# Allele frequency in loci which control coat colours in Hucul horse population

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**ABSTRACT**: The objective of the study was to determine the frequency of alleles which produce coat colours in Hucul horse population in Poland. The breed is included in the Global Strategy for Management of Farm Animal Genetic Resources, hence its gene pool should remain in unaltered state. Huculs are bay, black, blue dun, yellow dun, tobiano, and chestnut. Grey and chestnut Huculs have always been undesirable. The material consisted of all 1022 matings which resulted in subpopulations recorded in Studbook volumes. The recessive allele frequency was estimated as the square root of recessive genotype frequencies in *ASIP (A), MC1R (E), DUN (D), KIT (To region),* and *STX17 (G)* loci. The frequency in *A* and *E* loci in total parental generation was also estimated in test matings. Genotype distribution in the population was anticipated according to gamete frequency in sires and dams. Small Wahlund effect, $F_{ST}$ and $\chi^2$ values for allele distributions show that division into subpopulations did not influence the population genetic structure significantly. Mean recessive allele frequency in *A, E, D, To,* and *G* loci amounted to 0.521, 0.115, 0.878, 0.929, and 0.997, respectively, and in *A* and *E* loci it was similar to that assessed in test matings. More bay horses and fewer *D* diluted horses appeared in offspring than expected. *A, e, d,* and *To* allele frequency showed a rising tendency. The genetic structure in Hucul population is not constant and does not comply strictly with the preservation aim. Bay, non-diluted, and tobiano horses are preferred. The linkage between *MC1R* and *KIT* loci can make the selection against *e* allele difficult. Breeders' preferences may lead to undesired changes in the allele frequency. To avoid such risk, it is recommended to select horses strictly complying with the rules included in the breeding programme and mate the horses randomly from this aspect.

**Keywords**: genetic structure; conservation programme

Hucul horses are a primitive breed that derives from the East Carpathian Mountains and dates at the 19th century. The Polish Hucul Studbook is conducted as closed since the 1980s, hence from then there has been no inflow of foreign genes. In 1999, the Hucul breed was included in the Global Strategy for the Management of Farm Animal Genetic Resources, which aims at preserving the animal gene pool in unaltered state (Peters, 2003). The Hucul population in Poland is a few times larger than each one in the Czech Republic, Hungary, Slovakia, Romania, and Austria. Originally used as pack, saddle or draught horses under harsh circumstances and complying with high human requirements, the Huculs became tough, resistant to illnesses, fertile and long-living family leisure horses (Brzeski and Jackowski, 1998). The specific properties in Hucul body conformation like short scapulae and metacarpi may be connected with the mountainous habitat, in which they have been bred (Komosa and Purzyc, 2009). The Huculs' outstanding skills to move in difficult terrain and pass natural obstacles are verified in specific performance tests (Stachurska et al., 2006).

Possible changes in the Hucul population preserved and closed a short time ago should be monitored with regard to the genetic pool. The frequency of alleles producing coat colours which are inherited in a simple way, may be assumed as a kind of a marker of the trends, along with molecular markers which enable to consider only a small number of individuals mostly living nowadays. The colours established in a breed should be preserved as a distinct trait which is characteristic of the breed representatives, together with less specific polygenic properties. When the breed is preserved, maintaining the coat colour is still more important for traditional and cultural aspects. Most studies show that the coat colour is neither correlated with the horse performance and physiologic traits, nor with the temperament (Estes Worth, 1948; Dušek, 1980; Dring et al., 1981). An analysis of Thoroughbreds has proved that loci which control the appearance of eumelanin and grey pattern are not considerably related to the racing scores (Stachurska et al., 2007). However, horse breeders often prefer certain coat colours believing that the trait impacts the performance, or favour particular colours for aesthetic reasons. The information on the allele frequencies producing colours in a population is important also because it shows whether there is a threat of losing those alleles in the breed conserved.

The objective of the study was to determine the frequency of alleles which produce coat colours in the Hucul horse population in Poland, in the view of conservation the gene pool of the breed.

