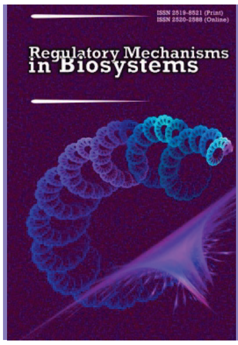


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# Comparative characteristics of polymorphisms of melanocortin 4 and ryanodine 1 receptor genes of Myrhorod pigs before and after the African swine fever outbreak

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One of the global problems that are anthropogenic in nature is the sharp decline in biodiversity, a special case of which is the reduction in the number of species of wild animals and the number of breeds of farm animals. The Myrhorod pig breed, due to its genetic and biological characteristics, is of great importance for the process of preserving the biodiversity of agricultural animals. The study's object was Myrhorod breed pigs, the study focused on the variations in the allele frequency of the RYR1 / SNP g.1843 C>T and MC4R / SNP c.1426 G>A genes that occurred in the Myrhorod breed as it recovered from the African swine fever epidemic. The age at which one pig reaches 100 kg (days); the average daily weight gain from 90 days to slaughter (g); the thickness of the backfat (mm); the length of the carcass (cm); the cross-sectional area of the longest back muscle at the level of the 6th and 7th thoracic vertebrae ($cm^2$); the slaughter output (%); and the yield of meat, fat, and bones from the carcass (%) are other quantitative changes in indicators of fattening and meat productivity. Significant alterations in the SNP marker MC4R polymorphism were observed during the restoration of the Myrhorod pig breed after the African swine fever epidemic. The frequency of the A allele dropped by 2.79 times to just 26.9% from 75.0%, which was 3.17 times higher than the frequency of the G allele until 2019. These alterations resulted from the forced crossing of the Myrhorod breed with the Pietrain breed, which has a high frequency of the G gene (83.0%), during the early phase of the breed's restoration in order to prevent close inbreeding. It is suggested that animals with the AA genotype should be preferred during further restoration in order to restore the Myrhorod breed to its original state. If crossbreeding becomes necessary again (to prevent excessive inbreeding), only breeds related to the Myrhorod (Berkshire, Poltava meat, Large White) should be used. The allele frequency in 2023 did not differ substantially from 2015 or 2019, based on the RYR1 DNA marker. The recessive T gene at the RYR1 g.1843 C>T locus was absent in 2023, confirming the stress resistance of the Myrhorod breed. When compared to the same indicator in the population of 2019, the average daily gain in the live weight of pigs belonging to the restored Myrhorod breed in 2023 was higher. Although this is a good development in terms of making profits by producing this breed of pig, these notable variations might point to important shifts in the polymorphism of genetic markers linked to growth rate. To determine the extent to which restoration has altered DNA markers linked to this productive attribute, more research is required to examine the frequency of alleles of genes linked to growth intensity.

*Keywords*: biodiversity; DNA-marker; MC4R; RYR1; growth; fattening traits; backfat thickness; meat index.

## Introduction

The Myrhorod breed of pigs is of great importance for the preservation of the biodiversity of agricultural animals. Biodiversity is a term that is now frequently used in research papers (Díaz & Malhi, 2022). The problem of preserving biodiversity exists alongside such global problems of our time as resource conservation and minimization of the negative impact of livestock production on the environment (Zos-Kior et al., 2020; Brockova et al., 2021; Zos-Kior et al., 2021). The benefits and roles provided by biodiversity form the foundation of human well-being and sustainable development (Mi et al., 2021). Primary production, nutrient cycling, air and water purification, reducing greenhouse gas emissions and climate change, crop pollination, food and genetic resource providing, disease prevention, and educational, cultural, and spiritual benefits are only a few of these services. As an illustration of the significant increase in attention to the topic of biodiversity conservation, we can cite the data of Chinese scientists (Mi et al., 2021), who note that at the beginning of the century, the number of scientific works devoted to biodiversity numbered several dozen, and in 2020 it almost reached 2000 publications. This is due to a significant increase in funding for this topic at the state level: the Chinese government has invested US$ 378.5 billion over the past two decades in 16 large-scale sustainable development projects (Bryan et al., 2018) and thus significantly slowed the loss of biodiversity (Liu et al., 2008). A similar situation is observed in other developed countries, for example in the USA (Weiskopf et al., 2020; Manfredo et al., 2021) and countries of the European Union (Pilotto et al., 2020; Hermoso et al., 2022). In Ukraine, scientists are also dealing with the issue of preserving the biodiversity of agricultural animals. As noted by Vishnevsky et al. (2017), the genetic resources of local breeds must be systematically maintained in the number of head necessary to preserve the gene pool, and the main criterion for evaluating animals of small breeds is not productivity, but reproduction of typical breed qualities and traits. Despite the understanding of the problem, there is currently a rapid narrowing of biological diversity in general and the diversity of farm animals in particular. Rapid genetic change is being produced by very efficient livestock selection programs thanks to advancements in processing power and statistical approaches. However, when the number of mating animals decreases, a high selection pressure also suggests a decrease in the effective size of the selected population. For example, intensive use of elite bulls through artificial insemination in cattle has led to a sharp decrease in the effective population size. Thus, a decrease in the genetic variance of chosen populations may represent the cost of selection efficiency (SanCristobal et al., 2006).

