Scientific basis of creation of genetically controlled pure hybrids of mulberry silkworm (Bombyx mori L.)

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DOI 10.66073/ReFocus-Conf/1610200

For citation: AKHMATJON YAKUBOV BAKIEVICH, ABDRIMOVA GULBAKHOR ERIMMATOVNA, ORIPOV OTABEK ORIPOVICH, UMBETBAEVA GULAYDA BAXADIR QIZI. Scientific basis of creation of genetically controlled pure hybrids of mulberry silkworm (Bombyx mori L.). In: Development of innovative climate-resilient technologies for monitoring and controlling water use efficiency and the impact of salinization on crop productivity and livelihoods in the Aral Sea region: Proceedings of the International Scientific and Practical Conference. Nukus, 2026. P. 754–759. DOI: 10.66073/ReFocus-Conf/1610200

Abstract. In this article, it is investigated that mulberry silkworm breeds genetically adapted to dry, hot and dusty air, low humidity mulberry leaves, local microflora for the climatic conditions of Uzbekistan. Pure hybrids genetically modified by gender were created and scientific research was carried out, preparation of pure hybrids of mulberry silkworm scientific basis of creating genetically controlled pure hybrids of mulberry silkworm with high heterosis, capable of manifesting its genetic potential, is described. The development of new selection systems of sex-marked breeds that can compete with the current regionalized breeds and simplify the production process of mulberry silkworm eggs and reduce the cost of raw silk production, showing high heterosis in terms of biological parameters, 100% pure preparation, highly productive industrial hybrids were created. In this, the productivity indicators of the gene-marked breeds in the world collection of mulberry silkworms of the Research Institute of Sericulture of Uzbekistan were analyzed, some valuable indicators of the breeds selected for research were improved, the problem of butterflies not emerging from the cocoons in the gene-marked breeds was studied, pure hybrid combinations among the gene-marked breeds. It is scientifically based that the created, genetically controlled pure hybrids were tested in the laboratory and in production conditions, using the methods used in the selection and breeding works in all development periods of the dominant gene mulberry silkworm breeds, and the selection work was carried out based on movement activity, and new hybrids were created.
Keywords: mulberry silkworm, egg, cast, cocoon, bud, butterfly, breed, hybrid, heterosis, female, male, gene, temperature, humidity, light, mulberry leaf, silkiness, worm viability, metric number, hop silk, spinning, ♀ – female gender, ♂ – male gender, silk fiber.

Nowadays, ready-made silk fabrics are produced in large quantities in countries with developed silk processing industry, such as the People's Republic of China, India, Japan, South Korea, Italy, France, England, the USA and Uzbekistan. Due to the global climate changes in the world, special attention is being paid to the creation of genetically controlled new breeds and completely pure hybrids that are resistant to sudden changes in daily temperature, heat-resistant, able to absorb mulberry leaves with a low content of water, and resistant to various local microflora that cause diseases. “...our republic is one of the leading countries in the production of silk fibers, its share of production is 1.2 percent, the yield of cocoons from 1 box of seeds is 59-60 kg.”[1] Currently, preparation of genetically controlled gene-marked pure hybrids suitable for rapidly changing natural

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and climatic conditions, providing 18-20% more silk fiber, conducting scientific research aimed at improving their technological properties, such as cocoon yield and metric number, are considered urgent problems worldwide.

In the world, a number of researches aimed at the selection of mulberry silkworm on the basis of the leading economically valuable traits and the development of new modern breeding methods, as well as new technologies for preparing mulberry silkworm eggs and increasing silk productivity are being conducted. Nevertheless, solving the problem of creating genetically controlled pure hybrids common to silk producing countries is an important research area. Early egg color breeding eliminates complex, labor-intensive and mis-breeding the cocoon, and significantly reduces time, money and manual labor. At the same time, scientific and practical research on preparing high-quality pure hybrid seeds from non-mixed purebred groups, ensuring high manifestation of heterosis power on economically valuable traits, and increasing the productivity of silk raw materials is of great importance.

Decree of the President of the Republic of Uzbekistan No. PF-6059 dated October 7, 2020 “On measures for the further development of cocooning and cattle breeding in the Republic of Uzbekistan” and No. PQ-3472 dated January 12, 2018 “On measures for the further development of the sericulture industry in the Republic”, these scientific researches serve to a certain extent in the implementation of the tasks specified in the decision and other normative documents [1,2,3,4,5,6].

