# Peculiarities of the development of the gene pool of crossbred carps of the first breeding generation reproduced by the synthetic breeding method

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**Abstract**. This study presents an analysis of the genetic characteristics of first-generation hybrid carp created by synthetic selection based on Ukrainian carp breeds: Nyvka carp (small-scaled intrabreed type of the Nyvkivka factory line of the Ukrainian breed of frame carp, *Cyprinus carpio*) and Antoniny-Zozulenets carp (Antoniny-Zozulenets intrabreed type of the Ukrainian framed carp breed, *Cyprinus carpio*). An analysis of protein and enzyme polymorphic systems showed clear genetic differentiation between the parental lines and the pattern of marker inheritance in their reciprocal hybrids. Five allelic variants were found in all studied groups of framed carp at the transferrin (Tf) locus: Tf A, Tf B, Tf C1, Tf C2, Tf D. At the albumin (ALB) locus, the allele A was the most common in all studied groups of framed carp. At the esterase (EST) locus, the fast-migrating allele EST F (0.525) prevailed only in the group of the Nyvky carp, while the allele EST S prevailed in other groups. At the posttransferrin (pTf) locus, the fast-migrating pTf F allele was predominant in all studied groups. At the prealbumin (prAlb) locus, the fast-migrating pTfA allele was predominant in the Nyvka carp, while the slow-migrating allele pTf B was predominant in all other studied groups. Genotype analysis showed that out of fifteen possible variants of the TF locus, nine genotype variants were found in the studied carps. The genotypes $BC_2$, $C_1D$, and DD were absent in all studied groups. The homozygous genotype $C_1C_1$ was predominant in Antoniny-Zozulenets and crossbred carps. In the Nyvka carp group, the highest frequency of occurrence was in the homozygous genotype BB. The EST locus was represented by three genotypes - FF, FS, and SS, among which heterozygous FS predominated for the studied groups, but the homozygous FF genotype was absent in the Antonino-Zozulenets carp group. As for the ALB locus, the heterozygous genotype AB was dominant, while the homozygous genotype BB was absent in all studied groups. Analysis of the average heterozygosity for all loci in all carp groups showed higher values of actual heterozygosity ($H_{obs}$) relative to expected heterozygosity ($H_{exp}$), indicating their high potential for genetic variability. In terms of the level of average heterozygosity in all studied groups, higher values of $H_{obs}$ were found compared to the expected value.
**Key Words**: carp, crossbreeding, population genetic monitoring, transferrin, albumin, esterase, genetic markers, heterosis, heterozygosity.

**Introduction**. The geographical peculiarities of a significant part of Europe, in particular the presence of natural water bodies adapted to artificial fish cultivation and the historical traditions of the human population, indicate that carp has been a traditional object of cultivation for the last three centuries (Balon 1995 2004; Horváth et al 2022). Currently, the share of its commercial production in global aquaculture is about 8.0% (Tang et al 2020). In Eastern European countries, particularly in Poland (Guziur et al 2003; Jezierski & Leszczyńska 2003; Lehoczky et al 2018), Hungary (Bakos & Gorda 2001), and the Czech Republic (Kohlmann et al 2005; Hulak et al 2010), where carp culture is most widespread, a number of highly productive lines have been developed over a long period through selective breeding. Some of them, thanks to artificial selection, have become widely distributed and are used as a basis for interline crosses or for creating new hybrid crosses.

Selective breeding with carp in Ukraine was initiated by O. I. Kuzoma, based on the structuring of local populations (Oleksienko & Hrytsynyak 2007). The studies were conducted in several areas, including survival, increasing growth rate, increasing fecundity and early maturity, resistance to diseases, and various negative environmental factors. As a result of the works carried out by Ukrainian scientists, Ukrainian framed and scaled carp lines and their structural units were created: Lyubin, Antoniny-Zozulenets, Nesvych, Galician and Nyvka carps (Hrytsynyak et al 2022).

Given that the breeding process is a continuous mechanism of creative development of an object of breeding to improve economic and useful parameters (Li 2000; Lou 2006; Fu et al 2014), an integral part of promising selective breeding research can be a study of the effectiveness of industrial crossbreeding of different carp lines (Suzuki & Yamaguchi 1980; Bakos et al 1997). Since each line has its own characteristics and structure aimed at preserving genotypes and consolidating productive parameters (Krasnopolska 2021). Therefore, it is worth crossing lines and fish populations that are distant from each other in terms of ecological and geographical conditions and have a high degree of genetic differentiation, which allows predicting a high level of heterozygosity in the offspring (Hrynzhevsky et al 2006; Bekh et al 2014).