According to researchers (Megens et al., 2008), three-quarters of local or traditional breeds are either extinct or neglected, while many native pig breeds in Europe have undergone significant alteration. On a global scale, five breeds are common: Large White, Landrace, Duroc, Hampshire and Pietrain, on the basis of which the main synthetic commercial genotypes of pigs used in industrial pork production were created (Khramkova & Povod, 2017). Numerous local pig breeds with low productivity were abandoned as a result of intense selective breeding and the genetic development of a small number of pig breeds. Local pig breeds, on the other hand, offer an invaluable genetic resource since they are better suited to their unique environmental circumstances and food supplies. Compared to modern breeds, they have superior fatty acid content, a greater lipogenic capability, and the ability to deposit more fat. Adipose tissue of local pig breeds shows a greater propensity for adipocyte hypertrophy and hyperplasia. When local pig breeds are compared to modern pig breeds, these traits can be explained by increased de novo fatty acid production, accelerated adipogenesis, and unique lipid mobilization. Studies using transcriptome and proteomic analyses of subcutaneous fat tissue identify a number of gene groups that are different between contemporary pig breeds and native breeds. Modern pig breeds showed upregulation of genes involved in extracellular matrix development and mitochondrial energy metabolism in subcutaneous adipose tissue, whereas local breeds showed upregulation of genes and proteins involved in lipogenesis, desaturation, immune response, and fatty acid transport. Studies on transcriptome and proteome have shown that genes and proteins associated in adipogenesis, lipogenesis, desaturation, and other processes are upregulated in cases of intramuscular fat (Poklukar et al., 2020).

The Myrhorod breed of pigs is a typical representative of small local breeds that are on the verge of extinction due to a number of reasons (Vashchenko et al., 2019). The Myrhorod breed was recognised in 1940, and its maximum number fell in the 1960s. The Myrhorod breed is characterized by some unique biological and economic features, which are currently not in demand in production, but the situation may change if production conditions and consumer demand change. Such features of the Myrhorod breed include, first of all, the extremely high content of intramuscular fat at the level of 6.56 ± 1.37% (Shcherban & Vovk, 2014). It is known that the content of intramuscular fat affects the tenderness and energy value of meat. For comparison, half-breeds of the Myrhorod breed with the Large White breed had 6.29 ± 0.58% fat in the muscles; hybrid animals ♀½ Myrhorod × ♂½ Pietrain – 2.98 ± 0.37% intramuscular fat; and animals with ¼ Landrace breed (♀M × ♂(½M * ½L)) – had 2.61 ± 0.31% intramuscular fat ($P < 0.05$, the difference is significant compared to purebred Myrhorod pigs). Thanks to this content of intramuscular fat, the meat of Myrhorod pigs is juicy and has an excellent taste. Although growing Myrhorod pigs under the conditions of industrial technology is not economically feasible, marble pork meat is in demand even now as a niche craft product.

The lard of Myrhorod pigs also has some differences in chemical composition compared to the lard of domestic animals. The content of hygroscopic moisture in the tallow of purebred animals was 8.8–26.0% lower compared to the tallow of animals obtained as a result of crossing with industrial breeds. In addition, the fat of purebred Myrhorod pigs recorded the lowest melting point (30.70 ± 0.38), which may indicate a lower content of unsaturated fatty acids (Shcherban & Vovk, 2014). It is worth noting that foods containing monounsaturated and polyunsaturated fatty acids are more beneficial for human consumption than those containing saturated fatty acids. Once in the blood, molecules of saturated fatty acids have a tendency to connect with each other, which can cause certain health problems for consumers of such a product. In contrast, unsaturated fatty acids do not form compounds in the blood, which allows them to pass freely through the arteries (Brych et al., 2020).

In addition to the above, another characteristic of the Myrhorod breed is its ability to use grass and bulky feed in relatively large quantities (Vashchenko et al., 2019). This is due to the fact that the breed is based on local pigs, which digest fiber better.

However, despite the valuable biological features of the Myrhorod breed, its population has steadily decreased over the past 30 years (Vashchenko et al., 2019). A real challenge for the preservation of the Myrhorod breed was the outbreak of African swine fever on the only breeding farm that was engaged in breeding of this breed. Since 2019, the restoration of the population was started using livestock preserved in non-breeding farms and collection herds. As of January 1, 2020, compared to the beginning of the breed's recovery, the population had increased by 1.7 times (Tsybenko & Vashchenko, 2020). But at the same time, the threat of extinction of the breed remains. So, today, the vast majority of animals are kept on one farm, their number remains insignificant, the level of kinship among the animals of the existing stock is very high.

An important role in the restoration of the Myrhorod breed has been played by molecular genetic methods of population research. The task of our work was to restore the population with the ratio of allele frequencies as close as possible to the original Myrhorod breed. At the same time, scientists have also tried to determine and take into account the associations of genes with productive traits, which were known from previous studies before the outbreak of African swine fever. Everything was known about allele frequencies and genetic variability in the population of pigs of the original Myrhorod breed for 25 gene loci of quantitative traits (Sarantseva et al., 2016; Vashchenko et al., 2019).

MC4R is one of the important DNA markers associated with the high fat thickness characteristic of the Myrhorod breed. The frequency of the A allele at the locus (SNP c.1426 G>A) in the population of 2018 was 0.750, which distinguished the Myrhorod breed from modern breeds, in which the G allele usually predominates (Vashchenko et al., 2019). SNP marker MC4R c.1426 G>A, according to the data of a significant number of studies (Davoli et al., 2012; Calta et al., 2022; Vashchenko et al., 2023), related to lard thickness and intensity of feed consumption in pigs. Such an effect is due to the fact that the mutation in this locus disrupts normal G protein coupling in the MC4R signaling to adenylyl cyclase (Ovilo et al., 2006). According to the above-mentioned authors, selection by locus (SNP c.1426 G>A) should take into account the purpose of the line with which work is being conducted. Since choosing the A allele will result in bigger and fatter pigs, but with a lower yield in premium cuts, lighter-colored meat, and a changed fatty acid profile that will tend to saturation, none of the alleles would clearly have an advantage. However, selection to increase the frequency of allele G in sire lines would be sufficient to improve the output of premium cuts and the color of the meat, while positive selection of allele A in dam lines might be beneficial to aid in improving the pigs' reproductive ability. By using these strategies, the proportion of heterozygous individuals for the MC4R gene in commercial crossbred pigs would rise, increasing the homogeneity of carcasses with respect to the impacted productive and quality features, which would be highly valued by the meat market (Ovilo et al., 2006).

