AGRICULTURAL UNIVERSITY - PLOVDIV

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GENEALOGICAL STRUCTURE OF THE DANUBIAN HORSE BREED, ITS PLACE IN THE NONIUS STRUCTURE AND DIRECTION OF DEVELOPMENT, IN THE CONTEXT OF AN OVERALL CONCEPT OF BREED DEVELOPMENT

ABSTRACT

ON A DISSERTATION FOR THE AWARD OF AN EDUCATIONAL AND SCIENTIFIC DEGREE
"DOCTOR"

OF SCIENTIFIC SPECIALTY: "BREEDING OF AGRICULTURAL ANIMALS, BIOLOGY AND BIOTECHNOLOGY OF REPRODUCTION"

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The dissertation is written on 299 pages, of which Literature review - 44 pages, "Aim and tasks" - 2 pages, "Materials and methods" - 7 pages, "Results and discussion" - 209 pages, "Summary" - 5 pages, "Conclusions" - 2 pages, "Recommendations" - 1 page, "Literature" - 26 pages. A list of 9 publications related to the dissertation work and 21 of their citations attached.

427 sources are cited, 87 of which are in Cyrillic. The material is illustrated with 93 figures and 34 tables.

The paper was discussed at a meeting of the extended departmental council at the Department of "Animal Science" of the Faculty of Agriculture of the Agricultural University - Plovdiv, No. 11/07.12.2023 and aimed for defense.

The defense of the dissertation will take place on 12.03.2024 year, from 11^{00} hours at the VI Audience of the Agricultural University - Plovdiv, at a meeting of the Specialized Scientific Jury, appointed by the Rector of the Agricultural University - Plovdiv with Order No. RD-RD-16-1299/ 18.12.2024 composed of:

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The defense materials are available on the website of the Agricultural University - Plovdiv, www.au-plovdiv.bg and in the library of the Agricultural University - Plovdiv, Plovdiv, 12 Mendeleev Blvd. Note: The numbering of the tables and figures used in the abstract does not correspond to that in the dissertation.

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INTRODUCTION

The Danubian horse breed is created in the former stud farm "Clementina" near the town of Pleven, on the basis of the Nonius breed section established after 1924. It is recognized in 1951 by Decree No. 631 of the Council of Ministers. When creating, along with the purebred breeding of the Nonius breed, absorptive and partially reproductive crossbreeding with stallions of this breed with Anglo-Arabian, Arabian and half-bred English mares is applied. Over the years, the import of breeding animals of the Nonius breed is mainly from Hungary, as well as from Yugoslavia and Czechoslovakia.

In the 1990s, like other livestock industries, horse breeding collapsed, and almost all national breeds were classified as "endangered" or "endangered with extinction" according to the FAO classification. As for the Danubian horse breed, the number of mares decreased to critical levels, the family structure was broken, some lines ceased to exist. A turning point in the history of the breed was the establishment of the National Horse Breeding Association in 1999. Since then, although with difficulties, specialists and breeders of the Danubian breed had been making efforts to preserve and develop it. Some of the lines had been restored. Import of stallions began. That created new problems. Expanding the genealogical basis of the breed could probably be done by importing stallions from the Nonius breed or other breeds that took part in the creation of the Danubian horse breed, but at the same time the uniqueness of the breed had to be preserved. On these issues, as well as on the overall strategy for the development of the breed, there is no consensus.

It is obviously necessary to put the future development of the Danubian horse on a scientific basis. The breed development strategy will largely depend on the state of the genealogical structures, genetic diversity, the ability to control inbreeding, etc. The current research is devoted to the clarification of these issues, using classical zootechnical methods and modern DNA technologies.

AIM AND OBJECTIVES

The aim of the research is to assess the current state of the Danubian horse breed, its genealogical structures and their relationship with the structure of the Nonius breed, as a prerequisite for the development of a concept for breeding activities and future development of the breed based on phylogenetic, genealogical, molecular-genetic and population-genetic analyses.

To achieve the aim, the task set is to conduct the following experiments and analyses:

1. Phylogenetic analysis of the Danubian horse breed and its genealogical structure from the end of the 1980s.
    1.1. Analysis of the conditions for the development of the breed
    1.2. Genealogical analysis of a linear structure of the Danubian horse breed and its phylogenetic development
    1.3. Genealogical analysis of the family structure of the Danubian horse breed and its phylogenetic development
2. Molecular genetic analysis of the Danubian horse breed
    2.1. Conducting microsatellite analysis of marker loci
    2.2. Conducting sequencing analysis of mitochondrial genetic markers
3. Molecular-genetic and population-genetic analysis of the Danubian horse breed
    3.1. Analysis of genetic structure and genetic diversity of the Danubian horse breed and its intrabreed structures by DNA-nuclear marker loci. Similarities and distances between lines
    3.2. Analysis of genetic similarities and distances of the Danubian horse breed with populations of the Nonius breed, breeds and populations of Bulgarian horse breeds.
    3.3. Comparative analysis of the genetic structures and phylogenetic analysis of the Danubian horse breed and Nonius breed with the Pleven horse and the Eastern Bulgarian horse breeds in accordance to and with Bulgarian local populations - Rilo-Rhodope, Stara Planina, Karakachan horse breed, according to mtDNA sequences
4. Analysis and guidelines of the Danubian breed breeding activities

MATERIALS AND METHODS

The present research is carried out on the basis of the registered breeding horses for the period 1924 - 2022 in the stud zootechnical books of the "Klementina" stud farm and the pedigree book of the Danubian horse.

For **the phylogenetic analysis**, the following is used: original pedigree certificates of the imported stallions and mares; factory stud books, annual zootechnical lists, primary zootechnical documents kept at the Clementina stud farm; state stud books; pedigree books of the Nonius breed; the database of the National Horse Breeding Association (NHBA), etc.; own observations, as a participant in the organization and control of the breeding activities in horse breeding as an animal scientist at the "Khan Asparuh" stud farm, executive director of the NHBA, expert in horse breeding, head of the "Horse Breeding" department and executive director of the Executive Agency for Selection and Reproduction in Animal Breeding. The data for the exterior parameters was processed statistically with IBM SPSS Statistics program.

The microsatellite analysis is performed at the GeneControl GmbH laboratory (Germany) on mane and/or tail hair follicle samples taken from 166 animals. 15 microsatellite marker loci were examined: AHT4, AHT5, ASB2, ASB17, ASB23, HMS1, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10 and VHL20 approved by International Society and International Society for Animal Genetics (ISAG), Champaign, Illinois, USA).

Population-genetic and statistical analysis. Fisher's method is used in the analysis of microsatellite markers. The probability of random breeding for each line is estimated by Chi-square (χ^2) and likelihood ratio (G^2) tests regarding the presence of significant deviation of the population structure from the Hardy-Weinberg equilibrium, for each locus (Smouseetal., 2017) . Indices of genetic difference between individual lines are determined using the program GENEALEX 6.5. Genetic distance between lines is calculated according to Nei (Takezaki & Nei, 1996). PHYLIP 3.69 software is used with 1000 replicates for each locus (Felsenstein, 2009). The PHYLIP 3.69 program is also used to construct a phylogenetic tree (Neighborhoodjoining method), which was visualized using MEGA11 (Molecular Evolutionary Genetics Analysisversion 11) (Tamuraetal., 2021). The effect of migration and gene flow on the genetic structure of the analyzed populations is estimated between each pair of populations according to the model of neutrality and minor mutations (Slatkin 1985). Genetic distances between populations are estimated using (Ds) standard genetic distance (Nei 1973) and DA distance (Neietal., 1983). STRUCTURE 2.3.4 software is used to assess the degree of differentiation of the lineages (Pritchard et al., 2010).

Monte Carlo simulations are performed using ten replicates for each value of K (2–12), where K is the number of clusters tested, with 20 replicates for each K using an admixture model and independent allele frequencies. To determine the optimal number of clusters (K), we apply the two log-likelihood methods of Prichard et al. (2010) as well as the ΔK value of Evanno et al. (2005). The number of clusters is determined and visualize using the Structure Harvester v 0.6.94 application (http://taylor0.biology.ucla.edu/structureHarvester/ (Earl et al., 2012). For multiple replicate alignments for each K and facilitating the interpretation of cluster analysis results (Kopelman et al., 2015), the online-based software Clumpak (http://clumpak.tau.ac.il/ .

For HVR, D-loop mtDNA sequencing analysis are used: hair follicles from 30 horses of the Nonus breed, from the stud farm "Mata"

(Debrecen, Hungary); 70 of Danubian horse; 15 by Serbian Nonius, from the stud farm "Karadjordjevo" (Karadjordjevo, Bachka Palanka municipality, Vojvodina autonomous province). DNA is isolated from hair follicles using a commercial DNA purification kit - GeneMATRIX Tissue (Cat. No. E3550, EURx Ltd., Gdansk, Poland) according to the manufacturer's instructions. The samples are incubated overnight at 56°C with continuous shaking. The quality and quantity of isolated DNA is verified spectrophotometrically and on 1% agarose gel electrophoresis, then visualized using a UV trans-illuminator gel documentation system, after staining with SimpliSafe ™ (cat. no. E4600; EURx Ltd., Gdansk, Poland). The isolated DNA is stored at -20° C. Based on a horse-specific mtDNA reference X79547 (Xiufeng & Arnason 1994), a 665 bp fragment of the mtDNA D-loop region (hypervariable region I, HVR1) is amplified (from 15.434 nd to 16.098 nd), by using primers: F15453 5'-CACCCAAAGCTGAAATTCTAC-3 and R16078 5'-ATAACACCTTATGGTTGCTG-3' (Hristov et al. 2017). All PCR reactions are performed with 10 ng of template DNA in a final volume of 50 μL (NZYTaq II 2×Colourless Master Mix, cat. no. MB354; NZYTech, Lda. - Genes and Enzymes, Lisbon, Portugal). PCR conditions are as follows: initial denaturation at 94°C for 5 min; 30 cycles - denaturation at 94°C for 30 seconds, primer hybridization at 50°C for 30 seconds, extension at 72°C for 1 minute and final extension at 72°C for 10 minutes. Successfully amplified products are purified using a commercial GeneMATRIX PCR/DNA Clean-Up Purification Kit (Cat. No. E3520; EURx Ltd., Gdansk, Poland) and sequenced in both directions using a PlateSeq kit (Eurofins Genomics Ebersberg, Germany).

The resulting mtDNA sequences are processed manually and aligned with the MEGA7 program (Kumar et al. 2016) using the complete mitochondrial reference sequence X79547 (Xiufeng & Arnason 1994). After processing, DNA fragments of about 640 bp (encompassing the proline tRNA-Pro gene and the start of the D-loop, HVR1 region) are used for phylogenetic analysis. The obtained sequences are deposited in the genetic database of the National Center for Biotechnology Information (GenBank, NCBI https://www.ncbi.nlm.nih.gov/) under the numbers MG420898-MG420955 (Danubian horse), MG420956-MG420978 (Nonius) and MG420979-MG420990 (Serbian Nonius).

DNA sequences are analyzed for the presence of polymorphic positions (SNPs - single nucleotide substitutions), after which the haplogroup belonging to each animal is determined according to the classification of Achilli et al. (2012).

The phylogenetic links between the obtained haplotypes are visualized using the program Network 10.0 (www.fluxusengineering.com). Principal component analysis (PCA) is visualized using XLSTAT software (MS Excel), according to Achilli et al. (2007). The number of polymorphic positions (S), the number of haplotypes (H), haplotype diversity (Hd), nucleotide diversity (π) and the average number of nucleotide differences in the population (K) are calculated using the program DnaSP6.0 (Rozas et al. , 2017). Nonsynonymous (dN) and synonymous (dS) mutation values are calculated and compared using the Z test (at $P < 0.05$ values are considered significant) according to the method of Nei & Gojobori (1986), with Jukes and Cantor correction . The D value of the Tajima test (Tajima 1989) and the D and F values of the Fu and Li test (Fu & Li 1993) are analyzed using the program DnaSP6.0, to evaluate the neutral theory of evolution (Rozas et al. 2017). When presenting the results of the conducted research, we also include the results of our previous research on the other two modern Bulgarian horse breeds - Pleven (n=11, GenBank Acc. no. MK465427-MK465437) and East Bulgarian (n=39, GenBank Acc. no. MK465388) -MK465426) (Hristov et al., 2020), as well as the three breeds/populations of autochthonous horses – Karakachan (n=45), Rilo-Rhodope (n=43) and Stara Planina (n=33) (GenBank Acc. no. KU601624 - KU601744) (Hristov et al., 2017). The aim is to detect genetic similarity or differences of the profile of the Danubian horse with the mentioned breeds.

RESULTS AND DISCUSSION

1. PHYLOGENETIC ANALYSIS OF THE DANUBIAN HORSE BREED AND ITS GENEALOGICAL STRUCTURE FROM THE END OF THE 80'S OF THE XX-th CENTURY.

Conditions for the development of the breed

The period after 1990 may be defined as the third period of the development of the Danubian horse. It coincided with the social and political changes that occur in the country, which were directly related to the new emerging economic conditions. The considered period was characterized by the regression of Bulgarian horse breeding and livestock breeding, in general. The activities with the first private owners of horses began after 1995, under the leadrship of the District Selection Centers of the National Station for Selection and Reproduction in Animal Breeding. Stud farms in the country were completely unprepared to work under the new economic conditions. The state of the Danubian horse breed reached the lowest point of development since its creation. A turning point was the establishment of the National Horse Breeding Association in 1999.

Relationship of the Danubian horse to the Nonius breed after 1990.

The analysis of the current state of the Danubian horse shows that the breeding scheme, from the creation of the breed to the present, has not been changed.

In the 1990s, the breeding stallions belong to five genealogical lines - by the stallions: Zdravko and Lider, which refer to N XXIX – A of the Nonius breed; Hrabar, of Line XXXI – B of Nonius; Torpedo, from line N XXXVI -C of Nonius; Kalifa, from line N IX through N III from line D of Nonius. In 2004, NHBA imported 2 foals - Nonius IV-21 Matróz (Matros) and Nonius IV-25 Mester (Mester), sons of Nonius XVII-30 from the line of Nonius N XXIX - A. In 2016, the Association of Horse Breeders in Bulgaria (AHBB) carry out a second import, from Serbia, of a stallion K. Nonius IX-3, which was used for one mating season. In the same year, the stallion Nonius IV-122 Rablot is imported from Hungary. Matroz, Mester and Rablot are half-sibs by father and belong to the line of N XXIX - A., thus a new line of stallion Erno is created (Fig. 1.). The link of the Danubian horse family structure to the Nonius breed after 1990 is: through the families of Nonius mares existing as of 2020, the foundation mares of the Danubian horse breed (Katya, Mara Yu, Serdika Yu, Stefa and Fraila) and through the imported, after 1990 mare K.N – 781 Deverica, of the Nonius breed.

In 2019, the first export of a stallion of the Danubian horse breed to Hungary took place. That was stallion Kalin I, which is provided under a contract to Hortobagi stud farm for the expansion of line D. As part of the exchange, the NHBA receive a female horse, a daughter of Kalin I - Nonius - 56 Harmony, foaled on 22.02.2021.

1. Genealogical analysis of the linear structure of the Danubian horse breed and its phylogenetic development

As a starting point in the analysis of the latest period of the development of the Danubian horse, we accept the report of Karaivanov et al. (1989). The authors consolidate that at the time of the analysis there are 4 lines of Zdravko, Lider, Hrabar and Kalifa, and the lines of Durtsash, Iskar, Record and Novak have only of historical significance. An opportunity to develop the stallion Torpedo line has also been established. Later, Burzev et al. (2007) report that the Torpedo line is recovered.

In the present dissertation, the phylogenetic development of the Danubian horse breed after 1990 is analyzed, and some elements from the previous period are used mainly in a comparative aspect.

Stalion N XXIX “A”line

The beginning of the oldest line in our country is established in 1925 with the import from Hungary of stallion **Zdravko** (Nonius XXIX-6) and 4 mares. Along with his mother, the mare Nonka (Nonius XXXIII-6), a foal is also imported - the future stallion Nish XXIX-7, son of Nonius XXXIX. In 1950 **Vit** (Nonius XLIII-4), **Dunav, Titan** (Nonius XLIII-6), **Durtsash** and **Factor** are imported from Hungary; in 1956 from Czechoslovakia - **Lider** (Nonius XXX–1); in 2004 **Matroz and Mester**, and in 2016 - **Rablot.** Thus, at the moment of the analysis, out of 6 active lines, 4 are from line A. Thus, the line is most widely represented in the Danubian horse, as well as in the Nonius breed in Hungary.

1.1 Stallion Zdravko /Nonius XXIX-6/ line

1.2 The stallion Zdravko line has been developed up to the VI genealogical level (fig. 2). The foundation sire was foaled in 1922 at Mezohegesh Stud and is imported from Hungary at the age of 3. 22 sons are obtained from him (Karaivanov, 1975), but in the next four generations the line developed through the line Zritel-Zund-Zlin-Zar. In the V generation, an attempt is made to expand, but in the VI generation, there is again only one extension of the line - Zdravko.

Already from the I generation, the consolidation of the stallions of the line begins (Fig. 3). In the II generation, the average change in parameters is proportional to the foundation sire's parameters, but Zund's height is 4.5 cm greater than Zdravko's, and body length and chest girth - by 10 cm each. That lead to a priority increase in stretch and massiveness, and the tendency is preserved in the next generation as well (Fig. 3). The mares of the line involved in the maintenance of the family structure of the breed are not significantly different in height and body length from the stallions, the average difference being less than one percent (Fig. 4). Stallions have a significantly ($P<0.05$) more well-developed chest and more massive bones.

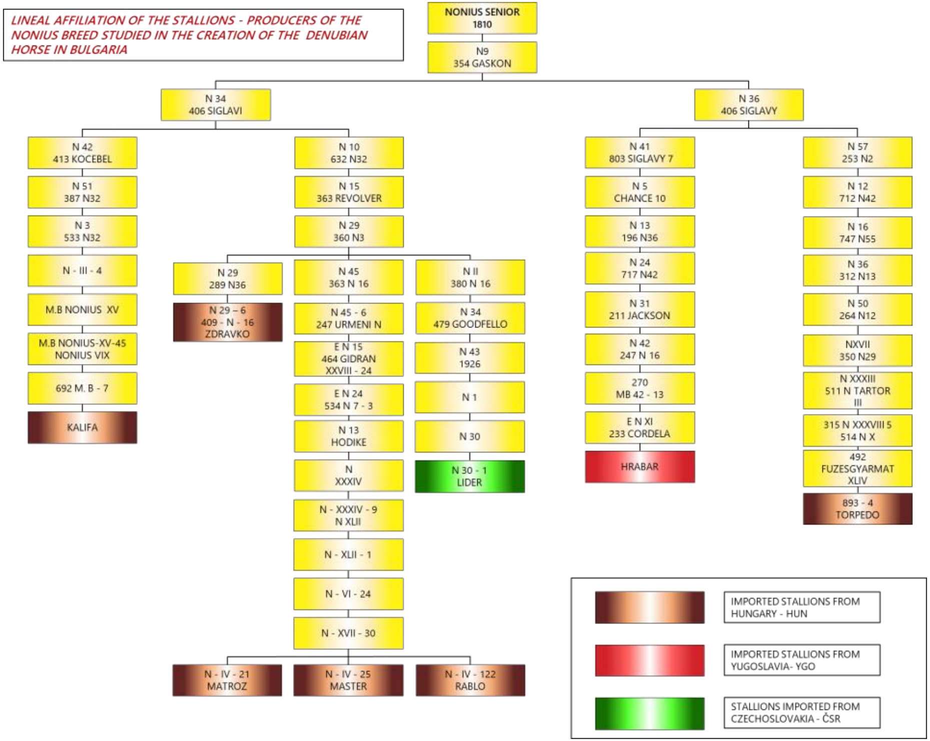


Fig. 1. Lineal affiliation of the stallions of the Nonius breed, which participate in the creation of the Danubian horse breed

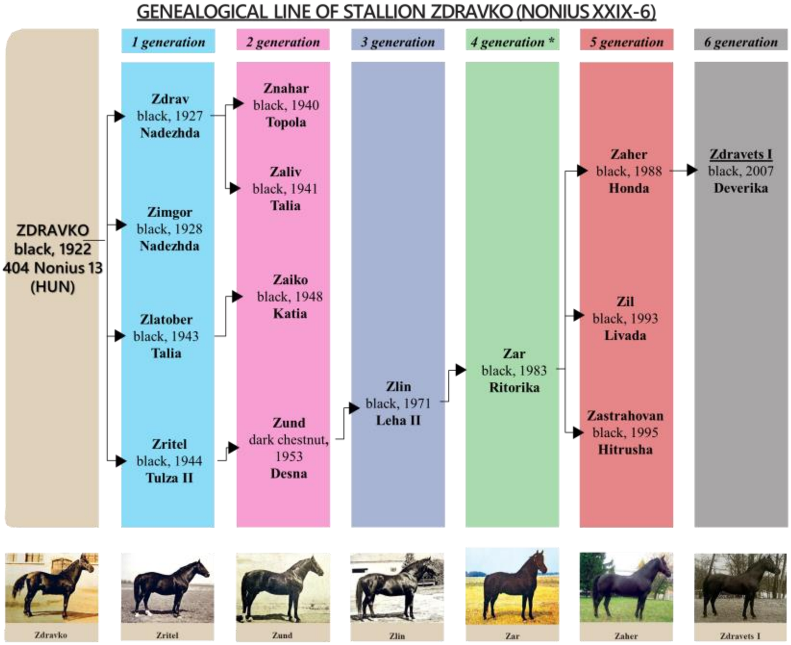


Fig. 2 Genealogical line of stallion Zdravko (Nonius XXIX-6)

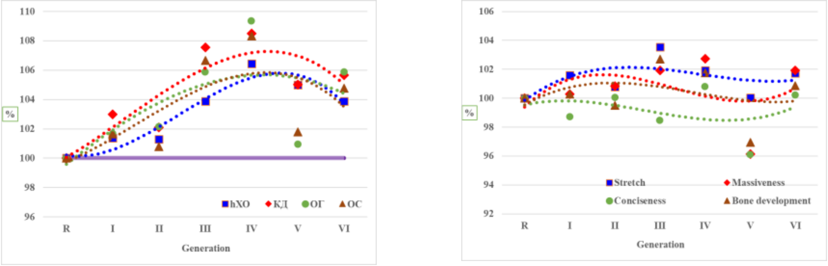


Fig. 3 Dynamics of exterior dimentions and constitution indices in the ancestors of the Zdravko line (Nonius XXIX-6), % compared to the foundation sire.

The stallions of the Zdravko line significantly contribute to the development of the family structure of the breed: Zar is the father of Ziha, Zahra, Zohena and Znaharka – continuing the family of the mare Mara Yu, of Zlatarka and Zakachka 2 of the family of Zhandarma, of Zlataritsa from the family of Leska II. Zaher's daughters Zahira I and Zlatka I are in the I generation of the newly formed family of Homotka, and Zahira continuing the family of Zhandarma. Zil's daughter - Zetka I, from the VI generation of the mare Katya - have 2 descendants in the VII generation - Teresa I and Lizabeth.

Zastrahovan is the sire of the mare Zakana I – continuing the family of Mara Yu in the VIII generation. Zagalo is the sire of the mare Zakrila – the foundation mare of a new family in the breed. Two daughters of Zdravets I - Zahara and Zaha, continue the family of the mare Stefa in the VII generation.

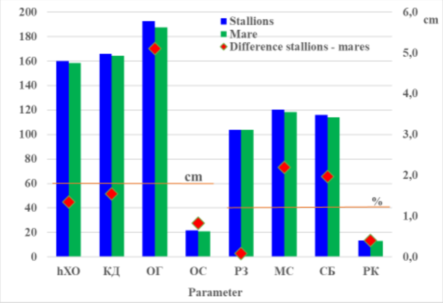


Fig. 4. Exterior measurements and conformation indices in stallions and mares from the Zdravko line (Nonius XXIX-6) included in the genealogical structures of the breed

The Zdravko line can be saved with purposeful work. Currently, one stallion continues the line, but there is preserved cryopreserved semen from his sire as well. There are only 6 representatives of the line, but they are from 5 families – Zhandarma, Stefa, Mara Yu and Zakrila with one mare each, and Homotka - two, and they are daughters of 4 stallions - Zaher - three and one each of Zagalo, Zastrahova, and Zdravets I. More or less blood from the line have 69 out of a total of 113 mares, active at the time of the analysis, including 23 with more than 25% of the bloodline.

