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GENETIC DIVERSITY IN CZECH HAFLINGER HORSES

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SUMMARY

The Haflinger as a small moutain horse breed originated from the South Tyrol district as a cross of Alpen Mountain breeds with Araber. This breed was expanding to Czech Republic during the last 25 years. The aim of this study was to analyse genetic diversity within the population using microsatellite markers. A total of 95 alleles have been detected. The highest frequency 88.18% showed allele 101 (HTG 6). The heterosigosity varied from 0.25 (HTG 6) to 0.84 (VHL 20), genetic diversity reached 0.6–0.8. The heterozygosity of the whole population studied is F_{IS}= -0.013. The average effective number of allele per locus was 2.93 with standard deviation 1.54, with minimal and maximal level 1.30 and 7.83, respectively. Average polymorphism information content per locus was 0.608 with standard derivation 0.146, with minimal and maximal level 0.208 and 0.824, respectively. The results showed that breeding program of Czech Haflinger is optimal, including optimized mating strategies. The diversity of the population Czech Haflinger, based on a small number of microsatellites, seems to be sufficient.

Key-words: microsatellite data, homozygosity by loci, genetic diversity

INTRODUCTION

The inbreeding coefficient is defined as the probability that, in a locus sampled randomly in a population, a pair of alleles is identical by descent with respect to a base population where all alleles are independent (Wright, 1922). The consequences of inbreeding are the loss of genetic variation, accumulation of recessive lethal genetic mutations and worsening of performance in production traits and fertility. Therefore, evaluating genetic diversity and relationship within and amongst populations of animals is a prerequisite for developing meaningful breeding programmes.

Inbreeding coefficients usually have been calculated from a pedigree, and the probability that a pair of alleles is identical by descent is estimated from statistical expectations. However, the recent availability of methods of molecular genetics has opened opportunities for using genomic information in animal breeding.

The development of tools for the analysis of DNA taking place in the last few decades has increased enormously the capacity to characterise variation within breeds. The microsatellites have been markers of choice to study genetic variation in the recent years. Based upon sites in which the same short sequences is repeated multiple times, they present a high mutation rate and codominant nature, making them appropriate for the study of both within- and between –breed genetic diversity.

The Haflinger horse is a breed of horse developed in the South Tyrol region during the late 19th century. This breed is a product of Alpen Mountain breeds with Araber cross. Until recently, the molecular genetic diversity has not been studied and no information on this diversity has been available for Czech Haflinger horse. The objective of this study is to determine the genetic diversity in the Czech Haflinger horse based on microsatellite markers.

MATERIAL AND METHODS

Blood samples were collected randomly from 369 horses in Czech Haflinger population within a 12-year period (2000-2012). Genomic DNA was isolated from the whole blood using the NucleoSpin Blood Kit (Clontech Laboratories, Palo Alto, CA, USA). Genotyping included 13 microsatellite loci (VHL20, HTG4, AHT4, HMS7, HTG6, AHT5, HMS6, ASB2, HTG10, HTG7, HMS3, HMS2 and HMS1) scattered at 8 chromosomes.

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Allele frequencies, observed heterozygosity, genetic diversity [heterozygosity expected assuming Hardy-Weinberg equilibrium (HWE)], an HWE test and genetic distances were estimated across the different loci and lines using the TFPGA 1.3 software package (Miller, 1997).

Heterozygosity in the whole studied population was evaluated by within-population inbreeding estimate also known as fixation index (F_{IS}) at each microsatellite locus. F_{IS} were computed by the FSTAT program (Gaudet, 2001) according to the following formula:

$$F_{IS} = 1 - \frac{H_O}{He}$$

where: H_0 – observed heterozygosity, H_e - expected heterozygosity. Effective number of allele were calculated by the following formula:

$$effective\ number of\ \ alllele = \frac{1}{1 - D_j}$$

where: D_j – genetic diversity of locus *j*. The polymorphic information content was calculated using PICcalc (Nagy, 2012).

