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Original Paper

Genetic diversity in five Czech native horse breeds assessed using microsatellite markers

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The aim of the present study was to analyse the genetic diversity of the endangered horse breeds kept in the Czech Republic. A set of 13 microsatellites was used for genotyping 349 Silesian Norikers, 397 Norikers, 552 Czech-Moravian Belgian horses, 271 Old Kladrubers (175 greys, 95 blacks) and 241 Hucul horses. The proportion of obtained heterozygosity indicates no major loss of genetic diversity within analyzed breeds. The Wright's FST and genetic distances indicated genetic segregation of both colour varieties of the Old Kladruber breed and small genetic distances between draft horse breeds. Moreover, the membership probability outputs showed that the frequencies of alleles varied across the three main regions. First region is represented by draft horse breeds, second region is represented by Old Kladruber horse and the last is represented by Hucul breed. The study provides data and information utilizable in the management of conservation programs in order to reduce inbreeding and to minimize loss of genetic variability.

Keywords: admixture, endangered breeds, horse, loss of genetic diversity

1 Introduction

Genetic diversity studies in domestic animals focus on evaluating genetic variation within and across breeds mainly for conservation purposes. The evaluation of genetic diversity between livestock breeds is an important prerequisite for developing effective and meaningful breed conservation programs. An effective management of farm animal resources requires comprehensive knowledge of the breeds' characteristics including data on population size and structure and within and between breed genetic diversity. In animal breeding, the knowledge of genetic characterization and genetic structure is the first step in breed conservation and may have implications for future breeding strategies and management plans. The analysis of the genetic structure of a population can be carried out using genealogical or molecular information. In the case of missing or incomplete pedigree, it would be better to use molecular information to characterize a population; moreover, the molecular information indicates the additional relatedness between animals appearing as founders in the pedigree (Delgado et al. 2014).The aim of this study was to analysis of genetic diversity within and between five endangered Czech horse breeds.

2 Materials and methods

Data

In the present study 1809 individuals from five endangered Czech horse breeds were used: 349 Silesian Norikers (SN), 397 Norikers (N), 552 Czech-Moravian Belgians (CMB), 270 Old Kladruber horses (175 greys – OKg, 95 blacks OKb) and 240 Huculs (H). Data were provided by the Association of Horse Breeder Unions as an umbrella organization of individual breeders. The oldest individual was born in 1978, whereas the youngest animals were born in 2016. The total set of 13 microsatellite markers (AHT4, AHT5, ASB2, HMS1, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10, and VHL20) recommended for parentage testing by the International Society for Animal Genetics (ISAG) and Equine Genetics Standing Committee was used for the analysis.

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Genetic diversity

Genetic variability within populations was characterized as allele frequency, mean number of alleles, observed heterozygosity (H_O) and genetic diversity, which is often called expected heterozygosity (H_e) (Weir 1996). Testing of the Hardy-Weinberg equilibrium and the Wright's fixation indices: F_{IS} – reduction in heterozygosity of an individual due to non-random mating within its subpopulation, F_{ST} – reduction in heterozygosity of subpopulation due to random genetic drift (fixation index), and F_{IT} – reduction in heterozygosity of an individual due to non-random mating and population subdivision relative to total population (overall inbreeding coefficient) were evaluated using the GenAlEx program (Peakal and Smouse, 2012). The analysis of molecular variance was done using the PEGAS package (Paradis, 2010). Genetic differences among individuals and between populations were evaluated by Nei's distances (D_A) (Nei et al., 1983), which assume differences caused by mutations and genetic drift. These indices provide more reliable results specifically for microsatellite data.

Population structure and genetic relationship

Subsequently, to determine genetic structure and to infer genetic admixtures, a discriminant analysis of principal components (DAPC) implemented in the Adegenet R package (Jombart and Ahmed 2011) was used for microsatellite data. The DAPC approach proposes an optimum distribution of individuals into predefined groups in relation to the discriminant function of principal components. An optimum number of clusters was defined by the *K*-averaging algorithm that makes use of the Bayesian information criterion. In addition, the DAPC was used to assign individuals and to obtain the membership probability which presents the overall genetic background of an individual. A trade-off between the power of discriminant analysis and overfitting of the given analysis was assessed by the α-score (Jombart and Ahmed 2011).