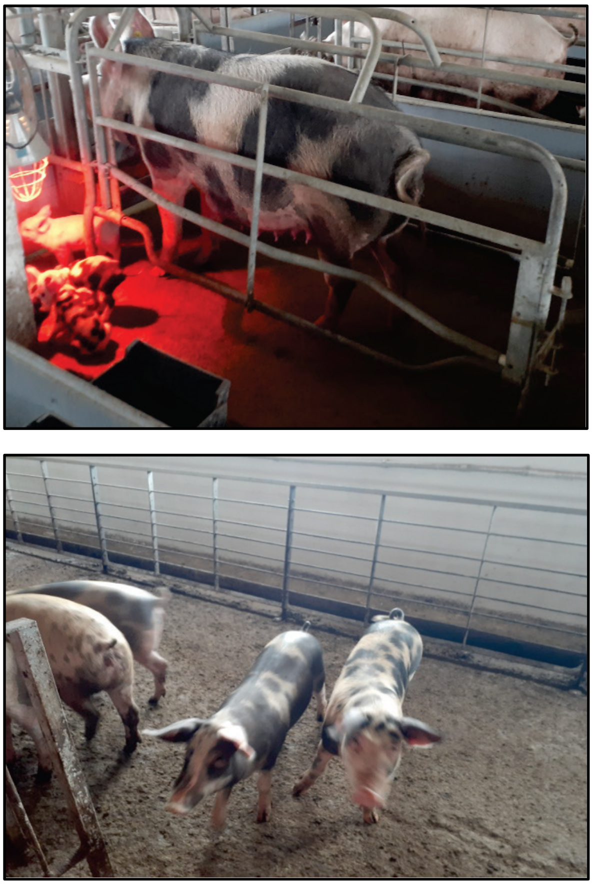
Another genetic feature of the Myrhorod breed before the outbreak of African swine fever was the absence or a very low frequency (no more than 4 percent) of a recessive allele of a DNA marker RYR1 (g.1843C>T SNP) (Vashchenko et al., 2019). Ryanodine receptor 1 gene (RYR1) is associated with a hereditary disease of pigs – hypersensitivity to stress, which in an extreme form can turn into malignant hyperthermia (Fujii et al., 1991). This mutation affects the health, welfare, and carcass and meat quality in slaughter pigs. According to research Cobanovic et al. (2019) RYR1 Nn pigs outweighed NN pigs in regards to live, hot, and cold carcass weights as well as average lifetime daily weight growth ($P < 0.05$). Furthermore, Nn pigs had better ($P < 0.05$) loin muscle thickness and lean meat content and lower ($P < 0.05$) backfat thickness, confirming the beneficial effect of the recessive n gene on carcass quality.

It was also found that RYR1 Nn pigs have a more favorable daily feed intake, increased feed conversion efficiency, and, consequently, higher growth rate and meatiness compared to NN pigs (Fàbrega et al., 2003; Silveira et al., 2011; Cobanovic et al., 2019). Fàbrega et al. (2003) and Cobanovic et al. (2019) consider that the faster metabolism is the reason for better feed utilization and the higher carcass lean content, as well as greater ability of stress-carrier pigs to assimilate proteins, and a lower predisposition for fat deposition.

However, the purpose of our work was not breeding to obtain more productive pigs, but the analysis of genetic changes that occurred in the population after the outbreak of African swine fever and the selection of pigs with a genotype as close as possible to that of the original Mirgorod breed.

## Materials and methods

During the research, we followed the requirements of "The European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasburg, 1985), the Order of the First National Congress of the Bioethics (Kyiv, 2001), and the law of Ukraine "On the protection of animals against ill-treatment" No. 3447-IV as of 21/02/2006 last amended on 04/08/2017. We obtained the approval for the study from the Committee for the Maintenance and Use of Animals of the Institute of Pig Breeding and Agroindustrial Production. Limitations on the capacity of experimental animals to meet their physiological and ethological requirements were minimized throughout the entire experiment. Only when absolutely required during estrus and during the lactation period following farrowing was fixed maintenance of sows implemented (Fig. 1a). The animals' overall health was monitored on a regular basis. Fattening was carried out in group cages of 9 heads in a box (Fig. 1b).



a

b

**Fig. 1.** Maintenance of experimental pigs of the restored Myrhorod breed at the State Enterprise "Experimental Farm named after the Decembrists": *a* – fixed maintenance of sows during lactation period, *b* – maintenance of experimental pigs during fattening

The objects of the study were pigs of the Myrhorod breed belonging to the research and production department of the Institute of Pig Breeding and Agro-Industrial Production of the National Academy of Agrarian Sciences and the State Enterprise "Experimental Farm named after the Decabrists".

The subject of the study was the changes in the allele frequency of the MC4R / SNP c.1426 G>A and RYR1 / SNP g.1843 C>T genes that occurred in the Myrhorod breed during its recovery after the outbreak of African swine fever. And also, quantitative changes in indicators of fattening and meat productivity: age of reaching 100 kg (days); average daily gain from the age of 90 days to slaughter (g); backfat thickness (mm); length of longitudinal half of carcass (cm); cross-sectional area of the longest back muscle at the level of the 6–7th thoracic vertebra ($cm^2$); slaughter output (%); yield of meat, fat and bones from the carcass (%).

