



Genetically Modified Silkworm Breeds (*Bombyx mori* L.) with Sexual Dimorphism at the Initial Stage of Ontogenesis

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Authors' contributions

This work was carried out in collaboration among all authors. Authors EAL and BAM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AM and NA managed the analyses of the study. Author FA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The purpose of the work was to evaluate the breeds of the world silkworm collection of the Scientific Research Institute of Sericulture (SRIS) labelled by sex at the stage of eggs of silkworm by biological characteristics for their use in hybridization. The eggs of silkworm of these genetically modified breeds can be easily separated by color (by sex) before reaching the imago stage. The work was carried out in the laboratory of genetics and breeding of the silkworm of the SRIS on the materials of the living collection of the silkworm in 2020-2022. The search and selection of breeds for hybridization was carried out by the ranking method. The ranking method consists in the ability to establish a relationship between the features, which are expressed by the order of the place occupied by each member in the aggregate, i.e. the place of rank in the variation series. Hybrids from breeds labeled by sex at the stage of silkworm eggs with 100% purity of preparation, with the maximum manifestation of heterosis in reproductive and biological indicators, differ in: a

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high number of normal eggs in a clutch of C-12xC-10 - 614pcs, C-5xC-13 - 598pcs, C-12xC-14 - 598 pieces; caterpillar viability - C-12xC-10 - 96,5%, C-5xC-13 - 96,0%, C-12xC-14 - 96,2%; content of raw silk, - C-12xC-10 - 22,2%, C-5xC-13 - 22,0%, C-12xC-14 - 20,3%.

By the methods of traditional breeding selection at all stages of development, improved lines of silkworm breeds C-5, C-10, C-12, C-13, C-14 were bred in terms of reproductive indicators, hatching of silkworm eggs, the viability of caterpillars, weight and content of raw silk of cocoons.

Keywords: *Silkworm; eggs of the silkworm; caterpillar; cocoon; butterfly; translocation; female; male; sex regulation; chromosome; genetic engineering.*

1. INTRODUCTION

A silkworm is an excellent object for developing the genetic foundations of breeding farm animals. Traditional quantitative selection of this object has achieved impressive results, but this report is devoted to the problem of increasing the productivity of the silkworm through genetic engineering at the chromosomal level.

Engineering at the level of individual chromosomes includes work on the artificial regulation of sex in silkworms. One of them is the method of marking breeds with various genes responsible for the color of the serous shell of eggs [1,2,3].

A total of more than 400 mutant traits have been detected and artificially obtained in the silkworm. These genes were distributed across all 28 pairs of non-sex and sex chromosomes.

By the action of nuclear radiation, it was possible to tear off a fragment of the 10th autosome with the dominant $+W_2$ gene and transplant (translocate) it onto the female-determining W -chromosome (Image 1).

Since the female silkworm has two different sex chromosomes, the W -chromosome gets into one-half of her eggs ready for fertilization, and the Z -chromosome gets into the other half. All

spermatozoa carry a Z -chromosome. The combination of W - and Z -chromosomes during fertilization determines the development of female traits, and the combination of ZZ gives the male sex.

The rearrangement of chromosomes is strictly transmitted from generation to generation. The non-transplanted piece of the autosome in subsequent generations was replaced by the normal-sized 10th autosome that came from the father (Fig. 1). As a result, the females of the new line had two normal autosomes of the 10th pair and an additional piece of the same pair of autosomes transplanted onto the W -chromosome. The increase in the hereditary material of the 10th autosomal pair to three chromosomes (trisomy) did not affect the viability of the females at all, but their weight decreased somewhat. If all the non-transplanted autosomes of the 10th pair in females (Fig.1) and males (Fig. 1) will have recessive w_2 genes, then it is not difficult to understand that only eggs developing into females will contain the dominant $+w_2$ gene that got into them together with the W chromosome, and, consequently, these eggs will be dark, while eggs developing into males will not have a single dominant gene of dark color and therefore will remain white (Fig.1). Now, according to the color of the egg, each family is unmistakably divided into females and males.