Research methods and materials

The methods of morphological, biological, technological, production and statistical analysis, as well as “Main methodological rules of mulberry silkworm breeding” and “Agrotechnics for the care of white cocoon breeds of mulberry silkworm” were used in the research work.

Various breeds and hybrids stored in the world collection of silkworms at the Scientific Research Institute of Silk were used in experimental work aimed at the scientific basis of creating genetically controlled pure hybrids of the mulberry silkworm.

W_2W_2, W_3W_3, W_5W_5 genes with sex chromosome W were translocated from the breeds in the world collection of the Silk Research Institute, and 12 breeds of mulberry silkworm were selected. According to the genetic modification of these breeds, depending on the color of the eggs, they were divided into male (light colored) and female (dark colored) genes.

These are breeds C-5, C-5pr.g., C-6, C-10, C-12, C-13, C-14, «Belokokonnaya 1» (whitish), «Belokokonnaya 2» (whitish), SANISH 8, SANISH 9.

Information on sexed breeds at the egg stage was retrieved from the catalogue.

The researched breeds are determined by different genes for egg color. The color of male eggs of different breeds is also different. For example, C-5 W_2W_2, C-5 PR. g. W_2W_2, C-13 W_2W_2, “Belokokonnaya 1” W2W2, SANISH 9 W2W2 breed eggs are light yellow, C-12 W_5W_5, “Belokokonnaya 2” W5W5 breed brown, C-6 W_3W_3, C-10 W_3W_3, C-14 W_3W_3, “Belokokonnaya 1” W3W3, SANISH 8 W3W3, dark brown in breeds.

Worms of the sexed breeds are white, some are masked and crescent-shaped, and only C-5 in the right shape as its own. Gus worms have a bluish color due to their transparent skin.

Breeding works with gene breeds at the egg stage were carried out according to the “Basic methodical manual of breeding work with mulberry silkworm” [7,8,9,10]. Some changes were made, taking into account the genetic characteristics of breeds defined by gender. Breed families, defined by sex at the egg stage, were incubated separately by sex. At the egg stage, families with low reproductive performance, gender imbalance and low egg survival rate were separated and sorted. For feeding in the second year of worms, 110 males and 110 females (dark, oozing) from each family were counted and reared together.

In the worm stage, worms with uneven development and low viability were removed. Analysis of families was conducted on a sample of 30 cocoons (15 females and 15 males). Families with very low silkiness, cocoon and shell mass were separated and removed. Analysis of cocoons for each breed was conducted separately for males and females. According to family analysis, cocoons with large

shell, high silkiness, and cocoons suitable for cocoon breeds in terms of shape and grain size were selected for preparation of the starting material batch.

By the method of outbreeding, the best performing breeds were crossbred. After individual selection, remaining cocoons from breeding families were used to prepare super elite and hybrid eggs. Industrial hybrids were reared under production conditions with an egg mass of 3 to 10 grams.

300-400 cocoon samples were collected from all reared families or cocoons from all returns, and technological analysis of purebred and hybrid cocoons was carried out.

The main feature of the selection work in the selected breeds of silkworms at the egg stage is that each batch of these breeds is divided into genes based on the color of the eggs, and they are counted as black and white eggs. For further work, laying eggs were taken in the ratio of 50%♀:50%♂, and the rest were separated. During the rearing period, each family was formed with 110 females and 110 males. Later, to eliminate the task of dividing the elephants by gene, the female and male genes were kept separately.

After 2 hours from the start of revival, when a sufficient number of worms (about 300 pieces) appeared on the leaf, they were separated (with the leaf) and they were fed by the usual accepted method.

The formation of families, or returns, was carried out in the third instar, in which the most active worms were used. During selection, passive (weak) worms were separated, thereby eliminating worms with lethal genes in their genotype.

A classification method was used to identify mulberry silkworm breeds for use in research [11,12,15]. The classification method is based on the ability to determine the relationship between the indicators of silkworm breeds, in which the main indicator is the sum of the indicators of each breed, and based on it, the place in the variation series is determined. In this case, not the absolute values of variable indicators, but the place occupied by each breed according to the sum of indicators was classified.