## MATERIAL AND METHODS

This study conducted with the use of documentary data did not require approval of animal procedures. The material consisted of all 1022 matings which resulted in consecutive subpopulations of horses recorded during 42 years in all eight volumes of the Polish Hucul Studbook. The horses are registered in the Studbook as adults after selection. The selection aims at preserving the established Hucul model with the attributed coat colours and improving the horse's movement, without changing the breed type. The Huculs are bay, black, yellow dun, blue dun (also called mouse dun or grulla), some have the tobiano pattern and some are chestnut. No red duns have been recorded. According to the current breeding program, grey Hucul horses cannot be registered though before establishing the Hucul standard phenotype, the grey colour was allowed in the population (Tomczyk-Wrona, 2010). Moreover, of chestnut Hucul horses, only mares which fulfill special requirements can be registered in the Studbook. Horses of all the coat colours recorded in the Studbook were included in the study. Colour shades were not considered. The coat colour percentage was determined in horses recorded in the Studbook volumes.

The analysis of the allele frequency concerned *ASIP, MC1R, DUN, KIT* (with regard to the inversion determining the tobiano pattern) and *STX17* loci. Phenotypes produced by particular genotypes are presented in Table 1. *ASIP* (*A*) and *MC1R* (*E*) loci on ECA22 and ECA3, respectively, control the so-called basic colours (Marklund et al., 1996; Rieder et al., 2001). The bay colour is produced by *A_E_*, black by *aaE_* and chestnut by _ _*ee* genotypes (Adalsteinsson, 1974). Dun colours result from *D* completely dominant dilution allele, whereas the basic colours are recessive (*dd*). The yellow dun is the diluted bay (*A_E_D_*), the blue

Table 1. Coat colour phenotypes produced by particular genotypes

| Phenotype | Loci | | | | |
|---|---|---|---|---|---|
| | *A (ASIP)* | *E (MC1R)* | *D (DUN)* | *To (KIT)* | *G (STX17)* |
| Bay | *A_* | *E_* | *dd* | *toto* | *gg* |
| Black | *aa* | *E_* | *dd* | *toto* | *gg* |
| Chestnut | _ _ | *ee* | *dd* | *toto* | *gg* |
| Yellow dun | *A_* | *E_* | *D_* | *toto* | *gg* |
| Blue dun | *aa* | *E_* | *D_* | *toto* | *gg* |
| Red dun | _ _ | *ee* | *D_* | *toto* | *gg* |
| Tobiano | _ _ | _ _ | _ _ | *To_* | _ _ |
| Grey | _ _ | _ _ | _ _ | _ _ | G_ |

dun is the diluted black (*aaE_D_*), and the red dun is the diluted chestnut (_ _ *eeD_*) (Van Vleck and Davitt, 1977; Adalsteinsson, 1978; Craig et al., 1985). *DUN* (*D*) locus is mapped to a group of microsatellites on ECA8 (Bricker et al., 2003; Rieder, 2009). The bay, black, yellow dun, and blue dun horses are called eumelanic, i.e. carrying *E* allele which causes eumelanogenesis. Phaeomelanin is also produced in them, except in the blacks. The chestnut and red dun horses are phaeomelanic i.e. they have solely phaeomelanin in the coat. Recent studies provide evidence for inversion on ECA3 which is a causative mutation for the tobiano spotting pattern. The inversion begins approximately 70 kb downstream of the *KIT* gene (Brooks et al., 2007; Haase et al., 2008, 2009). The tobiano pattern is inherited as a completely dominant trait and can appear in each colour, e.g. a bay-tobiano horse is of the genotype *A_E_To_*, whereas horses without the pattern are *toto* (Trommershausen-Smith, 1978; Bowling, 1987). *STX17* (*G*) locus on ECA25 controls the appearance of grey pattern: completely dominant *G* allele produces the pattern in every colour and *gg* horses are not grey (Salisbury, 1941; Henner et al., 2002; Locke et al., 2002; Swinburne et al., 2002; Rosengren Pielberg et al., 2008). The bay, chestnut, blue dun, and yellow dun shades are presumably caused by a few modifiers and to some extent by the heterozygosity or homozygosity in *A*, *E*, and *D* loci (Stachurska et al., 2002; Stachurska and Brodacki, 2003).

Because of the linkage between *E* and *To* loci, as well as lack of data on the main colour not registered in the tobianos and greys, results of mating horses with tobiano and grey patterns were not included in the overall analysis of *A*, *E*, and *D* loci. The allele frequency was determined in stallions and mares assumed to be offspring subpopulations recorded in consecutive volumes of the Studbook, as well as in the entire offspring generation and the entire parental generation, with regard to the gender. The allele frequencies were calculated as non-weighted means in males and females. To determine the Wahlund effect and $F_{ST}$, non-weighted mean allele frequency in subpopulations and variance were estimated (Jorde and Ryman, 1990; Gillespie, 2004).