Feeding of pigs during the fattening period was carried out in accordance with the norms (Provatorov et al., 2007). When the animals were weaned, they weighed between 20 and 30 kg live weight. They were fed feed that contained 17.6 MJ of exchangeable energy, 1.26 kg of dry matter, 239 g of crude protein, and 12.4 g of lysine per day. After being removed from rearing and placed on fattening, the pigs received a diet that was adjusted depending on their live weight (Table 1).

**Table 1**
Nutritional value of the daily ration experimental pigs for fattening

| Components of ration | Weight of pigs, kg | | | | | | |
|---|---|---|---|---|---|---|---|
| | 40–50 | 50–60 | 60–70 | 70–80 | 80–90 | 90–100 | 100–120 |
| Metabolic energy, MJ | 24.5 | 29.0 | 32.4 | 34.8 | 38.2 | 40.7 | 43.5 |
| Dry matter, kg | 1.80 | 2.13 | 2.38 | 2.56 | 2.81 | 2.99 | 3.20 |
| Crude protein, g | 293 | 347 | 388 | 387 | 424 | 451 | 483 |
| Digestive protein, g | 229 | 272 | 303 | 302 | 332 | 353 | 378 |
| Lysine, g | 14.7 | 17.4 | 19.4 | 18.6 | 20.4 | 21.7 | 23.2 |
| Methionine + cystine, g | 8.70 | 10.2 | 11.4 | 11.0 | 12.0 | 12.8 | 13.7 |
| Threonine, g | 9.50 | 11.3 | 12.6 | 12.1 | 13.2 | 14.1 | 15.1 |
| Tryptophan, g | 2.65 | 3.13 | 3.50 | 3.33 | 3.65 | 3.89 | 4.16 |
| Crude fiber, g | 108 | 128 | 143 | 179 | 197 | 209 | 224 |
| NaCl | 10.4 | 12.4 | 13.8 | 14.8 | 16.3 | 17.3 | 18.6 |
| Calcium, g | 15.1 | 17.9 | 20.0 | 20.7 | 22.8 | 24.2 | 25.9 |
| Phosphorus, g | 12.6 | 14.9 | 16.7 | 17.2 | 18.8 | 20.0 | 21.4 |
| Iron, mg | 157 | 185 | 207 | 207 | 228 | 242 | 259 |
| Copper, mg | 21.6 | 25.6 | 28.6 | 30.7 | 33.7 | 35.9 | 38.4 |
| Zinc, mg | 216 | 256 | 286 | 307 | 337 | 359 | 384 |
| Manganese, mg | 144 | 170 | 190 | 205 | 225 | 239 | 256 |
| Cobalt, mg | 2.16 | 2.56 | 2.86 | 3.07 | 3.37 | 3.59 | 3.84 |
| Iodine, mg | 0.41 | 0.49 | 0.55 | 0.59 | 0.65 | 0.69 | 0.74 |
| Selenium, mg | 0.45 | 0.53 | 0.60 | 0.64 | 0.70 | 0.75 | 0.80 |
| Carotene, mg | 10.4 | 12.4 | 13.8 | 13.3 | 14.6 | 15.5 | 16.6 |
| Vitamin A, thousand IU | 5.22 | 6.18 | 6.90 | 6.66 | 7.31 | 7.77 | 8.3 |
| Vitamin D, thousand IU | 0.52 | 0.62 | 0.69 | 0.67 | 0.73 | 0.78 | 0.83 |
| Vitamin E, mg | 52.2 | 61.8 | 69.0 | 74.2 | 81.5 | 86.7 | 92.8 |
| Vitamin B1, mg | 4.14 | 4.90 | 5.47 | 5.12 | 5.62 | 5.98 | 6.40 |
| Vitamin B2, mg | 5.40 | 6.39 | 7.14 | 7.68 | 8.43 | 8.97 | 9.60 |
| Vitamin B3, mg | 25.2 | 29.8 | 33.3 | 35.8 | 39.3 | 41.9 | 44.8 |
| Vitamin B4, g | 1.80 | 2.13 | 2.38 | 2.56 | 2.81 | 2.99 | 3.20 |
| Vitamin B5, mg | 104 | 124 | 138 | 148 | 163 | 173 | 186 |
| Vitamin B12, µg | 41.4 | 49.0 | 54.7 | 58.9 | 64.6 | 68.8 | 73.6 |

For the purpose of animal typing using the melanocortin 4 receptor gene, samples of pig bristles were obtained from 26 animals. Genetic research was conducted in an accredited laboratory of the Institute of Pig Breeding and Agroindustrial Production. With "Chelex 100" (Bio-Rad Laboratories, Inc., USA) genomic DNA was isolated from pig bristles (Korinnyi et al., 2005). To type DNA, the PCR-RFLP method was utilized (Hlazko et al., 2001). A fragment of the MC4R gene (MC4R / SNP c.1426 G>A / 2-nd exon / NCBI accession number rs 178554175 / Asp >Asn) consisting of 220 bp was amplified using a pair of specific primers: forward: 5'- TGATTCAGGATCTATTGCTACTA -3' and reverse: 5'- TATACTGTCGCTTGTGCTTAAG -3' (Kim et al., 2006). PCR reactions were performed in 25 µL (final volume) of the mixture containing 10–100 mg of genomic DNA, 200 nM of forward and reverse primers, 2.5 mM MgCl2, 0.25 mM of each of the dNTPs and one unit of the recombinant Taq DNA Polymerase (Thermoscientific, EU). PCR amplification program: 95 °C – 2 minutes; 30 cycles: 95 °C – 30 s, annealing of primers 52 °C – 30 s, 72 °C – 105 s; 72 °C – 7 min. PCR was performed in thermocycler "Tertsyk-2" (DNA Technology, RF).