1.2 Stallion Lider /Nonius XXX-1/ line

Lider is the only foundation sire in the Danubian breed lines imported from Czechoslovakia. The stallion's mother and father are imported to Czechoslovakia from Hungary. In our country, Lider is imported in 1956, and it is the only purebred Nonius imported to our country, which refers to the most typical representatives of the large Nonius (Karaivanov, 1975). The line is developed up to the VII generation (Fig. 5). Out of Lider's numerous progeny,

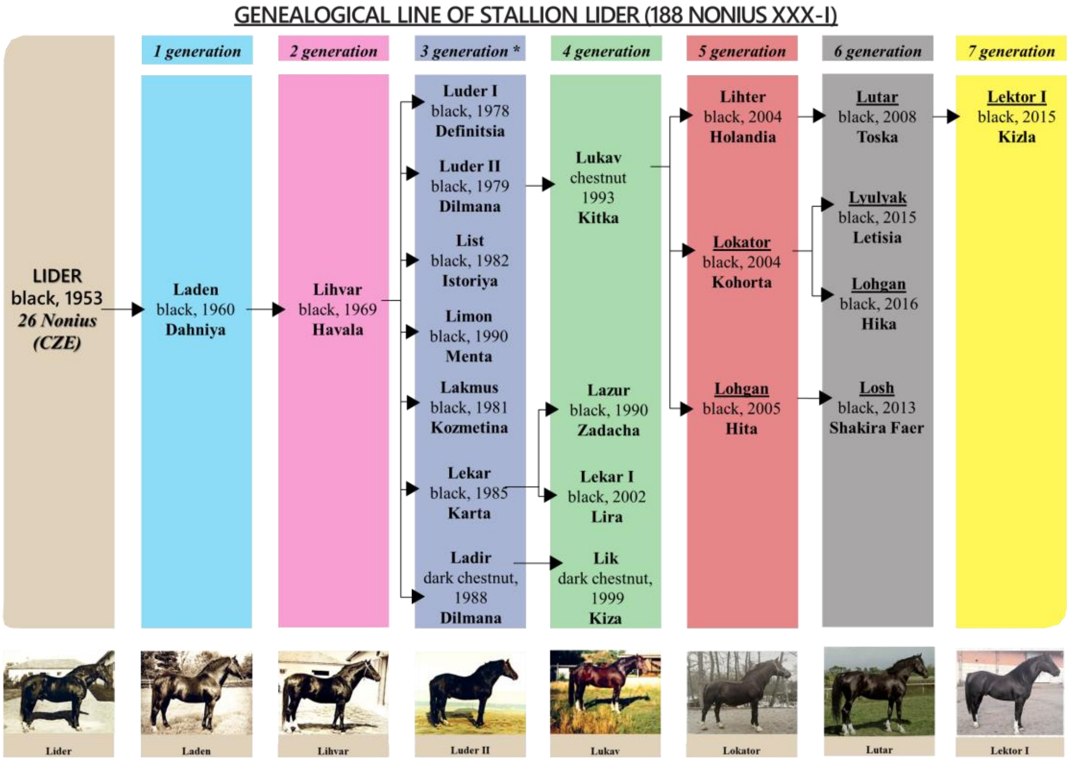


Fig. 5. The genealogical line of stallion LIder (188 Nonius XXX-I)

at present, of importance to the breed are his daughters: Ludenita I, foundation mare of the powerful branch of Mara Yu's family, to which belong 20 of all 22 living mares of the family; Ludenita, to whose branch belong all modern mares of the mare Katya family; Ladna and Lekha II – branches of the Fraila family to which 11 of the 16 current representatives of the family belong. Out of a total of 16 sons of Lider used as sires (Karaivanov et al., 1989), and only stallion **Laden** continues the line, and in the next generation - his son **Lihvar**. In the III generation, an attempt is made to extend the line, but to this day only descendants of **Luder II** remain.

Unlike the Zdravko line, in the Lider line, some of the exterior parameters of the foundation sire are largely preserved. The body length and withers height gravitate around the line of the foundation sire, with certain, inconsistent deviations in individual generations (fig. 6.). The rest of the parameters have lower values. More importantly, with Lider, after the initial more considerable dynamics (in the II generation), the average values of the exterior parameters change in parallel. This leads to the preservation of body proportions. In terms of body stretch, the trend is similar to that of Zdravko - increasing.

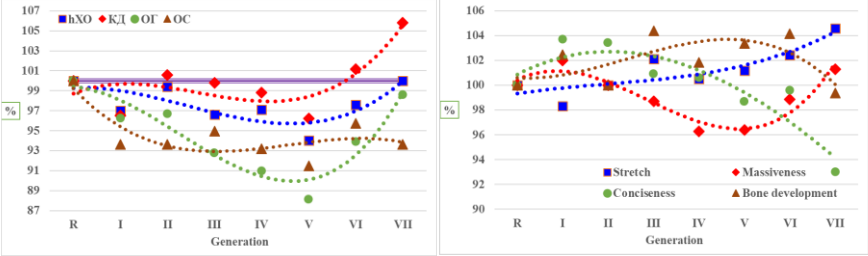


Fig. 6. Dynamics of exterior dimensions and conformation indices of the Lider (Nonius XXX-1) line

In the last two generations, after the use of smaller stallions in the V generation, a certain rounding up is noticed. The VI and VII generations of the line are still in development.

The mares forming the family structure of the breed are smaller than the stallions (Fig. 7). The most significant difference is the chest girth - 10.5 cm ($P<0.01$), but the other differences are also reliable - withers height 2.19 cm ($P<0.05$), body length - 4.94 cm ($P<0.001$) and by the cannon bone girth – 1.05 cm ($P<0.001$). Due to the mentioned differences, stallions are more elongated ($P<0.001$) and more massive ($P<0.05$) than mares, with better developed bones ($P<0.001$).

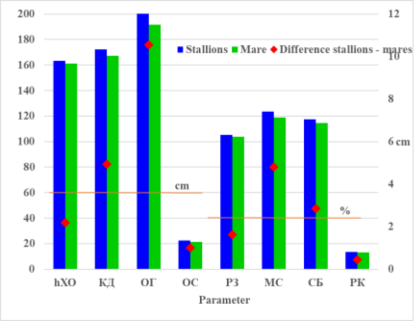


Fig. 7. Exterior dimentions and conformation indices of stallions and mares of the LIder line ((Nonius XXX-1)) included in the genealogical structures of the breed

The Lider line has significant potential for development. At the time of the research, 5 stallions are available, as well as 23 mares, daughters of sires from the line, in all families: Mara Yu, Katya, Fraila, Zhandarma and Hala - 3 each– 2, Leska – 2, and Serdika, Stefa, Homotka, Klara, Zakrila and Deverika 1 each.Out of 113 modern representatives of the breed, 80 have blood from the line, and in addition to 23 mares from the line (bloodline ≥ 50%), another 22 have bloodline ≥ 25%.

1.3 Stallion Erno /Nonius XVII-30/ Line

Erno's line is formed by his sons Matroz N IV-21 and Mester N IV-25, imported by National Horse Breeding Association in 2004, and Rablot N IV-122 imported in 2016 (fig.8). **3665 Nonuis XVII-30 Erno /N IV t m/** was foaled in 1996 at Mezohegesh Stud. On his mother's side, Erno has blood from the thoroughbred English stallion Anblick.

The II generation of Erno's family is formed by the sons of Matroz and Mester - **Metezh and Matador**, and the third - by the sons of Matador **Momento and Maklaran**.

It is characteristic of Erno's line that it is extremely smooth as an exterior. In the first two generations, the exterior parameters fluctuate around those of the foundation sire with deviations of up to 2% (Fig. 9). The only major deviation is in the range of the cannon bone which already in the I generation grows by 1.5 cm, but does not change after that. The stallions of the last generation have a longer body length and reduced chest girth, but the difference with the previous generation is insignificant. The weak dynamics of the exterior parameters, do not change drastically the body proportions (Fig. 9), with the exception of the bone development index.

At present, the line includes 11 mares - 3 each in the family of Mara Yu and the newly formed family of Homotka; in the other new family - of the mare Hala and one each in the families of Stefa, Leska II and Zhandarma.

Fig. 8. Genealogical line of stallion Erno (Nonius XVII-30)

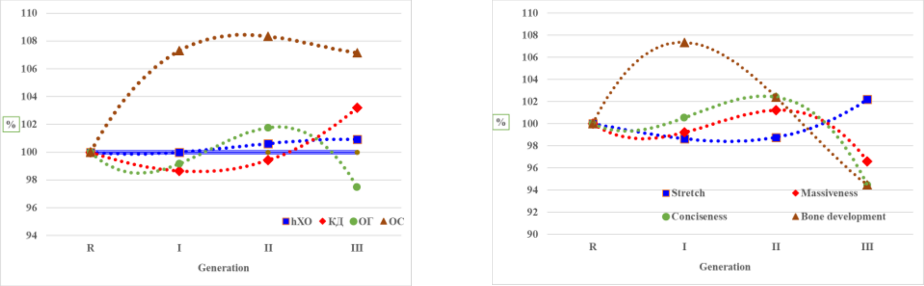


Fig. 9. Dynamics of exterior dimentions and conformation indices in the offspring that continue the line of the stallion Erno (Nonius XVII-30).

Erno's line has potential for development, but the genealogical base needs to be expanded, due to the high genetic similarity and inbreeding in the line, evident from a structural pedigree of modern representatives of the line (Fig. 10).

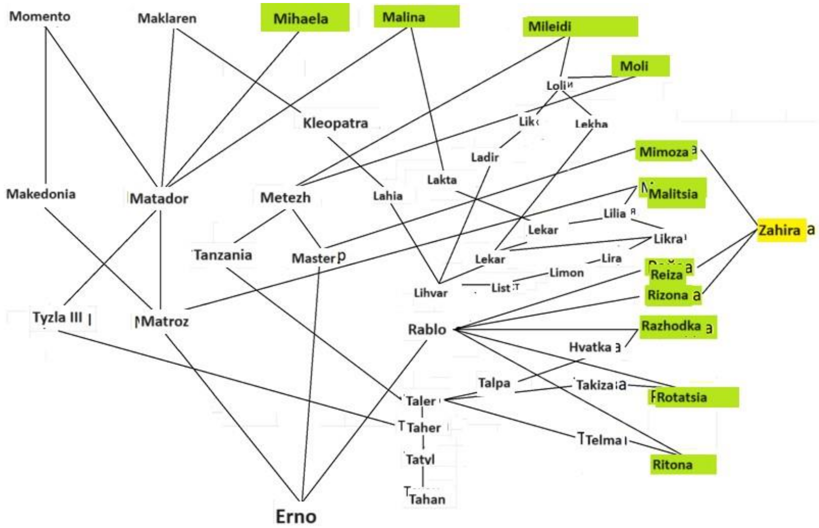


Fig. 10. Structural Pedigree of Modern Representatives of the Stallion Erno Line.

Stallion N XXXI "B" Line

The beginning of the "B" line in Bulgaria is set with the import of the stallion Novak /Nonius XXXI-13/ from Hungary in 1924. Later, from this line, from Yugoslavia are imported stallions Hrabar /E. Nonius XI-26/, Iskar /K. Nonius XVII-11/, Hercules /E. Nonius XI-30/ and Doiran /E. Nonius XI-5/. Hrabar and Iskar extract genealogical lines. The influence of the other three stallions is only through the maternal composition in the breed. In the initial stage, Iskar's line develops well, but a suitable offspring to continue the family of the foundation mare are not found and by the 2nd generation the line became extinct. Today, line "B" is successfully developed only through the descendants of stallion Hrabar.

1.4. Stallion Hrabar /E. Nonius XI-26/ line

Hrabar was foaled in 1935. Prof. Karaivanov (1975) notes that Hrabar has a "very harmonious body" and in terms of type fully meets the requirements that are set for the breed. At the present moment, the Hrabar line has been developed to the V generation, with all the stallions starting from the stallion Hisar, from the II generation of the line (fig. 11). After Hissar, in subsequent generations, the line develops with several branches, which ensures its stability.

In the present structure of the breed, along with the significant number of stallions, the line is also widely represented through the maternal input. Stallion Heliosine, is the sire of the mare Hedesa, whose branch is the only one in the mare's family

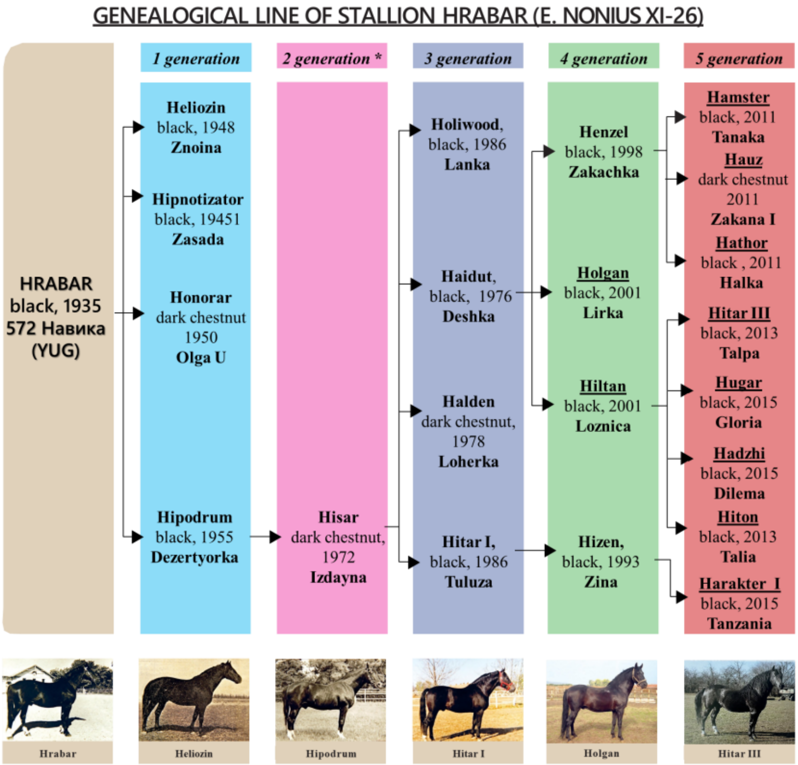


Fig. 11. Genealogical line of stallion Hrabar (E. Nonius XI-26)

Serdica, who has reached the present day. Hippodrum and his son Hissar, are fathers of the mares Hladka I and Helia 70, whose branches are the only ones to continue the family of the mare Katya. The modern structure of the mare Mara Yu family has its roots entirely in stallions from the Hrabar line. Stallions from the Hrabar line are also the foundation sires of all modern branches and the Zhandarma family. The family of Leska II in the V generation is continued only by the mare Hartia I (by Hissar), and is currently represented by 3 daughters of the stallion Hilton. In the family of Fraila, the line of Hrabar is included – later, in the V generation, out off two daughters of Haidut and in the VI - with a daughter by Holgan. In the newly created families, the Hrabar line is represented by 2 daughters by Hizen, in the family of Deverika, and 7 daughters by Holgan in the family of Klara. The stallions from Hrabar's line are the fathers of the foundation mares of two new families in the breed - Homot – out off Homotka and Hlapak – out off Hala. The wide representation of the line is explained by the fact that for fourteen mating seasons at the stud farm, only Hissar stallion has been used as a sire.

In the development of the Hrabar line, initially there is an aspiration for enlargement, which is most pronounced in relation to the "volume" of the body, expressed by the girth of the chest (Fig. 12.).

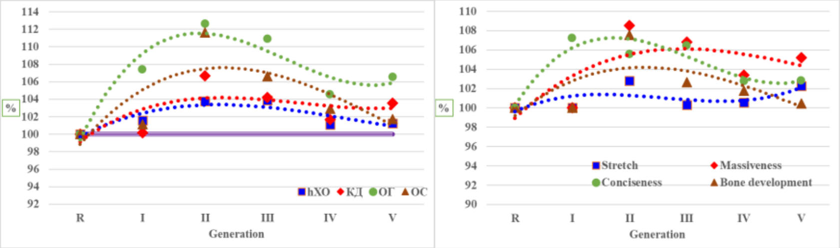


Fig. 12. Dynamics of exterior dimentions and body constitution indices in the offspring that continue the line of stallion Hrabar (E. Nonius XI-26).

In the subsequent generations, the parameters gradually return to those of the foundation sire of the line, being slightly higher. The modern representatives of the line are more elongated and shorter than the representatives of the I generation, but the differences are insignificant (Fig. 12.). The difference in massiveness is bigger in between them, as well as compared to the foundation sire of the line. Stallions and mares do not differ significantly in appearance (Fig. 13), but the differences in some parameters, although small, are reliable. Stallions are significantly longer (P<0.01), with a better developed chest (P<0.01) and more massive bones (P<0.001). Their bodies are more stretched (P<0.05), more massive (P<0.01) and with a better developed bone system (P<0.001).

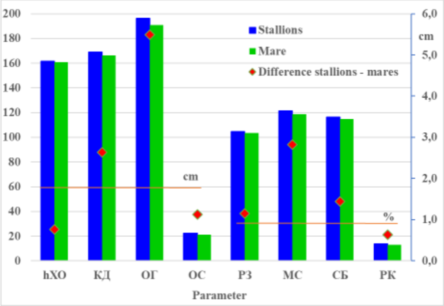


Fig. 13. Exterior dimentions and body constitution indices of stallions and mares from the stallion Hrabar (E. Nonius XI-26) line

In general, at the present time the line of stallion Hrabar can be defined as the main line in the breed. It is the basis of the development of 8 of the 12 modern families, there are 10 stallions and 23 mares active in 7 families.

N XXXIV „C” Line

From the "C" line, in our country, Rudnik, Record, Titan and Torpedo are used as breeding stallions. All are imported from Hungary - Rudnik in 1935, and Record, Titan and Torpedo in 1950. 2 lines are formed - Record and Torpedo. The first one is extinct in the III generation. Thus, at present, line "C" in our country is represented only by the line of stallion Torpedo.

1.5. Stallion Torpedo line

Torpedo was foaled on 22.03.1944. In our country, he is included in breeding the following year after import, as part of the stud farm "Clementina". The line is developed up to the VI generation (Fig.18.). After the I generation, 2 or more stallions are left as possibly suitable to continue the line, but subsequently the development is by one stallion - Tahan→Tatul→Teheran→Taler.

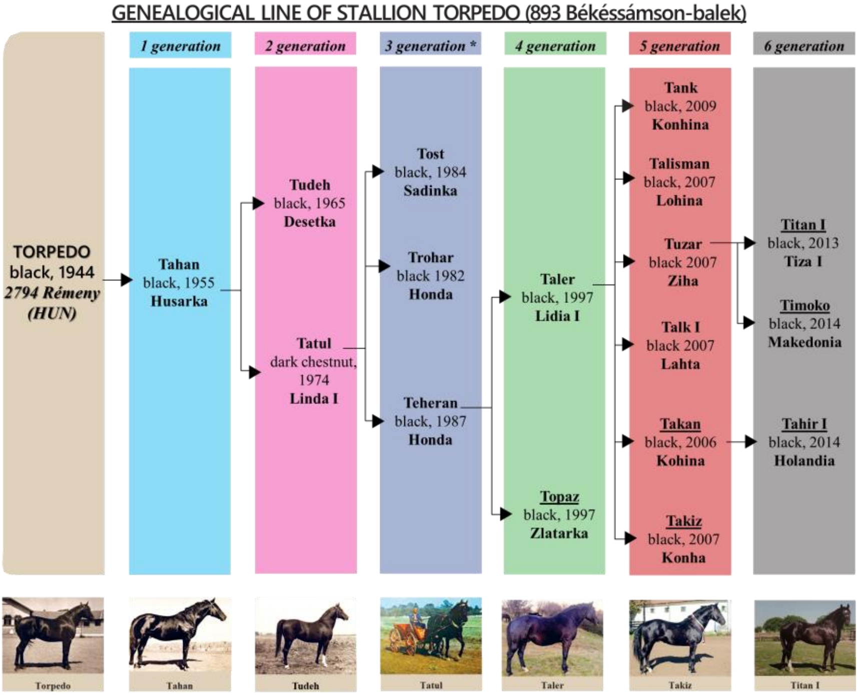


Fig. 14. Pedigree of stallion Torpedo (BÉKÉSSÁMSON-BALEK)

For the development of the line and the breed, in general, the following are essential: stallion **Tatul,** from whose 2 daughters Tosca and Tresca are the only modern representatives of the mare Fraila family; **Teheran**, which is highly overbearing in exterior. His daughter

Tiha continue one of the two modern branches of the mare Katya's family; Tanaka and her daughter Litva II are the only living representatives of Serdika Yu's family; Turka continues the family of the mare Stefa, through the branch of the mare Kala; Tanzania continues the primary branch of the mare Nozharka, in the VIII generation of the mare Zhandarma family.

At the current stage, the line also has representatives in the families of: Katya (via Talc I); Mara Yu- 4 daughters of Taler and one each of Talc I and Talisman; Fraila- 2 daughters by Topaz; Zhandarma- 4 daughters by Takiz and one by Takan; Leska II - two daughters by Taler, and all modern representatives of the family are descendants of the Tudeh stallion. In the new families the line is represented by one daughter each of Takan in the families of Deverika and Zakrila; one daughter each by Tahir I in the families of Zakrila and Hala.

The stallions from the Torpedo line are extremely consolidated in exterior from the III to VI generation, with the only exception being the stallion Timoko, who is larger. In general, after a certain reduction in the length of the body - up to the II generation and an increase in cannon bone girth up to the III, significant fluctuations in the external measurements of the stallions are not observed (fig. 15.). This also leads to the preservation of body proportions in the line. Generations differ significantly only in the bone development index (P<0.05).

The differences in exterior measurements and body proportions of stallions and mares from the line are small, but some of them are reliable (fig. 16). The biggest difference is in breast girth, where stallions outperformed mares by 5.7 cm (P=0.001).

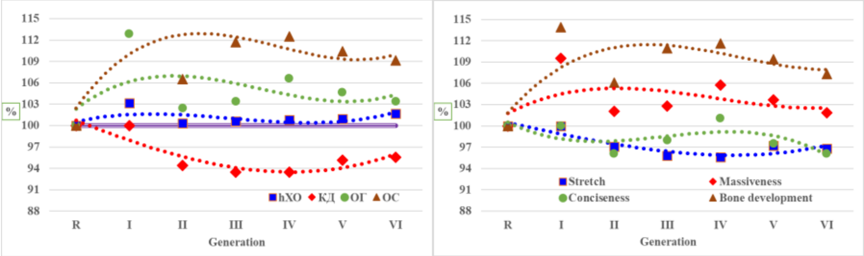


Fig. 15 Dynamics of exterior dimensions and conformation indices in offsprins that continue the line of stallion Torpedo (BÉKÉSSÁMSON-BALEK)..

A reason for the high evenness of the line is probably the high genetic similarity and level of inbreeding evident from the structural pedigree of the last generation (Fig. 17)

At this stage, inbreeding, which is not at strikingly high levels, has no negative consequences, but the process must be monitored.

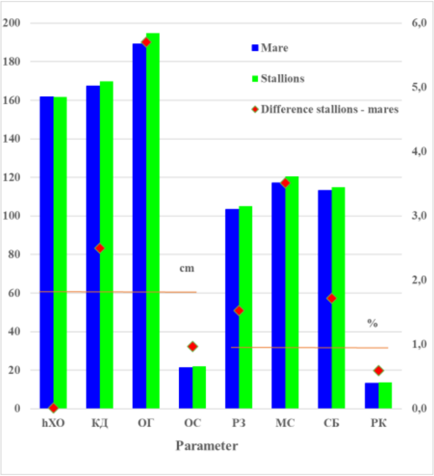


Fig. 16 Exterior dimensions and conformation indices in stallions and mares of the stallion Torpedo line (BÉKÉSSÁMSON-BALEK).

extremely carefully and at the first manifestations of inbreeding depression to look for sources of diversity.

In general, the line has good prospects for development. In addition to the number of stallions, the line has 23 broodmares. Another 12 mares have ≥25% blood.

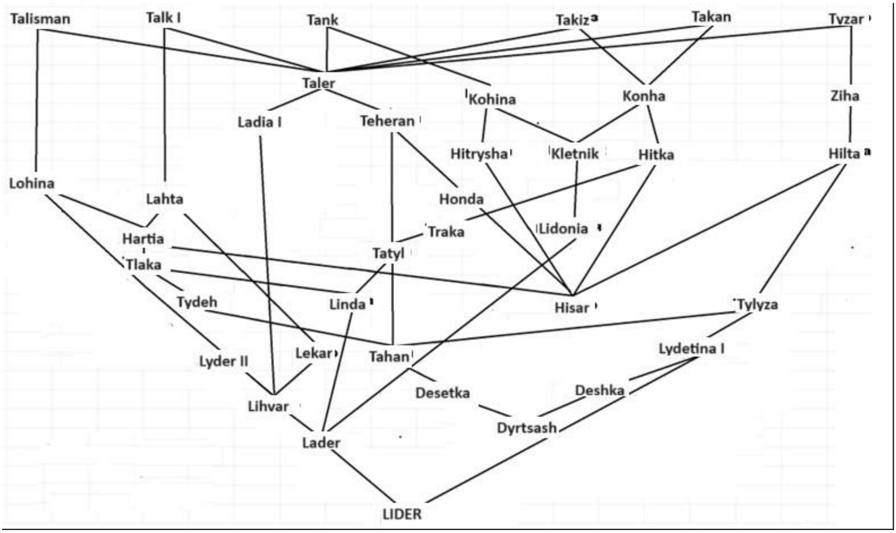


Fig. 17 Structural Pedigree of the V Generation of Stallion Torpedo (BÉKÉSSÁMSON-BALEK) Line.