The frequency of recessive *e*, *d*, *to*, and *g* alleles was estimated as the square root of the chestnut (*ee*), nondiluted (*dd*), nontobiano (*toto*), and nongrey (*gg*) genotype frequency. The frequency of recessive *a* allele was calculated as the square root of the sum of black, blue dun, and *aaee* chestnut frequency (Stachurska and Brodacki, 2008). To esti-

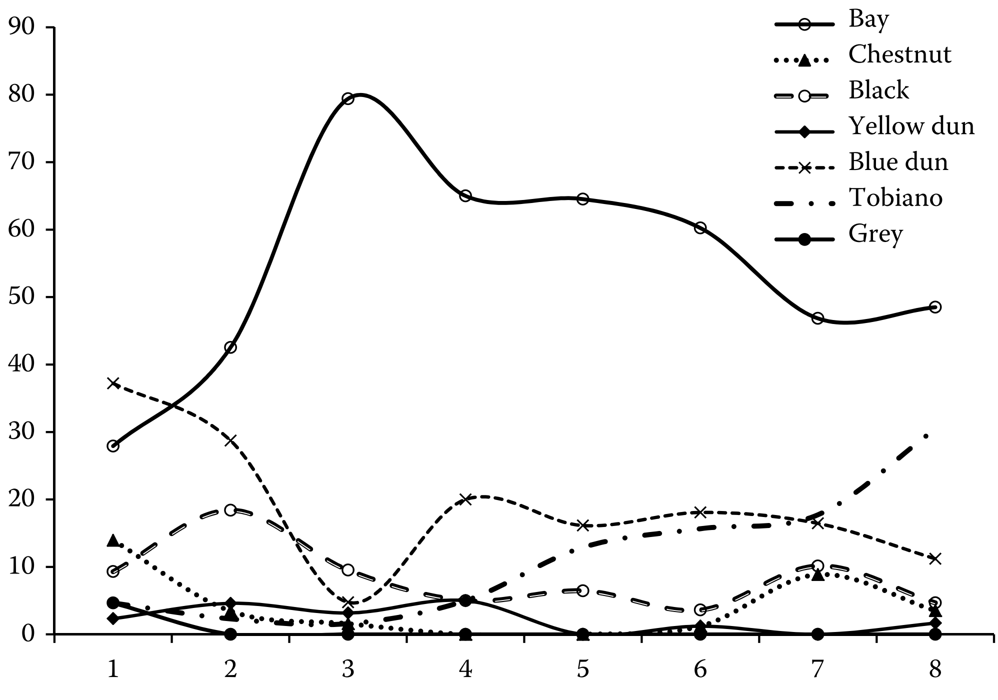

Figure 1. Percentage of variously coloured Hucul horses registered in the Studbook volumes (subpopulations) Significant differences in numbers of variously coloured horses (considering groups of at least 5 horses) between the 2nd–3rd volumes ($P < 0.01$), 4th–5th volumes ($P < 0.05$), and 7th–8th volumes ($P < 0.01$)

mate the latter, it was assumed that the ratio of *aaee* chestnut to _ _*ee* chestnut frequency agrees with the ratio of black and blue dun frequency to the total frequency of bays, yellow duns, blacks, and blue duns. The frequency of alleles in the *A* and *E* loci was also estimated on the basis of test matings to verify whether the frequencies calculated on the basis of recessive genotype frequency are correct. In the *A* locus, bay and yellow dun horses (*A_*) mated to black and blue dun horses (*aa*) were considered. In this case the chestnuts were not included. They were so few that they could not alter the result significantly. In the *E* locus, eumelanic (*E_*) horses mated to phaeomelanic (*ee*) horses were taken into account. On the basis of gamete frequency in sires and dams, genotype distribution in the population was anticipated. The expected and actual genotype distributions were compared.

To verify whether matings were random with respect to the coat colour, numbers of variously coloured dams covered by sires of a given colour were compared.