RESULTS AND DISCUSSION

The total number of alleles detected at 13 microsatellite loci in the Czech Haflinger population was 86. Microsatellites were highly polymorphic. The average number of alleles per microsatellite locus was 8.25 (±2.6) with a range of 4 to 13. Higher number of alleles for each locus suggested that all the markers used were appropriate to analyse genetic diversity. A more appropriate measure of genetic variation within a population was gene diversity (Nei, 1987). An estimated average for the observed heterozygosity across microsatellite loci was relatively high 0.656, while the estimated mean value of genetic diversity was 0.663. The heterozygosity observed for each of the microsatellites ranged from 0.217 for the HTG6 microsatellite to 0.844 for the VHT20 microsatellite. As with heterozygosity, the lowest value of genetic diversity was found out in the HTG6 microsatellite (0.228) and the highest value of genetic diversity was achieved via the VHL20 microsatellite (0.872). This corresponds to the value of the effective size of the alleles. Big difference was noticed between the total number of alleles and effective number allele by all loci. This difference indicates again the increase of homozygosity in population. These values are relatively high and therefore the population appeared sufficiently heterogeneous. Tekezaki and Nei (1996) determined that for markers to be useful measuring genetic variation, they should have an average heterozygosity ranking form 0.3 to 0.8 in the populations. General information on differences and aggregate statistics is shown in Table 1. High level of polymorphism is also the average value of polymorphic information content (PIC=0.61). PIC is calculated with the total number of alleles and allele frequencies in a population. If PIC value is higher than value 0.75 the locus becomes much more informative. This level is not exceeded at most loci. Statistically conclusive deviation from HWE was detected only at VHT20 ($P<0.01$) loci. Other loci were in agreement with HWE. Similar values of the observed heterozygosity and genetic diversity were also found out in Spanish Celtic horses (Canon et al., 2000), Lipizzaner horses (Achmann et al., 2004), German draft horses (Aberle et al., 2004) and Biłgorai horses (Zabek et al., 2005). Conversely, Iwanczyk et al. (2006) reported that the values of heterozygosity and genetic diversity of Polish heavy horses were considerably lower.

The heterozygosity of the whole population studied is F_{IS} =-0.013±0.031 whereas the average value of F_{IS} reached a negative number. According to Hamilton (2009) it can be concluded that there was no reduction of heterozygosity. However, this value is close to zero. This detected nonsignificant values indicating genetic variability increase in the population. The increased value of heterozygosity corresponds to reality that Czech Haflinger is open population for gene flow from other Haflinger populations. However, the utility of molecular marker information is rather limited, especially if high-quality pedigrees are available (Toro et al., 2009). The value of estimated populations parameters, which are considered as measure of inbreeding based on marker data, are very different from inbreeding coefficient estimated by Majzlík et al. (2012) based on pedigree information (Fx=0.84% with variation from 0 to 4.69%). The estimated inbreeding coefficient based on pedigree analysis in the 2012 does not correspond with molecular data. The molecular inbreeding measured with a handful of molecular markers is not necessarily a good predictor of the genealogical or genomic inbreeding, that there are problems in estimating genomic heterozygosity using only a few molecular markers (Toro et al., 2009).

Table 1. Characteristics and summary statistics for microsatellite loci analysed in the population of the Czech Haflinger Horses

Locus	No. of alleles	Observed heterozygosity	Genetic diversity	Chromosomal Location	Effective number of allele	F_{IS}	PIC	Hardy Weinberg equilibrium
VHL20	11	0.8442	0.8723	30	7.83	-0.031	0.824	p = 0.0000
HTG4	7	0.6112	0.6005	9	2.50	0.011	0.535	p = 0.8347
AHT4	9	0.7571	0.7826	24	4.60	-0.036	0.718	p = 0.1918
HMS7	8	0.7059	0.6658	1	2.99	0.057	0.653	p = 0.2794
HTG6	8	0.2171	0.2283	15	1.30	-0.051	0.208	p = 0.4496
AHT5	8	0.7378	0.7391	8	3.83	-0.002	0.700	p = 0.3903
HMS6	5	0.6749	0.7092	4	3.44	-0.054	0.611	p = 0.1040
ASB2	13	0.7198	0.7391	15	3.83	-0.024	0.675	p = 0.9142
HTG10	9	0.7278	0.7147	21	3.51	0.019	0.687	p = 0.5908
HTG7	4	0.6079	0.6250	4	2.67	-0.031	0.560	p = 0.5263
HMS3	9	0.6993	0.6984	9	3.32	-0.001	0.653	p = 0.1698
HMS2	11	0.6599	0.6630	15	2.97	-0.006	0.610	p = 0.4054
HMS1	13	0.5642	0.5788	15	2.37	-0.029	0.475	p = 0.5319
Mean	8.25	0.6559	0.6628	-	2.97	-0.013	0.608	-
SD		0.150	0.152	-	1.54	0.031	0.149	-

CONCLUSION

The chosen set of microsatellite markers has confirmed the high polymorphism and its usefulness in estimating genetic diversity by Czech Haflinger. The results of the analysis based on microsatellite data show a high heterozygosity in the population. The diversity of the population Czech Haflinger, based on a small number of microsatellites, seems to be sufficient.

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