Line of Stallion N IX through N III Line "D"

From the line D of the Nonius breed, which is formed from a branching of N IX through N XXXIV and N III, only stallion Kalifa is used in the breeding activities in our country. It was imported from Hungary in

1950. This is the last import before the breed is officially recognized in 1951.

1.3. Stallion Kalifa Line

At the beginning of the present study, the line of Kalifa, known in our country under the name Kalif, is developed to the V generation (Fig. 18.). The first two generations are described by Karaivanov (1975), Karaivanov et al. (1989), Burzev et al. (2007).

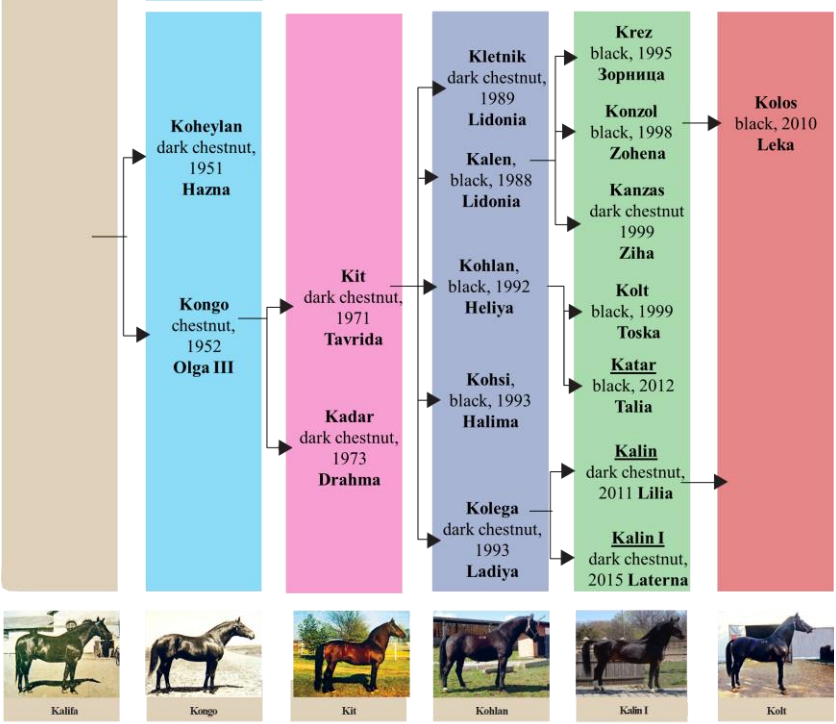


Fig. 18. Genealogical line of stallion Kalifa (Kalif)

The stallions of the **III generation** were foaled at the Georgi Dimitrov stud farm, but were used as sires after 1989, mainly in the private sector. An exception is the stallion Kletnik, who is used at the stud farm from 1995 to 1999.

The III generation is represented only by five sons of the stallion **Kit**. Kit also left a large number of daughters forming the family structure of the breed. One of the two branches of the family of the mare Katya - of Helia 70, continues only through Kit's daughter - Kohorta. Out off Kit's daughters Klima and Kala, two of the three modern branches of the mare Stefa family develop. Daughters of Kit form separate branches in the families of: Mara Yu- Cleopatra; Fraila- Kuta; Zhandarma - Colona. Through Kit-Kiza's daughter, the only surviving branch of Serdika Yu developed.

From the III generation, the most important are Kletnik, Kolega and Kohlan, which form their own branches. Kletnik is used for breeding at the stud farm for 12 years. In addition to the sons, the main section of the pedigree book records 6 of his daughters - three continues the family of the mare Mara Yu. Kletnik's daughters Kohina and Konha are mothers of three offsprings that continue stallion Torpedo line, in the V generation. Kolega is the most actively used stallion of the line, in the private sector. 20 horses are registered from him, of which, in addition to his sons, 5 daughters are left for breeding - three - continue the family of the mare Fraila, the other 2 are modern representatives of the two separate branches - Vezhda and Nozharka, in the family of Zhandarma. **Kohsi** is the father of the mare Klara - the foundation mare of a new family in the breed.

The IV generation is formed by 7 stallions from three branches. From the first branch - of Kletnik, male progeny in the V generation is left only by stallion **Konzol**. He also leaves 3 daughters - one in Fraila's family and 2 in Katya's. **Kanzas** leaves one daughter, Kegla, who is a maternal grand-daughter of the English thoroughbred stallion Gadular. She leaves 3 daughters - in the XI generation of Zhandarma's family. **Krez** leaves 11 foals, with 2 of his daughters forming the I generation of the newly created mare Klara family.

Kohlan's sons Kolt and Katar are out of mares from the Torpedo line. Like Kletnik's sons, they are similar in appearance, with Katar being slightly larger. In terms of body proportions, the two stallions are identical. The Kolt has 42 foals registered. In the main section of the pedigree book, two of his sons are recorded - Kalofer out of mare Lucretia and Kadir out of mare Doza and two daughters - Kohorta - Iskar, the family of the mare Mara Yu continues; Kalifornia is in the II generation of the newly created family of Homotka I. **Katar** has 6 registered horses as two males and two females are recorded in the pedigree book of the breed. His daughter Kohezia is the only representative in the VII generation of the mare Mara Yu family, from the primary branch of the mare Deshka.

Kolega's sons Kalin and Kalin I are out of mares from the Lider line. They are relatively similar in exterior measurements, but differ in body proportions. **Kalin** is a typical representative of the breed, with a specific temperament. His sons are recorded in the main section of the genealogical book. Katan (out of Thalia) and Kolgan (out of Lidia I). Two of his daughters are part of the I generation of the new family of mare Zakrila, and the third - Katy, is currently the only representative of the family in the II generation.

Kalin I (Kolega - Laterna) was foaled on 08.03.2015. In 2018, he is placed under contract at Hortobagy Stud in Hungary for two mating seasons. Currently, the stallion has no registered offspring in Bulgaria.

Stallions of the V generation are still young. Kolos has one registered daughter and Katan - a male horse out off Lidia I.

In terms of exterior dynamics, the Kalifa line does not differ significantly from the rest of the Danubian horse lines. Initially, there is a desire to consolidate, and then to retain the achieved desired parameters (Fig. 19).

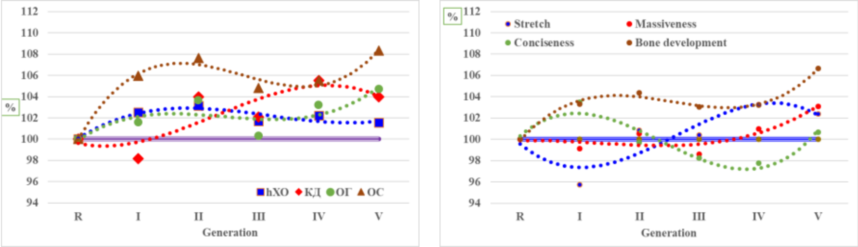


Fig. 19 Dynamics of exterior measurements and conformation indices in the offspring that continue the stallion Kalifa line

In the line, the most significant is the increase in the massiveness of the bones, while the length and volume of the body do not change significantly. The height in the II generation even decreases, and then increases, with which the proportions of the conformation of the III generation are stabilized. Stallions of the last generation are slightly more massive and compact, compared to the foundation sire of the line, but with better developed bones.

Stallions are larger than mares (Fig. 1.6.3.), but only the differences in breast girth (5.34 cm, P<0.01) and cannon bone (0.98 cm, P<0.001) are significant. This resulted in significant differences in mass (P<0.01) and bone development index (P<0.01).

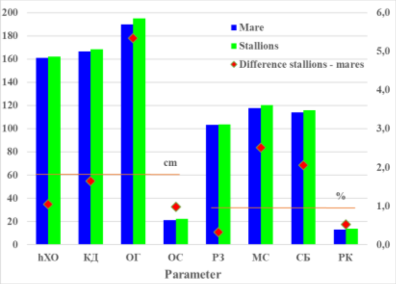


Fig. 20 Exterior dimensions and conformation indices in stallions and mares of the stallion Kalifa line.

The Kalifa line has significant development potential. Besides 4 stallions that can lay the beginning of new branches, there is a significant number of male horses. There are 22 breeding mares, divided into 6 families.

1. Genealogical analysis of the family structure of the Danubian horse breed and its phylogenetic development.

There is a direct relationship between the linear and family structure of the breed and their sustainable development is a guarantee for the existence of the breed. The last comprehensive study of the family structure of the Danubian horse was made by Prof. Karaivanov (1975). In his doctoral dissertation, the author examines in detail the structure of the significant, at that time, families - of mares Nonka and Norvegia from the Nonius breed, as well as Leska II, Zhandarma, Isabella and Alexina - of Bulgarian origin. Later (Karaivanov et al., 1989), the information is supplemented, the development of the families of the mares Nonka, Norvegia, Leska II, Zhandarma and Isabella is examined, the family of Alexina is excluded and two new families are included - of mares Mara Yu and Fraila, imported from Yugoslavia.

Today, the family structure of the Danubian horse has preserved the approach established during the creation of the breed, that the foundation mares of families should only be mares of the Nonius breed, Bulgarian local and local improved mares. After 1990, 5 new families are established, one of mare Deverika, of the Nonius breed, and 4 of improved local mares - Zakrila, Klara, Homotka and Hala. In our study, in addition to the analysis of the development of the mentioned families, we also include previously unresearched families of mares from the Nonius breed, which take part in the first stage of the creation of the breed: - mare Katya, originating from Hungary and mares Stefa and Serdika Yu, originally from Yugoslavia.

1.1. Family of the mare Katya.

Katya is the only mare originating from the Nonius breed, imported from Hungary, whose family continues to this day. The mare was foaled on 03.05.1937. By origin, she belongs to the stallion line N XXXIII, from mother 659 Lina and father 358 N XXXIII-12. Katya is used for breeding from 1942 to 1953 - 12 mating seasons. She gave birth to 9 foals, of which 6 are females. her family is developed up to the VII generation (Fig. 21).. **The I generation** is formed by the two daughters out of the foundation mare - Zakalena and Zakana. In **the II generation**, the family is developed only through the daughters of Zakalena, Difuzia and Dozora, referring to the line of Durtsash. In the III generation there is one mare - the only daughter of Dozora - Ludetina. In practice, after this generation, Katya's line is represented only by descendants of

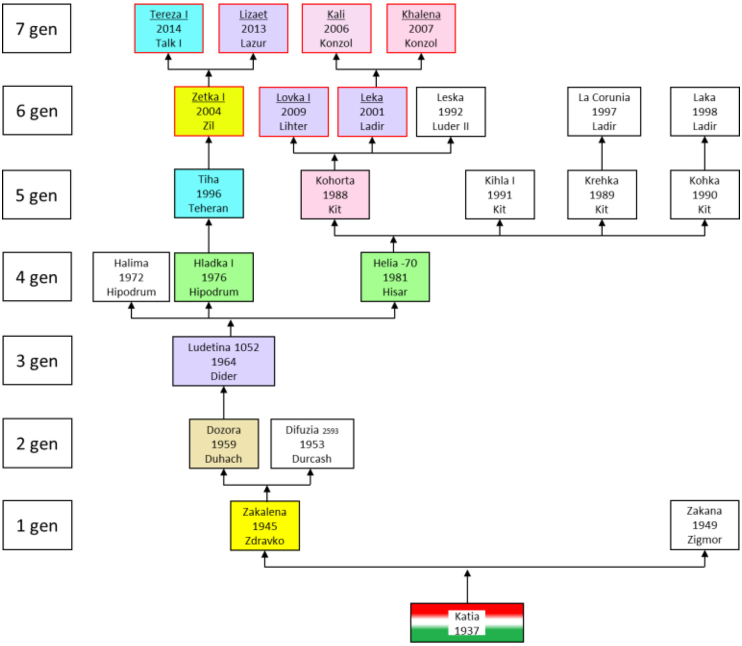


Fig. 21 Scheme of the family of mare Katya.
*Underlined mares are alive at the time of the study

Ludetina who can be identified as a foundation mare of a new family. The offspring that continue the family in the IV generation are daughters only by stallions from the Hrabar line. Two stationary stallions are also left from this selection. In the next two generations, the family expands, but in the 5-th it continues to be on a narrow genealogical basis - 5 mares - originating out of two mothers and by two fathers.

In the V generation, a certain consolidation of the exterior is achieved - the maximum differences are: - body height - 3 cm, body length - 7 cm, cannon bone girth - 0.7 cm. The biggest difference is the chest girth - 9 cm, but this is less than 4.8%. All parameters are higher than those of the foundation mare by about 5% (Fig. 22), and the body proportions are identical to hers.

In the next three generations there is a certain "scattering". The height of the animals is preserved, the body length and the cannon bone girth - increase, and the girth of the chest decreases. As a result, the mares are more stretched, narrower and shallow, with coarser bones. It should be noted, however, that the increase in stretch in the three generations was from 1.5 to 2.94%, in massiveness from -1.61 to -2.29%, in bone development - from 0.42 to 2, 16%.

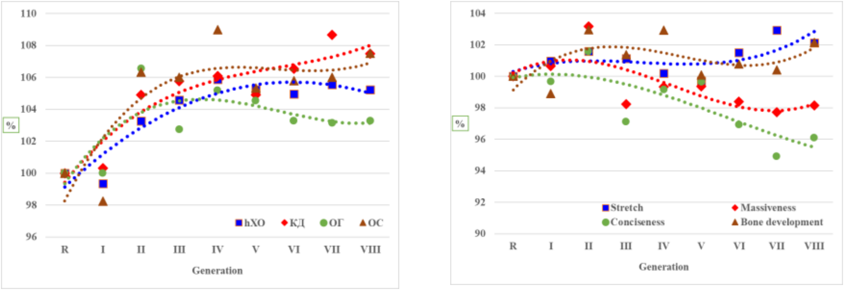


Fig. 22 Generational dynamics of exterior dimentions and conformation indices of mares from the Katya family

In **the VI generation** of Katya's family, in addition to the number, the genealogical base also expanded - 6 mares are left, from 4 mothers and 4 fathers, from two lines. In **the last VII generation**, the family is represented by 4 mares, but 3 of the mares from the previous generation are alive and an increase in numbers is possible.

In general, **regarding the exterior** of the mares of Katya's family, it can be noted that after solidification in the II generation, in the next six, drastic changes in exterior measurements are not observed. The "format" of the body is similar.

The family has good potential for development. Seven mares - 3 from the VI generation and all from the VII-th are alive. The mares are extremely close in exterior parameters. This is a good basis for consolidation of the breed, assuming that these are the optimal parameters.

The family is plastic enough. The mares of the family can be used in schemes for inline breeding and strengthening the qualities of the Kalifa, Lider, Zdravko and Torpedo lines, as well as for maintaining the genetic diversity of the breed by crossing with stallions from all lines, except for the Lider line . (Table 1).

Table 1. Bloodline of the separate lines of the breed in the living mares of Katya family

Mare	Local mare	Line					
		Zdravko	Durtsash	Hrabar	Lider	Kalifa	Torpedo
Cali	0,78	0,78	1,56	6,25	28,13	62,50	
Khalena	0,78	0,78	1,56	6,25	28,13	62,50	
Leka	1,56	1,56	3,13	12,50	56,25	25,00	
Lovkal	1,56	1,56	3,13	12,50	56,25	25,00	
Zetka I	1,56	51,56	3,13	12,50	6,25		25,00
Lizabeth	0,78	25,78	1,56	6,25	53,13		12,50
Tereza I	0,78	25,78	1,56	6,25	3,13		62,50

1.1. Family of mare Serdika Yu

Serdika Yu was foaled in Bulgaria out off mother Siva, a Nonius breed that was imported from Yugoslavia. At the time of import, Siva is pregnant with stallion K. Nonius 41. Serdika Yu is chestnut in colour. According to the parameters of the exterior, it refers to the Small Nonius. She is used for breeding from 1944 to 1955. She gives birth to 9 foals, eight of them by stallion Durtsash.

The first generation is formed by 3 of the daughters of the foundation mare (Fig. 23), but Krasimira is disapproved and removed from the asset of the stud farm. In general, the exterior parameters and proportions of the foundation mare's conformation are preserved (Fig. 24). The greater height comes from Krasimira, which does not influence the family in subsequent generations.

In **the II and III generations**, there is a tendency to increase the height of the mares. In the first, represented by 4 mares, the change in the height of the family comes from the daughters of Heliosin - Hedona and Hedesa. Heliosin is a large stallion, with a height at the withers of 164 cm. In the case of his two daughters, the height is not inherited intermediately, but is closer to that of the father, with Hedesa being shorter than him by only 1 cm.

Breeding activities in the II generation of the family is an example of unsuccessful work. The mares left have a heterogeneous exterior. In the wandering for the selection of stallions, out of 2 mares with a total of 25 mating seasons, 3 female offspring remain, from which, after continuing the same approach in the **III generation**, the family is continued by only one daughter of Hedesa. The daughter of Hedona - **Aheloia**, as a daughter of an English thoroughbred stallion, is the tallest mare in the family. Attempts to somehow strengthen her parameters by insemination with stallions of the English thoroughbred and Hanoverian breeds were unsuccessful. The selection of an English thoroughbred stallion can also be defined as unsuccessful for

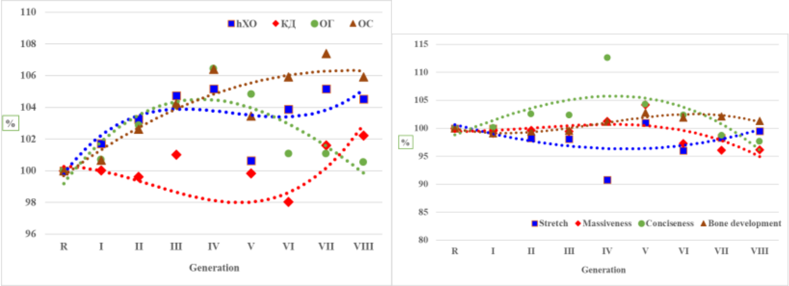


Fig. 24 Generational dynamics of exterior dimensions and conformation indices of mares of Serdika Yu's family

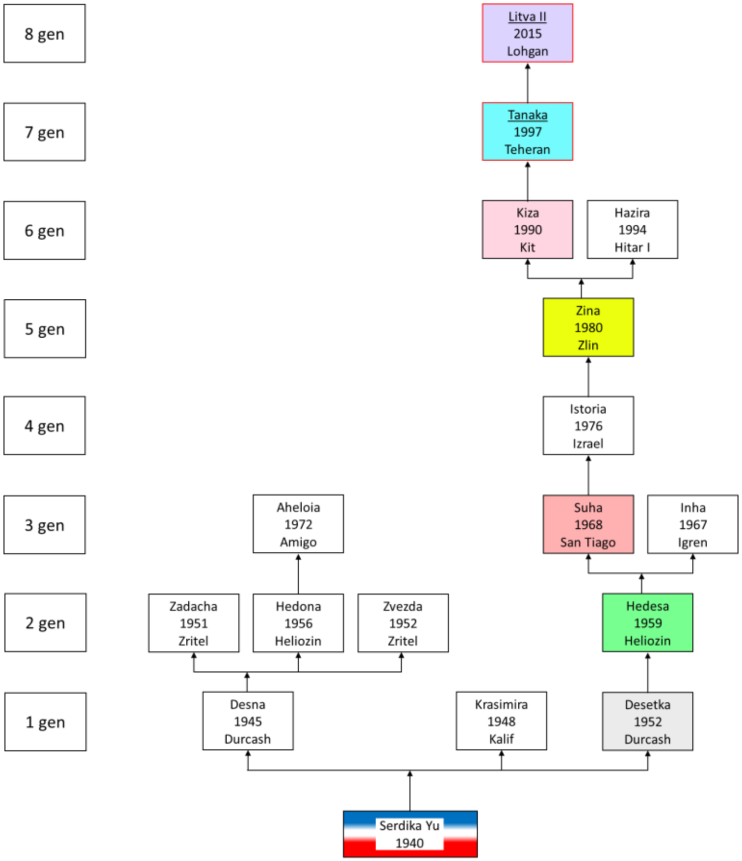


Fig. 23 Scheme of the family of mare Serdika Yu

mare Hedessa. Her daughter **Suha** - in 16 years of breeding activities gives birth to 6 foals, of which 5 are male - all of them disapproved. Thus, from the continued unsuccessful work with the family in the IV generation, there remains one mare Istoria by stallion Israel (Iskar), from whom one daughter **Zina** is left. Combinatorial ability of Istoria with Zlin is bad.

Unsuccessful selection in the IV generation returns the growth in the V to the initial position, but in the last three generations, it stabilizes at about + 5% of the initial one. A significant generational dynamic is also observed in relation to the breast girth - which initially increases, and after the IV generation returns and gravitates around that of the foundation mare. Weaker is the dynamics of the body length, moving around the initial value, and of the cannon bone girth, increasing steadily up to the IV generation, and then remaining at similar levels. As a result of these changes, the mares of the last generations are similar in body proportions to the foundation mare, with a certain bias towards a decrease in massiveness and compactness and an increase in the "heaviness" of the bones.

In general, it can be noted that the work with the family of Serdika Yu was conducted too chaotically. Stallions of different breeds are used in the selection - Nonius, Russian Trotter, Thoroughbred English, Hanover and from a significant number of lines of the Danubian breed (Table 2). It is encouraging that in the last two generations, proper combinations have been found and the exterior parameters are transmitted steadily.

Table 2 Bloodline of the separate lines of the breed in the living mares of Serdika Yu family

Mare	**Serdica Nonius**	**Line**							
		Durt sash	**Hra bar**	**Sant yago**	**Is kar**	**Zdrav ko**	**Ka lifa**	**Li der**	**Tor pedo**
Tanaka	0,781	0,781	1,563	3,12	6,250	12,5	25,5	0	**50**
Litva II	0,391	0,391	0,781	1,56	3,125	6,25	12,5	**50**	25

2.3 Family of mare Stefa

Stefa was foaled in 1934 in Yugoslavia, at the stud farm "Karadjordjevo", out off mare 350 Nula and by sire K. Nonius XXIV. The mare is imported inseminated by K. Nonius XLVII, in 1939. According to the parameters of the exterior, it refers to Small Nonius. She is included in breeding in 1939 and is used for 14 mating seasons, she gives birth to ten foals, of which eight are females. Thanks to Stefa's good breeding activity, the family is based on a broad genealogical basis (the daughters are by 5 stallions), but subsequently developed through 2 branches (Fig. 25). In **the I generation**, six mares are used for breeding, and two of them, daughters of two foundation sires of lines - Zalska by stallion Zdravko and Istina by Iskar, are important for the future development of the family. The mare transmits steadily her traits in her offspring, and at this stage, Stefa's line is practically the only continuing through her descendants.

The **II generation** mares are from 5 different lines – Herzana (Hrabar), Doza II (Durtsash), Faza (Factor), Zaika (Zdravko) and Kitka (Kalifa). On the one hand, this is good, as it reduces the risk of unwanted inbreeding, but on the other hand, the animals of the generation are too heterogeneous in appearance. In general, a consolidation of animals has begun, which continues until the VI generation (Fig. 26).