SAS/Genetics™ Software (2003) procedure was used to assess the frequencies and to compare the distributions of phenotypes, genotypes, and alleles with $\chi^2$ test. Formulae to calculate Wahlund effect, variance, and $F_{ST}$ were applied according to Hartl and Clark (2007).

## RESULTS

The total Hucul population is mainly bay (51.0%), tobiano (21.5%), and blue dun (14.8%). Considering most numerous coat colour horse groups, changes occurred between the 2nd and 3rd volumes of the Studbook ($P < 0.01$), the 4th and 5th volumes ($P < 0.05$), as well as the 7th and 8th volumes ($P < 0.01$; Figure 1). Grey horses were recorded only in the 1st volume (4.7%). The number of tobianos has been increasing from the 5th volume. In the Hucul subpopulation included in the latest volume, 48.5% bays, 30.4% tobianos and 11.2% blue duns have been recorded. Some black, chestnut and yellow dun horses have also occurred (4.7, 3.5, and 1.7%, respectively).

The frequency of recessive *a* allele in horses recorded in the Studbook was changing in the successive subpopulations and totally decreased from 0.782 ± 0.049 to 0.471 ± 0.022 (Figure 2). The recessive *e* allele occurred in horses registered in the first three volumes, it did not occur in the 4th and 5th volumes and was observed again becoming still more frequent in the next volumes (0.182 ± 0.017 in the 8th volume). The recessive *d* allele frequency equaled to 0.719 ± 0.054 in the 1st volume and increased with fluctuations to 0.933 ± 0.011. The 3rd and 4th volumes, as well as the 6th and 7th vo-

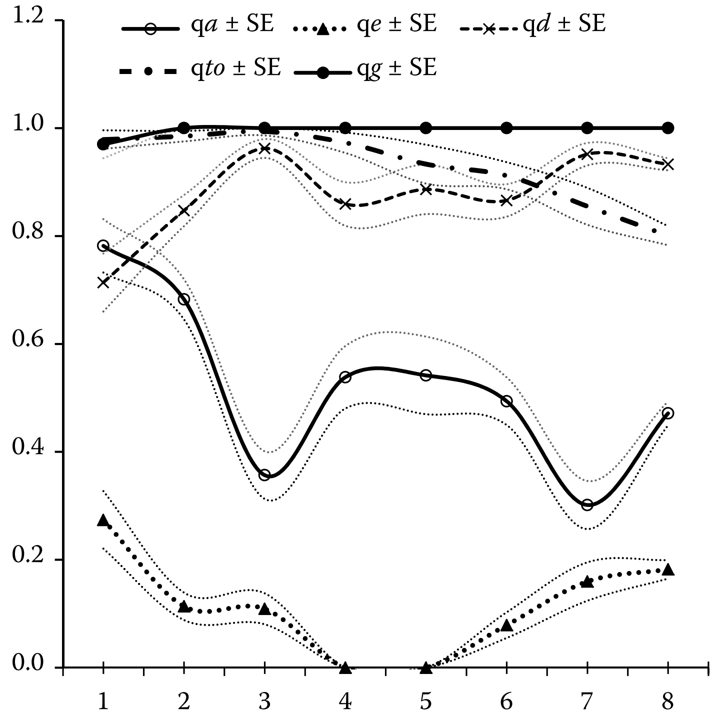

Figure 2. Frequency (q) and standard error (SE) of recessive alleles in Hucul horses registered in the Studbook volumes (subpopulations). Significant differences in *A* and *a* allele distribution between the 2nd–3rd, 3rd–4th, 6th–7th, 7th–8th volumes ($P < 0.01$); *E* and *e* allele distribution between the 1st–2nd, 3rd–4th ($P < 0.01$), 5th–6th, 6th–7th volumes ($P < 0.05$); *D* and *d* allele distribution between the 1st–2nd ($P < 0.05$), 2nd–3rd ($P < 0.01$), 3rd–4th, 6th–7th volumes ($P < 0.05$)