For these works, single crossbreeding is used, sometimes in the early stages, using several groups with the desired characteristics. The offspring obtained are reproduced in themselves, and then they undergo intensive selection in one of the selected directions, for example, growth rate, disease resistance, high productivity, scale cover, etc. (Zeng et al 2017; Hermawan et al 2024). Reproductive crossbreeding and its variant, synthetic selection, have been used in the breeding of many carp lines (Bekh 2011), while in the crossbreeding of various species of sturgeon (Káldy et al 2020), Chinese carps (Lamer et al 2010; Zhang et al 2013), some salmonids and tilapia, it has led to the creation of new interspecific forms that are absent in natural water bodies (Diedericks et al 2021; Devlin et al 2022). Studies using the synthetic breeding methods in Ukrainian carp culture have been conducted over the past 10-15 years. In particular, the hybrid potential of crosses between Lyubin and Antoniny-Zozulenets, Galician and Antoniny-Zozulenets, as well as Galician and Lyubin carps has been studied. These experiments were based on the study of the heterosis phenomenon and they demonstrated that commercial yields were 6.1-21.4% higher compared to that of their parental lines (Grishin et al 2018; Kurinenko & Syrovatka 2022; Krasnopolska & Kurinenko 2023).

At the same time, in modern aquaculture, population genetic monitoring is becoming an integral part of the selection process. It allows tracking changes in the genetic profile of populations resulting from artificial selection and various crossbreeding systems. The use of protein polymorphism and DNA marker analysis methods provides an effective solution to strategic tasks in fish gene pool management (Hulak et al 2010; Bielikova et al 2021; Stetsyuk et al 2021). The analysis of protein markers provides insight into the genetic history of a population, as they reflect alleles that have been tested for viability by natural selection. By studying electrophoretic variants of proteins, fish breeders can not only measure the genetic variability of carps but also track how it changes under human influence. This is an indispensable way to confirm the pedigree and ensure that the stock remains genetically pure during breeding (Hrytsyniak et al 2008; Hrytsyniak et al 2021).

The main protein markers used in genetic studies are albumin (ALB) (Hutapea et al 2023), esterase (EST) (Yang & Gui 1999; Leticia & Gerardo 2008) and transferrin (Tf) (Zakin 1992). ALB performs a transport function by carrying organic compounds and is a source of nitrogen for building. Genetic variations in ALB have been found in many fish species (Schussler 2000; Hrytsyniak et al 2021). TF in the genome of all fish species is represented by a single locus. In most fish species, the Tf locus is polymorphic, and the number of alleles can range from 2 to 13 (García-Fernández et al 2011; Stafford & Belosevic 2023). ESTs are the most popular biochemical marker used in aquaculture to analyse various biological characteristics. This marker is also used to distinguish species and clarify taxonomic status and is a key component in biochemical genetic analysis (Wu & Wang 1992).

The heterozygosity index is also critically important for analysing the genetic variability and stability of carp stocks. The use of protein markers to determine this level allows not only to assess the current state of the gene pool, but also to identify the real impact of selection measures on the genetic structure of the population, ensuring reliable control over its variability (Stetsyuk et al 2021).

Given that synthetic selective breeding is based on the heterosis phenomenon, selection consequently leads to a significant increase in productivity but threatens the long-term stability of the population by destroying genetic variability (Riga 2024). Consequently, the application of this method requires careful management to balance genetic diversity with commercial improvement. Therefore, studying the combinatorial ability for the level of heterosis effect in carps of different genesis when combined in industrial crossbreeding, taking into account genetic parameters, is an important step towards improving the theoretical foundations of selective breeding in aquaculture and requires scientific studies.

**Material and Method**. The study was conducted at fish farms in the Kyiv (State Enterprise "Experimental Farm Nyvka") and Khmelnytskyi regions (PJSC "Khmelnytskrybhosp"), located in the Forest-Steppe zone of Ukraine. Two types of Ukrainian carp were selected for crossing: Antoniny-Zozulenets carp (Antoniny-Zozulenets intrabreed type of the Ukrainian framed carp breed, *Cyprinus carpio*) and Nyvka carp (small-scaled intrabreed type of the Nyvkivka factory line of the Ukrainian breed of frame carp, *Cyprinus carpio*). The Antoniny-Zozulenets carp was originally created in the 1950s by crossing the local Antoniny carp and Galician carp lines with a mirror type of scale cover. The Nyvka carp was bred in 1993 by crossing the Ukrainian framed carp with the Frasinet carp. Selection efforts aimed to develop highly productive lines with improved commercial qualities, including reduced scale coverage, a high back, and a meaty physique. The enriched genetic heredity of these carp lines provides them with earlier sexual maturation, high fecundity, survival, and growth rate compared to their parental forms (Bekh 2011).