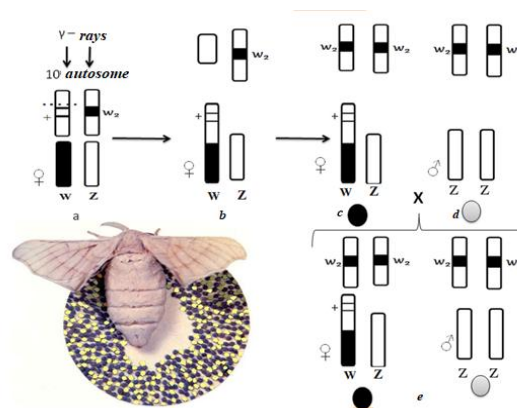


Image 1. Scheme of breeding a silkworm breed labeled by sex at the egg stage

Currently, the Scientific Research Institute of Sericulture is developing a "Device with software and technologies for dividing eggs of sex-labeled silkworm breeds". It is assumed that the device will be put into production in the near future [4]. Therefore, silkworm hybrids with 100% purity of preparation, created using genetically modified breeds, marked by sex with the color of eggs, prepared for introduction in the next 5 years, will be in demand and successful in production.

Sericulture all over the world is based on the production of only first-generation hybrids for the manifestation of heterosis. The heterosis effect is manifested mainly in the signs of two categories - viability and quantitative signs. High viability leads to increased resistance to diseases and extreme conditions, accelerated growth and development, fertility, as well as an increase in indicators of many quantitative signs, including those of economic importance [5,6,7]. This is also facilitated by a favorable combination of polymer-acting genes controlling quantitative traits during hybridization.

Silkworm moths mate immediately after leaving the cocoons and thus produce a purebred breed. To avoid mating within each breed, it is necessary to separate its females and males from each other in advance, even before leaving the cocoons, in order to then cross the females of one breed with the males of another. Tens of millions of individuals are subject to gender separation. Meanwhile, the methods of separating breeding material by sex for the purpose of hybridization are either inaccurate or very laborious.

The analysis of industrial grain showed that it contains only 20-25% of hybrid eggs, while the remaining eggs are the parent initial breed [8].

Clogging of hybrid silkworm eggs with the original parent breeds reduces the yield so much that further introduction becomes impossible. However, the development and improvement of sericulture is unthinkable without the precise preparation of hybrid eggs for industrial purposes. This is achievable only if the elite material is accurately divided into separate groups of females and males. The problem can be solved by genetic methods, namely, the use of genetically modified, so-called, sex-labeled breeds in hybridization [9].

The living collection of the silkworm Scientific Research Institute of Sericulture (SRIS) contains 12 breeds labeled by sex at the egg stage.

These breeds can serve as a material for creating pure hybrids. The creation and implementation of such hybrids can bring tangible income to producers due to the maximum use of the heterosis effect in 100% pure high-silk-bearing hybrids of the first generation.

The aim of the work was to evaluate the breeds of the world silkworm collection of the SRIS labeled by sex at the eggs stage according to biological characteristics for their use in hybridization.

2. MATERIALS AND METHODS

The work was carried out in the laboratory of genetics and breeding of the silkworm of SRIS on the materials of the living collection of the silkworm in 2020-2022.

The live collection of the silkworm SRIS [10] contains 12 breeds labeled by sex at the egg stage: C-5 W_2 , C-5 W_2W_2 , C-6 W_3W_3 , C-12 W_5W_5 , C-13 W_2W_2 , C-14 W_3W_3 , Belokokonnaya-1 W_2W_2 , Belokokonnaya-1 W_3W_3 , Belokokonnaya-2 W_5W_5 , SANIISH-8 W_3W_3 , SANIISH-9 W_2W_2 . Breeds with translocation of the W-chromosome of the eggs W_2W_2 have a straw color with male embryos, eggs W_3W_3 – brown, W_5W_5 -dark brown. Some of these breeds have been used before and are now being used in breeding and scientific work and in the creation of hybrids with different practical orientations [11].

The search and selection of breeds for hybridization were carried out by the ranking method [12]. The ranking method consists in the ability to establish a relationship between the features, which are expressed by the order of the place occupied by each member in the aggregate, i.e., the place of rank in the variation series. At the same time, the processing does not include the absolute values of the varying features, but the ordinal places or ranks occupied by the members in the aggregate for each of the correlating features. The best indicators of signs occupy the first places, then – according to the descending values of indicators.