Research results and their discussion

Breeding egg-stage species from the IITI world collection were maintained alive by feeding on a worm grouping scheme. This, of course, leads to a decrease in their main valuable economic indicators. In hybridization, it is necessary to carry out selection-breeding works with breeds in order to fully reveal their potential and improve their reproductive and biological indicators.

In the effective conduct of selection and breeding work, it is necessary to take into account the genetic parameters of the indicators being selected. This helps to correctly predict the results of the selection and to take the right direction.

Good intergenerational transmission of parameters such as silkiness, butterfly hatching percentage, cast mass, shows that mass selection for these parameters is effective. The effectiveness of selection on egg viability, cocoon shell mass, cocoon thread length and thinness is slightly less. Selection for worm viability and cocoon mass does not give the expected effect, because the appearance of these indicators depends mainly on the conditions of feeding the worms. It was found that the feeding conditions of the worms, including the quality and quantity of mulberry leaves, the feeding season and the diversity of the conditions in the regions, affect the occurrence of genetic diversity. In a changing environment, the transmission of a trait and the effectiveness of selection also change. If the quantity and quality of the leaves are not changed, the selection process is accelerated and the efficiency of the selection increases [13,14,18,19].

If a strict selection method is implemented in all stages of mulberry silkworm development (egg, larva, pupa, butterfly), the indicators can change for the better. For example, during egg aestivation, selection of the best batches was carried out according to the following parameters: according to the number of normal eggs in the batch, batch mass, single egg mass and 1:1 ratio of genes. The reproductive parameters of the studied breeds are shown in Table 1.

Table 1. Reproductive indicators of eggs of the studied breeds (2022-2023)

№	Breeds	Date	Normal number of eggs, pcs		Mass of normal eggs, mg		Weight of one egg, mg	
			$\overline{X}_{\pm S}\overline{x}$	Cv	$\overline{X}_{\pm S}\overline{x}$	Cv	$\overline{X}_{\pm S}\overline{x}$	Cv
1	C-5	2022	540±5,0	13,4	290±3,1	15,4	0,538±0,003	4,1
		2023	493±6,9	18,3	255±3,5	17,7	0,516±0,014	8,9
2	C-10	2022	492±6,7	18,3	246±4,0	21,7	0,501±0,603	4,6
		2023	447±9,7	18,2	206±5,8	23,2	0,464±0,007	12,6
3	C-12	2022	624±15,0	13,2	324±7,9	13,3	0,520±0,065	5,4
		2023	459±15,9	19,0	202±7,8	21,2	0,437±0,005	6,6
4	C-13	2022	545±13,0	13,5	298±3,0	15,4	0,550±0,020	9,5
		2023	604±6,9	16,8	186±4,2	22.4	0,456±0,015	10,3
5	C-14	2022	544±15,8	13,8	298±3,3	15,9	0,548±0,021	4,2
		2023	466±7,8	17,2	234±4,4	19,4	0,503±0,006	12,2
6	Ipakchi 1 (control)	2022	654±6,6	10,7	354±7,9	15,2	0,542±0,003	4,1
		2023	690±7,0	11,2	320±8,0	16,1	0,542±0,103	6,2

According to the data in Table 1, among breeds defined by gene, the highest C-12 in the number of eggs in the cast (624 pieces), the smallest C-10 (492 pieces), the highest C-12 in the mass of normal eggs (324 mg), in the smallest C-10 (246 mg) breeds, which are slightly less than the control Ipakchi 1 breed (624 pcs, 354 mg). But since these differences are very small, in general, it can be said that the reproductive indicators of the gene-specified breeds are at the level of the indicators of the control option.

The high coefficients of variation in the reproductive indicators of the gene breeds indicate that the possibilities of the genetic variability of the breeds have not disappeared yet, if the breeding and selection work is continued, there are opportunities to further improve and stabilize the indicators in the future.