Four experimental groups were created based on the crossbreeding of these brood fish:

Experiment I – ♀ Antoniny-Zozulenets carp × ♂ Antoniny-Zozulenets carp (♀AZC × ♂AZC);

Experiment II – ♀ Sparsely scaled Nyvka carp × ♂ Sparsely scaled Nyvka carp (♀SSNC × ♂SSNC);

Experiment III –♀ Antoniny-Zozulenets carp × ♂ Sparsely scaled Nyvka carp (♀AZC × ♂SSNC);

Experiment IV – ♀ Sparsely scaled Nyvka carp × ♂ Antoniny-Zozulenets carp (♀SSNC × ♂AZC).

Age-1+ carps were grown in polyculture with Chinese carps at a stocking density of 1,500 fish $ha^{-1}$ in 0.5-6.5 ha ponds. They were fed a complex diet consisting of artificial feeds and feed mixtures based on grain crops, soybeans and sunflower meal, with a protein content of at least 20%.

The genetic structure of the studied groups of carp was assessed using the frequency distribution of alleles and genotypes of loci encoding the following proteins and enzymes: Tf, ALB, EST, prealbumin (prAlb), and posttransferrin (pTF). Blood samples from age-1+ fish of different genesis were collected *in vivo* from the caudal vein in the fall of 2024. Heparin was used as a coagulant at the rate of 25 IU $mL^{-1}$ of blood. The collected samples were then centrifuged for 10 min at 3,000 rpm, and plasma was collected in separate tubes. The distribution of proteins and enzymes was carried out by polyacrylamide gel electrophoresis with subsequent histochemical staining. Electrophoretic analysis is based on the fact that different proteins differ from one another in terms of molecular weight, structure, molecular shape, and the magnitude of their surface electrostatic charge. Consequently, different proteins move at different speeds in a uniform electric field, meaning they have different mobilities (Peakall & Smouse 2012). This method allowed for high-quality electrophoretic separation and reproducibility of the protein spectrum. The method allowed for the typing of alleles of the Tf, Alb, Est, prAlb, and pTF loci on a single gel plate.

In order to determine whether the experimental groups are in genetic equilibrium, the $x^2$ statistical criterion was used, which was applied according to the Hardy-Weinberg law. Statistical significance was considered at: $p < 0.05$, $p < 0.01$, $p < 0.001$. The data obtained were processed with a special macro GenAlE×6.5 for MS Excel.

The heterosis effect was calculated as the percentage by which a certain parameter of the local group exceeded that of the original lines (Basovsky et al 2001).

$$HI = \frac{CI \cdot 100}{PI}, \%$$

Where: HI - heterosis index; CI - crossbreeding index; PI - parental line index.

The obtained data were subjected to statistical processing using standard computer programs for one-way analysis of variance (ANOVA).

**Results**. Five allelic variants were found in all studied groups of framed carp at the Tf locus: Tf A, Tf B, Tf C1, Tf C2, Tf D. The most frequently observed was the Tf B allelic variant (0.525) in the group of the Sparsely scaled Nyvka carp group. The least frequently observed was the Tf D locus (0.032) in the Antoniny-Zozulenets carp group (Fig. 1). In the crossbred groups, the most frequently observed was the Tf C2 locus (from 0.380 to 0.400). The most frequently observed was Tf C1 (from 0.380 to 0.435) in hybrid forms of carp and Antoniny-Zozulenets carp, while TfB (0.525) was in the Nyvka carp.

As for the ALB loci, the allele A had the highest frequency (from 0.597 to 0.660) in all studied groups of framed carps. For the EST locus, the fast-migrating allele EST F (0.525) prevailed only in the group of Nyvka carp, while the allele EST S prevailed in other groups with a frequency range from 0.600 to 0.660. At the pTf locus, the fast-migrating allele pTf F (from 0.500 to 0.580) was predominant in all studied groups. For the prAlb locus, the fast-migrating allele pTfA (0.650) prevailed in the group of the Sparsely scaled Nyvka carp, while the slow-migrating allele pTf B (from 0.548 to 0.600) prevailed in all other studied groups.