Table 2. Overall statistic characteristics in Hucul population

| Locus | Number of horses | Mean recessive allele frequency[†] | SE | $\chi^2$[††] | Wahlund effect | Variance | $F_{ST}$ |
|---|---|---|---|---|---|---|---|
| *A* | 604 | 0.521 | 0.014 | 4.53 | 0.043 | 0.022 | 0.086 |
| *E* | 604 | 0.115 | 0.009 | 1.93 | 0.015 | 0.007 | 0.046 |
| *D* | 604 | 0.878 | 0.009 | 1.19 | 0.011 | 0.005 | 0.032 |
| *To* | 1020 | 0.929 | 0.006 | 2.67 | 0.009 | 0.004 | 0.037 |
| *G* | 1022 | 0.997 | 0.002 | 0.36 | 0.000 | 0.000 | 0.003 |

[†] non-weighted mean of subpopulations recorded in consecutive Studbook volumes

[††] differences in allele distributions in particular loci considering all subpopulations not significant ($P > 0.05$)

SE = standard error

lumes differred the most, considering *a, e,* and *d* allele frequencies. The frequency of the recessive *to* allele was continuously decreasing from the 3rd volume (0.994 ± 0.007) to the 8th volume (0.801 ± 0.018), though the differences between the successive subpopulations were not significant. The lowering tendency of the *to* frequency went hand in hand with increasing *e* allele frequency. The *g* allele frequency was less than one (0.976 ± 0.002) only in horses recorded in the 1st volume.

Mean recessive allele frequency in *A, E, D, To,* and *G* loci in subpopulations was highly differentiated and amounted to 0.521, 0.115, 0.878, 0.929, and 0.997, respectively (Table 2). The difference between the recessive and dominant allele distributions in particular loci considering all subpopulations was not significant. The heterozygosity reduction resulting from the division into subpopulations, estimated with Wahlund effect, was low in *E, D,* and *To* loci (1.5, 1.1, and 0.9%, respectively). In *A* locus, the Wahlund effect amounted to 4.3%. The heterozygosity reduction estimated with $F_{ST}$ exceeded 5% only in the case of *A* locus and in other loci it was lower.

The *a* and *e* allele frequencies assessed in test matings and converted into the whole parental generation approximated to those estimated on the basis of the recessive genotype frequency (Table 3). The frequency of the recessive *a* allele was lower in the entire progeny than in the parental generation ($P < 0.01$). The *a* allele in dams was more frequent than in sires ($P < 0.01$). The recessive *e* and dominant *E* allele distributions in the parents and in the offspring were alike, whereas

Table 3. Mean recessive allele frequency (q) in loci controlling the coat colour in Hucul horses in parents and progeny

| Horse group | *A* locus | *E* locus | *D* locus | *To* locus | *G* locus |
|---|---|---|---|---|---|
| Sires | 0.532 B | 0.081 C | 0.866 F | 0.832 K | 1.000 – |
| Dams | 0.646 B | 0.211 C | 0.806 F | 0.923 K | 0.999 – |
| Parents in total | 0.589 H | 0.146 – | 0.836 I | 0.877 – | 0.999 – |
| Parents according to test matings[1] | 0.581 | 0.153 | | | |
| Male progeny | 0.507 – | 0.074 J | 0.906 – | 0.867 – | 0.999 – |
| Female progeny | 0.558 – | 0.249 J | 0.883 – | 0.893 – | 0.999 – |
| Progeny in total | 0.532 H | 0.161 – | 0.894 I | 0.880 – | 0.999 – |

Recessive and dominant allele distributions within one locus in different horse groups marked with the same capitals differ at $P < 0.01$

[1] allele frequencies assessed on the basis of test matings do not differ significantly from those estimated on the basis of recessive genotype frequency ($P > 0.05$)

Table 4. Expected and observed genotype distribution in progeny

| Distribution | Genotypes | | | | |
|---|---|---|---|---|---|
| | *A_E_ddtotogg* | *__eeddtotogg* | *aaE_ddtotogg* | *____D_totogg* | *______To_gg* |
| Expected | 0.34 F | 0.01 B | 0.17 | 0.25 C | 0.23 |
| Observed | 0.43 F | 0.03 B | 0.18 | 0.13 C | 0.23 |

Expected and observed genotype distributions marked with the same capitals differ at $P < 0.01$

in both female groups the *e* allele frequency was greater than in respective male groups ($P < 0.01$). The *d* allele was more frequent in the progeny than in the parents and in sires compared to dams ($P < 0.01$). In *To* locus, the recessive allele frequency was greater in dams than in sires ($P < 0.01$), though the parental and offspring generations did not differ in this regard.