Then, according to the minimum score of the considered features, a list of breeds (or one breed) of high rank with the best indicators is established.

The traditional breeding selection was carried out with the selected breeds according to the "Basic methodological provisions of breeding work with

silkworms" [13], and the method of selection by motor activity was also used, which involves the selection of the most mobile newly hatched caterpillars and the most active male butterflies [14].

All quantitative results were subjected to biometric processing by the Plokhinsky N. A. method [15]. A parametric statistical criterion was used - the Student's t-criterion built on the basis of the parameters of this set of X and S^2x and representing the functions of these parameters, which was due to the need to calculate the

sample characteristics of the average value and the indicators of variation. The compared samples were taken from normally distributed aggregates.

3. RESULTS AND DISCUSSION

When selecting breeds to create 100% pure hybrids, the breeds labeled by sex at the egg stage were ranked according to the viability of caterpillars, weight and content of raw silk. The results are shown in Table 1.

Table 1. Ranks of breeds labeled by sex at the stage of silkworm eggs with an assessment of biological indicators

No	Name of breeds	Viability of caterpillars,		Cocoon weight, g.		Content of raw silk, %		Total points	Place by min. the amount
		Absolute unit	Rank	Absolute unit	Rank	Absolute unit	Rank		
1	SANIISH-8 W ₃ W ₃	90,8	1	1,41	10	15,8	10	21	7
2	SANIISH-9 W ₂ W ₂	79,5	10	1,59	4	20,1	8	22	8
3	Belococoonaya-1 W ₂ W ₂	89,2	2	1,51	6	20,0	9	17	5
4	Belococoonaya-2 W ₅ W ₅	83,8	8	1,62	2	20,6	3	18	6
5	Sovetskaya-6 W ₅ W ₅	86,9	6	1,63	2	20,3	7	16	4
6	C-5 W ₂ W ₂	84,4	7	1,64	1	23,9	1	10	1
7	C-10 W ₃ W ₃	88,6	3	1,43	9	23,0	5	17	5
8	C-12 W ₅ W ₅	88,5	4	1,53	5	23,7	2	11	2
9	C-13 W ₂ W ₂	88,6	3	1,44	8	22,8	4	15	3
10	C-14 W ₃ W ₃	88,2	5	1,46	7	23,4	3	15	3

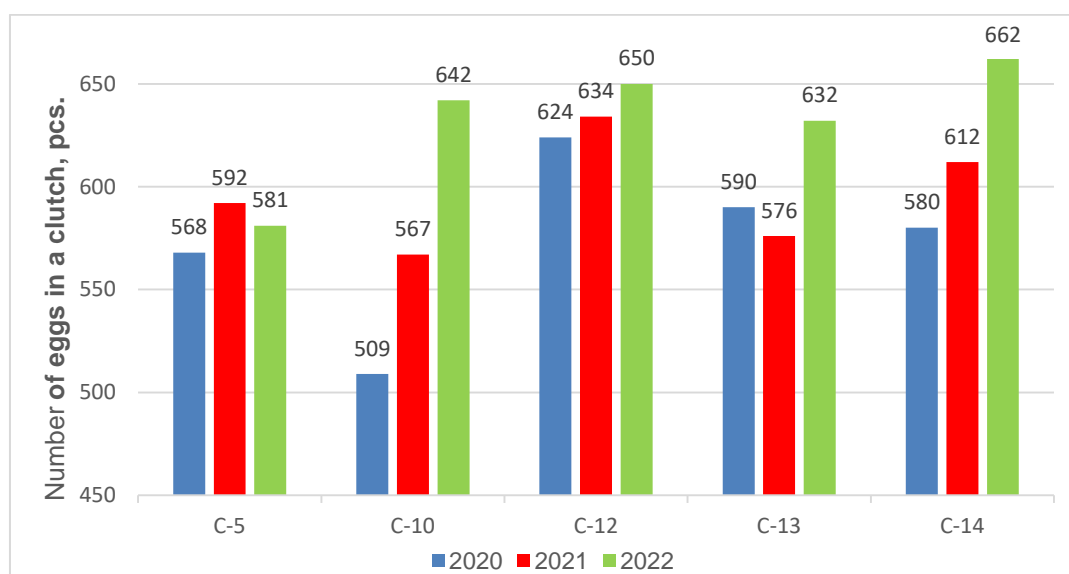


Fig. 1. The number of eggs in the clutch of families selected for incubation, by year

