

Summary of the doctoral thesis by *MSc Marta Pasternak*, entitled:

THE GENETIC BASIS OF PIEBALD COAT COLOUR IN HUCUL HORSES

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In recent years, the piebald coat colour has become popular with breeders of many horse breeds. In Hucul horses, the tobiano piebald-spotting pattern may appear in one of three forms: minimal, classic or maximal (extreme). The minimal variant, which sometimes occurs only as white spots on legs, may result in the animal being wrongly classified as a horse of uniform colour with white markings. In Hucul horse breeding it is particularly important to accurately determine the genes responsible for the presence of piebald coat colour and markings, because the breed standard specifies that markings disqualify these horses from breeding. Because it is not allowed to register animals with markings in the herd book, the problem associated with correct verification of this trait has become even more relevant. Passing the minimal pattern to offspring may lead to the elimination from breeding of more valuable tobiano piebald horses whose confusing phenotype will be classified as markings.

The objective of this study was to analyse polymorphisms associated with the presence of piebald coat colour in Hucul horses, and to estimate the number and make a detailed description of the horses whose coat colour was wrongly described because phenotypic differentiation of markings from the minimal tobiano pattern was not possible. The results obtained were used to verify the coat colour records of the investigated animals in the breeding documentation (passport).

The study used blood samples collected from 242 Hucul horses with different colour patterns, originating from both state-owned studs and private breeders. DNA isolated from blood was analysed to identify inversion of chromosome 3 (PCR method) and MspI polymorphism in intron 13 of the KIT gene (PCR-RFLP method). The horses were divided into 3 study groups: the first group included piebald horses, the second were horses of uniform colour with markings, and the third consisted of single-coloured horses without

markings. Each group of horses was subdivided into 3 subgroups designated 0, 1 and 2, where the digit indicating subgroup number stood for the number of piebald parents in the pedigree.

The results of analysis of inversion of chromosome 3 confirmed that it is a direct factor in determining the presence of piebald (tobiano) colour in the Hucul horses under study. No inversion was found in any of the single-coloured animals without markings, regardless of the number of piebald parents, whereas inversion appeared in all of the piebald horses. Inversion was also identified in 18% of animals (11 out of 61) from the group of horses described in the passport as single-coloured with markings, which showed how important it is to distinguish between markings and the minimal piebald pattern. In fact, the horses with wrongly described coat colour have the Tobiano gene, which is phenotypically identified as the minimal pattern (MIN*); it is also known as crypto-tobiano and may give a false impression that the horse has markings, leading to a wrong description of the horse. This problem becomes even more serious considering the breed standard, which specifies that Hucul horses with markings should be disqualified from breeding.

The results of analysis of MspI polymorphism and KM1 allele associations showed that they were significantly ($p < 0.01$) correlated to tobiano piebald colour in the studied horses, but the presence of this allele in the group of single-coloured horses and single-coloured horses with markings ruled it out as a marker for identification of tobiano coat colour. The higher frequency of the KM1 allele in the single-coloured Hucul horses under study compared to the horses of other breeds studied by other authors, is indicative of between-breed differences in this trait.

The analysis also demonstrated a significant ($p = 0.01906$) linkage disequilibrium between MspI polymorphism and inversion of chromosome 3 observed in the studied population of horses. The genotype variant was found to have no statistically significant effect on the size of white spots in the studied horses with tobiano piebald colour, either for inversion of chromosome 3 ($p > 0.05$) or for the MspI polymorphism ($p > 0.05$).

Based on the results obtained, it is concluded that the minimal (MIN*) tobiano piebald pattern is often wrongly classified as normal markings when making a description of the horse for the passport. This conclusion is supported by the fact that as much as 52.4% of the animals with the minimal (MIN*) tobiano pattern had their coat colour wrongly identified. Therefore, when describing the horses, it is necessary to pay special attention to animals with piebald ancestors whose coat colour is questionable. The most reliable way for correct classification of coat colour is to analyse DNA for the inversion of chromosome 3,

which will conclusively confirm or rule out the presence of the tobiano gene in a given animal. At the same time, this would make it possible to keep breeding the horse, which despite having the minimal pattern, could produce foals characterized by the classical pattern in the next generations. It would also be appropriate to perform more detailed studies to determine the factor controlling the extent of white spots in piebald horses and in single-coloured horses with markings; this would enable this trait to be properly directed in breeding.

Key words: Hucul horses, breed standard, white markings, piebald, tobiano
MspI polymorphism, chromosome 3 inversion

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