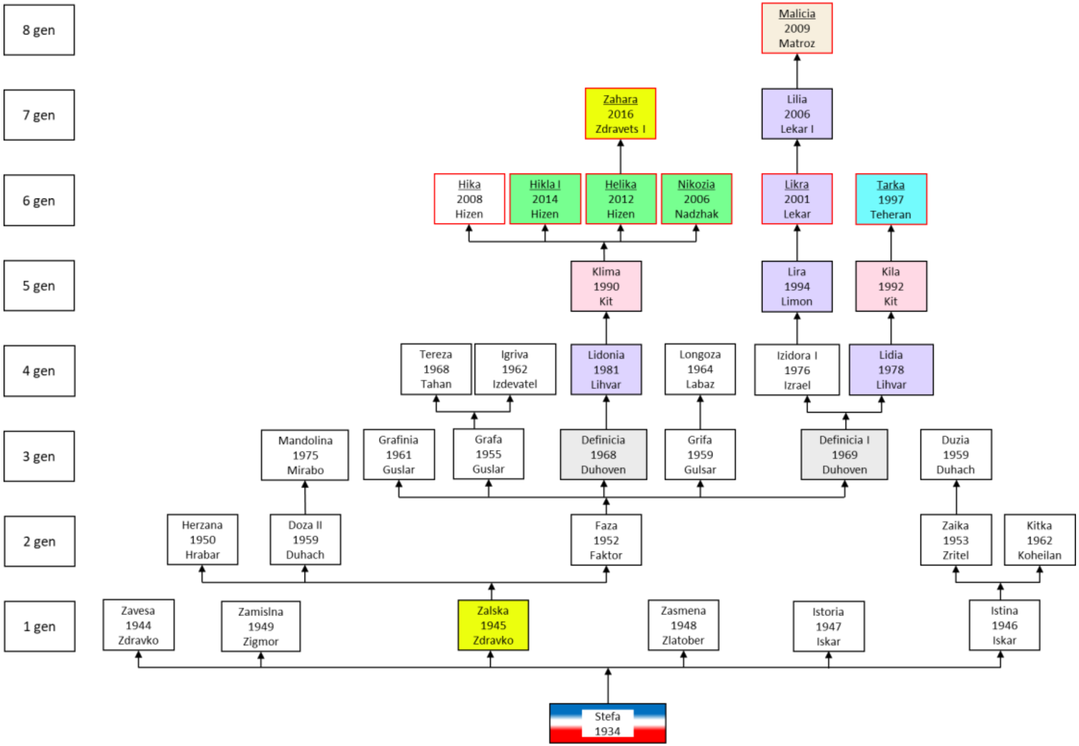


Fig. 25 Scheme of mare Stefa family

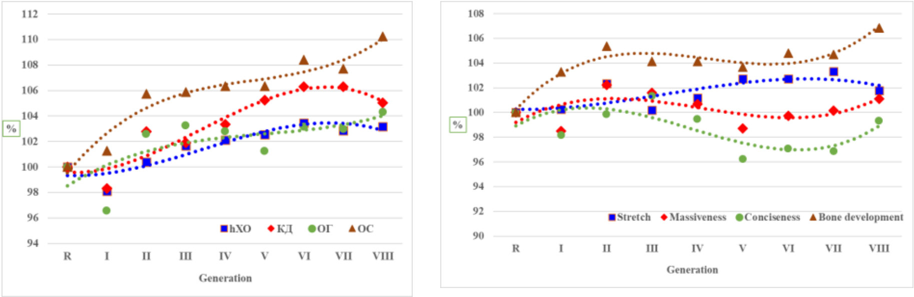


Fig. 26. Generational dynamics of exterior dimentions and conformation indices of Stefa family mares

Initially, mostly enlarged are the bones, but then, within four generations, the cannon bone girth practically does not change. In contrast, the body length increased consistently from the III to the VII generation, compared to a significantly lower increase in height. This leads to an elongation of the body (Fig. 26). The weakest, after an initial increase within 2-3%, is the dynamics of the chest girth. This, compared to an increase in body height and length, leads to a decrease in compactness and massiveness, with the compactness index decreasing more significantly.

The **III generation** is represented by the daughters out off 3 mares – Duzia of Zaika, with whom the branch of Istina ends; – **Mandolina**, the last of the Doza I branch of Zalska and 6 daughters out off mare Faza. Mandolina is a daughter by the English thoroughbred stallion Mirabeau.

In **the IV generation**, both the number of dam mares and the broad genealogical basis are preserved - 6 mares out off 4 dams, but here ends the existence of 2 branches – that of mares Grafa and Griffa. The daughters out off the full sisters Definitsia and Definitsia I - both **Lydonia** and **Lidia**, are similar in appearance to their mothers and to each other. The reason is probably in the stable transmission of heredity from mare Faza, since the father of the two mares, Lihvar (Lider), do not impose his traits, and it is clearly a matter of poor individual combinatorial ability. In **the V generation**, the number of mares is reduced and the genetic base of the family narrowed. It is represented by 3 mares (Fig. 25), and the genetic similarity between Klima and Kala is Rxy=37.5%, and between Klima and Lira and between Lira and Kala - Rxy=9.375%. In exterior measurements, the mares differ significantly, but the desired body proportions have been found, which with slight changes are maintained in subsequent generations. In **the VI generation**, the family is expanded once again - 6 mares are left for breeding. However, the genetic basis remains narrow - three of the mares - Hika, Hikla I and Helica are full sisters (Rxy=50%). They have one half-sister, Nicosia, with whom Rxy is 25%. The four mares are related to the other two, with a genetic similarity of 37.5% with mare Tarka and 9.375% with Likra. Lycra and Tarka are similar to each other - Rxy=2.72%. Mares of the VI generation are equal in terms of exterior parameters and body proportions. Individual variation is within narrow limits. **The VII generation** is represented by two mares, the **VIII -** by one, but both are in the process of formation, as there are living representatives of the VI generation.

The mare Stefa's family has significant **potential for development**. Within the last six generations, the exterior parameters are inherited stably, and the variation is within 4%. The proportions of the body are inherited even more stably from the V to the VII generation. Mares of the family can be used widely in the population but extremely carefully. Likra has 75% blood from Lider, but there is no "blood" from 4 of the other modern lines, and the participation of Zdravko line in the formation of its genotype is insignificant (Table 3).

Nicosia can practically be used for mating with stallions of all lines. Hika, Helica and Hikla I - can be used to reproduce and stabilize the heredity of Kalifa and Lider lines, as well as to maintain genetic diversity by using stallions from the Torpedo, Erno and Zdravko lines for insemination. Zahira can be used in the same way, but without mating with Zdravets I. Malvina can be used for selection with stallions from the lines of Zdravko, Kalifa, Hrabar and Torpedo. The plasticity of the line is extremely high, and the selection will depend exclusively on the selection objectives.

Table 3. Bloodline of the separate lines of the breed in the living mares of Stefa family

		Mare							
		Likra	Nicosia	Hika	Helika	Hikla I	Zahara	Malicia	Tarka
	Nonius	1,563	1,563	1,563	1,563	1,563	0,781	0,391	1,563
Line	Durtsash	6,25	6,25	6,25	6,25	6,25	3,125	1,563	6,25
	Factor	3,125	3,125	3,125	3,125	3,125	1,563	0,781	3,125
	Zdravko	1,563	1,563	1,563	1,563	1,563	**50,78**	0,391	1,563
	Kalifa		25	25	25	25	12,5		25
	Hrabar		0	**50**	**50**	**50**	25		
	Lider	**75.0**	12,5	12,5	12,5	12,5	6,25	43,8	12,5
	TEH		**50**						
	Torpedo								**50**
	Iskar	12,5						3,125	
	Erno							**50**	

2.4. Family of mare Mara Yu

The development of Mara Yu's family up to the IV generation is described in detail by Karaivanov et al. (1989). In practice, the development of the family, of the mare imported from Yugoslavia, begins with her daughter Shumka (Fig. 27). In the following generations, the family is expanded, with mares from three branches formed by Shumka's daughters - Dashka, Deshka and Deshka I reaching the V generation. In the V generation, the branch of Deshka I is interrupted by mare Galina, daughter of the thoroughbred English stallion Garant.In **the V generation**, five of the eight representatives of the family are daughters of the stallion Hisar (Hrabar), four of them out off mare Tuluza (Teheran) and one - out off Zornitsa (Zdravko). Two more of the mares are Hissar's granddaughter - Zahara, through his daughter Holandia and Hazena, through his son Hitar I. In this way, in the mares of the V generation, which leave offspring of the family in the next generation, the bloodline from Hisar's line is from 25 to 50% (Table 4). Saturation continues in part of the mares in the VI generation, with the bloodline content reaching 65.63% - in mare Hilka and even up to 75% - in Hazena II. The remaining mares of Mara Yu's family, to a lesser (3.12%) or greater (up to 37.5%) degree, have Hrabar bloodline.

The mares of the generation are relatively of the same type. They are similar in height and length, so the body stretch index varies within 2.5%. More significant are the differences in chest girth, which affects the indices of massiveness (up to 12.8%) and compactness (up to 14.2%).

In the **VI generation**, the genealogical basis of the family is extended. The eight mares are from 3 established branches of the family - Zornitsa, Holandia and Tuluza and from 4 lines - Hrabar, Lider, Zdravko and Kalifa. This negatively affects the consolidation of the exterior. In the **VII generation**, the family is represented by 11 mares, daughters of 7 mothers and 8 fathers from 4 lines – Kalifa, Lider, Torpedo, Erno, as well as one English thoroughbred stallion. This variety also leads to greater differences in the exterior, compared to the previous generation, but it is lower than observed in the first ones. It should be noted that the estimated variation is based on the initial low variation of the exterior parameters in the family.

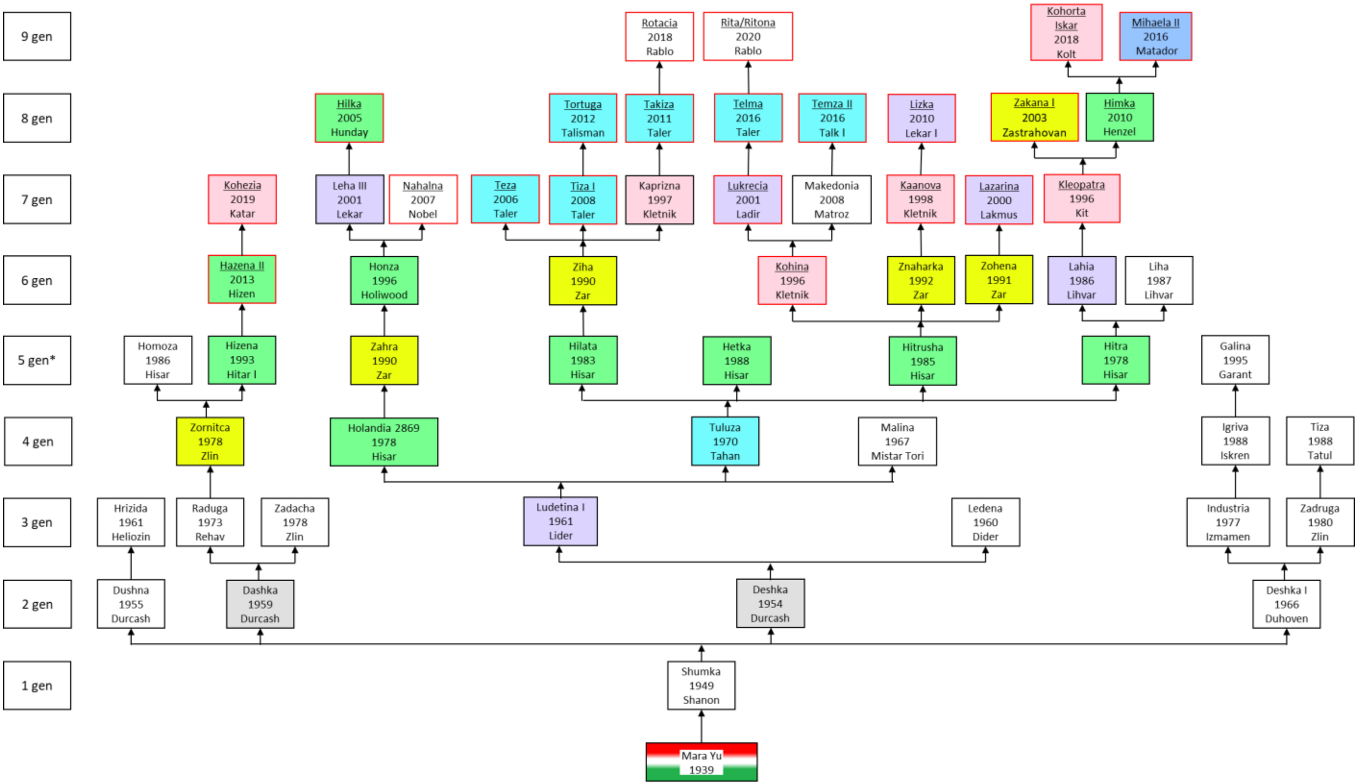


Fig. 27 Scheme of Mare Mara Yu family

Table 4. Bloodline of the separate lines of the breed, in the living mares of Mara Yu family of

Mare	**Shumka**	**Line**								
	Nonius	**Durtsash**	**Rehav**	**Zdravko**	**Kalifa**	**Hrabar**	**Lider**	**TEH**	**Torpedo**	**Erno**
Konhina	3,125	3,125			**50,0**	25,00	6,250		12,50	
Hazena II	3,125	3,125	6,250	12,5		75,00				
Kazanova	1,563	1,563		25,0	**50,0**	12,50	3,125		6,250	
Lazarina	1,563	1,563		25,0		12,50	**53,13**		6,250	
Lukrecia	1,563	1,563			25,0	12,50	**53,13**		6,250	
Teza	1,563	1,563		25,0		12,50	3,125		**56,25**	
Tiza I	1,563	1,563		25,0		12,50	3,125		**56,25**	
Kohezia	1,563	1,563	3,125	6,25	**50,0**	37,50				
Zakana I	0,781	0,781		**50,0**	25,0	6,250	14,06		3,125	
Hilka	0,781	0,781		6,25		**65,63**	26,56			
Lizka	0,781	0,781		12,5	25,0	6,250	**51,56**		3,125	
Himka	0,781	0,781			25,0	**56,25**	14,06		3,125	
Takiza	0,781	0,781		12,5	25,0	6,250	1,563		**53,13**	
Tortuga	0,781	0,781		12,5		6,250	1,563		**78,13**	
Telma	0,781	0,781			12,5	6,250	26,56		**53,13**	
Temza II	0,781	0,781			12,5	6,250	1,563		**53,13**	25,0
Mihaela	0,391	0,391			12,5	28,13	7,031		1,563	**50,0**
Kohorta Iskar	0,391	0,391			**62,5**	28,13	7,031		1,563	
Rotacia	0,391	0,391		6,25	12,5	3,125	0,781		26,56	**50,0**
Rita	0,391	0,391			6,25	3,125	13,28		26,56	**50,0**
Nahalna	1,563	1,563		12,5		31,25	3,125	**50,0**		
Kleopatra	1,563	1,563			**50,0**	12,50	28,13		6,250	

In **the VIII generation**, mare Mara Yu family is represented by 7 mares, which originate from 6 mothers from 4 formed branches - the mares Honza (Hilka), Cleopatra (Zakana I and Himka), Ziha (Tortuga and Takiza) and Hitrusha (Telma, Temza and Lizka), and 4 lines – by Hrabar (2), Zdravko (1), Lider (1) and Torpedo (4). VIII generation mares are similar in appearance. The difference in height at the withers is up to 4 cm, in the cannon bone girth - up to 1 cm, in body length - up to 6 cm. The mares are slightly stretched, massive and compact, with well-developed bones, preserving the body proportions of the previous generation. In the **IX generation** of the family, as of 2022 there are four registered mares. The generation is in formation process.

In general, in terms of the ***exterior, Mara Yu's family*** has developed too dynamically, inconsistently, and it seems that until today, the optimal parameters have not been found (Fig. 28).

With 22 living mares from the VI to the IX generation, the family has significant development potential. Due to the high blood percentage of the individual lines and a high degree of inbreeding, in some of the mares, the selection must be extremely precise and targeted.

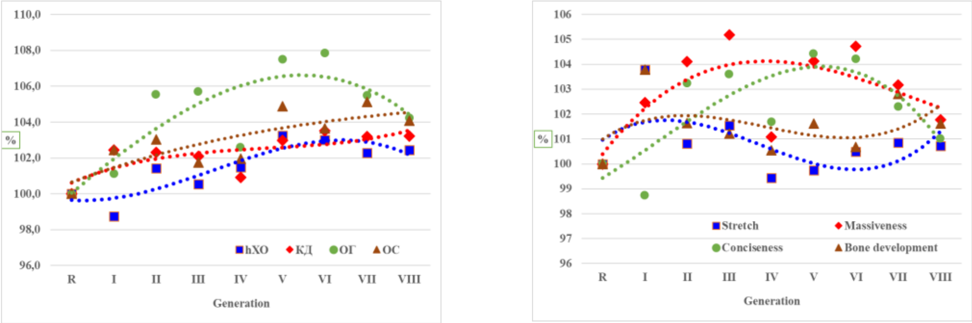


Fig. 28 Generational dynamics of exterior dimentions and conformation indices of mares from Mara Yu family

1.5. Family of mare Fraila

The mare Fraila family is firstly described by Karaivanov et al. (1989). The mares up to the III generation are included, with the exception of the mare Tosca, being however, the main mare that continues the family (Fig. 29). In **the IV generation**, apart from the mares Lotaria I (with which the development of the Moda branch with Mistar Tori ends) and Lazarina (a dead end branch of Zestra) described by Karaivanov et al. (1989), 5 more mares are left in the following years.

In the **V generation**, the family includes 5 mares. Lolita is the last mare from the branch of Tendency I. The remaining 4 are out off 3 mothers - Kuta, Laterna and Litva I, and by two fathers - Haidut and Kolega, whose father is the grandfather of Kuta's daughters - Hanka and Hokla maternally. Because of this, the genetic diversity in the V generation is greatly reduced (Table 5.). The genetic similarity between Hanka and Khokla is 50%, between Clementina and Kalina II - 34.35%, and between Hanka and Khokla with Clementina and Kalina II - 9.63%. The selection of Kuta with Hajdut is an attempt of homogenous selection that turns out to be unsuccessful. Their two daughters are shorter than their parents, with less developed chest and bones. The combination of mares from the Lider-Laterna and LitvaI lines with Kolega from the Kalifa line is more successful, as a heterosis effect is observed according to certain signs.

In the last **VI generation**, an attempt is made to expand the genetic diversity. Although the five mares are out off 4 mothers and by 4 fathers from 4 lines, there is no particular effect. The most significant diversity is brought by stallion Topaz, whose grandfather - Tatul is found in the 3rd genealogical line of mares of the VI generation. Among the others - the father of one of the mares - Holgan is a son of the grandfather of two others, and stallion Konzol - the father of Kihana is the nephew of Kolega, who is the grandfather of 3 of the 5 mares in the generation

In contrast to most families, in which a tendency to massiveness is observed, in the Fraila family, the tendency is a reverse one (Fig. 30). The more significant increase is in the cannon bone girth from the I to II generation, but in the next generations the parameter decreases by more than 4.5%. The height at the withers after the II generation decreases within 2-3%. Mares of the VI generation are more equal than the V one, especially in terms of height, but in other measurements the differences are more significant.

As of 2022, the family is represented by a sufficient number of mares, but due to the narrowed genetic diversity, selection must be conducted with extreme care. Potential for increasing genetic diversity are the lines of Erno and Zdravko, with whom the family has no blood relation (Table 5). At this stage it is advisable to stop using stallions from the Kalifa line. Stallions from the Hrabar line can be used in selection with mares from the Laterna branch.

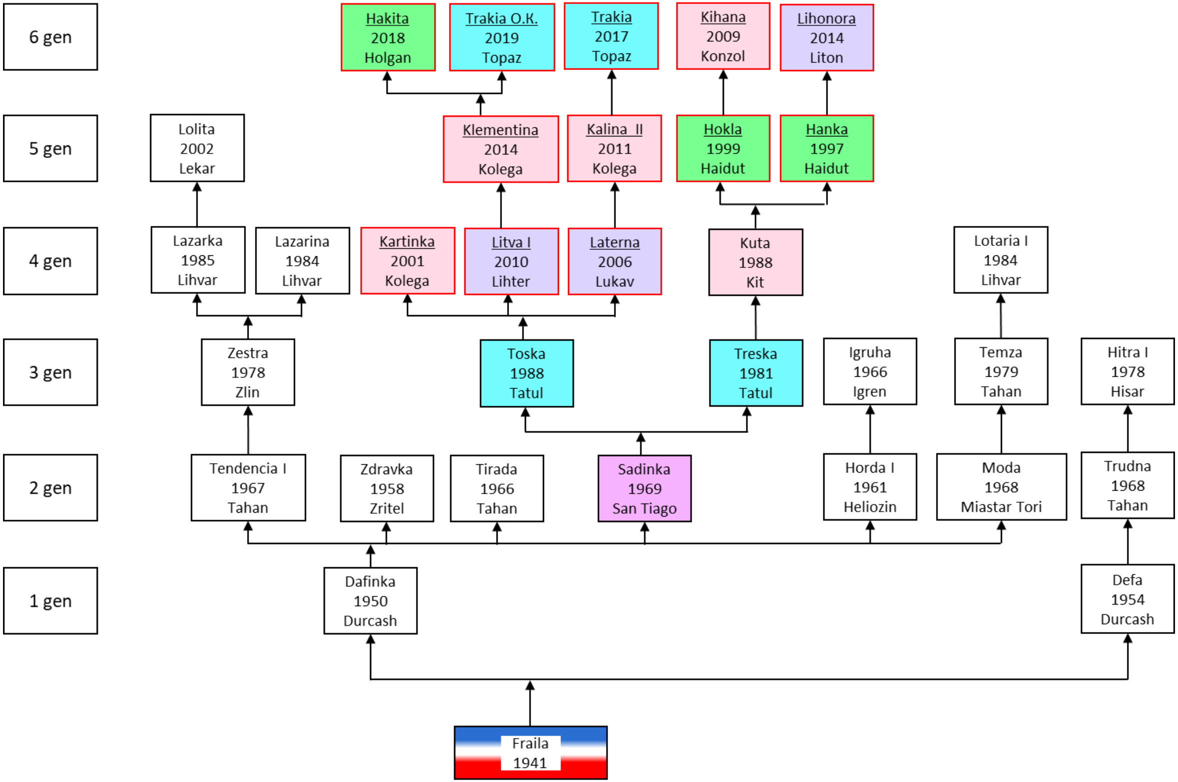


Fig. 29 Scheme of mare Fraila family

Table 5. Bloodline of the individual lines of the breed in living mares of the **Fraila** family

Mare		**Line**					
	Nonius	**Durtsash**	**Kalifa**	**Hrabar**	**Lider**	**TEH**	**Torpedo**
Kartinka	6,250	6,250	**50,0**			12,500	25,00
Litva I	6,250	6,250			**50,0**	12,500	25,00
Laterna	6,250	6,250			**50,0**	12,500	25,00
Hanka	3,125	3,125	25,0	**50,0**		6,250	12,50
Hokla	3,125	3,125	25,0	**50,0**		6,250	12,50
Kalina II	3,125	3,125	**50,0**		25,0	6,250	12,50
Klementina	3,125	3,125	**50,0**		25,0	6,250	12,50
Trakia	1,563	1,563	25,0		12,5	3,125	**56,25**
Trakia O.K	1,563	1,563	25,0		12,5	3,125	**56,25**
Hakita	1,563	1,563	25,0	**50,0**	12,5	3,125	6,250
Lihonora	1,563	1,563	12,5	25,0	**50,0**	3,125	6,250
Kihana	1,563	1,563	62,5	25,0		3,125	6,250

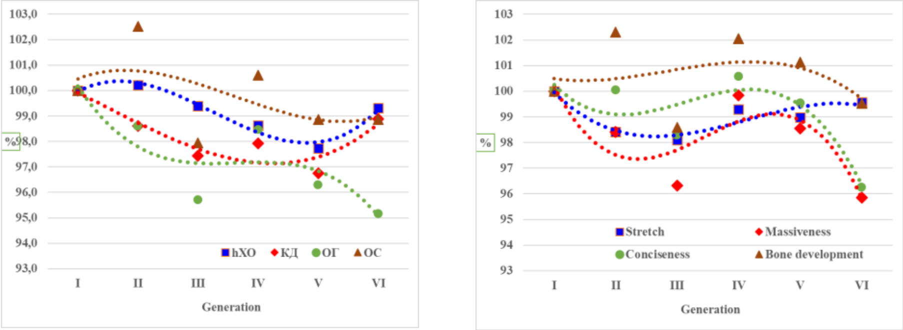


Fig. 30 Generational dynamics of exterior measurements and conformation indices of Fraila family mares

2.6 Family of the mare Deverica

Deverica is a purebred mare of the Nonius breed. She is imported pregnant from Serbia, in 2005. This is the first mare of the breed, imported after the recognition in 1951st of the Danubian horse. By body type, Deverika belongs to the small Nonius. In the I generation, the family has one representative, the daughter of Deverika by Kalen - Katerina, and in the second – out off 4 of her daughters by 3 sires from 3 lines (Fig. 31).