Table 1 shows that the lowest scores and, accordingly, the highest ranks are scored by the breeds C-12 W_5W_5 , C-13 W_2W_2 , C-14 W_3W_3 .

Only strict selection for three years at all stages of the development of the silkworm (eggs, larva, pupa, imago) can contribute to a change in indicators for the better. For example, Fig. 1 shows the number of eggs in the clutch of families selected for incubation, by year.

Fig. 1 shows that the number of eggs in the clutch of breed C-10 increased from 509 pieces at the beginning of selection in 2020 to 642 pieces in 2022, and breed C-14 - from 580 pieces to 662 pieces. However, in the C-12 breed, the number of eggs in the clutches of families selected for incubation has changed slightly – from 624 eggs in 2020 to 650 eggs in 2022, that is, by 26 eggs, in the C-5 breed – from 568 eggs in 2020 to 581 eggs in 2022.

It is known from studies [16] that the number of eggs in a clutch is closely correlated with the mass of eggs in the clutch and to a lesser extent with the hatching of silkworm eggs. The results of our studies are consistent with these observations (Fig. 2).

Fig. 2 shows that the best recovery of the eggs is observed in breeds C-12 - 98.2%-98.7% and C-13 98.0%-98.9%, the worst in breeds C-5 - 95.1%- 94.9%. It is known that egg hatching in silkworms, according to [17], belongs to a group of traits with an average degree of heritability and depends not only on the genotype of the breed but also on the conditions of egg storage.

The best egg-hatching families of sex-tagged breeds were selected for feeding. Biological indicators of the fed families are given in Table 2.

As can be seen from Table 2, the viability of the caterpillars of all the breeds taken into work remained quite high for three years and ranged from 90,0% to 98,7%. Sex-labeled breeds differ from the usual ones by the presence of chromosomal rearrangement in their genomes. Therefore, these breeds react more sensitively to any changes in the conditions of maintenance [18]. High coefficients of variation from 8,0 to 15,2 of the sex-labeled breeds indicate a large variability of such an indicator as the viability of caterpillars.

Extremely low viability heritability coefficients (h_2) are observed in agricultural plants and animals. But low heritability is largely determined by a small proportion of the genotypic component in the phenotypic realization of the trait [16,14,19]. Relatively smaller, but also quite high Cv have adaptively significant features, such as the weight of the cocoon, silk shell, and eggs, as well as the percentage of egg production. The variability of the content of raw silk, an adaptively less significant trait, is characterized, on the contrary, by the lowest Cv [17]. The coefficients of variation in the viability of the unlabeled Ipakchi1 breed (average) are significantly lower - from 4,8 to 7,7. This indicates the stability and balance of its genome on this trait. Of the five studied breeds, high content of raw silk is observed in C-14 (23,8%), C-13 (23,4%), and C-15 (21,2%).

