inbreeding coefficient	0.367	0.192

MICROSATELLITES ANALYSIS



ORDERED LISTS OF MEAN KINSHIP (MK) BY SEX:

Rank	MALES	FK	FEMALES	FK
1	ESPANHO	0.323	BAIO	0.323
2	ESPANHO	0.323	VICINHA	0.323
3	ESPANHO	0.323	YELA	0.323
4	ESPANHO	0.323	YELA	0.323
5	ESPANHO	0.323	YELA	0.323
6	ESPANHO	0.323	YELA	0.323
7	ESPANHO	0.323	YELA	0.323
8	ESPANHO	0.323	YELA	0.323
9	ESPANHO	0.323	YELA	0.323
10	ESPANHO	0.323	YELA	0.323
11	ESPANHO	0.323	YELA	0.323
12	ESPANHO	0.323	YELA	0.323
13	ESPANHO	0.323	YELA	0.323
14	ESPANHO	0.323	YELA	0.323
15	ESPANHO	0.323	YELA	0.323
16	ESPANHO	0.323	YELA	0.323
17	ESPANHO	0.323	YELA	0.323
18	ESPANHO	0.323	YELA	0.323
19	ESPANHO	0.323	YELA	0.323
20	ESPANHO	0.323	YELA	0.323
21	ESPANHO	0.323	YELA	0.323
22	ESPANHO	0.323	YELA	0.323
23	ESPANHO	0.323	YELA	0.323
24	ESPANHO	0.323	YELA	0.323
25	ESPANHO	0.323	YELA	0.323
26	ESPANHO	0.323	YELA	0.323
27	ESPANHO	0.323	YELA	0.323
28	ESPANHO	0.323	YELA	0.323
29	ESPANHO	0.323	YELA	0.323
30	ESPANHO	0.323	YELA	0.323
31	ESPANHO	0.323	YELA	0.323
32	ESPANHO	0.323	YELA	0.323
33	ESPANHO	0.323	YELA	0.323

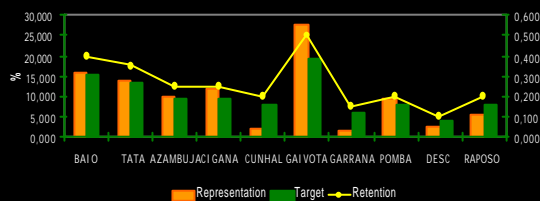
15 PAIRS SELECTED Resulting: GeneD=64.741 FGE=1.418

Rank	MALES	FK	FEMALES	FK
1	ESPANHO	0.323	BAIO	0.323
2	ESPANHO	0.323	YELA	0.323
3	ESPANHO	0.323	YELA	0.323
4	ESPANHO	0.323	YELA	0.323
5	ESPANHO	0.323	YELA	0.323
6	ESPANHO	0.323	YELA	0.323
7	ESPANHO	0.323	YELA	0.323
8	ESPANHO	0.323	YELA	0.323
9	ESPANHO	0.323	YELA	0.323
10	ESPANHO	0.323	YELA	0.323
11	ESPANHO	0.323	YELA	0.323
12	ESPANHO	0.323	YELA	0.323
13	ESPANHO	0.323	YELA	0.323
14	ESPANHO	0.323	YELA	0.323
15	ESPANHO	0.323	YELA	0.323

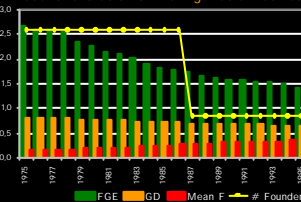
Number and proportion of matings selected for each animal:

Rank	MALES	FK	FEMALES	FK
1	ESPANHO	0.323	BAIO	0.323
2	ESPANHO	0.323	YELA	0.323
3	ESPANHO	0.323	YELA	0.323
4	ESPANHO	0.323	YELA	0.323
5	ESPANHO	0.323	YELA	0.323

FOUNDER ALLELE REPRESENTATION



Recent evolution of main genetic metrics



DISCUSSION & CONCLUSIONS

The usual problems of small populations and the reproduction strategy used on this breed led to an evident unequal representation of founders on the living population, extremely low value for FGE (both actual and potential) and high values of inbreeding coefficients. Two of the founders are no longer represented at the living population and some under-represented founders are at great risk of loss.

The potential FGE (Founder Genome Equivalents) of 2.6 as compared with the actual 1.4, the increasing Mean F (Mean Inbreeding Coefficient) and the decreasing GD (Gene Diversity) reflects the urgency of an improvement of the genetic management of the breed.

The subpopulation GERMANY, although with only 5 founders, retains better values for the genetic metrics analysed. The 15 most genetically important animals, males and females, are from this subpopulation. Thus, breeding pairs recommended to produce offspring (15) on the overall population do not include animals from PORTUGAL.

The age of some breeders selected (> 20 years) may suggest the importance of artificial reproduction techniques in such conservation plans.

The subpopulation GERMANY presents few alleles per locus which is explained by the founder effect, although no alleles were lost by genetic drift since its foundation in 1975. Nevertheless, the levels of Heterozygosity (He and Ho) are higher than in the subpopulation Portugal, due to the mating strategy adopted by German breeders, using more than one stallion in each breeding year.

The subdivision of small and closed populations followed by further immigration between subpopulations can be pointed as a tool for genetic conservation.

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