The amplification fragment of the MC4R gene was restricted with the enzyme Taq I (Thermo Fisher Scientific, Lithuania) at 65 °C – 3 hours, which caused the appearance of restriction fragments corresponding to the following genotypes of the MC4R gene: AA – 220 bp, AG – 220, 150, 70 bp, GG: 150, 70 bp (Fig. 2).

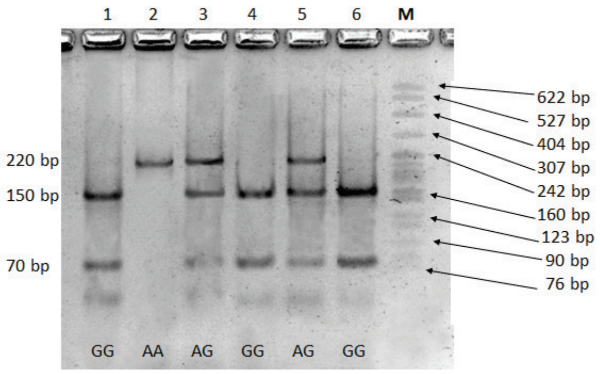



**Fig. 2.** Results of MC4R gene restriction in 3.5% agarose gel: *1*, *4*, *6* – animals with a genotype GG; *3*, *5* – animals with a genotype AG, *2* – animal with a genotype AA; *M* – marker of molecular weight pBR322/MspI

Amplification of the RYR1 gene fragment (137 bp) was carried out using the primers of the following structure (Balatsky et al, 2015): F: 5`-GTGCTGGATGTCCTGTGTTCCCT- 3`, R: 5`- CTGGTGACATAG-TTGATGAGGTTTG - 3`. PCR amplification program: 1 cycle of denaturation 94 ºC – 4 minutes, 35 cycles – denaturation 94 ºC – 1 minute, annealing 68.5 ºC – 1 minute, elongation 72 ºC – 1 minute, 1 cycle – final elongation 72 ºC – 4 minutes. The size of the amplification and restriction fragments was determined using a marker pUC19DNA/MspI ("Thermo scientific", USA).

HhaI-polymorphism of the studied fragment is caused by the presence of one restriction site for the specified enzyme, which occurs as a result of the C/T transition and leads to the formation of restriction fragments with a length of 84 and 53 bp. The presence of one restriction site for the specified enzyme, which occurs as a result of the C / T transition and forms restriction fragments with a length of 84 and 53 bp, makes it possible to detect the HhaI polymorphism of the studied segment.

The coefficient of inbreeding of pigs of the Myrhorod breed, which remained after the outbreak of African swine fever in 2019, was determined according to the method of S. Wright in the modification of Kislovsky described in the work of Voitenko (2018).

Meat qualities of fattening young animals were determined after the slaughter of experimental animals when they reached a live weight of 100 kg. Carcasses were split in half lengthwise with a circular saw. The thickness of bacon after slaughter was measured with a metal ruler with an accuracy of ± 0.25 mm. The length of the carcass after slaughter was measured with a measuring tape with a division value of 1 mm from the front edge of the growth of the pelvic bones to the front edge of the first cervical vertebra. Cross-sectional area of the longest back muscle at the level of the 6–7th thoracic vertebrae was determined by measuring the area of the muscle imprint on the calculus using a planimeter (Standart-M, UA). After the slaughter of the experimental animals, the carcasses were deboned and the mass of muscles, fat and bones was determined on an electronic scale with an accuracy of ± 0.05 kg. After that, the percentage ratio of the mass of different tissues was calculated. Slaughter yield (%) was defined as the percentage ratio of the carcass weight without internal organs (lungs, liver, heart and gastrointestinal tract) to the pre-slaughter weight of the animal. The meatiness index was defined as the ratio of the cross-sectional area of the longest back muscle at the level of the 6–7 thoracic vertebrae to the cross-sectional area of backfat at the same level.

Data processing was done with SAS/STAT(R) 15.1 (SAS Institute Inc., USA, 2018) software. The tables present the arithmetic mean values along with the corresponding standard errors (x ± SE). The Chi-square test was used to calculate the reliability of differences in the frequency of alleles between pig breeds. If the expected frequency values in the contingency tables were <10, the Yates correction was applied to the Chi-square test. Analysis of variance (ANOVA) followed by the Tukey HSD test were used to see whether there were any significant differences between the groups. Differences were deemed significant when $P < 0.05$.

**Results**

As a result of the typing of pigs of the Myrhorod breed according to the DNA marker MC4R / SNP c.1426 G>A in 2023 and the comparison of the results obtained with those obtained in 2015 and 2019, it was established that in the studied herds, which represent 90% of the entire breed as a whole, the frequency of alleles A and G has changed significantly. If before the outbreak of the African plague (2015), the frequency of allele A was 3.17 times higher compared to the frequency of allele G, then in 2023 we observe the opposite situation: the frequency of allele G is 2.72 times higher compared to the frequency of allele A (Table 2).