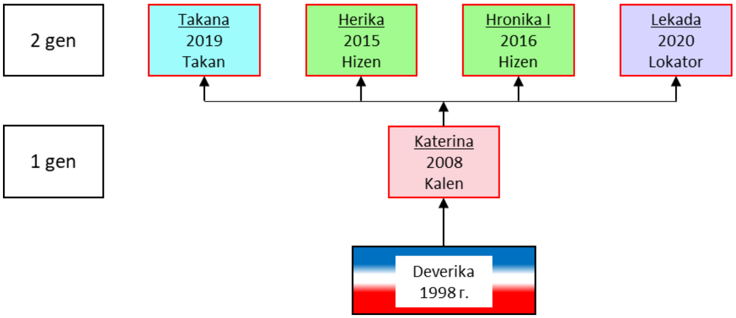


Fig. 31 Scheme of mare Deverica family

2.7. Family of mare Zhandarma

Zhandarma's family is the most numerous in the structure of the Danubian horse. It includes 102 mares in XII generations (Fig. 32) and continues to exist and develop for almost 100 years. Karaivanov (1975) and Karaivanov et al. (1989), present the family in detail up to the VI generation, and from VII - only 2 mares are described. From the very beginning, the family relied on a broad genealogical basis. The three daughters of Zhandarma, forming the I generation, are from three different fathers, from two lines Zdravko and Vit, from *Line N XXIX (A)*, and Novak - from *Line Nonius XXXI (B)*. In this connection, Karaivanov (1975) writes "Here it would be more correct to speak of a related group of three families". The use of stallions from almost all lines of the breed (Table 32), as well as purebred stallions from the Nonius and Thoroughbred English horse, do not lead to serious dynamics in the exterior (Fig. 33).

However, like most of the other kinship groups, a certain increase of massiveness is noticeable, which starts more significantly from the VI generation.

Of the three branches of the family, up to the III generation, the Zalozhba branch developed the most. Probably an unsuccessful selection with stallions from the line of Iskar and Kalifa reduces it to 4 representatives in the IV generation. In the V one, an unsuccessful attempt to restore is made, through the offspring that continues the Zdravko-Zund line, as well as by the inclusion of stallions of previously unused, in the selection in the family, lines - Lider and Torpedo. In the VI generation, the Hrabar line returns again, through which the branch develops

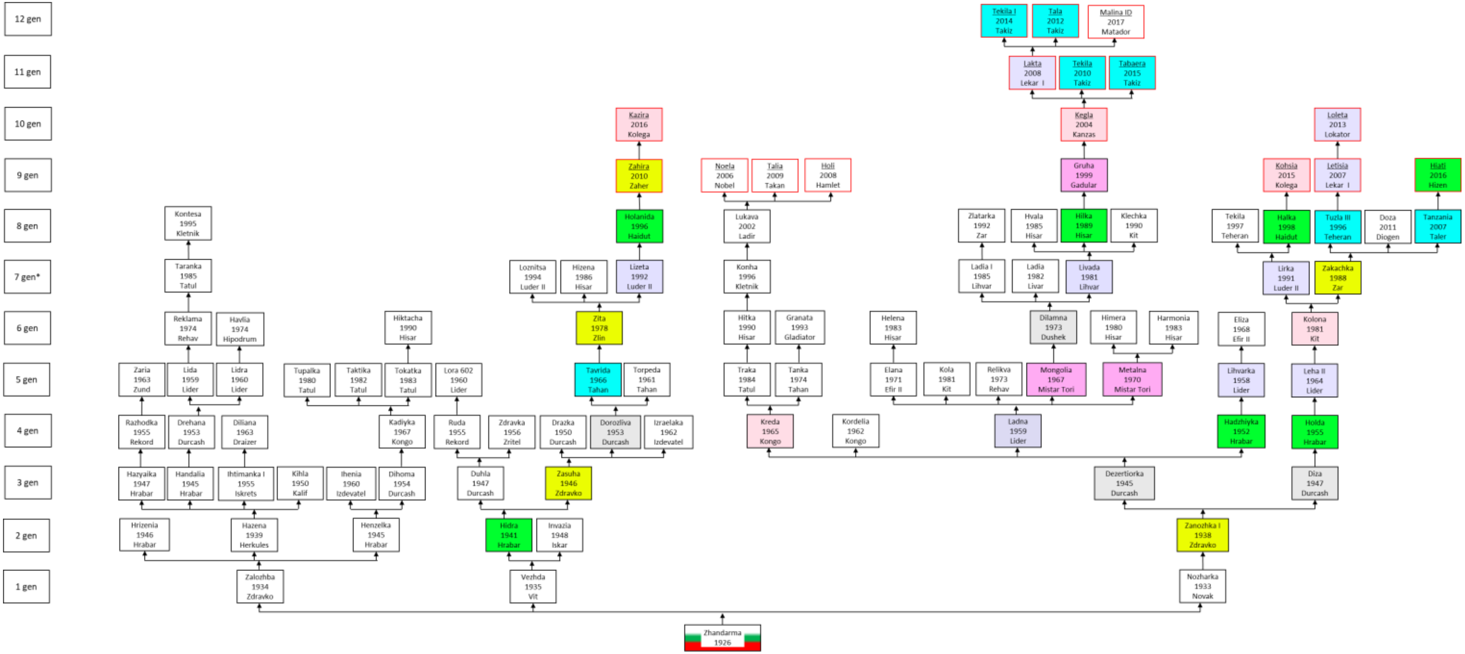

12 gen
11 gen
10 gen
9 gen
8 gen
7 gen*
6 gen
5 gen
4 gen
3 gen
2 gen
1 gen
Tekila I 2014 Takiz
Tala 2012 Takiz
Malina ID 2017 Matador
Lakta 2008 Lekar I
Tekila 2010 Takiz
Tabaera 2015 Takiz
Kazira 2016 Kolega
Kegla 2004 Kanzas
Loleta 2013 Lokator
Zahira 2010 Zaher
Noela 2006 Nobel
Talia 2009 Takan
Holi 2008 Hamlet
Gruha 1999 Gadular
Kohsia 2015 Kolega
Letisia 2007 Lekar I
Hiati 2016 Hizen
Kontesa 1995 Kletnik
Holanida 1996 Haidut
Lukava 2002 Ladir
Zlatarka 1992 Zar
Hvala 1985 Hisar
Hilka 1989 Hisar
Klechka 1990 Kit
Tekila 1997 Teheran
Halka 1998 Haidut
Tuzla III 1996 Teheran
Doza 2011 Diogen
Tanzania 2007 Taler
Taranka 1985 Tatul
Loznitsa 1994 Luder II
Hizena 1986 Hisar
Lizeta 1992 Luder II
Konha 1996 Kletnik
Ladia I 1985 Lihvar
Ladia 1982 Livar
Livada 1981 Lihvar
Lirka 1991 Luder II
Zakachka 1988 Zar
Reklama 1974 Rehav
Havlia 1974 Hipodrum
Hiktacha 1990 Hisar
Zita 1978 Zlin
Hitka 1990 Hisar
Granata 1993 Gladiator
Helena 1983 Hisar
Dilamna 1973 Dushek
Himera 1980 Hisar
Harmonia 1983 Hisar
Eliza 1968 Efir II
Kolona 1981 Kit
Zaria 1963 Zund
Lida 1959 Lider
Lidra 1960 Lider
Tupalka 1980 Tatul
Taktika 1982 Tatul
Tokatka 1983 Tatul
Lora 602 1960 Lider
Tavrida 1966 Tahan
Torpeda 1961 Tahan
Traka 1984 Tatul
Tanka 1974 Tahan
Elana 1971 Efir II
Kola 1981 Kit
Relikva 1973 Rehav
Mongolia 1967 Mistar Tori
Metalna 1970 Mistar Tori
Lihvarka 1958 Lider
Leha II 1964 Lider
Razhodka 1955 Rekord
Drehana 1953 Durcash
Diliana 1963 Draizer
Kadiyka 1967 Kongo
Ruda 1955 Rekord
Zdravka 1956 Zritel
Drazka 1950 Durcash
Dorozliva 1953 Durcash
Izraelaka 1962 Izdevatel
Kreda 1965 Kongo
Kordelia 1962 Kongo
Ladna 1959 Lider
Hadzhiyka 1952 Hrabar
Holda 1955 Hrabar
Hazyaika 1947 Hrabar
Handalia 1945 Hrabar
Ihtimanka I 1955 Iskrets
Kihla 1950 Kalif
Ihenia 1960 Izdevatel
Dihoma 1954 Durcash
Duhla 1947 Durcash
Zasuha 1946 Zdravko
Dezertiorka 1945 Durcash
Diza 1947 Durcash
Hrizenia 1946 Hrabar
Hazena 1939 Herkules
Henzelka 1945 Hrabar
Hidra 1941 Hrabar
Invazia 1948 Iskar
Zanozhka I 1938 Zdravko
Zalozhba 1934 Zdravko
Vezhda 1935 Vit
Nozharka 1933 Novak
Zhandarma 1926

Table 6. Bloodline of the individual lines of the breed in the living mares of the mare Zhandarma’s family

Mare	Zhandarma	Line									
		Durtsash	Vit	Zdravko	Kalifa	Hrabar	Lider	TEH*	Torpedo	Novak	Erno
Zahira	0,195	1,563	0,195	**57,03**		25,39	12,50		3,125		
Kazira	0,098	0,781	0,098	28,52	**50,00**	12,69	6,250		1,563		
Kegla	0,098	3,516		0,195	**50,00**	12,50	7,031	26,56		0,098	
Tabakera	0,049	1,758		0,098	25,00	6,250	3,516	13,28	**50,00**	0,049	
Tekila	0,049	1,758		0,098	25,00	6,250	3,516	13,28	**50,00**	0,049	
Lakta	0,049	1,758		0,098	25,00	6,250	**53,52**	13,28		0,049	
Malina ID	0,024	0,879		0,049	12,50	3,125	26,76	6,641		0,024	**50,00**
Taka	0,024	0,879		0,049	12,50	3,125	26,76	6,641	**50,00**	0,024	
Tekila I	0,024	0,879		0,049	12,50	3,125	26,76	6,641	**50,00**	0,024	
Noela	0,195	0,781		0,391	14,06	6,25	25,00	**50,00**	3,125	0,195	
Holi	0,195	0,781		0,391	14,06	**56,25**	25,00		3,125	0,195	
Tilia	0,195	0,781		0,391	14,06	6,25	25,00		**53,12**	0,195	
Koxsia	0,195	0,781		0,391	**56,25**	26,56	15,62			0,195	
Leticia	0,195	0,781		12,89	6,25	1,563	**53,12**		25,00	0,195	
Loleta	0,098	0,391		6,445	3,125	0,781	**76,56**		12,50	0,098	
Haiti	0,195	0,781		12,89	6,25	**51,56**	3,125		25,00	0,195	

**TEH – thoroughbred English Horse*

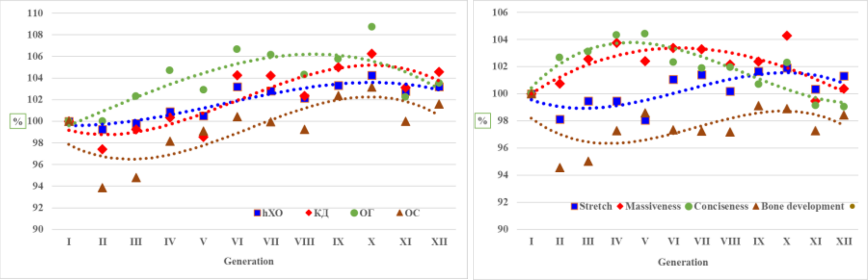


Fig. 33. Generational dynamics of conformation indices of mare Zhandarma’s family

at the beginning, to the II generation, but this time unsuccessfully, and in the VII generation there is one mare.

The Zalozhba branch is terminated in the VIII generation. The reasons for termination of work with the branch are not clear. From Fig. 34 and 35 it is evident that the mares have a correct exterior. In the VI and VII generations, the height at the withers is closest to the average, and in the 8th, the general trend of growth follows. The rest of the exterior parameters are also closest to the average, and what's more, they have the weakest and purposeful dynamics, compared to the other branches.

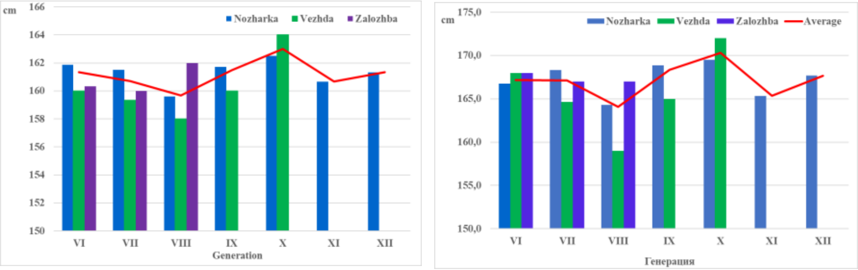


Fig. 34. Generational dynamics of height at withers and body length of mares of the primary Zhandarma family branches

The branches of the mares Vezhda and Nozharka continue to develop to this day, with the latter being more developed. Regarding the exterior, the **Vezhda** branch is developing most dynamically. From the VI to the VIII generation, the size of the mares decreases, and then increases sharply (Fig. 34-35). The turn comes in the IX generation from the mare Zahira, who is a daughter of her half-brother Zaher. The close inbreeding has no negative effect. During the first two generations

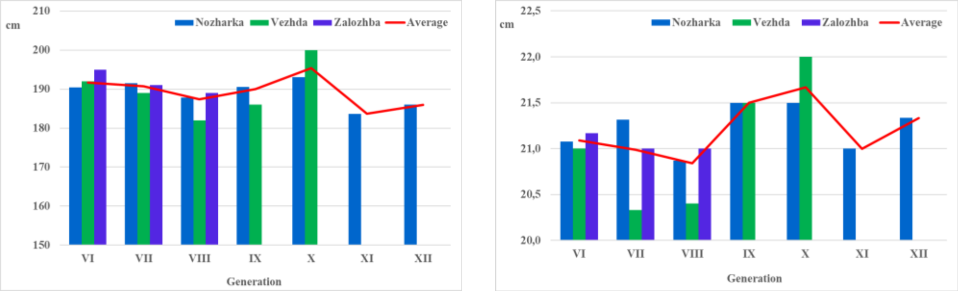


Fig. 35 Generational dynamics of breast girth and cannon bone girth of mares from the primary Zhandarma family branches

the branch of **Nozharka** develops relatively modestly. In the III generation, two branches are separated. The one of Diza, in the next three generations, developes through one mare each, daughters of sires from 3 lines – Hrabar, Lider and Kalifa, and from the VII generation, the number of mares progressively increases. From the VI to the VIII generations, the external parameters of the mares from the branch change inconsistently, and from the VIII to the X they have a consistent tendency to increase (Fig. 36-37).

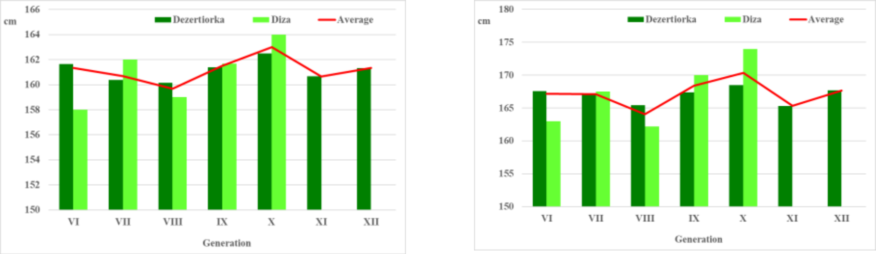


Fig. 36 Generational dynamics of height at the withers and body length in mares from the branches of the Nozharka branch

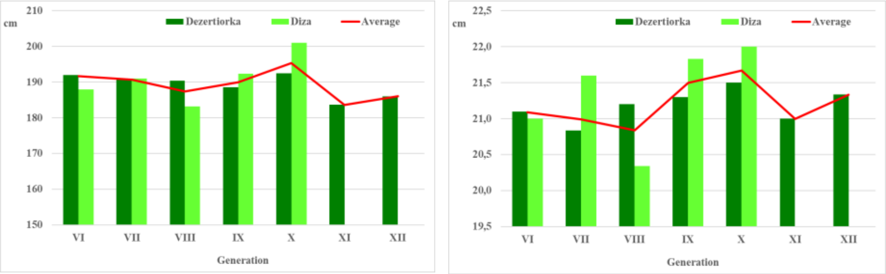


Fig. 37 Generational dynamics of breast girth and cannon bone girth in mares from the forks of the Nozharka branch

The second branch of the **Desertiorka** mare is currently the most strongly represented of all the branches of the family. It has been placed on a broad genealogical basis since the IV generation of the family, as the mare has left 4 offspring, daughters by stallions from 3 lines - one each from Hrabar and Lider and 2 from Kalifa. In the V generation, 3 branches are formed, from one daughter from each line. In terms of the exterior, the branch of the Desertiorka develops in variable directions, but the dynamics are within small limits.

The family has a good development perspective. It develops on a broad genealogical basis. At the time of the analysis, 16 mares are alive - from 2 large branches - Vezhda and Nozharka - separated eleven generations ago, with the development of 2 branches of Nozharka - from the III generation; 2 branches of the Desertiorka - separated from the IV generation. The representatives of the family have blood from different lines, which makes it possible to use them both to strengthen the heredity of the individual lines - of Zdravko (Zahira), Kalifa (Kohsia, Kazira, Kegla), Hrabar (Haiti, Holi); Lider (Leticia, Loleta, Lakta), Torpedo (Talia, Taka, Tekila I, Tabakera, Tekila), Erno (Malina SA), and to maintain genetic diversity. In this regard, the representatives of the family with high blood from the English thoroughbred horse breed (Noela, Kegla, Tabakera, Tekila, Lakta) can also be used.

2.8. Family of mare Leska II

The family of Leska II is the oldest in the structure of the Danubian breed - it has been developing for nearly 100 years. It is represented by 47 mares in eight generations (Fig. 38). Karaivanov (1975) and Karaivanov et al. (1989) analyzed the status of the family up to the V generation. At the end of the eighties of the last century, the family is at risk of being extinct. It is preserved thanks to the mare Hartia I, kept in KZ Klementina, and then developed through her two daughters Lohina and Lahta. Homogeneous selection is used to restore the family and preserve its traits as much as possible. Lahta and Lohina are created according to the same scheme – Lekar and Luder II are grandsons of Laden, and Hartia is his great-granddaughter. The inbreeding in both mares is low -Fx- 1.56%, but they are extremely close in appearance. The daughters of the two mares form the **VII generation**, and the genealogical base is maximally extended. The seven mares are by 6 sires of 2 breeds - Danubian *(Zar, Taler, Hilton)* and English Thoroughbred (*Digay, Gold Spiid, Diogen*). In the **VIII generation**, the family is represented by three mares.

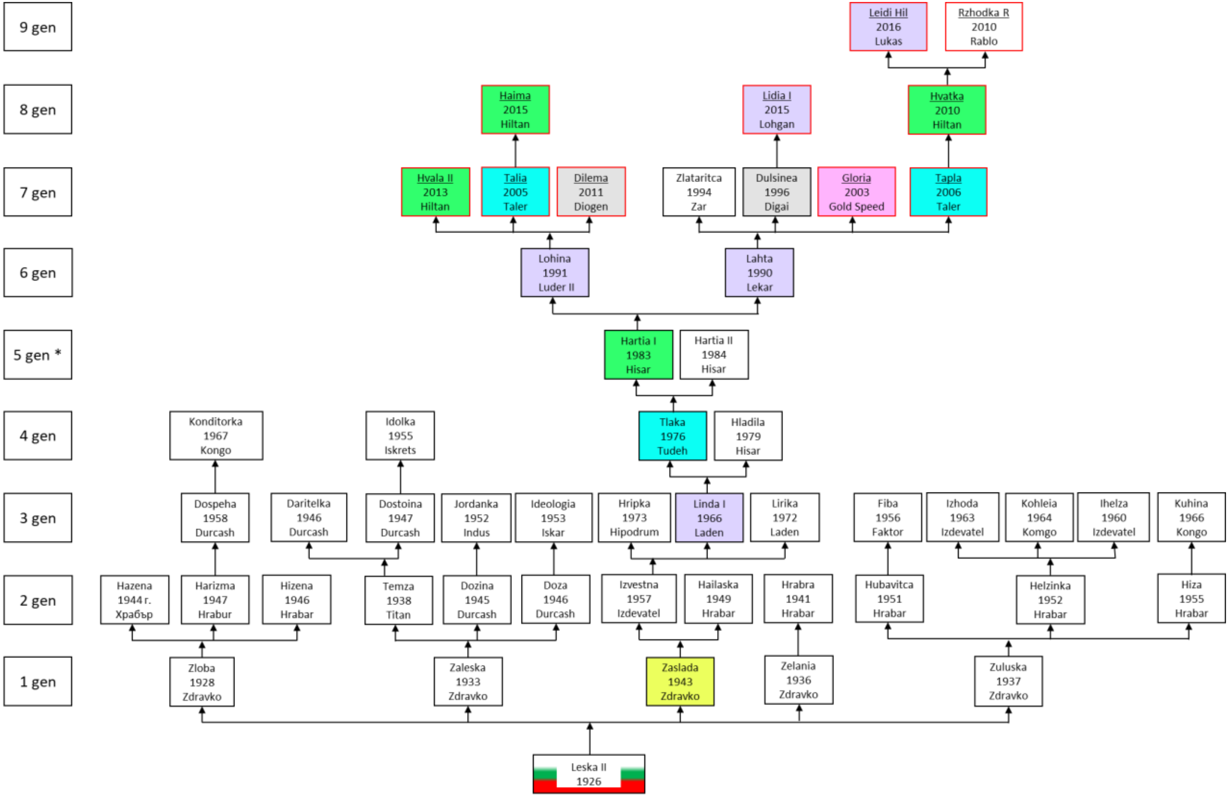


Fig. 38. Scheme of mare Leska II family

Considering the exterior, the mares of the VIII generation return to the parameters and proportions of the Hartia II, with the only major difference being the cannon bone girth. Due to the different change directions of individual indicators, the dynamics of the average values seem wide (Fig. 39), but in fact it are within 2-3%. Only the body length in the last two generations and the cannon bone girth – in the last one, the difference is greater, but the formation of these generations is not over. The differences in body proportions are greater, the reasons for which are the change in the parameters forming the conformation indices of some generations being in opposite directions.

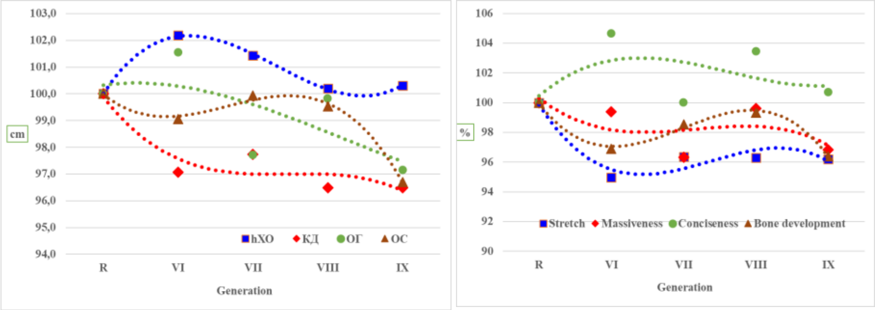


Fig. 39. Generational dynamics of exterior dmentions and conformation indices of Leska II mares

The mares of the family have blood from four of the six modern lines of the Danubian horse (Table 7).

Table 7. Bloodline of the separate lines of the breed in the living mares of Leska II family

Mare	Leska	Line						
		Zdravko	Hrabar	Lider	TEH	Torpedo	Iskar	Erno
Gloria	0,781	0,781	12,50	28,12	**50,0**	6,250	1,563	
Talpa	0,781	0,781	12,50	28,12		**56,25**	1,563	
Talia	0,781	0,781	12,50	28,12		**56,25**	1,563	
Dilema	0,781	0,781	12,50	28,12	**50,0**	6,250	1,563	
Hvala II	0,781	0,781	**62,50**	28,12		6,250	1,563	
Hvatka	0,391	0,391	**56,25**	14,06		28,12	0,781	
Lidia I	0,391	0,391	6,250	**64,06**	25,0	3,125	0,781	
Hatma	0,391	0,391	**56,25**	14,06		28,12	0,781	
RazhodkaR	0,195	0,195	28,12	7,031		14,06	0,391	**50,0**
Leidi Hil	0,195	0,195	28,12	**57,03**		14,06	0,391	

The distribution by lines is relatively even - 3 mares each have the highest bloodline from Hrabar and Lider lines, 2 from Torpedo and 4 from Kalifa. The inclusion of stallions from the Zdravko and Erno lines in the selection would increase the genetic diversity of the family, as well as of the two lines, which have a small number of representatives.