Table 2. Biological indicators and coefficients of variation of researched breeds (2022)

№	Breeds	Liveness		Cocoon mass, g		Shell mass, mg		Silkiness, %	
		$\overline{X}_{\pm S}\overline{x}$	Cv	$\overline{X}_{\pm S}\overline{x}$	Cv	$\overline{X}_{\pm S}\overline{x}$	Cv	$\overline{X}_{\pm S}\overline{x}$	Cv
1	C-5	91,8±1,2	15,1	1,43±0,02	4,1	297±4,4	6,9	20,8±0,2	5,2
2	C-10	97,7±1,5	13,2	1,45±0,02	4,2	279±6,6	6,7	19,3±0,7	10,3
3	C-12	98,5±1,7	8,5	1,46±0,02	4,1	278±7,0	6,6	19,1±0,3	4,5
4	C-13	94,3±2,5	13,0	1,45±0,02	5,5	306±0,7	6,9	21,1±0,3	6,9
5	C-14	92,5±3,3	14,8	1,48±0,21	6,6	320±6,4	48	21,6±0,2	4,5
6	Ipakchi 1 (control)	92,0±0,8	7,7	1,55±0,03	8,0	347±5,0	8,8	22,4±8,3	5,9

According to the data in Table 2, the viability of worms of the breeds used in the research is quite high, ranging between 91.8% and 98.5%. It should be noted that the viability of C-5, C-10, C-12, C-13, C-14 gene-marked worms is not significantly different from the viability indicator of Ipakchi 1 worms, which is not marked by gene, in the control option this indicator is 92.0%.

Gene-marked breeds differ from normal breeds in that they have rearranged chromosomes in their genomes. Therefore, gene-marked breeds show a quick reaction (response) to any change. It is known from the results of many studies that, in good experimental conditions, the biological indicators of gene-specific breeds are at a good level, but in changing, unstable conditions, genetically variable breeds tend to show poor results. The high coefficient of variation of the gene breeds (from

DOI 10.66073/ReFocus-Conf/1610200

8.5 to 15.1) indicates that a parameter such as worm viability has a large variability and proves that future selection work on this parameter is possible.

According to our research that vigor has high variability is further supported by the low heritability coefficient (h_2) of vigor in agricultural plants and animals. It is known that the low level of heritability is explained by the small amount of more genotypic components in the phenotypic occurrence of the trait [16,17].

The traits that have an adaptive effect on the vitality index of worms are: cocoon, shell and egg masses, where the value of Cv is relatively high. Silkiness is a less significant variable-adaptive indicator, on the contrary, it is characterized by the lowest Cv.

The transmission of cocoon mass to the generation is moderate, and the effectiveness of the mass selection method on this indicator is considered very low. The fertility of the method of selection for worm viability and cocoon mass is also very low, since these indicators mainly depend on the conditions of care and feeding of worms. According to some studies, the conditions of care and feeding of worms, including the quality and quantity of mulberry leaves, seasonal and regional conditions, affect the genotypic diversity of worms. Under changing environmental conditions, the degree of transferability of indicators and the effectiveness of selection also change. If the conditions for feeding and caring for future generations of maternal offspring are kept optimal and stable, the intensity of selection will increase, and selection processes will accelerate.

After reaching the expected level in terms of gene-specific breeds, productivity and technological indicators, we started work on creating hybrids, as a result of which reciprocal hybrids of type A x B and B x A were created.

It is known from some studies that when genetically distinct forms are crossed, heterosis can occur, which causes a sharp increase in some indicator at the expense of different genes. But this situation may not occur in cross-breeding of closely related forms, in which the indicators develop not under the control of non-adaptive genes, but under the control of slowly acting genes.

In other words, the parameters of the characters change under the general influence of the alleles passed down from the parents. Therefore, the value of such indicators is equal to the arithmetic mean value of parents. But in heterozygous organisms, the indicators of quantitative traits are often high, and vice versa, even if they are genotypic stability in inbred generations, this indicator decreases. To some extent, this phenomenon can be explained from a genetic point of view, that is, in inbred systems, non-eliminated and newly formed polylethals pass to the homozygous state, while in hybrids they are absorbed by normal alleles.

Conclusions

In conclusion, unlike other insects, the mulberry silkworm feeds only on mulberry leaves. The quality and quantity of nutrients in mulberry leaves depend on mulberry varieties and agrotechnics of their care. In our research, we suggest the selective use of feed leaves with large, thick leaf plates and optimal biochemical composition to increase productivity and quality indicators of mulberry silkworms.

Our republic has a unique climate, which differs from the climate of countries with high temperature and low humidity located in the Eastern and South-Eastern regions. Therefore, when feeding silkworms, it is necessary to choose effective varieties of mulberry, to prepare special worm houses separately and to ensure the necessary temperature and humidity in them.

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