**Table 2**
Dynamics of SNP MC4R allele frequencies
in the Myrhorod breed of pig over the years

| The year of determining the genotype by marker | n | Frequency of allele | | P (compared to 2015) |
|---|---|---|---|---|
| | | A | G | |
| 2015[1] | 50 | 0.760 | 0.240 | – |
| 2019[2] | 38 | 0.750 | 0.250 | 0.8785 |
| 2023 | 26 | 0.269 | 0.731 | $5.19*10^{-9}$ |

*Note*: [1] – data for 2015 for comparison of allele frequencies are taken according to Balatsky et al. (2015); [2] – data for 2019 for comparison of allele frequencies are taken according to Vashchenko et al. (2019).

It should be noted that in 2019, a year after the outbreak of the African swine fever, a high frequency of the A allele was still preserved in pigs that were found in farms that bought breeding animals from the SE "Experimental Farm named after the Decembrists". However, due to the small number of livestock remaining in the breed and its high degree of consanguinity (from 25% to 70% according to the Wright-Kyslovsky coefficient), at the first stage of restoration Myrhorod pigs were crossed with the Pietrain breed, which is characterized by a high frequency of the allele G (Table 3).

**Table 3**
Comparison of the frequency distribution of the MC4R gene in the recovered Myrhorod breed and breeds common in Ukraine and the world

| Breed | n | Frequency of allele | | P (compared to Myrhorod) |
|---|---|---|---|---|
| | | A | G | |
| Myrhorod | 26 | 0.27 | 0.73 | – |
| Ukrainian Large White 1[1] | 100 | 0.87 | 0.13 | $7.54*10^{-19}$ |
| Ukrainian Large White 3[1] | 100 | 0.75 | 0.25 | $9.25*10^{-11}$ |
| English Large White[1] | 100 | 0.55 | 0.45 | $3.08*10^{-4}$ |
| Pietrain[1] | 9 | 0.17 | 0.83 | 0.578 |
| Poltava meat breed[1] | 40 | 0.40 | 0.60 | 0.123 |
| Ukrainian Spotted Steppe[1] | 20 | 1.00 | 0.00 | $1.70*10^{-12}$ |
| Ukrainian White Steppe[1] | 10 | 0.80 | 0.20 | $1.31*10^{-4}$ |

*Note*: [1] – data for comparing other breeds with the Myrhorod breed are taken according to Balatsky et al. (2015).

In order to prevent the loss of the gene pool of the Myrhorod breed, only breeds related to the Myrhorod breed were used in further work on its restoration. These include the Poltava meat breed (75% of the sows that formed the basis of the Poltava meat breed belonged to the Myrhorod breed), as well as the Berkshire and Large White breeds (boars of both breeds were used at the initial stages of the Myrhorod breed). In further work on the restoration of the breed, the task is to increase the frequency of this allele to the values characteristic of the original Myrhorod breed by means of selection for breeding of animals with the A allele of the MC4R / SNP c.1426 G>A marker. In this context, the term "original Myrhorod breed" refers to the population that existed before the outbreak of African swine fever.

As we can see from Table 3, according to the allele frequency of the MC4R / SNP c.1426 G>A locus, the Myrhorod breed currently differs significantly not only from various types of large white breed, but also from other local breeds of Ukraine, Ukrainian Spotted Steppe and Ukrainian White Steppe.

No significant differences were found for this productive trait between groups of animals of 2019 and 2023 (Table 4).

**Table 4**
Comparison of productivity of Myrhorod pigs in different years (x ± SE)

| Performance indicators | Year | |
|---|---|---|
| | 2019 | 2023 |
| Age of reaching 100 kg, days | 203.42 ± 3.56 | 193.22 ± 3.45* |
| Backfat thickness, mm | 31.01 ± 1.33 | 30.89 ± 0.38 |
| Average daily gain from the age of 90 days to slaughter, g | 598.06 ± 10.07 | 632.56 ± 12.48* |
| Length of longitudinal half of carcass, cm | 94.17 ± 1.94 | 93.50 ± 0.32 |
| Cross-sectional area of the longest back muscle at the level of the 6–7th thoracic vertebrae, $cm^2$ | 32.99 ± 1.20 | 32.33 ± 0.27 |
| Pre-slaughter weight, kg | 99.28 ± 0.29 | 101.67 ± 0.71 |
| Carcass weight, kg | 73.67 ± 0.55 | 73.64 ± 0.30 |
| Slaughter output, % | 74.20 ± 0.65 | 72.46 ± 0.54 |
| Cross-sectional area of the backfat at the level of the 6–7th thoracic vertebra, $cm^2$ | 48.24 ± 0.38 | 45.94 ± 0.46*** |
| Meat index, units | 0.684 ± 0.009 | 0.704 ± 0.007 |
| Carcass content: meat, % | 49.22 ± 0.45 | 50.40 ± 0.32* |
| fat, % | 41.89 ± 0.57 | 40.59 ± 0.25* |
| bones, % | 8.89 ± 0.17 | 8.98 ± 0.11 |
| n | 18 | 18 |

*Note*: the difference is significant (ANOVA followed by Tukey HSD test): * – at P < 0.05; *** – at P < 0.001.

The most significant changes in the productivity of pigs studied in 2019 and 2023 are observed in the area of backfat at the level of the 6–7th thoracic vertebrae. Although its height did not decrease significantly, the cross-sectional area decreased by 2.3 $cm^2$, or by 5.01%. This, in turn, led to an increase in the yield of meat from the carcass by 1.18 percentage points and a decrease in the yield of lard by 1.30 percentage points. Also, a significant difference between pigs of the Myrhorod breed was recorded for fattening quality traits: the age at which the weight of 100 kg was reached and average daily gains during the fattening period. Purebred pigs of the Myrhorod breed, in the second or third generation of the pedigree of which Pietrain pigs were present, had average daily gains of 34.5 g, or 5.45% more compared to the purebred pigs studied in 2019. According to the RYR1 g.1843 C>T gene polymorphism, the situation in the Myrhorod breed has practically not changed over the past eight years (Table 5).