2.9. Family of mare Zakrila

Zakrila was foaled on April 12, 2006, in the village of Staroseltsi, Pleven region. Her mother is a local improved mare with a black color, and the father is a Zagalo stallion from the Zdravko line. In **the 1st generation**, the family is represented by 5 daughters of the foundation mare (Fig. 40), by 4 stallions from 3 genealogical lines - Kalifa, Torpedo and Lider. The five daughters of Zakrila have inherited the main traits of the Danubian breed and have the potential to develop the family. **In generation II**, there is one mare at this stage.

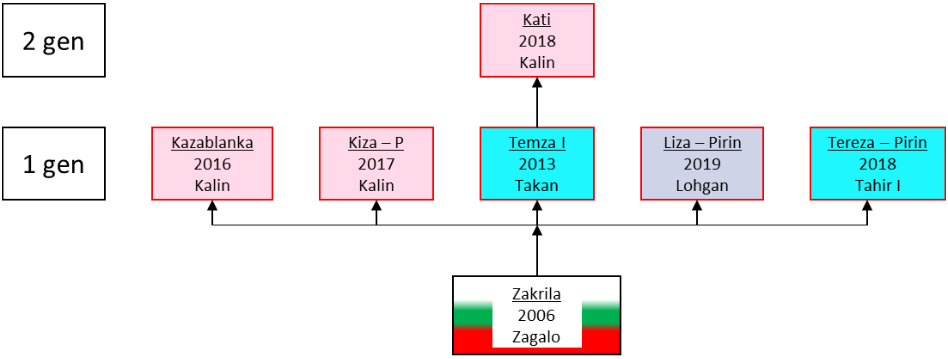


Fig. 40. Scheme of mare Zakrila family

2.10. Family of mare Klara

Klara was foaled on 25.02.2002. She is out off a local mare and by stallion Kohsi, from the Kalifa line. The mare is typical of the breed, surpassing even some purebred mares in appearance. Klara also has a very high reproductive capacity. She is used for breeding in 10 mating seasons, 8 foals are born, of which six are female forming the **I generation** (Fig. 41). **II generation**, currently is represented by 5 mares, 4 of which are stallion daughters. Obviously, when forming the II and subsequent generations, the genealogical base must be expanded. Seven of the ten living mares of the family are daughters of the stallion Holgan; and full sibs are Hanika, Hecuba-Bov and Haika; Klara-Nia and Karinka; Hannka, Holka-Bov and Hinka.

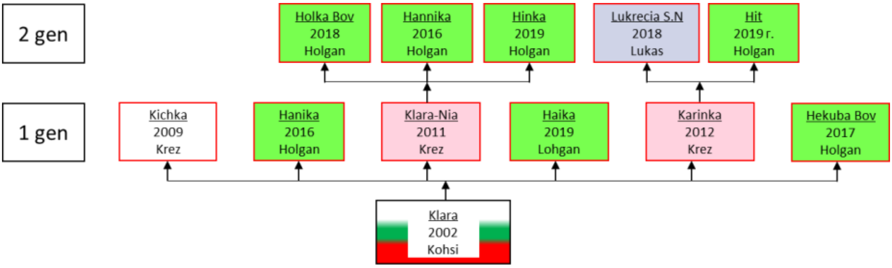


Fig. 41. Scheme of mare Klara family

All II generation mares have 25% Krez blood. Klara- Nia and Karinka are inbred by Kit- Fx-3.125%.

2.11. Family of mare Homotka I

Homotka I was foaled on 04.05.1995 in the village of Stolnik, Sofia region. She comes from a local Bulgarian mare and sire Homot (*Hisar - Mandolina)*. The mare is black in color, approaching the type of the breed. She has three mating seasons, gives birth to three daughters, forming the **I generation** (Fig. 42).

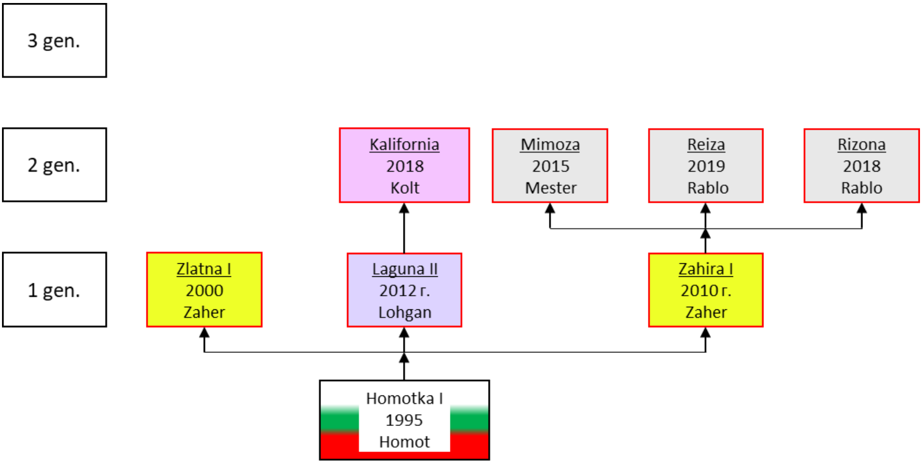


Fig. 42. Scheme of mare Homotka I family

The II generation of the family is formed by 4 mares, 3 from Erno's line. In the family, it is possible to form three branches - the mares of the I generation. At this stage, the genealogical basis is narrow - two of the three mares from the first and two of the four from the II generation are full sisters, and the three daughters of Zahira I are from two half-brothers: Mester /Nonius IV-25/ and Rablot /Nonius IV -122/ .

2.11. Mare Hala’s family

Hala was foaled on 16.05.1996 in the village of Mirovyane, Sofia region. It comes from a local mare and father - stallion Hlapak *(Hisar - Livada)*, who was a stud horse for Sofia region. In type, she is similar to the Danubian horse. She inherited the black color of her coat from her father. The newly formed family is developed up to the III generation (Fig. 43).

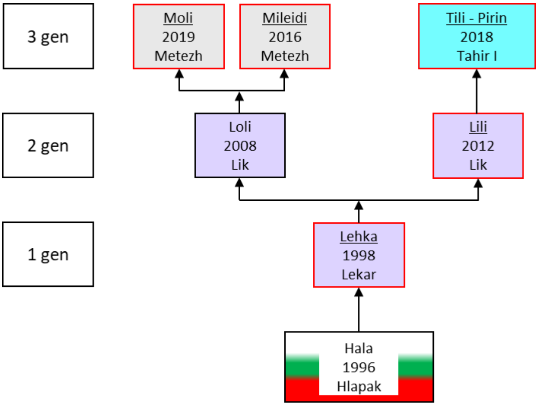


Fig. 43. Scheme of mare Hala family

The families of Deverika, Zakrila, Klara, Homotka and Hala are in the process of formation. They can also be joined by mares recorded in the additional section of the pedigree book, which have the potential to create families and are under the supervision of the breeding organization, such as **Zakala, Tesla, Lorca, Zlata and Nilma.**

2. GENETIC STRUCTURE AND GENETIC DIVERSITY OF THE DANUBIAN HORSE BREED AND ITS GENEALOGICAL LINES, ACCORDING TO MICROSATELLITE LOCI

To characterize the genetic diversity in the Danubian breed and populations of the Nonius breed, we use 15 microsatellite loci. The calculated PIC (0.73±0.15) and Shannon's information index (2.22±0.07) (Table 8.) show that they are suitable for this purpose. The Danubian breed is characterized by high polymorphism of the studied loci. A total of 184 alleles are identified in the 166 animals studied. The mean number of alleles (Na) ranged from 10.0 (ASB17) to 13.33 (HMS3 and HMS7). The overall mean number of alleles is higher than that of Nonius. The mean effective number of alleles (Ne) is 9.48±0.42, ranging from 4.8 at the ASB17 locus to 11.28 at the HMS7 locus.

The observed heterozygosity (Ho) ranged from 0.75 (AHT4) to 0.96 (ASB23), and the expected (He), a fundamental indicator of genetic diversity, is high. Heterozygous deficiency

Table 8. Main parameters characterizing the structure and genetic diversity of the Danubian horse, according to the studied loci

Locus	Na	Ne	PIC	Ho	He	I	F_{IT} ^{a}	F_{IS}	F_{ST} ^{a}	D_{ST}	H_{T}	G_{ST}
AHT4	11.17	8.62	0.81	0.75	0.74	1.98	0.158	0.012	0.148	0.611	0.885	0.131
AHT5	11.50	9.28	0.78	0.88	0.84	2.16	0.040	−0.046	0.082	0.509	0.915	0.066
ASB2	11.33	9.20	0.77	0.91	0.85	2.18	−0.008	−0.073	0.061	0.363	0.904	0.044
ASB17	10.00	4.80	0.69	0.94	0.79	1.85	−0.146	−0.201	0.047	0.158	0.823	0.032
ASB23	11.83	6.77	0.74	0.96	0.85	2.13	−0.077	−0.132	0.049	0.260	0.890	0.033
HMS1	12.67	9.96	0.65	0.80	0.79	2.19	0.119	−0.016	0.132	0.657	0.910	0.116
HMS2	12.50	10.26	0.69	0.86	0.83	2.24	0.066	−0.033	0.096	0.575	0.918	0.079
HMS3	13.33	10.74	0.73	0.92	0.86	2.36	0.009	−0.059	0.065	0.452	0.923	0.048
HMS6	12.50	9.59	0.67	0.83	0.81	2.19	0.060	−0.022	0.119	0.661	0.920	0.103
HMS7	13.33	11.28	0.75	0.86	0.89	2.42	0.068	0.028	0.041	0.277	0.927	0.023
HTG4	12.67	9.80	0.71	0.89	0.85	2.26	0.022	−0.051	0.069	0.438	0.913	0.053
HTG6	12.83	9.86	0.68	0.77	0.82	2.23	0.151	0.058	0.099	0.559	0.912	0.081
HTG7	12.67	10.55	0.72	0.92	0.86	2.31	0.002	−0.064	0.062	0.423	0.919	0.046
HTG10	12.83	10.63	0.75	0.90	0.89	2.38	0.025	−0.015	0.040	0.277	0.927	0.022
VHL20	13.17	10.90	0.73	0.91	0.87	2.36	0.025	−0.032	0.055	0.385	0.924	0.037
Mean (SE)	12.29 (0.44)	9.48 (0.42)	0.73 (0.15)	0.87 (0.02)	0.84 (0.02)	2.22 (0.07)	0.037 (0.021)	−0.043 (0.016)	0.078 (0.009)	0.459 (0.04)	0.907 (0.007)	0.061 (0.009)

Na - number of alleles; Ne -number of effective alleles; PIC- polymorphism index; Ho- observed and He- expected heterozygosity; I- information index of Shannon; Fit^{a} *- interpopulation coefficient of inbreeding, Fis - intrapopulation coefficient of inbreeding, Fst*^{a} *- coefficient of genetic differentiation; Dst - standard genetic distance; Ht -total genetic diversity; Gst -genetic diversity in each population*

(He > Ho) was found only in loci HMS7 and HTG6. In general, the Danubian breed has a high genetic diversity (0.84). The breed is not threatened by inbreeding depression - the total inbreeding coefficient (F_{IT}) is close to zero.

The intrapopulation coefficient of inbreeding (F_{IS}) - an indicator of the share of inbreeding in subpopulations from the total inbreeding, for none of the markers is higher than 0.1, which confirms the low level of inbreeding and the absence of heterozygous deficiency in the sample of the six lines of The Danubian breed. The genetic diversity between F_{ST} lines (0.078±0.009) is significantly lower than the intrapopulation one. The 6 Danubian horse lines studied are "very similar" to each other (F_{ST} ≤.0.15). This result confirms the data from the genealogical analysis, which shows that the lines do not breed in isolation and only a small proportion of the stallions and mares have a higher percentage of blood from one or another line.

The calculated average D_{ST} value describing the diversity among the six lines is 0.46. The overall average value of the G_{ST} coefficient, determining the genetic differentiation, is 0.061, i.e. only 6.1% of the total genetic diversity is due to differences between the lines.

In all lines, a Hardy-Weinberg population equilibrium test (HWS tests) is performed for each studied locus (Table 9). The results show that only in the Torpedo line there is no deviation from HWS. The presence of heterozygous deficiency is found in loci HTG6 (Kalifa, $P<0.05$), ASB23 (Hrabar, $P<0.01$), and HTG10 (Lider, $P<0.05$).

In the Table 10. the main parameters for assessing the genetic diversity in the Danubian horse lines are presented. The average number of alleles, on the studied loci, varies from 4.20 in the Zdravko line to 14.60 in the Lider and Kalifa lines. A unique allele with a frequency of 2.9% is found in the Kalifa line at the ASB17 locus. The observed heterozygosity ranges from 0.65 in the Zdravko line to 0.94 in that of Torpedo, while the expected one is again the lowest in the Zdravko line (0.57) but the highest in the Lider and Kalifa lines (0 ,91). The number of alleles with a frequency ≥ 5% is the lowest in Zdravko's line and the highest in Torpedo's - almost 11 alleles.

From the values for Nei's minimum genetic distances (DA) (Table 11), it is evident that the lines of Lider and Kalifa (DA=0.171) and Lider and Torpedo (DA=0.188) are genetically closest.

The highest genetic distance is between Zdravko and Erno (DA

Table 9. Hardy-Weinberg equilibrium (HWS) test for the studied microsatellite loci in the six lines.

Locus	Line					
	Zdravko	Erno	Torpedo	Lider	Kalifa	Hrabar
AHT4	0.803	0.148	0.455	0.375	0.561	0.874
AHT5	0.892	0.932	0.495	0.578	0.915	0.270
ASB2	0.785	0.141	0.762	0.823	0.629	0.835
ASB17	0.046*	0.987	0.979	0.959	0.713	0.000***
ASB23	0.075	0.064	0.357	0.559	0.996	0.006**
HMS1	0.991	0.028*	0.162	0.654	0.002**	0.370
HMS2	0.214	0.797	0.849	0.720	0.796	0.292
HMS3	0.977	0.074	0.377	0.320	0.305	0.121
HMS6	0.909	0.842	0.553	0.169	0.645	0.921
HMS7	0.695	0.658	0.209	0.475	0.374	0.325
HTG4	0.509	0.699	0.446	0.159	0.467	0.471
HTG6	0.062	0.587	0.774	0.887	0.011*	0.052
HTG7	0.370	0.874	0.249	0.382	0.393	0.275
HTG10	0.222	0.193	0.877	0.047*	0.575	0.837
VHL20	0.386	0.109	0.803	0.429	0.788	0.792

*P< 0,05, **P <0,01, ***P<0,001

Table 10. Main parameters characterizing the structure and genetic diversity of the studied loci in the Danubian horse lines

Line	Na	Ne	I	Ho	He	NPA	No DA
Zdravko	4,20	2,73	1,07	0,65	0,57	-	3,06
Erno	13,93	10,72	2,45	0,91	0,89	-	7,47
Torpedo	14,33	11,19	2,50	0,94	0,90	-	10,73
Lider	14,60	11,57	2,54	0,92	0,91	-	9,13
Kalifa	14,60	11,40	2,53	0,93	0,91	1,00	9,13
Hrabar	12,07	9,29	2,19	0,88	0,83	-	8,73
Mean	27,67	12,29	2,22	0,89	0,87	-	8,04

Na - number of alleles; Ne -number of effective alleles; Ho- observed and He-expected heterozygosity; I- information index of Shannon; number of alleles unique to the line (NPA) and NoDA average number of alleles for which the frequency is equal to or lower than 5%

1.142). This is also confirmed by the F_{ST} values (from 0.103 to 0.122), and it should be noted that the Zdravko line is the most distant from all other lines. Hrabar's line is similarly distant from Zdravko's line and the other lines, being closer to the latter.

Table 11. Comparison Nei matrix of genetic distance (above diagonal) and F_{ST} values (below diagonal) between pedigree lines of the Danubian horse.

Line	**Zdravko**	**Erno**	**Torpedo**	**Lider**	**Kalifa**	**Hrabar**
Zdravko		1.142	1.055	0.960	1.023	0.660
Erno	0.122		0.206	0.259	0.247	0.533
Torpedo	0.118	0.010		0.188	0.214	0.541
Lider	0.115	0.012	0.009		0.171	0.538
Kalifa	0.117	0.012	0.010	0.008		0.542
Hrabar	0.103	0.034	0.034	0.034	0.034	

Using the program STRUCTURE software v0.6.94 (Irvine, CA, USA), based on the level of "admixture" in each individual, by using the correlated allele frequency model, the genetic structure of the Danubian breed is determined. The results of the Delta K analysis show that the optimal number of genetic clusters demonstrating maximum similarity is at K = 3 (44. a, b). In Fig. 45. the lines and each individual within them are graphically represented.

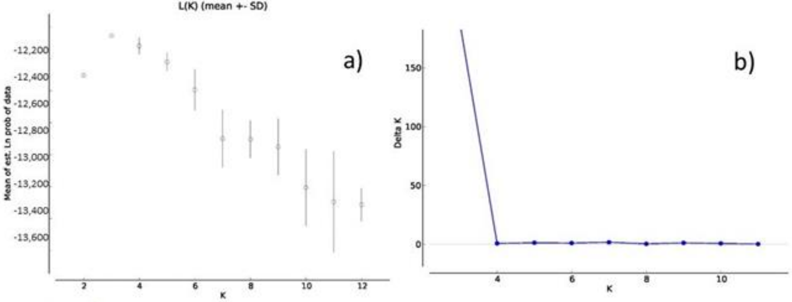


Fig. 44. Delta K values of the STRUCTURE analysis of six lines of the Danubian horse breed. (a) Delta K calculated from Evanno et al. (2005), is plotted against the number of modeled gene pools (K). (b) The highest probability Delta K are observed for K = 3.

Based on the studied marker loci, the lines are differentiated into three genetic groups. In cluster one fall the individuals mainly from the Zdravko line (colored blue), in the second - of Erno, Torpedo, Lider and Kalifa - (green color; K = 3), in the third - to the lines and Hrabar (red color). The grouping made by STRUCTURE was also confirmed by principal coordinate analysis (PCoA) (Fig. 46.).

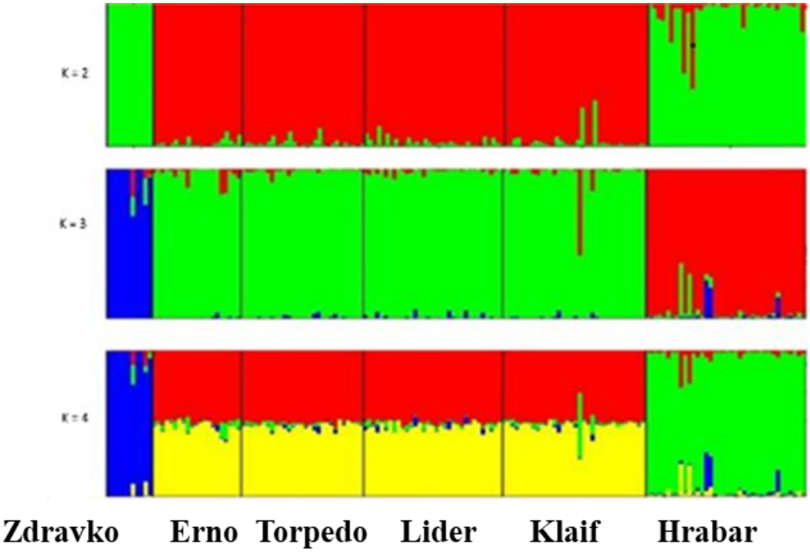


Fig. 45. Genetic structure of six pedigree paternal lines of the Danubian horse based on 15 SSR marker data.

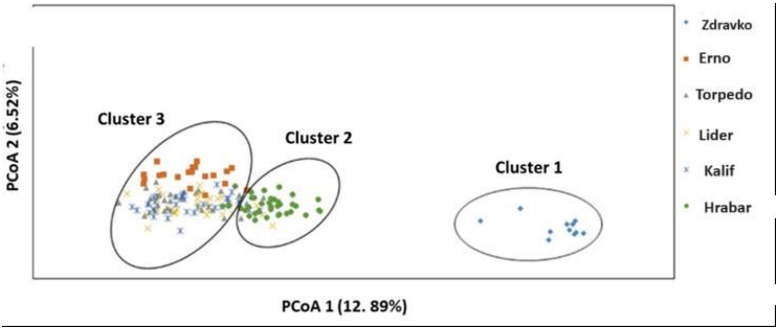


Fig. 46. Population structure of six paternal lines of Danubian horses genotyped with 15 microsatellites by means of principal coordinate analysis (PCoA) using GenAlEx 6.5 software. director Coordinates 1 and 2 are drawn for individuals (on forms I indicate which genealogy they were from).

PCoA divides individuals into 3 clusters. Principal coordinates (PC) 1 and 2 explain 12.9% and 6.5% of the variance in the genotype data, respectively.

3. GENETIC SIMILARITY AND DISTANCES OF THE DANUBIAN HORSE BREED WITH POPULATIONS OF THE NONIUS BREED, BULGARIAN HORSE BREEDS AND POPULATIONS

To establish the genetic similarity and differences of the Danubian horse with the Nonius breed, the Pleven horse and the East Bulgarian horse (Hristov et al., 2020), three local populations – Stara Planina, Rilo-Rhodope, Karakachan horse (Hristov et al., 2017) and wild prehistoric horses, we use a mitochondrial analysis. It is fit for the purpose because mitochondria are passed down through the maternal line only. The analysis of the 640 bp sequences obtained from the D-loop region show a high value of haplotype diversity, the average value of Hd for the three studied breeds being 0.886. 12 haplotypes are found in the Hungarian and Serbian populations of Nonius, and 13 in the Danubian Horse (Table 12).

Table 12. mtDNA polymorphism and neutrality tests in Hungarian Nonius, Danubian horse and Serbian Nonius.

Population	**S**	**Eta**	**K**	**H**	**Hd ± SD**	**π ± SD**	**Fu and Li's D test**	**Fu and Li's F test**	**Tajima's D**
Hungarian Nonius	55	58	13,557	12	0,909 ±0,036	0,02282 ± 0,0030	-1,1409	-1,1183	-0,5402
Serbian Nonius	45	49	19,121	12	1,000 ± 0,034	0,0312 ±0,0029	0,8042	0,9211	0,8184
Danubian horse	16	16	2,445	13	0,748 ± 0,047	0,0152 ±0,0017	-1,5900	-1,5957	-0,8846

S – number of polymorphic positions, Eta – total number of detected mutations, K – average number of nucleotide differences, H – number of haplotypes, Hd – haplotype diversity, π – nucleotide diversity. Values in the last three columns are statistically insignificant at the 0.05 significance level.

The obtained results show the presence of one to six haplotypes in the studied breeds from a total of 51 different haplotypes, which refer to 9 HGs (Table 13.). Only one haplotype co-occurs in Hungarian and Serbian Nonius (Hap55), and Hap24 haplotype co-occurs in Danubian Horse and Serbian Nonius. The results demonstrate a different genetic structure in the studied populations.

The estimated value of Tajima's D test and Fu and Li's D and L tests are negative in Nonius and Danubian horses, suggesting an increase in population size.

Table 13. Frequency of detected HGs in the Danubian horse, Nonius and Serbian Nonius populations.

Haplogroups (HGs)	Nonius		Serbian Nonius		Danubian Horse		Total	
	n	%	n	%	n	%	n	%
D	0	0,0	8	66,7	0	0,0	8	8,6
O'P	6	26,1	1	8,3	2	3,5	9	9,7
G	3	13,1	2	16,7	26	45,0	31	33,3
A	0	0,0	1	8,3	1	1,7	2	2,2
L	0	0,0	0	0,0	12	21,0	12	12,9
C	0	0,0	0	0,0	1	1,7	1	1,1
Q	1	4,3	0	0,0	3	5,3	4	4,3
E	3	13,0	0	0,0	0	0,0	3	3,2
M	10	43,5	0	0,0	13	21,8	23	24,7

Haplogroups are defined according to the classification of Achilli et al. (2012).

Unlike them, with the Serbian Nonius the values are positive.

The majority of the examined samples fall into **HG G**. Common to all breeds is also **HG O'P**, and **HG M**, is not observed in the Serbian Nonius. Along with groups common to breeds, there are also specific ones for each breed. In the Serbian Nonius, it is the rare **HG D**, in the Danubian horse - **HG L**, and also the rare **HG E** is found only in the Hungarian Nonius.