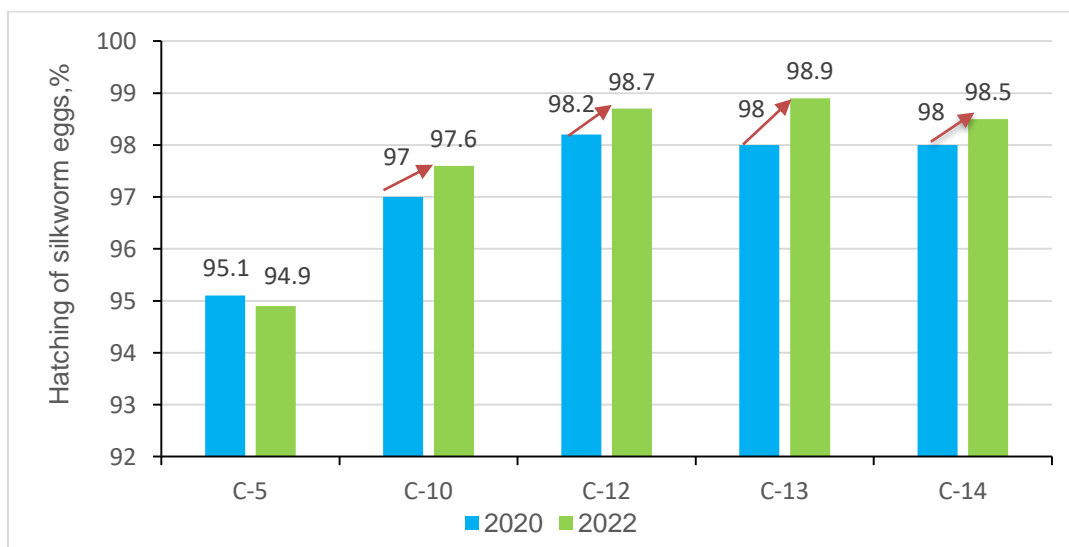


Fig. 2. Hatching of silkworm eggs in sex-tagged breeds at the beginning and at the end of the selection

Table 2. Biological indicators and coefficients of variation of the studied breeds by year

No	Breeds	Years	Viability of caterpillars, %		Weight cocoon, g		Weight shells, mg		Content of raw silk, %	
			$\bar{X} \pm S^2x$	C_v	$\bar{X} \pm S^2x$	C_v	$\bar{X} \pm S^2x$	C_v	$\bar{X} \pm S^2x$	C_v
1	C-5	2020	91,8±1,2	15,1	1,53±0,02	4,1	333±4,4	6,9	20,8±0,2	5,2
		2021	92,5±2,2	14,9	1,54±0,02	4,8	326±3,4	5,7	21,2±0,17	4,4
		2022	90,0±2,0	14	1,50±0,02	4,0	320±3,2	5,1	20,3±0,2	4,2
2	C-10	2020	97,7±1,5	13,2	1,45±0,02	4,2	276±6,6	6,7	19,3±0,7	10,3
		2021	96,6±1,9	13,3	1,45±0,02	6,5	280±3,7	7,7	19,3±0,19	5,5
		2022	95,5±1,6	13,1	1,40±0,02	5	280±4,2	6,9	20,0±0,2	6,1
3	C-12	2020	98,5±1,7	8,5	1,46±0,02	4,1	276±7,0	6,6	19,1±0,3	4,5
		2021	98,1±1,5	8	1,45±0,02	6,9	270±4,0	8,2	18,6±0,16	5
		2022	98,0±1,6	8	1,40±0,02	5,1	270±5,0	7,3	19,3±0,2	4,7
4	C-13	2020	94,3±2,5	13	1,75±0,02	5,5	404±0,7	6,9	23,1±0,3	6,9
		2021	93,6±2,4	13,5	1,62±0,02	5,3	376±3,9	6,2	23,2±0,16	3,8
		2022	90,6±2,0	13	1,62±0,02	5	320±3,4	6,1	23,4±0,2	3,2
5	C-14	2020	92,5±3,3	14,8	1,48±0,21	6,6	346±6,4	48	23,6±0,2	4,5
		2021	91,8±3,0	15,1	1,50±0,02	6,6	360±3,4	3,4	23,8±0,19	4,5
		2022	90,9±3,0	15,2	1,48±0,02	6	350±3,5	3,3	23,7±0,2	4,1
6	Ipakchi 1 (average)	2020	92,0±0,8	7,7	1,55±0,03	8	331±5,0	8,8	22,4±8,3	5,9
		2021	92,8±1,1	4,8	2,00±2,03	5,7	421±8,1	8,2	21,0±0,2	4,5
		2022	94,3±2,0	6,1	1,44±0,03	6	291±9,2	10	20,6±0,2	5,2