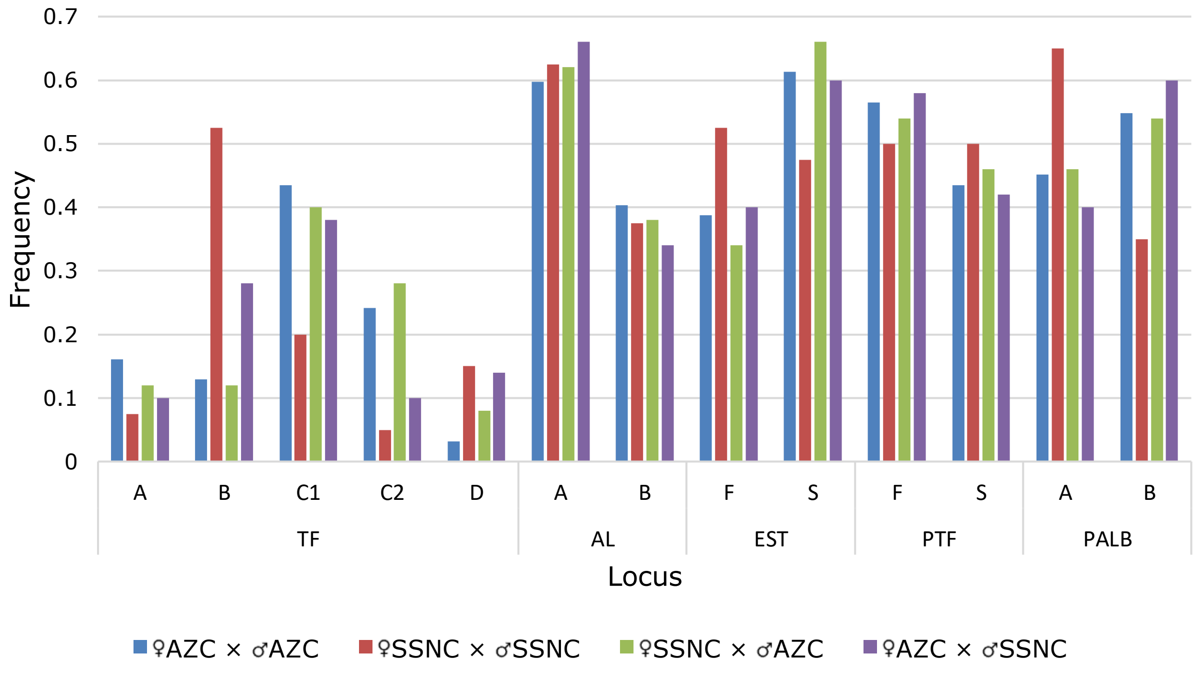


Figure 1. Distribution of allele frequencies in leather carp of different genesis. (Note: The letters A, B, $C_1$, $C_2$, D, F, and S denote genotype variants at the Tf, ALB, EST, prAlb, and pTF loci.)

Table 1

Distribution of actual (Go) and expected (Ge) genotypes, (n=25)