When comparing the Nonius and the Danubian horse with the Pleven and East Bulgarian breeds, significant differences are found, as genetic similarity, with regard to some HG, is observed only concerning the Danubian horse (Fig. 47.). Bulgarian horse breeds share the common **HG L**, which is observed with high frequency in all three breeds, but is not found in Nonius and Serbian Nonius. In the East Bulgarian horse, the highest frequency is **HG Q**, followed by **HG L** and **A**. In the Pleven horse, the European **HG L** is the most frequent, followed by the Middle East **HG C**. In the populations of the East Bulgarian and Pleven horses, we also found the very rare **HG N**.

The analysis of three local horse populations - Rilo-Rhodope, Stara Planina and Karakachan - show a huge diversity of almost all known HG except C, F and R (Fig. 47). West Eurasian HGs B, D, M and L have the highest frequencies. The HGs A, J, I, O'P and Q are also high frequencies, but they are not equally spread between the three populations. In the case of the Karakachan and the Staroplani Horse, the local genetic profile of the populations is preserved. In contrast, the Rilo-Rhodope horse population shows significant genetic introgression from East Eurasian as well as East Asian horse populations. In the case of the Karakachan and the Staroplani Horse, the local genetic profile of the populations is preserved. In contrast, the Rilo-Rhodope horse population shows significant genetic introgression from East Eurasian as well as East Asian horse populations.

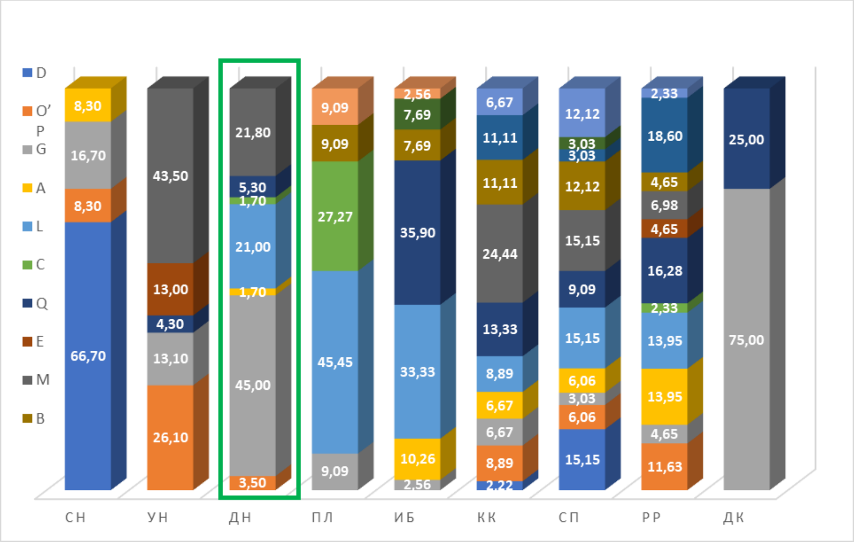


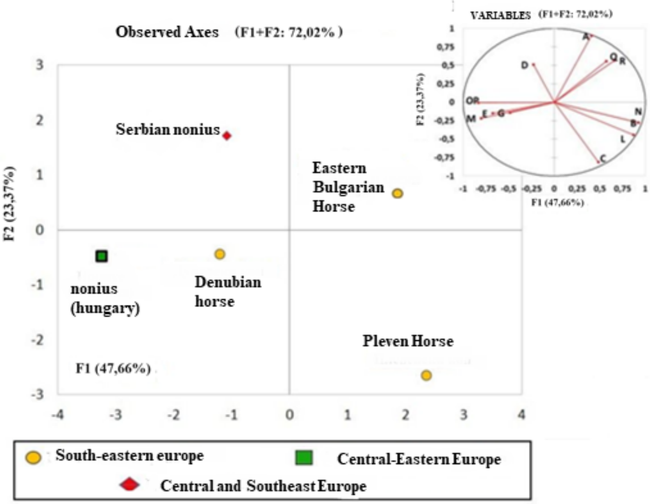
Fig. 47. Comparative diagram of the mitochondrial profiles of ancient wild horses inhabiting today's territories of Bulgaria (DC(ДК)), modern Bulgarian breeds - Danubian horse (DN(ДН)), Pleven horse (PL(ПЛ)), East Bulgarian horse (IB(ИБ)), local national populations – Stara Planina horse (SP(СП)), Rilo-Rhodope horse (RR(РР)) and Karakachan horse (KK(КК)), Hungarian Nonius and Serbian Nonius. The classification of HCG is according to the nomenclature of Achilli et al. (2012).

The Rilo-Rhodope horse seems to be a typical example of a population created through the influence of populations that are genetically isolated (genetic admixture). are not evenly distributed among the three populations. A preserved local genetic profile of the populations is observed in the Karakachan and Stara Planina horses. In contrast, the Rilo-Rhodope horse population has significant genetic introgression from East Eurasian as well as East Asian horse populations. In the

Danubian horse and the other two newly created breeds, some of the genetic diversity of the local breeds has been lost (Fig. 47).

As we noted, HG L has been preserved in all three of our modern breeds, Danubian, East Bulgarian and Pleven, despite the successive crossing of local populations with introduced breeds.

In order to graphically display and summarize the information regarding the frequencies of the different HGs in the studied breeds, we performed a principal component analysis (PCA) (Fig. 48). From the figure it can be seen that the Nonius and the Danubian Horse are in the same quadrant, since they have the same HGs - M, G and O'P, i.e. the two breeds show a higher genetic similarity to each other than to the Serbian Nonius. The other modern Bulgarian breeds are visualized in completely different quadrants - East Bulgarian and Pleven horses, which is a proof of a completely different genetic profile related to the way the three Bulgarian breeds are created.



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- **Fig. 48** PCA plot representing the genetic profile in three modern Bulgarian horse breeds, Nonius and Serbian Nonius, based on mtDNA HGs frequencies.

An explanation of the results presented so far can be sought **in the origin of local and modern breeds**. In the literature review, we focus in detail on the modern hypotheses about the places of origin of domestication of the horse - the Central Asian steppes, the Iberian Peninsula, Anatolia and Southwest Asia, the Pontic-Caspian steppes (the Yamna culture, Fig. 49) and Southern Siberia (the Athanasius culture). According to the generally accepted "steppe hypothesis", the western steppe herders (Yamna culture) and the settlers from Afanasius culture made a major contribution to the domestication of the horse. The territory of modern Bulgaria is a border region of these centers of horse domestication.

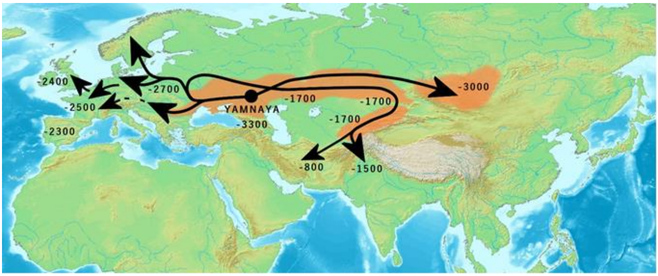


Fig. 49. Spread of the Yamna culture (Anthony 2007; 2017; Narasimhan et al., 2019; Nordqvist & Heyd 2020)..

The discoveries in recent years of the earliest presence of domesticated horses in Bulgaria during the Early Bronze Age in the area of Urdoviza, at the end of the 3rd millennium BC, is an indicator of the possible participation of the local population and wild local forms in the process of domestication of horses on our lands.

As far as the Nonius breed, an unusually high frequency is the HG M, which is present at a frequency of about 7% in the modern and about 17% in the ancient European horse populations (Achilli et al., 2012). The HG is of typical European origin. It is found in samples from the Mesolithic/Neolithic era (5200-4900 BC) and later - during the Neolithic/Bronze Age of the Iberian Peninsula (Cieslak et al. 2010; Lira et al. 2010). Its high frequency common in Nonius is often explained by the participation of Spanish mares in its creation, as well as by the fact that the direct ancestors of the Hungarian horses are the local Spanish horses from the early Bronze Age.

Found with high frequency in the Nonius breed, other HG - O'P, is typical for the Middle East. Its high frequency at Nonius is explained by the participation of Arabian and Andalusian mares in the breeding process during its creation. The presence of the Central Asian HG G and the rare HG E in the Nonius population may have appeared during the migrations of people of the Yamna culture (with already domesticated horses) into the central and eastern Carpathian Basin during the Early Bronze Age (Klejn et al., 2018; Tassi et al., 2018) or during the migration of the ancient Hungarians from the Eastern

European steppes to the Carpathian basin at the end of the 9th century AD. (Fóthi et al. 2020). It is interesting to note that the two HGs are, however, allochthonous to the Caucasus region, as they are not established in this territory before 2000 BC. In this regard, it can be assumed that they are probably introduced at the end of the 3rd millennium BC. from the Pontic-Caspian steppe in the Caucasus and Anatolia via the Transcaucasian route (Guimaraes et al., 2020). After 2200 B.C. the genetic profile of wild horses in ancient Anatolia undergo significant changes - 13 new mitochondrial HGs appeare during the Bronze and Iron Ages (Fig. 50).

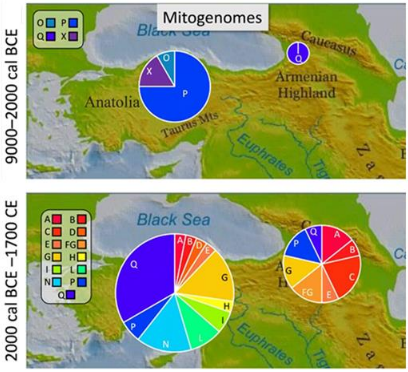


Fig. 50. Mitochondrial diversity in ancient horses before and after 2000 BC. Evolution of mitochondrial HGs in Anatolia and the South Caucasus. The size of the circles is proportional to the number of samples studied (Guimaraes et al., 2020).

The least data on the formation of the breed exists about the **Serbian Nonius.** Sequence analysis of part of the mitochondrial D-loop region shows the presence of the very rare HG D. It occurs in about 4.5% in modern and 1.4% in ancient samples from Europe (Achilli et al., 2012). In ancient Asian samples, the frequency of HG D is significantly above 10% (Achilli et al., 2012). This gives reason to assume the Asian origin of this genetic line. Its appearance on the Balkan Peninsula is possible via two routes through Southeast Europe (Bosphorus and Balkans) and through the Caucasus.

HG D is also present in the populations of **the autochthonous Bulgarian horses - Karakachan (2.22%) and Stara Planina (15.15%).** These observations confirm the hypothesis of the ancient origin of this HG and its presence in local horse breeds.

The Nonius is the main breed in the creation of the Danubian horse, but genetic markers transmitted through mitochondrial (female) inheritance show a specific genetic profile of the Danubian horse, distinguishing it both from the Nonius populations from Hungary and Serbia, and from the other two modern Bulgarian breeds - East Bulgarian horse and Pleven horse (Fig. 51).

Mitochondrial analysis of the Danubian breed shows an extremely high frequency of HG G - 45%. This is logical, considering its high frequency in Nonius and participation of female animals of this breed in the creation of the Danubian horse. A large number of local and locally improved mares are also involved in the creation of the Danubian breed, and from Fig. 47

. it is evident that HG G, local populations, as well as in samples of remains of ancient horses in Bulgaria. The other HG with a high frequency in the Danubian horse is HG M. The frequency of the HG in the Danubian breed and in the Nonius breed is 3 to 7 times higher than in modern European breeds. Like HG G, the high frequency of HG M in the Danubian horse population is probably related both to the participation of Nonius mares in the formation of the breed and to the participation in all studied contemporaries of modern local mares, as evidenced by the high frequency of HGs in modern local populations. Another HG with a high frequency in the Danubian horse is **HG L**, which, together with M, is typical of West Eurasian horse populations. Its presence in the population of the Danubian horse is not related to the influence of Nonius (in which it was not found), but from the local horses, which is indicated by its presence with a high frequency in The East Bulgarian and Pleven horses, as well as the autochthonous Bulgarian horse breeds (Fig. 47).

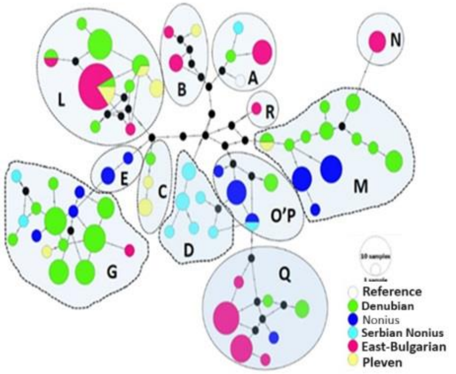


Fig. 51. phylogenetic network, based on sequence analysis of the D-loop region in the Danubian horse, Eastern Bulgarian horse, Pleven horse, Nonius and Serbian Nonius breeds

Mitochondrial genotypes G and Q, typical for the Danubian, Eastern Bulgarian and Pleven horse breeds, are probably of ancient local origin from the early period of domestication, as evidenced by their presence in the genotypes of ancient wild horses inhabiting our lands. In all likelihood, the presence of these HGs in indigenous and modern Bulgarian horse populations represent autochthonous genetic lines, due to the presence of domestication processes in this geographic region.

3. Analysis and guidelines of the breeding activities for the Danubian horse breed

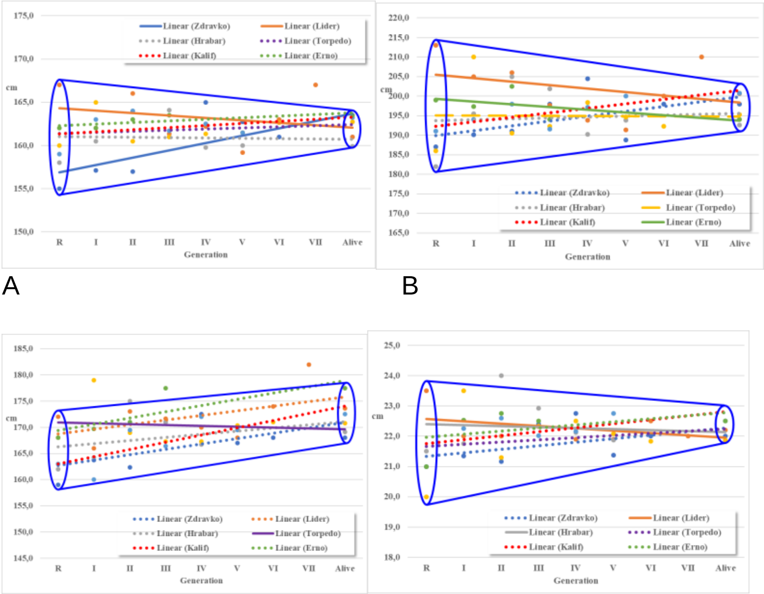
The Danubian horse is a unique national breed with a specific mitochondrial profile that demonstrates its relationship with modern Serbian (**haplotype A**) and Hungarian (**HG M**) Nonius populations, but also the differences between them. HG L and C found in the Danubian horse are not found in the Nonius, and HG **D**, found with a high frequency in the Serbian Nonius and **E** – characteristic of the Hungarian Nonius are not found in the Danubian horse. The uniqueness of the breed, on the one hand, is due to the breeding activities, on the other hand, it is also related to the preservation of the gene pool of the local mares that participated in its creation. HG L is found in all local populations and the newly created Bulgarian breeds, but it is not found in Nonius, as in the breeds from the national gene pool, the haplotype has high frequencies. This once again shows the uniqueness of the newly created Bulgarian breeds, as well as the importance of local populations in their formation. It also emphasizes the stability of the gene pool of the local populations over time, which is probably related to ensuring the adaptability of the created breeds to the local ecological and economic conditions.

The genealogical analysis shows that, although small in number, the Danubian breed has a well-established genealogical structure that guarantees sustainable development of the breed. When ***working with the lines,*** the disadvantage of leaving 1-2 stallions at a time as sires is overcome, which lead to the strengthening of the linear structure. Stallions from the main lines are used evenly in the families.

In the first stages, the selection in the creation of the Danubian horse is generally aimed at rounding up the animals. Compared to the foundation sires and representatives of the I generations, modern stallions are taller, with a more elongated body, a better developed chest and more massive bones (Fig. 52).

In 5 of the 6 lines, the work in terms of height is directed to increase, and in Lider, which, as we noted, refers to the "big Nonius" - to decrease. This has led to a consolidation of height within 160-164 cm on average. Body length has increased in all lines. Compared to the smaller change in height, related to length, the body of the stallions of the last generations is more elongated, compared to the first ones (Fig. 53).

The lines are consolidated according to the body stretch index, with the differences ranging from 104.3% for the Zdravko line to 107.8% for the Lider line. In parallel with an increase in height and lengthening of the body, the selection is also aimed at consolidation of the depth and width of the body. In four of the six lines, the chest girth increases



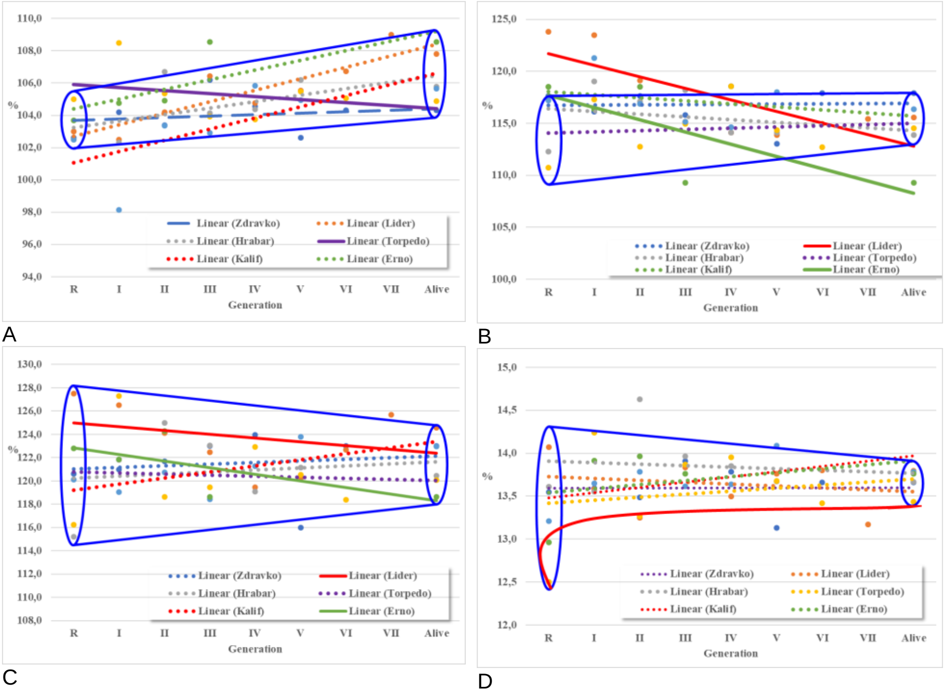
A B

C D

Fig. 52. Generational dynamics of height at withers (A), body length (B), chest girth and cannon bone girth in Danubian horse lines

and the increase is more significant than that of height and length. In the Erno and Lider lines, the average chest girth decreased by 5.0 cm and 12.4 cm, respectively. In modern representatives of the lines, the chest girth is consolidated at the level of 193-200 cm. The cannon bone girth increases on 5 of the 6 lines and only on the Lider line it decreases by 1.3 cm. From 20.0 to 23.5 cm in the first stages, in the current one, the cannon bone girth has been reduced to 21.85 cm 22.5 cm.

The unidirectional variation in exterior measurements also lead to unidirectional variation in body composition indices. Body mass index increased in 4 of the 6 lines and decreased in the Lider and Erno lines, consolidating at 119-125. The compactness index increases for the Zdravko, Hrabar and Torpedo lines, and decreases for the remaining 3 to reach average levels from 109.3% to 117.9%. After an increase in 5 of the 6 lines and a decrease in the Lider line, the highest consolidation is achieved in the bone development index - from 13.43% to 13.79%.



A B

C D

Fig. 53 Generational dynamics of indices for body stretch (A), massiveness (B), compactness (C) and bone development (D) in the Danubian horse breed lines

The analysis carried out shows that the purpose of the selection is to create animals of a "medium" type intermediate between the "small" and the "large" Nonius, but closer to the latter. In general, a breed consolidated in type and constitution is created, with specific exterior parameters and proportions of the body that differ slightly between individual lines.

As for **the linear structure** itself, initially, it is based on a not very broad basis. A large number of stallions are imported, but only four lines are formed, which Professor Karaivanov (1975) indicates that the main ones that make up the structure of the breed are the lines of Durtsash, Iskar, Zdravko and Hrabar.

Lines are established on a broad basis, and here the main question is why one or two stallions are left for continuing the line, which ultimately leads to the destruction of the line, such as those of **Durtsash and Iskar**. At the first, the foundation sire left 24 stallions and 77 mares (Karaivanov, 1975), but later the line is discontinued. The fact that even today, out of 113 mares, 67 have blood from the line speaks of its importance for the development of the breed. Unfortunately, it is 0.391% to 6.25% and the line is non-restorable. From the stallion Iskar, 38 mares and "approximately as many stallions" are used for breeding (Karaivanov, 1975). Of the mares currently in use, 13 have Iskar blood, mainly from the Leska II family, from 1.563% to 12.5% in one mare, which is grossly insufficient to take any action to continue the line. Some of the mares have preserved blood from other stallions used in the breeding process. In the family of the mare Stefa, there is still blood from stallion **Factor** - in 8 mares, up to 3.125%. Some of the mares from the Zhandarma family have blood from the stallion **Novak** - up to 0.195%, and two - blood from the stallion **Vit**. Two mares from Mara Yu's family have bloodlines from the stallion **Rehav** - one 6.25% and the other 3.125%.

Of the remaining 2 main lines, **Zdravko** line has a certain potential for survival and development, but this can only be done with a purposeful, well-developed strategy. Currently one stallion of VI generation continues the line, cryopreserved semen from his sire, and only 6 active mares, 3 of which are daughters of one stallion. Mares with 25% and slightly above this bloodline can also be used to restore the line. There are 16 mares from 5 families: Zakrila - 5, Homotka and Mara Yu - 4 each, Katya - 2 and Zhandarma - 1. Of these mares, 6 have blood more than 50% of the Kalifa line, with the same blood three mares from Erno's line, 5 from Torpedo's line, 2 from Lider's line. Among the active mares there are also 9 with 12.5% bloodline from the line, 5 with 6.25% bloodline and 32 with lower bloodline. All this creates an opportunity to place the selection on a broad basis and choose representatives, according to the goals of the breeding work.

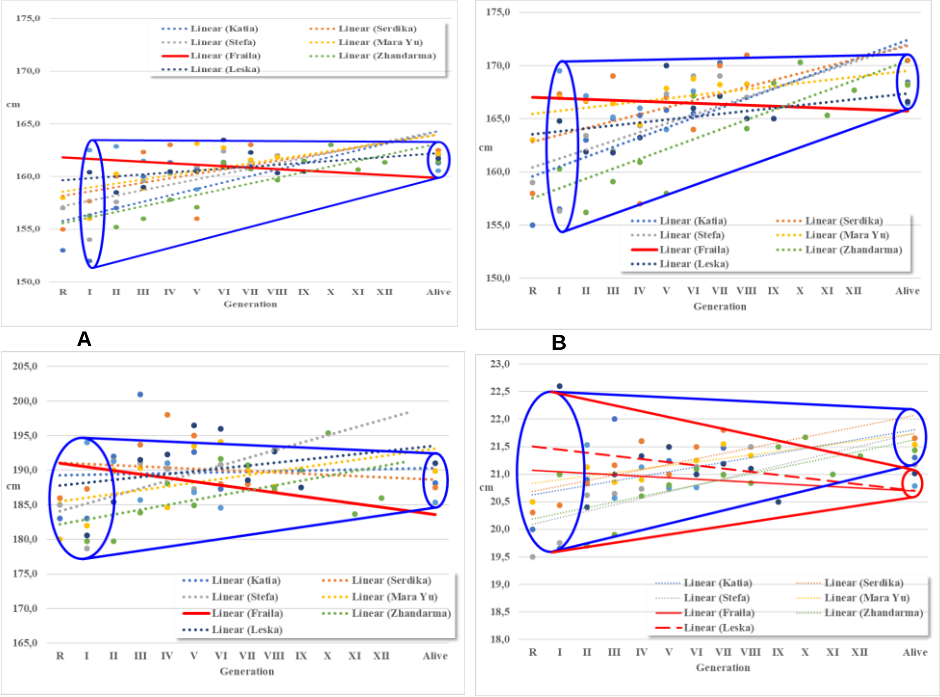
The only one of the "old" lines that has developed successfully since its inception is the line of the stallion **Hrabar.** It also passes through one stallion - in the II generation, but in the next ones, a larger number are left to continue the line and at least 3 branches are developed. At the present time, the stallion Hrabar line can be defined as the main line in the breed. The line has 10 active stallions. Out of 113 active mares - 88 have the Hrabar bloodline, of which 23 mares with bloodline 50% and more, 16 - with bloodline from 25 to 37.5%, 21 - with bloodline 12.5%, 19 with 6.25 % and the rest with a lower percentage. The mares of the line enter the structure of 11 of the 12 modern families of the breed and have a different bloodline than all the current lines.