The frequency of some genotypes observed in horses registered in the Studbook considerably differed from the frequency anticipated on the basis of gamete frequency in sires and in dams (Table 4). More bay and chestnut horses and fewer *D* diluted horses appeared than expected ($P < 0.01$).

Percentage of variously coloured dams covered by bay sires versus those covered by blue dun sires, as well as by bay versus tobiano sires differed (Table 5). For instance, bay sires covered more blue dun mares than blue dun or tobiano sires did. On the other hand, the percentage of bay dams mated to bay sires was similar to that mated to black, blue dun, and tobiano sires. No dams were covered by grey sires.

## DISCUSSION

Coat colours accepted in a breed result mainly from colours of its ancestors, though according to various breeding programmes certain colours are strongly preferred or excluded. Earlier studies indicate that in many horse breeds, even despite the fact that formally the coat colour is not a criterion in the selection, some colours are favoured (Brodacki and Stachurska, 2000; Stachurska and Brodacki, 2000, 2008). For instance, bay colour is preferred in Małopolski, Wielkopolski, and Thoroughbred horses. In Hucul breeding, after a period of a greater variability, the model of the horse was established and coat colour criteria were included in the con-

Table 5. Percentage of variously coloured dams covered by sires of particular coat colours

| Dams | Sires | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bay | | Chestnut | | Black | | Yellow dun | | Blue dun | | Tobiano | | Total | |
| | *N* | (%) | *N* | (%) | *N* | (%) | *N* | (%) | *N* | (%) | *N* | (%) | *N* | (%) |
| Bay | 231 | 46.6 | 0 | 0.0 | 12 | 48.0 | 7 | 77.8 | 80 | 45.7 | 149 | 47.6 | 479 | 46.9 |
| Chestnut | 11 | 2.2 | 4 | 100.0 | 2 | 8.0 | 0 | 0.0 | 10 | 5.7 | 23 | 7.3 | 50 | 4.9 |
| Black | 23 | 4.6 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 14 | 8.0 | 21 | 6.7 | 58 | 5.7 |
| Yellow dun | 5 | 1.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 0.6 | 8 | 2.6 | 14 | 1.3 |
| Blue dun | 151 | 30.5 | 0 | 0.0 | 11 | 44.0 | 2 | 22.2 | 40 | 22.9 | 62 | 19.8 | 266 | 26.0 |
| Tobiano | 74 | 14.9 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 28 | 16.0 | 50 | 16.0 | 152 | 14.9 |
| Grey | 1 | 0.2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 1.1 | 0 | 0.0 | 3 | 0.3 |
| Total[1] | 496 | 48.5 | 4 | 0.4 | 25 | 2.5 | 9 | 0.9 | 175 | 17.1 | 313 | 30.6 | 1022 | 100.0 |

Differences in distributions significant between dams covered by bay and blue dun sires ($P < 0.05$), as well as by bay and tobiano sires ($P < 0.01$); in the $\chi^2$ test, groups of below five dames and dams covered by chestnut and yellow dun sires were not considered

[1] total number of matings by sires of a particular colour and its percentage in the total number of matings

servation programme to maintain the breed status quo in this respect.

Small Wahlund effect, $F_{ST}$ and $\chi^2$ values for allele distributions indicate the population division into subpopulations did not cause significant heterozygosity deviation from the expected heterozygosity in the total population (Gillespie, 2004). This gave a good reason for analysing the population not only in subpopulations, but also totally, without the division. Low SE values of allele frequency show that the genetic drift and migration effect in the Hucul horse population in forty two years of documented breeding were weak. The result is similar to findings of the earlier investigation conducted on the allele frequency in loci controlling coat colour in big and stable Thoroughbred population (Stachurska and Brodacki, 2008). In the present study, the frequency of *a* and *e* alleles assessed on the basis of test matings similar to that calculated from the recessive genotype frequency documents the frequencies were estimated correctly.