It is known that the manifestation of quantitative signs in the silkworm is due to the combined effect of hereditary factors and environmental conditions [16,19]. Global climate changes around the world and in Uzbekistan, in particular, lead to the destabilization of natural conditions for the reproduction of the silkworm and the cultivation of mulberry. This inevitably affects the condition of the silkworm, depriving it of the opportunity to fully realize its genetic potential. In addition, the results of breeding and tribal selection largely depend on the degree of heritability of a particular trait and the correlations between the traits. In changing conditions of keeping caterpillars, changes in genetic parameters occur: variability, heritability, and correlation between traits, and the selection of breeding individuals by phenotype will not fully correspond to their genotype [20]. In our case, this is exactly what is observed: in changing conditions of feeding and maintenance, genotypes with a different reaction rate are selected.

Hatching of silkworm eggs refers to traits with an average degree of heritability, however, in our work, this particular trait reacted better than others to family selection - the hatching of silkworm eggs of all sex-labeled breeds exceeded the control (Table 2). The content of raw silk refers to traits with a high degree of heritability, however, only 2 breeds C- 13, and C- 14 have seriously increased their content of raw silk as a result of individual selection (Table 2).

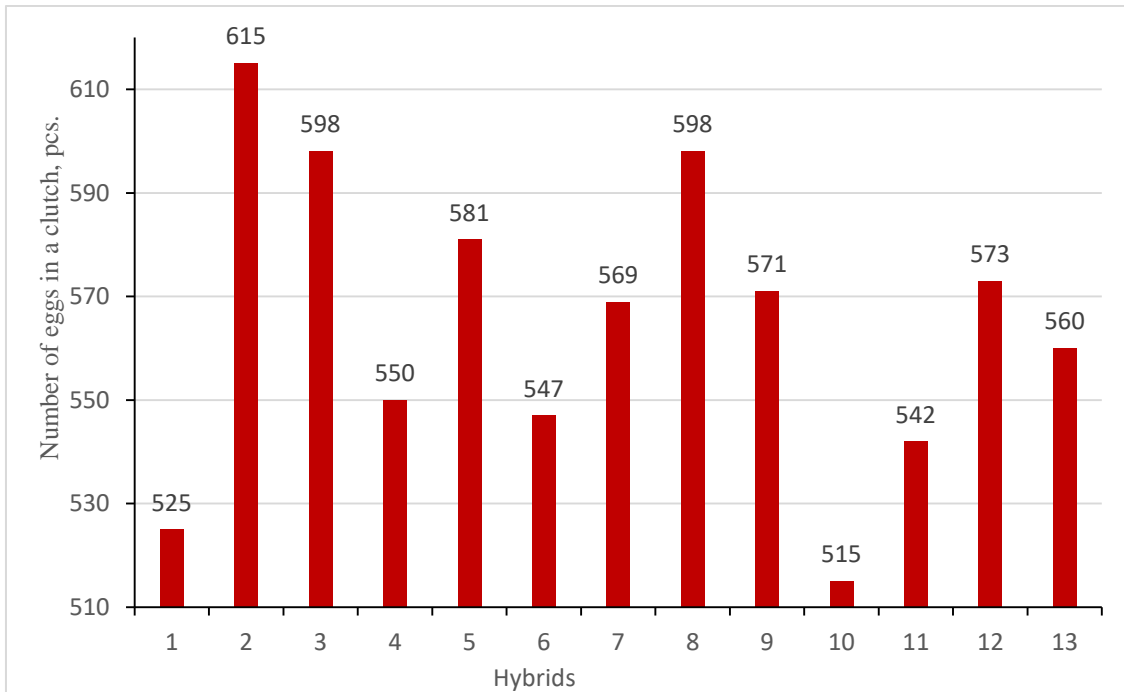
It is known that cocoons with an oval-elongated cocoon shape lend themselves best to unwinding. Therefore, crosses were carried out between breeds with an elongated cocoon shape - C-5, C-10, C-14 and oval - C-12, C-13: C-12xC-5, C-12xC-10, C-12xC-14, C-5xC-12, C-5xC-13, C-13xC-5, C-13xC-10, C-13xC-14, C-10xC-12, C-10xC-13, C-14xC-12, C-14xC-13. For clarity, Fig. 3 shows the number of normal eggs in a clutch in new hybrids in 2020.