| *Locus* | *Genotype* | *♀AZC × ♂AZC* | | *♀SSNC × ♂SSNC* | | *♀AZC × ♂SSNC* | | *♀SSNC × ♂AZC* | |
|---|---|---|---|---|---|---|---|---|---|
| | | *Go* | *Ge* | *Go* | *Ge* | *Go* | *Ge* | *Go* | *Ge* |
| TF | AA | 2 | 0.802 | 0 | 0.113 | 1 | 0.360 | 1 | 0.250 |
| | AB | 0 | 1.290 | 1 | 1.575 | 2 | 0.720 | 0 | 1.400 |
| | $AC_1$ | 0 | 4.355 | 1 | 0.600 | 0 | 2.400 | 0 | 1.900 |
| | $AC_2$ | 5 | 2.419 | 0 | 0.150 | 2 | 1.680 | 2 | 0.500 |
| | AD | 1 | 0.323 | 1 | 0.450 | 0 | 0.480 | 1 | 0.700 |
| | BB | 3 | 0.516 | 7 | 5.513 | 1 | 0.360 | 4 | 1.960 |
| | $BC_1$ | 2 | 3.484 | 2 | 4.200 | 0 | 2.400 | 2 | 5.320 |
| | $BC_2$ | 0 | 1.935 | 0 | 1.050 | 0 | 1.680 | 0 | 1.400 |
| | BD | 0 | 0.258 | 4 | 3.150 | 2 | 0.480 | 4 | 1.960 |
| | $C_1C_1$ | 10 | 5.879 | 2 | 0.800 | 9 | 4.000 | 8 | 3.610 |
| | $C_1C_2$ | 5 | 6.532 | 1 | 0.400 | 2 | 5.600 | 1 | 1.900 |
| | $C_1D$ | 0 | 0.871 | 0 | 1.200 | 0 | 1.600 | 0 | 2.660 |
| | $C_2C_2$ | 2 | 1.815 | 0 | 0.050 | 4 | 1.960 | 0 | 0.250 |
| | $C_2D$ | 1 | 0.484 | 1 | 0.300 | 2 | 1.120 | 2 | 0.700 |
| | DD | 0 | 0.032 | 0 | 0.450 | 0 | 0.160 | 0 | 0.490 |
| | | $X^2 = 31.087$; $p = 0.001^{***}$ | | $X^2 = 10.278$; $p = 0.416^{*}$ | | $X^2 = 29.524$; $p = 0.001^{**}$ | | $X^2 = 29.476$; $p = 0.001^{**}$ | |
| EST | FF | 0 | 4.645 | 2 | 5.513 | 1 | 2.890 | 2 | 4.000 |
| | FS | 24 | 14.710 | 17 | 9.975 | 15 | 11.220 | 16 | 12.000 |
| | SS | 7 | 11.645 | 1 | 4.513 | 9 | 10.890 | 7 | 9.000 |
| | | $X^2 = 12.366$; $p = 0.000^{***}$ | | $X^2 = 9.920$; $p = 0.002^{**}$ | | $X^2 = 2.838$; $p = 0.092$ | | $X^2 = 2.778$; $p = 0.096$ | |
| ALB | AA | 6 | 11.040 | 5 | 7.813 | 6 | 9.610 | 8 | 10.890 |
| | AB | 25 | 14.919 | 15 | 9.375 | 19 | 11.780 | 17 | 11.220 |
| | BB | 0 | 5.040 | 0 | 2.813 | 0 | 3.610 | 0 | 2.890 |
| | | $X^2 = 14.153$; $p = 0.000^{***}$ | | $X^2 = 7.200$; $p = 0.007^{**}$ | | $X^2 = 9.391$; $p = 0.002^{**}$ | | $X^2 = 6.635$; $p = 0.010^{*}$ | |
| pTF | FF | 9 | 9.879 | 6 | 5.000 | 6 | 7.290 | 6 | 8.410 |
| | FS | 17 | 15.242 | 8 | 10.00 | 15 | 12.420 | 17 | 12.180 |
| | SS | 5 | 5.879 | 6 | 5.000 | 4 | 5.290 | 2 | 4.410 |
| | | $X^2 = 0.412$; $p = 0.521$ | | $X^2 = 0.800$; $p = 0.371$ | | $X^2 = 1.079$; $p = 0.299$ | | $X^2 = 3.915$; $p = 0.048^{*}$ | |
| prALB | AA | 4 | 6.323 | 7 | 8.450 | 4 | 5.290 | 2 | 4.000 |
| | AB | 20 | 15.355 | 12 | 9.100 | 15 | 12.420 | 16 | 12.000 |
| | BB | 7 | 9.323 | 1 | 2.450 | 6 | 7.290 | 7 | 9.000 |
| | | $X^2 = 2.837$; $p = 0.092$ | | $X^2 = 2.031$; $p = 0.154$ | | $X^2 = 1.079$; $p = 0.299$ | | $X^2 = 2.778$; $p = 0.096$ | |

Note: The data obtained were processed using the GenAlE×6.5 special macro for MS-Excel; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Tf – transferrin; ALB – albumin; EST – esterase; pTf – posttransferrin; prAlb – prealbumin.

Analysis of the genotypes of the studied carp groups showed that out of fifteen possible variants of the TF locus, nine genotype variants were found in the Antoniny-Zozulenets and Nyvka carps, as well as in groups of crossbred origin. All studied groups of carp lacked the $BC_2$, $C_1D$, and DD genotypes (Table 1). The homozygous $C_1C_1$ genotype was predominant in the Antoniny-Zozulenets and crossbred carp groups, with the highest frequency of occurrence (from 3.610 to 5.879). In the Nyvka carp group, the highest frequency was observed in the homozygous genotype BB, with a frequency of 5.513.

The EST locus is polymorphic and represented by three genotypes - FF, FS, and SS, among which heterozygotes EST FS (from 9.975 to 14.710) prevailed for the studied groups, but the homozygous genotype FF was absent in the Antoniny-Zozulenets carp group.

In all studied groups, the heterozygous genotype AB (from 9.375 to 14.919) was dominant at the ALB locus, while the homozygous genotype BB was absent in all studied groups.

The studied polymorphism of the pTF and prALB loci showed the predominance of heterozygous genotypes in all groups of carp (Table 1).