Fluctuations in the percentage structure of variously coloured Hucul horses in subpopulations, particularly in the bay and blue dun horse numbers, as well as the growth of tobianos number have been the consequence of changes in the frequency of alleles in loci controlling the coat colour. The dominant *G* allele was easily eliminated because within the population registered in the Studbook the grey horses were very rare. Instead, the recessive *e* allele frequency in the total population has not decreased according to the breeding aim but has remained similar in the parents and the progeny. The frequency is indeed greater in females than in males, as the program allows, however, the increase observed in horses recorded in the 6th and subsequent volumes does not follow the limitations formulated in the breeding programme. The fact can be attributed to easy allowance of chestnut mares to the breeding and may be connected with the rising tobiano horse occurrence. The linked *MC1R* and *KIT* loci are separated by only 20–34 cM, hence the crossing over in that region may occur not often (Penedo et al., 2005). Theoretically, e.g. a bay-tobiano or black-tobiano sire carrying the recessive *e* allele linked with the *To* gene may disseminate the undesired *e* allele in the population, however, it is not possible to check it in the Studbook. The fact that the main colour in tobianos is not recorded makes scientific analyses difficult and the breeders are limited in predicting the foal's coat colour from a mating. It should be suggested to record the trait in all breeding documents.

The zero *e* allele frequency in horses registered in the 4th and 5th Studbook volumes arose due to estimating it on the basis of recessive genotype occurrence. In fact, some individuals were heterozygous. The *e* allele frequency assessed on the basis of test matings similar to that calculated from the recessive genotype occurrence provides evidence the selection against the *e* allele has not considerably altered the population genetic structure. In order to decrease the *e* allele frequency more effectively, it can be suggested to eliminate not only chestnut horses but also other carriers of the *e* allele from the breeding. To detect them, molecular testing may be used e.g. in *E_* sires.

Inversely proportional *a* and *d* allele frequencies in subpopulations indicate the more bay horses are recorded, the fewer blue duns appear and vice versa. Lower recessive *a* allele frequency in offspring than in parents shows that *A_* horses are actually favoured. The fact is also documented by more frequent *A* allele occurrence in sires than in dams. As it is known, sires are selected more strictly than dams, hence usually they are of a more desired genotype and the allele frequency in this group better illustrates breeders' aspirations. The *e* allele frequency is low, hence the *A* allele occurs mainly in *A_E_* genotypes of bay horses. The actual *A_E_* genotypes number higher than in the distribution anticipated on the basis of gamete frequency confirms that the *A* allele is preferred.

In turn, higher *d* allele frequency in offspring than in parents, as well as in sires compared with dams, indicates non-diluted *dd* horses are desired. The actual twice higher number of *D_* horses than expected on the basis of gamete frequency also documents this fact. *D_* genotypes produce diluted, so-called primitive colours. The differences in the allele frequency may show a slow depart from such colours towards non-diluted colours attributed to sophisticated breeds.

Although the allele distribution in *To* locus in parents and progeny was alike, as well as the *To_* expected and observed numbers of genotypes did not differ, smaller *to* frequency in sires than in dams shows a tendency of dominant allele preference. The tobiano colour rated among paint patterns is favourable in many show breeds. On the other hand, some registries disallow tobiano spotting erroneously believing the white patches will become larger and larger in consecutive generations

(Sponenberg, 1996). In Huculs, the tobiano pattern is appreciated, maybe because it is more flashy. Currently, tobiano Huculs have become numerically second group, smaller compared only to bays.

Hucul dams are not mated quite randomly, regardless of the coat colour. Mating so many blue dun dams to bay sires confirms the *dd* horses preference. It seems that breeders aim at reducing the diluted blue dun colour occurrence in the population. This results in the phenomenon that differently to other breeds (Brodacki and Stachurska, 2000; Stachurska and Brodacki, 2000), possible assortative matings of like with like are not more frequent than other matings. In the compared Thoroughbred population, dams are mated randomly, regardless of colour (Stachurska and Brodacki, 2008).

## CONCLUSION

The genetic structure in the Hucul population is not constant and does not comply strictly with the preservation breeding aim. The *A, d,* and *To* allele frequencies show a rising tendency, though there are no formal selection criteria as to colours produced by these genes. The linkage between *MC1R* and *KIT* loci can hamper the selection against the recessive *e* allele. Chestnut mares are allowed to the breeding too easily, whereas bay, non-diluted, and tobiano horses are preferred. Such preference may lead to important changes in the genetic structure of the population, which is not desired in the case of the breed included in the Global Strategy for the Management of Farm Animal Genetic Resources. The alteration of variously coloured horses percentage would be a visible change in the population. To avoid such risk, it is recommended to select horses strictly complying with the rules included in the breeding programme and mate the horses randomly from this aspect.

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