**Table 5**
Dynamics of SNP RYR1 allele frequencies
in the Myrhorod breed of pig over the years

| The year of determining the genotype by marker | n | Frequency of allele | | P (compared to 2015) |
|---|---|---|---|---|
| | | C | T | |
| 2015[1] | 50 | 0.950 | 0.050 | – |
| 2019[2] | 68 | 0.956 | 0.044 | 0.6179 |
| 2023 | 26 | 1.000 | 0.000 | 0.1659 |

*Note*: [1] – data for 2015 for comparison of allele frequencies are taken according to Balatsky et al. (2015); [2] – data for 2019 for comparison of allele frequencies are taken according to Vashchenko et al. (2019).

If in 2015–2019 the frequency of the recessive allele RYR1 T, which is associated with stress sensitivity, did not exceed 5.0%, then in 2023 this allele was completely eliminated from the population.

## Discussion

The vast amount of crossbreeding that occurs between porcine breeds in an attempt to increase productivity and improve meat quality has significantly altered the genetic makeup of the many pig breeds that are raised around the world. A genetic selection that aims for increased production has supplanted the genetic variability, which has been significantly decreased. These breeds are threatened by high rates of consanguinity and the resulting loss of biodiversity (Llambí et al., 2020). At the same time, local breeds of pigs are distinguished by valuable qualities primarily related to their resistance to diseases and high adaptability (Vashchenko et al., 2022).

As of 2019, the Myrhorod breed of pigs had a number of features of polymorphism of genetic markers that distinguished it from the five most common industrial breeds (Vashchenko et al., 2019). For example, in the Myrhorod breed, the frequency of the recessive T allele at the RYRI marker (SNP g.1843C>T), associated with stress sensitivity, was only 4%, and the remaining population as of 2023 is completely free of the mutant T allele. However, in the Pietrain breed, 100% of animals are carriers of the RYRI T allele (Balatsky et al., 2015). In order to improve the critical state of the effective population of the Myrhorod breed after the outbreak of African swine fever, a one-time crossbreeding with the Pietrain boars was used. The fact that after the use of this crossbreeding, it was possible to restore 100% frequency of allele C in the Myrhorod breed, which was inherent in the "original" Myrhorod breed, is explained by regular testing and strict selection of the obtained crossbreeds for this particular gene. Much attention was paid to this work precisely because one of the features of the Myrhorod breed was its resistance to stress, due to the almost 100% frequency of the C allele (Ivanov & Guk, 2019; Vashchenko et al., 2019). According to some researchers (Tor et al., 2001; Cobanovic et al., 2019), the recessive allele can have a positive effect on the productivity of pigs when the genotype is in a heterozygous state. In research (Tor et al., 2001) it was established that pigs with genotype Nn (CT) differed in better average daily gains at the later stages of fattening, in the period from 135 to 165 days they exceeded their counterparts with genotype NN by 91 grams per day, which was 9.92% (P < 0.01). In addition, the pigs with Nn (CT) genotype had higher predicted carcass lean content (+11.1 ± 3.7 g/kg) and higher proportion of ham in the carcass (+2.9 ± 1.4 g/kg). At the same time, better growth indicators were accompanied by a deterioration of meat quality: the muscles of Nn pigs showed a lower pH by 0.25 ± 0.05 (P < 0.01) 45 minutes after slaughter and a higher electrical conductivity as in the longest back muscle (+1.03 ± 0.17; P < 0.01) and in the semimembranosus muscle (+1.30 ± 0.33; P < 0.01). The differences were consistent with those previously reported (Wittmann et al., 1999) and confirmed that Nn pigs were more prone to PSE meat production than NN homozygotes. However, as already mentioned, the goal of our work was not to increase productivity, but to reproduce the stress-resistant Myrhorod breed as close as possible to the "original" population. For comparison, in the industrial Pietrain breed, which has high meat productivity, but also high stress sensitivity, a 100% frequency of the recessive allele T was found, thus, the now restored Myrhorod breed reliably differs from the Pietrain breed in terms of the frequency of alleles of the stress sensitivity marker RYR1. That is, we can conclude that the goal of restoration has been achieved for this gene.