Analysis of the correspondence of the actual distribution of genotypes to the expected one according to the Hardy-Weinberg distribution showed the absence of statistically significant deviations at the prALB locus in all studied groups, at the EST locus it was observed only in the crossbred groups, and at the pTF locus only in the group Nyvka carp ♀ × Antoniny-Zozulenets framed carp ♂. At all other loci in the studied groups of carp, a statistically significant difference in the actual distribution of the number of genotypes in relation to that expected according to the Hardy-Weinberg law was observed.

The study showed heterogeneity of genetic parameters at different loci based on the heterozygosity index in Antoniny-Zozulenets carp. In particular, a significant deficit of heterozygotes was found at the Tf locus (0.452 compared to the expected level of 0.720). In contrast, the opposite trend was observed at the EST and ALB loci, with a marked excess of actual heterozygosity ($H_{obs}$) exceeding the theoretically calculated values in both cases.

In the group of Nyvka carp, the expected level of heterozygosity was lower than the actual level at all loci, except for the TF locus (0.550 and 0.671, respectively) and pTf (0.400 and 0.513), where the opposite trend was observed. In the groups of ♂ Antoniny-Zozulenets × and ♀ Nvka carps, the actual level of heterozygosity at all loci was higher than expected, except for the Tf locus (Figure 2).

In the hybrid combination obtained from crossing an Antoniny-Zozulenets female and a scaleless male, a significant predominance of $H_{obs}$ over theoretically expected heterozygosity ($H_{exp}$) was recorded. In particular, these values were 0.760 versus 0.481 at the ALB locus, and 0.600 versus 0.458 at the EST locus, respectively.

Analysis of the average heterozygosity at all loci in all groups of carp showed higher values of $H_{obs}$ relative to the $H_{exp}$, indicating their high potential for genetic variability. In terms of the level of average heterozygosity in all studied groups, higher values of $H_{obs}$ were found compared to the expected ones (Figure 2).

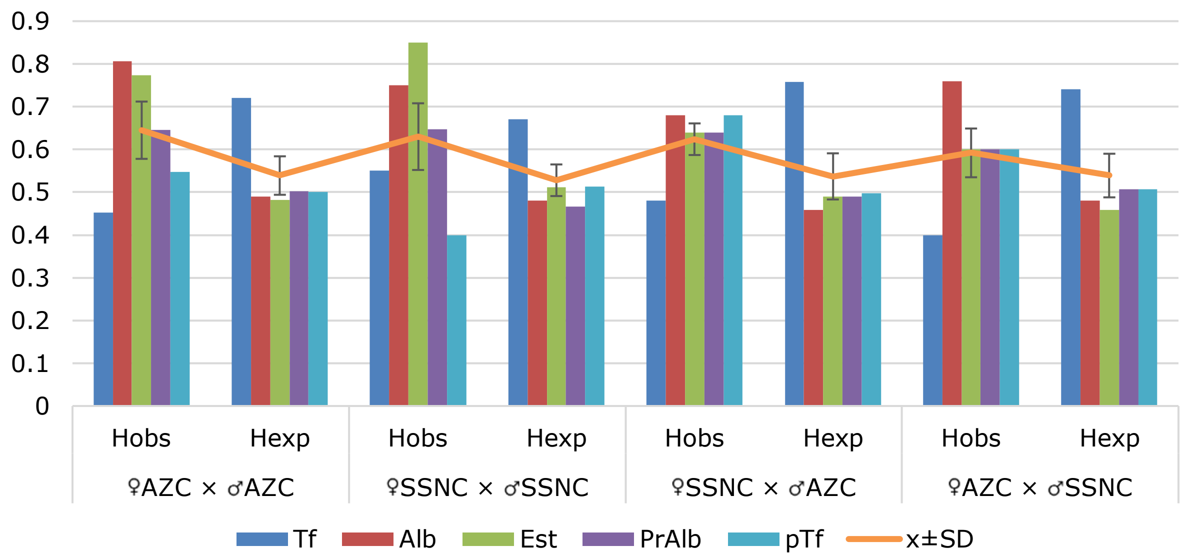


Figure 2. The level of observed ($N_{obs}$) and expected ($N_{exp}$) heterozygosity based on protein markers in framed carps of different origins.

According to average body weight, the heterosis effect when crossing the Nyvka carp with Antoniny-Zozulenets type brood fish was within the range of 106.6-108.8%. The use of Antoniny-Zozulenets type males in crossbreeding allowed increasing the individual body weight compared to the pure line. Accordingly, the heterosis index (HI) for the paternal line was 108.4%, and for the maternal line, 107.5%. A similar value of 107.5% was recorded for the maternal line in the crossbreeding of ♀SSNC × ♂AZC. In contrast, the lowest HI of 106.6% was recorded in the cross of ♀AZC × ♂SSNC (Fig. 3).