The newer lines - Lider, Kalifa and Torpedo - are also developing well. Currently, the **Lider** line is represented by 5 stallions and 23 mares, in all families of the breed. Another 57 mares have blood from the line, of which 22 – from 25 to 43.8%; 15 – from 12.5 to 15.62%; 9- from 6.25 to 7.03% and the rest below 3.6%. The **Kalifa** line is developed through 4 stallions and 22 mares, in all families of the breed. In addition to these mares, which have a bloodline of 50% and more, another 73 mares have bloodline from the line, incl. - 5 mares with a bloodline of 37.5%, 28 with a bloodline of 25%, 13 with a bloodline of 12.5 to 14.1%. Mares from the Kalifa line make up the modern genealogical structure of 9 of the 12 families of the Danubian breed. The **Torpedo** line is represented by 4 stallions and 22 mares in 10 of the 12 families of the breed. Another 41 mares have blood from the line: - 12 from 25 to 28.12%; 9 from 12.5 to 14.06%; 10 - 6.25% and the rest – with lesser percentage. The newest line, of stallion **Erno**, also has potential for development, and the genealogical base should be gradually expanded. Of the three imported stallions, half-brothers by father, today there are 2 active sires, grandsons of one of the three brothers. From the line there are 11 mares with 50% bloodline and one with 25% bloodline. Five of the mares are daughters of Rablot, 2 of Metezh, 2 of Matador and one each of Mester and Matroz. Initially, Momento and Maklaran can be used, and then, with the resulting male horses, inbreeding can be applied to Metazh, Mester and Rablot. With the Rablot branch, this can be done without the involvement of Momento and Maklaran, inside the branch, since some of the mothers are genetically far enough apart. The work with the line must be conducted extremely carefully, taking into account a possible increase in homozygosity not only on Erno, but also on the line of Torpedo and Lider.

In the creation and development of the Danubian horse, in addition to local mares of different bloodlines and the Nonius breed, attempts were made to use other breeds, such as the Russian Trotter horse, the Hanover and the Thoroughbred English horse. There are no modern representatives of the Danubian horse bloodline from the ***Russian Trotter breed and the Hanover breed**.* Stallions from the ***English Thoroughbred breed*** are included periodically in the reproductive process of the Danubian horse, 22 stallions are used. The effect of using the breed is diverse, but rather positive. Currently, there are 27 mares with bloodlines of the English Thoroughbred breed, of which 5 with bloodlines of 50% - 2 from Nobel and one each from the stallions Gold Spiid, Diogen and Najak. One of the mares has 25% blood of the breed, one with 26.56%. In 2 mares, the blood from the English stallions is 25 and 26.56%; in 6 mares - from 12.5 to 13.3%; in another 6 from 6.25 to 6.64% and the rest have lower blood levels.

The genealogical analysis of the families shows that in the "old" families breeding work is carried out at a high level. The families are either based on a broad genealogical basis (Zhandarama, Stefa) or it is gradually enriched (Serdika Yu, Mara Yu, Fraila, Katya, Leska II). Branches of different origins developed in the families. This makes it possible to currently use the main families both to continue and strengthen the traits of the main lines and to maintain the genetic diversity in the breed. When breeding the families, the principles laid down during the creation of the breed are respected - the foundation mares must be either local mares or Danubian mares, or from the Nonius breed.

In the initial stages, when creating the breed, work is generally aimed at enlarging the animals, the most significant being the increase in body length (Fig. 54).



A

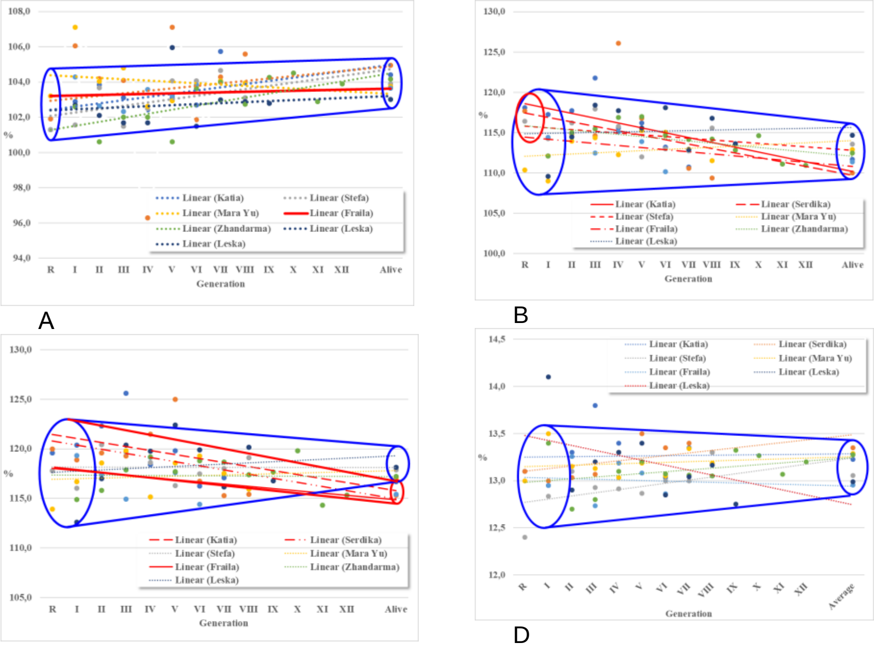
B

C

D

Fig. 54. Generational dynamics of height at the withers (A), body length (B), chest girth and cannon bone girth in Danubian horse breed families

To a lesser extent, the other main exterior parameters also increased. From the above it is clear that the aim is to preserve the proportions of the body and although it is slightly elongated, due to the priority increase in length, the differences in the stretch index between the above mentioned initial stages and those of living mares are lower (Fig. 55.).



A

B

C

D

Fig. 55. Generational dynamics of indices for body stretch (A), massiveness (B), compactness (C) and bone development (D) in the families of the Danubian horse breed

The chest girth also increases, but with lower values, which, compared to the more significant increase in the height of the withers and body length, leads to a multidirectional change in the indexes for massiveness and compactness, the changes are again in low limits. The massiveness increases in the families of Stefa, Zhandarma, Mara Yu and Leska, and decreases in the rest of the families. Compactness does not change in parallel with massiveness. It increases in the families of Zhandarma, Mara Yu and Leska, and decreases in the case of Stefa, Fraila, Katya 6.4% and Serdika. The cannon bone girth decreases in the Fraila family - by 0.2 cm, and with Leska by 1.6 cm. In the rest of the families, it increases from 0.4 cm for Gendarma to 1.7 cm for Stefa.

Thus, the index of bone development changes in low limits - it decreases in the families of Zhandarma and Leska and increases in mares from the other families. The different direction of changing the exterior parameters in individual families is aimed at consolidating the size and proportions in the breed, and it is largely achieved, which is also evident from the figures presented above. The Danubian horse is taller and more balanced in height from the Serbian Nonius, with a better developed chest but with thinner bones, but the mares of the breed are smaller than those in the stud farm at the Izvin station in Romania (Maftei et al., 2015). The Danubian mares are inferior in height to the Hungarian Nonius, they are significantly shorter, with a smaller chest girth, but with the same bone development (Bene et al., 2014). The "lightened" and "shortened" Danubian horse is more suitable than the Hungarian and Romanian Nonius for racing and showjumping. In general, it can be noted that the Danubian horse breed has a well-developed family structure. There are currently 12 families active - 7 of which, of the mares Katya, Serdika, Stefa, Mara Yu, Fraila, Zhandarma and Leska II, are participants in the breeding process. With the greatest perspective are the families of the mares of: Mara Yu - with 22 representatives, from 5 branches, divided into the V generation, with branches formed in VI, VII, VIII generations. The mares are daughters of representatives of all modern lines in the breed. Zhandarma - with 16 mares, from 2 branches, separated in the mid-1940s, with numerous subsequent branches - from the III, IV and subsequent generations. The modern representatives of the breed are from all existing lines, as well as an English Thoroughbred stallion. Fraila - with 12 representatives, from 2 branches, divided into the III generation and 4 modern branches. The mares are daughters by 8 stallions from 4 lines - Lider, Kalifa, Hrabar and Torpedo, Stefa - with 8 representatives - from three independent branches (Definicia, Isidora I, Lidia), daughters by 5 stallions, from 5 lines of Zdravko, Hrabar, Lider, Torpedo and Erno. The mares have an even, stably inherited exterprior. The families of Leska II - 10 mares from 2 branches, divided into the III generation, also have good prospects for development. The mares are daughters by 7 stallions, 2 of which are from the English Thoroughbred and 5 from the lines of Lider, Erno, Hrabar and Torpedo; Katya - 7 mares matched in exterior, from 4 lines - Zdravko, Lider, Kalifa and Torpedo. The family of the mare Serdika Yu, with 2 representatives at the moment, is threatened with extinction.

The stability and development of the breed is directly related to the generation interval, which indicates the time for the change of generations and is defined as the average age of the parents at the birth of their offspring for breeding. The generation interval in the Danubian horse lines averages from 7.44 years in the Erno line to 15.58 years in the Kalifa line, and the differences between generations in individual lines are significant. The average generation interval in the families is lower - 9.79 years, with the variation being on average from 8.88 in the family of Zhandrama to 11.44 years in the family of Fraila.

Between the conclusions drawn from the **genealogical and sequence analysis** of DNA of the Danubian horse, based on 15 microsatellite loci, there is no and cannot be sought an absolute parallel. The loci included in the study are suitable for studying the genetic diversity of the breed (PIC=0.73± 0.15). When using them, it was found that the Danubian breed has a high genetic diversity - Shannon's information index (I) is on average 2.22 ± 0.07, the observed heterozygosity (He) is 0.87 ± 0.02 and is higher than expected (He= 0.84 ± 0.02), in 13 of the 15 loci studied. The Nei coefficient for total genetic diversity is HT=0.907 (0.007). In the 166 animals studied, a total of 184 alleles are identified in 15 loci.

Based on the total (F_{IT}) and intrapopulation (F_{IS}) inbreeding coefficients, which have average values close to 0 - 0.037 ±0.021 (P = 0.004) and - 0.043 ±0.016 (P = 0.002) respectively, it is found that the Danubian breed is not at risk of inbreeding depression.

The lines have high intraline diversity. The average number of alleles, at the studied loci, varies from 4.20 in the Zdravko line to 14.60 in the Lider and Kalifa lines. In the latter, a unique allele is found at the ASB17 locus with a frequency of 2.9%. The observed heterozygosity ranged from 0.65 in the Zdravko line to 0.94 in the Torpedo line, and the expected heterozygosity ranged from 0.57 in the Zdravko line to 0.91 in Lider and Kalifa. These findings are made comparing the reported relatively high level of mating between relatives and genetic similarity among representatives of individual line and families.

However, the extremely high similarity (F_{ST} =0.078±0.009; D_{ST}, 0.46±0.04) between the lines found in the DNA analysis fully confirms the data from the genealogical analysis, where it is found that the lines are not bred in isolation and only a small some stallions and mares have a higher percentage of blood from one line or another. The closeness found between the lines of Lider and Kalifa (D_A =0.171) and Lider and Torpedo (D_A =0.188) is also beyond doubt. In the Table 14 is presented the percentage of mares from a given line (with bloodline ≥50% of it) with bloodline ≥ 25% and ≥ 12.5% of other lines.

Table 14. Percentage of mares of a given line with blood ≥ 25% above the diagonal and ≥ 12.5% below the diagonal, from another line

Line	Zdravko	Erno	Torpedo	Lider	Kalifa	Hrabar
Zdravko		16,7	20,7	10	20,7	13,3
Erno	0		27,3	11,8	0	5,88
Torpedo	6,9	12,1		24,4	22,7	6,7
Lider	13,3	2,9	11,3		26,7	15,2
Kalifa	6,9	0	18,2	6,7		46,7
Hrabar	10	16,7	13,3	28,3	13,3	

Thus, 26.7% of the mares from the Kalifa and Lider lines have ≥ 25% blood from the other line (Lider or Kalifa), and 6.7% have blood from 12.5 to 24.9%.

The microsatellite analysis also confirms the proximity of the lines of Torpedo and Lider, Torpedo and Erno, Kalifa and Torpedo. At the same time, a relatively high distance of Hrabar and Kalifa (D_A =0.542) is found, given that 46.7% of mares from both lines have ≥ 25% blood from the other and another 13.3% have blood from 12,5 to 24.9%. Particularly interesting is the separation of Zdravko's line into a separate cluster, given that the mares from this line have blood from all the others, as well as the established genetic similarity of Kalifa and Erno, given that the mares from both lines have no blood from the other.

The obtained results once again demonstrate that ***the data of DNA analysis alone cannot be used independently*** in the analysis of genetic processes and phylogeny of populations subjected to artificial selection, even less in small populations where the possibility of founder’s effect, random genetic drift and other elementary evolutionary events can have a huge effect. The purpose of selection is phenotypic change of animals, by combining the genotypes that determine it, in the parental forms. Microsatellites are not associated with a specific phenotype and are not subjected to parallel selection with biological and economic traits of individuals.

It is obvious that ***when developing breeding strategies, genealogical and microsatellite analyzes should be used in parallel, and the results of our study allow this complex approach to be applied in developing breeding strategies for the Danubian breed.***

The concept of integrated use of studbook data and molecular data to manage genetic diversity and optimize breeding plans is supported by a number of authors (Ayala et al., 2020; Goleman et al., 2021; Hauser et al., 2021), including in horse breeding (Dell et al., 2020; Mancin et al. 2020; Ivanković et al., 2021, Poyato-Bonilla, et al., 2022, etc.).

Irrespective of the methods used, the assessment of the population-genetic status of small and endangered populations is essential, but only the first step towards their preservation and development. For the management of genetic diversity, it is necessary to develop a comprehensive breeding concept for the breed.

The development and determination of the breeding objectives in the Danubian horse must be considered as a continuous process, which during a certain time interval, not longer than the generation interval, requires an analysis and, if necessary, a review and rethinking of the selection strategy, in the context of the development guidelines of horse breeding, in general and the population genetic status of the breed.

Our analysis shows that in the short term, the work with the Danubian horse should continue in the direction of preserving the available gene pool. For this purpose it is necessary:

- Strengthening the current and establishing new lines. Currently 4 lines are well developed in the breed, one is not yet developed and another is in danger of being extinct
- To expand the process of cryopreservation of semen to include stallions of all lines, with priority given to the most endangered ones. Attempt to preserve embryos from mares from endangered lines and families.
- In parallel with the development and strengthening of the "old" families, some of which have a relatively small number of representatives, to continue the development of the newly established ones, expanding their genealogical base.
- The mating plans should be developed on the basis of the genealogical and microsatellite analysis data obtained in the current development, which should be periodically updated.

The concept of long-term development of the Danubian horse should be oriented towards sustainable development of the breed, based on three components - "genetic sustainability", social and economic effect.

SUMMARY

Sequence analysis of the D-loop mtDNA region shows that the modern Danubian horse is a unique national breed, with a specific mitochondrial profile and high haplotype diversity Hd-0.748±0.047. The highest frequency is **HG G** (0.450), followed by **HG M** (0.218) and **HG L** (0.210). With smaller frequencies are HG **Q** (0.053), **O'P** (0.035), **A** and **C** with (0.017). Mitochondrial profiling demonstrates the relationship of the Danubian horse to modern Serbian (**haplotype A**) and Hungarian (**HG M**) Nonius populations, as well as the differences between them. The **HG L** (with a frequency of 0.210) and **C** (with a frequency of 0.017) found in the Danubian horse, does not occur in the Hungarian and Serbian Nonius, and HG **D,** found with a high frequency in the Serbian Nonius (0.667) and **E** – typical for the Hungarian Nonius (0.130) are not found in the Danubian horse. The uniqueness of the breed is related to the breeding activities - haplotype **O'P** is found in the Danubian horse, the two studied populations of Nonius and the local national populations, but it is not found in the Pleven and East Bulgarian horses; Haplotype **A** is found in the Danubian horse, the East Bulgarian horse, the local populations and the Serbian Nonius, but is not found in the Hungarian Nonius and the Pleven horse. Haplotype **M** is found in Danubian horse, Hungarian Nonius, national local populations, but not found in Pleven, East Bulgarian horse and Serbian Nonius. Haplotype **G**, found in three of the four samples of ancient horses, occurs in all breeds analyzed, but in the Danubian horse it has an unusually high frequency (0.45), significantly exceeding the frequencies in the rest. The uniqueness of the Danubian horse is probably also related to the preservation of the gene pool of the local mares that participated in its creation. HG **Q**, which in the Danubian horse has a higher frequency (0.053) than in Nonius (0.043), was found in the bone remains of ancient wild horses, in the local Rilo-Rhodope (0.163) and Sredna Stara Planina (0.091) populations, in the Karakachan horse (0.133), as well as with a high frequency in the East Bulgarian horse. Haplotype **C** is found only in Danubian, Pleven and Rilo-Rhodope horses. HG **L** is found in all local populations and the newly created Bulgarian breeds, but it is not found in the Nonius, and it should be noted that in the breeds from the national gene pool, the haplotype has high frequencies. This once again shows the uniqueness of the newly created Bulgarian breeds, as well as the importance of local populations in their formation. It also emphasizes the stability of the gene pool of the local populations over time, which is probably related to ensuring the adaptability of the created breeds to the local ecological and economic conditions.

Although small in number, the Danubian breed has a well-established genealogical structure that guarantees sustainable development of the breed. When working with the lines, the disadvantage of leaving 1-2 stallions at a time is overcome, which lead to the strengthening of the linear structure. Stallions from the main lines are used evenly in the families. In the breed, 6 lines are established and developed, of which 4 lines are sustainable and with significant potential for development. Those of the stallions: Hrabar (with 10 stallions and 23 mares), Kalifa (4 stallions and 22 mares), Lider (5 stallions and 22 mares) and Torpedo (4 stallions and 22 mares). The Zdravko stallion line has one male representative and cryopreserved semen from his sire. The line can be restored through the existing mares. There are 6 mares from the line with 50% blood, and 16 mares with 25%. In the newly established stallion Erno line (with 2 stallions and 11 mares), an opportunity for expansion is found, through the restoration of the branches of the stallions of the I generation, based on their female descendants. The effect of using stallions of the Thoroughbred English breed is rather positive. Two of the "old" families of the Danubian breed continue to exist thanks to the use of Thoroughbred English stallions. In the Danubian breed, 12 families are developing - 5 of which have been established in recent years. The breeding work with the families is carried out at a high level - the families are either based on a broad genealogical basis (Zhandarma, Stefa) or it is gradually enriched (Serdika Yu, Mara Yu, Fraila, Katya, Leska II). From the families participating in the breeding process, the families of: Mara Yu - with 22 representatives, from 5 separate branches, are steadily developing; Gendarme - with 16 mares, from 2 branches; Fraila - with 12 representatives, from 2 branches; Stefa - with 8 representatives, from 3 independent branches. The families of: Leska II (10 mares from 2 branches) also have good prospects for development, Katya (with 7 mares egalised in exterior, from 4 lines). The family of the mare Serdika Yu, with 2 representatives at the moment, is threatened with extinction. Breed selection is aimed at creating animals of the "medium" type, intermediate between the "small" and the "large" Nonius, but closer to the latter. As a result, a breed consolidated in exterior is created. In stallions, the average height at the withers is 161.4 ± 0.47 cm, body length - 171.4 ± 0.79 cm, chest girth 196.1 ± 1.20 cm, cannon bone girth - 22.08 ± 0.12 cm. Physiological indices are: for stretched body 106.2 ± 0.35%, for massiveness - 121.5 ± 0.68%, for compactness - 114.5 ± 0.67% and for bone development - 13.68 ± 0.06%. Respectively for mares, the values are: average height at the withers - 161.6 ± 0.29 cm, body length - 167.9 ± 0.49 cm, chest girth 189.2 ± 0.74 cm, cannone bone girth - 21 .28 ± 0.07 cm. Physiological indices are: for stretched body 103.9 ± 0.22%, for massiveness - 117.1 ± 0.42%, for compactness - 112.8 ± 0.41% and for bone development - 13.17 ± 0.04%.

The Danubian breed has a high genetic diversity. At 15 microsatellite loci suitable for studying genetic diversity (PIC=0.73±0.15), Shannon's

Information Index (I) averaged 2.22±0.07. The observed heterozygosity (No) is 0.87 ± 0.02 and is higher than expected (He= 0.84 ± 0.02) in 13 of the 15 studied loci. Nei coefficient for total genetic diversity $\mathbf{H_T}$=0.907 (0.007). In the 166 animals studied, a total of 184 alleles are identified in the 15 loci. The average number of alleles (Na) for all investigated microsatellite markers was 12.29± 0.44. The Danubian breed is not threatened by inbreeding depression. Total (F_{IT}) and intrapopulation (F_{IS}) inbreeding coefficients have mean values close to 0 - 0.037 ±0.021 (P = 0.004) and -0.043 ±0.016 (P = 0.002), respectively. The lines of the Danubian breed have a high intralineal diversity. The average number of alleles, on the studied loci, varies from 4.20 in the Zdravko line to 14.60 in the Lider and Kalifa lines. In the latter, a unique allele is found at the ASB17 locus with a frequency of 2.9%. Ho varies from 0.65 in Zdravko's line to 0.94 in Torpedo's, and Ne from 0.57 in Zdravko's line to 0.91 in Lider and Kalifa. The Danubian horse lines are genetically extremely similar to each other (F_{ST} =0.078±0.009; D_{ST} 0.46±0.04). This confirms the data from the genealogical analysis that the lines do not breed in isolation and only a small part of the stallions and mares have a higher percentage of blood from one or the other line. Only 6.1% (G_{ST} =0.061±0.021) of the genetic diversity is due to differences between lines. Genetically, the closest are the lines of Lider and Kalifa (D_A =0.171) and Lider and Torpedo (D_A =0.188), and the most distant are those of Zdravko and Erno (D_A =1,142), with Zdravko's line being the furthest from all lines. Hrabar's line is similarly distant from Zdravko's line and the other lines, being closer to the latter. These differences are also confirmed by the cluster analyzes made by the model of correlated allele frequency and PCoA, which separate the Zdravko and Hrabar lines into independent clusters.

CONCLUSIONS

1. The Danubian horse is a unique national breed, with a specific mitochondrial profile, the result of the breeding activities with the breed and the preservation of the gene pool of the local populations that participated in its creation.
2. The newly created Bulgarian breeds - Danubian horse, Pleven horse and East Bulgarian horse are genetically closely related to each other, to modern local populations and to prehistoric wild horses inhabiting today's territories of the country, which further emphasizes their uniqueness and the importance of the gene pool of local populations during their formation, probably related to ensuring adaptability to local ecological and economic conditions.

3. The newly created Bulgarian breeds have lost part of the mitochondrial diversity found in modern local horse populations.

4. The data from the mitochondrial analysis of prehistoric wild horses and of modern national breeds and populations gives reason to assume that today's territories of the country have been a place of domestication of the horse, as part of the western periphery of the Pontic-Caspian center.

5. The Danubian breed has a well-established genealogical structure that guarantees sustainable development of the breed. 6 lines and 12 families have been developed in the breed, five of which have been established in recent years.

6. When working with the lines, the disadvantage of leaving 1-2 stallions at a time is overcome. This led to the strengthening of the linear structure. Stallions from the main lines are used evenly in the families.

7. The selection of the breed is aimed at creating animals of the "medium" type, intermediate between the "small" and the "large" Nonius, with a bias towards the latter. As a result, a breed consolidated in exterior is created.

8. The conducted microsatellite analysis of 15 marker loci shows that the Danubian breed has a high genetic diversity and at this stage is not threatened by inbreeding depression.

9. The lines of the Danubian breed have a high intralineal diversity, but with an extremely high genetic similarity to each other, which requires maximum precision when developing the selection schemes.

10. Data from genealogical and DNA analyzes cannot be used independently to establish the origin, phylogeny and ongoing genetic processes in the population. When developing breeding strategies, they should be used comprehensively.

RECOMMENDATIONS

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1. The breeding organization must comply with the recommendations laid down in the objectives of the "concept for short-term development of the breed", set out in the study.

2. The breeding organization, together with the breeders of the Danubian horse, experts and scientific workers to develop a "Concept for the long-term development of the Danubian horse breed", with the results of the current study being included in the criteria for "genetic stability", which should be periodically updated.

3. When developing concepts for the development of the Danubian breed, a complex approach should be used taking data from genealogical and molecular genetic analyses.

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