However, the most significant change in the polymorphisms we studied that occurred in the Myrhorod breed over the past five years can be considered the change in the frequency of alleles in the MC4R / SNP c.1426 G>A marker. A missense mutation of MC4R (p.Asp298Asn) in pigs has been linked to growth and fatness; however, there is debate regarding the impact of Asp298Asn substitution on MC4R function, which limits its use in animal breeding (Zhang et al., 2020). Melanocortin-4 receptor, through binding to its ligands [e.g. a-melanocyte-stimulating hormone (a-MSH) and agouti-related protein (AgRP)], has been reported to play a pivotal role in the regulation of food intake, energy balance and fatness in mammals (Cone, 2005). Also, the melanocortin 4 receptor gene is a widely recognized marker that exhibits a strong relationship with growth, leanness, and feed intake (Kim et al., 2000). Pigs with the MC4R AA genotype tend to consume more feed and, as a result, to more deposition of subcutaneous fat. For example, in research Khalak & Gutyj (2020) it was found that animals with the AA genotype have a greater backfat thickness above the 6–7 thoracic vertebrae by 2.3 mm (P < 0.01), but heterozygous animals differ in better average daily gains and the age at which they reach a live weight of 100 kg animals with the AG genotype, respectively, by 38.3 g (P < 0.001) and 4.6 days (P < 0.01), in addition, the hybrids had a longer chilled carcass length by 2.2 cm (P < 0.001) and a greater length of the bacon half of the chilled half carcass by 2.9 cm (P < 0.001). Also, in our previous studies on the Myrhorod breed, a significant influence of the MC4R genotype on the thickness of the lard (P = 0.0188), cross-sectional area of the longest back muscle (P = 0.0487) and the age of reaching a weight of 100 kg (P = 0.0251) was established (Vashchenko et al., 2019). Like other researchers, MC4RAG turned out to be the desired genotype for obtaining better values of productive traits. The associative analysis conducted by us based on the animal typing data in 2023 confirmed the patterns previously established by us and other researchers: animals with the GG genotype had 1.78 mm, or 5.93% lower fat thickness ($P < 0.001$) and 0.89 mm, or 2.79% higher cross-sectional area of the longest back muscle ($P < 0.05$). Although according to our previous research (Vashchenko et al., 2019) and scientific research of other authors (Gozalo-Marcilla et al., 2021; Zhang et al., 2021; Tinh et al., 2023) MC4R / SNP c.1426 G>A is associated with the backfat thickness of pigs, no significant differences were found for this productive trait between groups of animals of 2019 and 2023.

The change in the frequency of alleles of the MC4R gene in 2023 compared to 2019 and 2015 was accompanied by a simultaneous decrease in the amount of lard, which is explained by the appearance of a significant percentage in the population of animals carrying the GG genotype, which, according to numerous studies, are characterized by less deposition of fat in the carcass (Fan et al., 2009; Fontanesi et al., 2013). At the same time, this situation is inconsistent with the concept of restoration and preservation of the Myrhorod pig breed, according to which the local disappearing breed does not need to be improved, because, on the contrary, its unique characteristics need to be preserved, even if they do not have economic advantages at this stage of the development of the industry (Vishnevsky et al., 2017; Vashchenko et al., 2019). In this regard, in further work with the breed, it is planned to select more animals that are carriers of the A allele for breeding.

The decrease in the area of fat above the 6–7 thoracic vertebrae was due to a decrease in the total fat content in the carcass by 1.3 percentage points ($P < 0.05$). Which is also probably caused by an increase in the frequency of the G allele in the population of the restored Myrhorod breed and is consistent with the data of other authors (Salajpal et al., 2009) obtained on three-breed hybrid pigs (Swedish Landrace x Large White sows mated with Pietrain boars).

It is obvious that when the content of fat in the carcass decreases, the content of muscle tissue increases simultaneously; in our studies, the yield of meat from the carcass increased by 1.18 percentage points ($P < 0.05$). These results are consistent with the data of studies on three-breed hybrids of the Large White, Landrace, and Pietrain breeds (Salajpal et al., 2009), where it was established that animals with the GG genotype prevailed in terms of meat content in the carcass of their counterparts with the AA genotype on 3.16 percentage points ($P < 0.01$) and analogues with the GA genotype – by 2.23 percentage points ($P < 0.01$).

In our research, no significant difference was found between Myrhorod pigs of different years in terms of carcass length, which is consistent with the data obtained by other scientists (Van den Maagdenberg et al., 2007) on three-breed hybrid pigs obtained as a result of the combination of two-breed sows (Large White x Landrace) and two-breed boars (Large White x Pietrain). These studies claim that MC4R gene polymorphism does not affect carcass length. Similar results regarding the lack of influence of MC4R genotypes on this trait were also obtained in studies on purebred pigs of Large White breed of Hungarian origin (Khalak et al., 2021).

In 2023, in our research, the increase in average daily gains in pigs of the Myrhorod breed compared to the similar indicator in the population in 2019 is also a positive change from the point of view of obtaining economic benefits from the breeding of the Myrhorod breed, but it may indicate significant differences in herds obtained as a result of restoration of the breed from the "original" population. The polymorphism of genes is related to the growth rate and requires further research in this direction to assess how significant changes have occurred in the Myrhorod breed according to other DNA markers associated with this productive trait.

## Conclusions

During the restoration of the Myrhorod breed of pigs after the African swine fever outbreak, significant changes occurred in the SNP marker MC4R polymorphism. The frequency of the A allele, which until 2019 was 3.17 times greater than the frequency of the G allele and was 75.0%, decreased by 2.79 times and is now only 26.9%. Such changes are due to the use at the first stage of restoration of the Myrhorod breed of forced (to avoid close inbreeding) crossing with the Pietrain breed, which is characterized by a high frequency of the G allele (83.0%). In order to return the Myrhorod breed to its original state, it is recommended that during further restoration, animals with the AA genotype should be preferably selected, and in the event of the need for crossbreeding again (to prevent excessive inbreeding), only breeds related to the Myrhorod (Berkshire, Poltava meat, Large White) should be used.

According to the RYR1 DNA marker, the allele frequency in 2023 did not change significantly compared to both 2015 and 2019. The Myrhorod breed is stress-resistant, which is confirmed by the absence in 2023 of the recessive T allele at the RYR1 g.1843 C>T locus.

The average daily increase in the live weight of pigs of the restored Myrhorod breed in 2023 increased compared to the similar indicator in the population of 2019. Although this is a positive change from the point of view of obtaining profits from breeding pigs of this breed, these significant differences may indicate significant changes in the polymorphism of genetic markers associated with growth rate. Further work is needed to study the frequency of alleles of genes associated with growth intensity in order to assess to what extent DNA markers associated with this productive trait have undergone changes in the process of restoration of the Myrhorod breed.

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