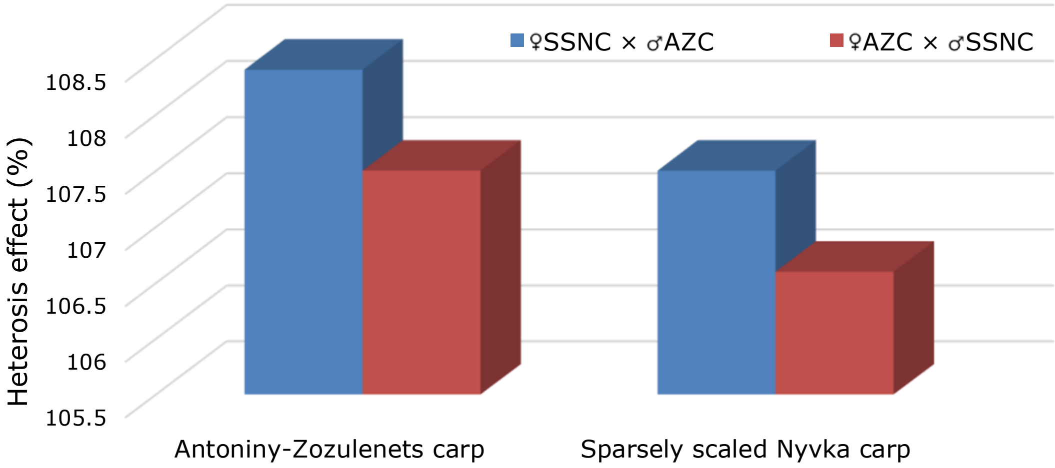


Figure 3. Heterosis effect in age-1 framed carps of different genesis in terms of seasonal weight gain.

These results indicate that the authors successfully selected the optimal ratio of parental lines, and the crossbreeding results suggest that the hybrids have high potential for high-yield production in future cultivation.

**Discussion**. In today's conditions, selective breeding activities are focused on studying the biological characteristics of offspring obtained from different interline and intraline groups (Vandeputte et al 2002; Hayden et al 2010; Liu et al 2024). The use of these crossbreeds allows obtaining offspring that combine the genetic traits of both parental lines. Enriched genetic variability contributes to increased productivity in offspring. At the same time, the positive manifestation of biological characteristics in first-generation hybrids can be the first link in the selection process, which will result in the development of a new line (Hulata 1995; Vandeputte et al 2004; Hulak et al 2010; Siwicki et al 2010). Thus, using genetic methods and applying various fish crossbreeding options, it is possible to significantly increase the productivity of industrial farming in a single farm in a relatively short period of time considering its environmental conditions and technological characteristics (Chevassus 1983; Scribner et al 2001). At the same time, the current stage of aquaculture development requires a transition from traditional breeding methods to systematic molecular-genetic monitoring (Gilyazetdinov & Tarasyuk 2012). Genetic studies using biochemical markers is fundamental to understanding the processes occurring in the gene pool of fish under the influence of artificial selection. The study of protein and enzyme polymorphism allows not only assessing the current state of lines, but also predicting their viability, avoiding inbreeding depression, and maximizing the benefits of combinatorial variability. It is this approach that provides the scientific basis for the creation of stable, highly productive crosses.

The results of the study show the dynamics of genetic parameters in the creation of synthetic carp lines in the first generation. Analysis of polymorphic protein and enzyme systems in the studied carp groups showed clear genetic differentiation between the parental lines and the nature of marker inheritance by their reciprocal offspring.

The detection of five allelic variants at the Tf locus indicates a high level of genetic polymorphism in the studied objects. Of particular interest is the predominance of the Tf B allele (0.525) exclusively in the Nyvka line, while the Antoniny-Zozulenets type and hybrids are characterized by the dominance of Tf $C_1$ and Tf $C_2$. The absence of genotypes $BC_2$, $C_1D$, and DD in all groups indicates the action of stabilizing selection or specific genetic consolidation of Ukrainian framed carps. The predominance of the homozygous C1C1 genotype in hybrids confirms the significant genetic contribution of the Antoniny-

Zozulenets type to the structure of the obtained hybrids, which is typical for carps of Ukrainian selection (Kotsar & Kuchma 2018; Mariutsa et al 2023).