3 Results and discussion

Genetic diversity

Each of the analyzed loci appeared as polymorphic and their alleles were present in or shared by all studied populations. The total number of alleles, average number of alleles on 13 microsatellite markers across all breeds and overall information about differences and total statistics are shown in Table 1. Statistically significant deviations from the Hardy-Weinberg equilibrium were found in all microsatellite loci. The parameters of observe heterozygosity (H_o), genetic diversity (H_e), allelic richness (N_A), effective number of alleles (N_e) and Wright's F_{IS} are present in Tables 2. The higher values of N_A and N_e were estimated for *H* breed. H_O and the F_{IS} index, indicate a sufficient proportion of heterozygosity across all breeds except *H* and *OKb*.

Genetic structure

Genetic differences between populations were tested by the pairwise F_{ST} coefficients and Nei's genetic distances (D_A) (Table 3). The largest distance was determined between *OKb* and *H* breeds (F_{ST} = 0.064) and the smallest between *SN* and *N* (F_{ST} = 0.004). To infer the population

Table 1 Characteristics of 13 microsatellite loci analyzed in five horse populations

	N	N_A	N_e	H_o	H_e	F_{IS}	F_{IT}	F_{ST}
VHL20	1,808	13	5.232	0.827	0.803	-0.030	0.042	0.069
HTG4	1,806	10	3.553	0.709	0.711	0.002	0.092	0.090
AHT4	1,808	20	5.147	0.802	0.803	0.000	0.044	0.044
HMS7	1,789	12	3.469	0.637	0.671	0.051	0.157	0.112
HTG6	1,804	12	1.946	0.483	0.474	-0.018	0.011	0.029
AHT5	1,797	14	4.233	0.776	0.761	-0.020	0.036	0.055
HMS6	1,807	11	3.076	0.699	0.673	-0.039	0.008	0.045
ASB2	1,801	19	4.361	0.771	0.758	-0.017	0.053	0.069
HTG10	1,772	14	3.522	0.669	0.701	0.045	0.168	0.128
HTG7	1,790	11	2.856	0.617	0.621	0.006	0.104	0.099
HMS3	1,741	16	3.485	0.694	0.694	0.000	0.046	0.047
HMS2	1,800	15	3.706	0.734	0.719	-0.021	0.057	0.077
HMS1	1,801	8	2.414	0.554	0.569	0.026	0.100	0.076

N – number of successfully genotyped individuals, N_A – number of alleles, N_e – effective number of alleles, H_o – observed heterozygosity, H_e – genetic diversity and F_{IT}, *n* = 1,806

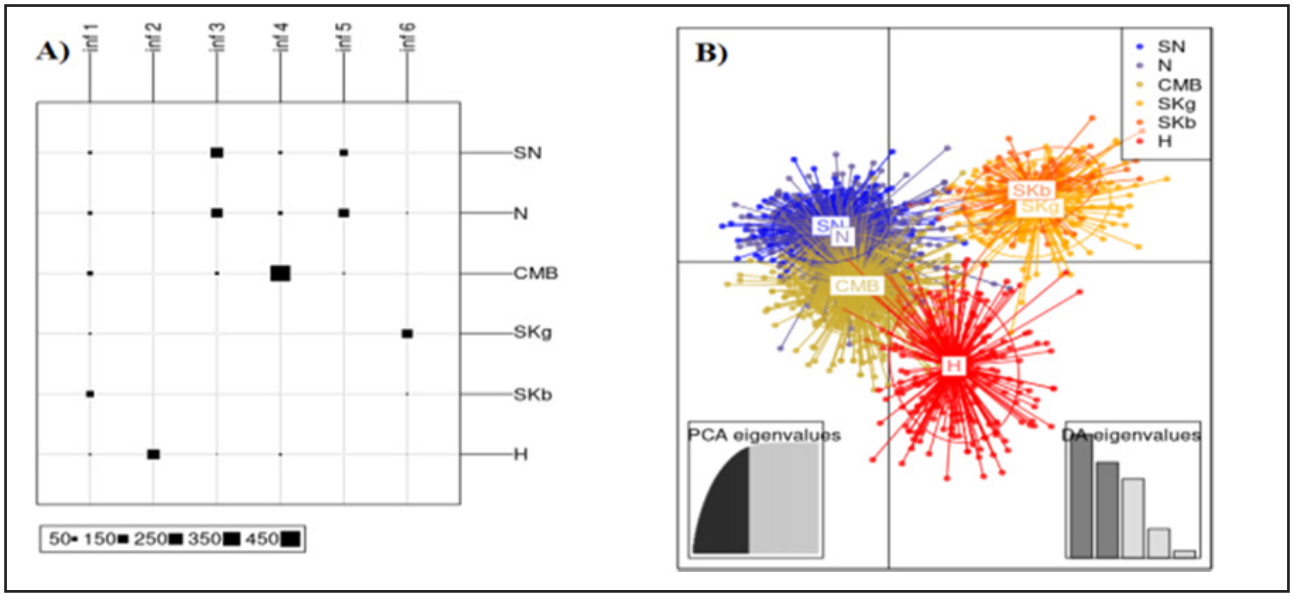

Figure 1 The Bayesian information criterion (BIC) statistic results referring to differentiation between inferred and original clusters (A) and the genetic clusters determined using discriminant analysis of principal components (B)