The EST locus showed an interesting pattern: the Nyvka line differs significantly from other groups in terms of the dominance of the "fast" EST F allele. At the same time, the high frequency of EST FS heterozygotes in all groups (especially in hybrids) indicates the predominance of combinational variability. The absence of FF homozygotes in Antoniny-Zozulenets carp may be a result of adaptation to specific environmental conditions, since ESTs often correlate with the ecological plasticity of a species. The results for the ALB and prAlb loci confirm the effectiveness of synthetic selection. The change of the dominant allele from "fast" pTf A (in Nyvka carp) to "slow" pTf B (in hybrids) demonstrates the redistribution of genetic material in $F_1$. The high frequency of the Alb A allele (up to 0.660) in all groups indicates its important role in physiological processes, in particular the transport function of blood proteins, which ensures intensive fish growth.

Analysis of the compliance of genotype distribution with Hardy-Weinberg law revealed significant statistical deviations for most loci. The stability of the prALB locus in all groups indicates its relative neutrality to selection processes at this stage. However, statistically significant differences in other markers indicate that the studied groups are under the active influence of artificial selection and targeted selection of parent pairs (Desvignes et al 2001; Hulak et al 2010).

A comparative analysis of $H_{obs}$ and $H_{exp}$ allows conclusions to be drawn about the genetic structure of the studied groups (Barrandeguy & Garcia 2021). A deficit of heterozygotes at the TF locus is observed in both the Antoniny-Zozulenets carp (0.452 vs. 0.720) and the Nyvka line. This is typical for natural population and closed breeding stocks and may be the result of genetic consolidation of the line or a limited number of brood fish involved in reproduction (Kurta et al 2016; Linløkken et al 2021). The genetic explosion in $F_1$ hybrids, most clearly as an effect of synthetic selection, was manifested in reciprocal crosses. The predominance of $H_{obs}$ over $H_{exp}$ at all loci (except for Tf) confirms the success of combining genetically distant parental forms, which is confirmed by a number of studies conducted with different fish species (Šimková et al 2022; Wohlfarth et al 1975). The advantage of direct cross in the combination of ♀ framed × ♂ sparely scaled is recorded by peak heterozygosity values (for example, for ALB, 0.760 versus 0.481). This indicates pronounced somatic heterosis, which usually correlates with growth energy and resistance to adverse environmental factors and has been repeatedly confirmed by studies with fish grown in controlled artificial conditions (Martinez-Bautista et al 2024). Higher rates of average $H_{obs}$ relative to expected in all groups indicate a high level of genetic plasticity obtained by crossing distant individuals (Ariyanto et al 2022). This allows concluding that the synthetic selection methods used in the creation of Antoniny-Zozulenets types and their crosses with Nyvka carp ensure the maintenance of an optimal balance between breed consolidation and the necessary level of genetic diversity.

The synthetic selection method based on combining the gene pools of the Nyvka and Antoniny-Zozulenets types led to the creation of hybrids with a unique genetic structure. The fact that the $H_{obs}$ in hybrid groups often exceeded the expected values (as noted in previous calculations), combined with the data obtained on allele frequency, indicates the phenomenon of superdominance. This gives reason to believe that first-generation reciprocal hybrids have increased adaptive potential. The genetic distance between the parental forms at the Tf and EST loci was sufficient for the heterosis effect to manifest itself, which is a key task in the creation of new highly productive carp hybrids.

The study allowed identifying the optimal combination of parental lines for producing highly productive hybrids with improved productive traits while maintaining high-quality physical characteristics. The results confirm the promise of using these hybrids as highly productive material for industrial fish farming, as they have significant potential for further selection.

**Conclusions**. Use of protein markers allowed establishing the genetic profiles of pure lines of Nyvka and Antoniny-Zozulenets carps as well as their reciprocal hybrids. A clear genetic distance between the groups was shown, and all studied groups were characterized by an excess of $H_{obs}$ compared to the theoretically expected one, which indicates high genetic variability and viability of the stock. Analysis of enzyme systems confirmed the presence of line-specific markers that allow identifying pure lines and their reciprocal crosses. Common to all studied groups is a high level of $H_{obs}$, which significantly exceeds the expected values. This indicates effective maintenance of genetic diversity in the selection process. Thus, the use of the selected marker systems will allow clear differentiation of pure lines according to their unique allelic profiles and control the level of their genetic consolidation. At the same time, this will make it possible to objectively assess the hybrid nature of the resulting offspring, predict the manifestation of the heterosis effect, and monitor the genetic contribution of each parental line to the gene pool of hybrids. The use of such genetic material in industrial farms will allow, due to the heterosis phenomenon, obtaining carp seeds with increased productivity and subsequently marketable products with a fleshy physique, high productive and gastronomic properties, and, accordingly, increasing the productivity of ponds and the economic efficiency of fish farms.

**Conflict of interest**. The authors declare that there is no conflict of interest.

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