genetic structure, DAPC was applied to genotyping data. The distribution of individuals according to the Bayesian information criterion (BIC) analysis showed that inferred clusters do not correspond to actual groups (Figure 1a). Based on the α-score (Jombart and Collins 2015), 32 PCA axes were left in DAPC. The four discriminate functions obtained correspond to 93% of variance. The first discriminate function clearly detected two major genetic clusters corresponding to draft horses and both colour variants of Old Kladruber horse. Using the first and the second discriminate function, a very close relationship was determined within two groups of breeds – draft horses and colour variety of Old Kladruber horse. Hucul breed is clearly separated from other analyzed breeds (Figure 1b), as expected.

Genetic variability, determined by means of microsatellite markers in endangered Czech horse breeds was analysed

Table 2 Genetic diversity across five horse populations

	MNA	N_e	H_o	H_e	F_{IS} (CI 95%)
SN	7.308 ±0.485	3.387 ±0.266	0.691 ±0.028	0.680 ±0.028	-0.017 (-0.041–0.007)
N	7.154 ±0.421	3.702 ±0.253	0.718 ±0.021	0.714 ±0.020	-0.006 (-0.024–0.012)
CMB	7.615 ±0.561	3.537 ±0.326	0.680 ±0.038	0.678 ±0.038	-0.004 (-0.026–0.018)
OKg	7.000 ±0.320	3.477 ±0.367	0.674 ±0.042	0.669 ±0.038	-0.004 (-0.024–0.016)
OKb	5.615 ±0.368	3.285 ±0.300	0.652 ±0.044	0.654 ±0.040	0.005 (-0.040–0.050)
H	11.846 ±0.823	4.303 ±0.410	0.726 ±0.027	0.739 ±0.026	0.016 (-0.009–0.041)

Mean number of alleles (MNA), effective number of alleles (N_e), observed heterozygosity (H_O), genetic diversity (H_e) and Wright's F_{IS} index with confidence intervals (95%)

Table 3 Wright's F_{ST} (above the diagonal) and Nei's minimum genetic distance (below the diagonal) per pair of breeds

	SN	*N*	*CMB*	*OKg*	*OKb*	*H*
SN		0.004	0.036	0.053	0.061	0.044
N	0.018		0.031	0.049	0.055	0.037
CMB	0.167	0.150		0.047	0.058	0.044
OKg	0.265	0.252	0.234		0.043	0.049
OKb	0.311	0.294	0.299	0.191		0.064
H	0.257	0.230	0.244	0.271	0.361	

in this study. All studied microsatellite markers showed high variability, and all markers were deviated from the Hardy-Weinberg equilibrium. Generally, the genetic diversity of microsatellite loci can be affected by many factors, including genetic drift, impact of selective breeding, effect of individual stallions and random effects. Estimated parameters of genetic diversity exhibited non-significant differences between draft horse breeds and between two colour variants of Old Kladruber horse, which fully corresponds to their breeding history.

Quite surprising is a fact that genetic differences estimated between draft horse breeds was smaller than between two colour variants of Old Kladruber horse. Genetic diversities within the studied breeds reached similar values to those obtained in the Old Kladruber horse by Kasarda et al. (2016). The determined values of genetic diversity were lower than e.g., those in other European endangered population – Polish Konik (Szwaczkowski et al. 2016). Observed heterozygosity and the coefficient of inbreeding measured by Wright's FIS index, indicate a sufficient level of heterozygosity in the studied populations except H and OKb breeds. The moderate F_{ST} value and the value in a genetic distance matrix (D_A) show a good genetic distance between the group of draft horses, both colour variants of Old Kladruber horse and Hucul breed. The H as original mountain horse breed with small body frame was never used in regeneration process of draft horses or in OKH breed.

4 Conclusions

This study gives an insight into the genetic structure and diversity of endangered breeds kept in the Czech Republic. The obtained results suggest a low level of differentiation as well as a high gene flow between draft horse breeds and colour variant of the OKH. The H breed is clearly separated from others. The low F_{ST} values between SN and N breeds can be explained by the commonly used crossing between these breeds. The results of this study should be applied to the conservation of gene resources of horses in the Czech Republic.

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