Fig. 3 shows that hybrids between sweat-marked rocks vary greatly in masonry size. The spread ranges from 515 eggs in C-10xC-13 to 615 eggs in C-12xC-10. The largest number of eggs in the clutch is observed in hybrids C-12xC-10 - 615 pieces, C-12xC-14 - 598 pieces, and C-5xC-13 - 598 pieces. The smallest number of eggs - in hybrids C-12xC-5 - 525 pcs, C-10xC-13 - 515 pcs, C-14xC-12 - 542 pcs. The number of normal eggs in a clutch of 7 out of 12 hybrids turns out to be higher than the control one - 560 pcs. The content of raw silk of cocoons is shown in Fig. 4.

Fig. 4 shows that the content of raw silk of cocoons of 8 out of 12 new hybrids exceeds the control, that is, it is above the red line of the control indicator - 21,1%. The best silk-bearing hybrids were C-12xC-10 - 22,2%, C-5xC-13 - 22,0%, C-13xC-14 - 21,8%. Thus, the best hybrids turned out to be: egg laying size - C-12xC-10, C-12xC-14, C-5xC-13, caterpillar viability - C-14xC-12, C-14xC-13, C-10xC-13, content of raw silk of cocoons - C- 12xC-10, C-

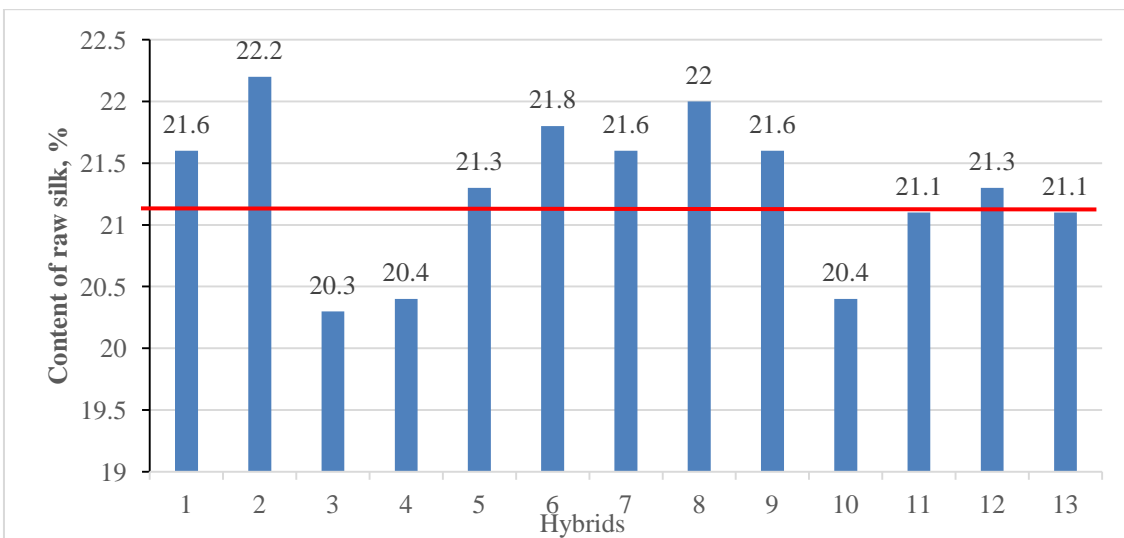
5×C-13, C-13×C-14. According to the totality of data, the best hybrids are hybrids C-12×C-10, C-5×C-13, and C-12×C-14. Considering that reciprocal hybrids are economically more profitable for industrial breeding than hybrids of one direction, it was decided to recommend hybrids C-12×C-10, C-10×C-12 for the

introduction. Moreover, the hybrid C-10×C-12 is not much inferior to the direct hybrid in almost all indicators. For a real assessment of the properties of hybrids C-12×C-10, C-10×C-12, Table 3 contains their main economically valuable characteristics.



1- C-12×C-5; 2- C-12×C-10; 3- C-12×C-14; 4- C-5×C-12; 5- C-5×C-13; 6- C-13×C-5; 7- C-13×C-10; 8- C-13×C-1; 9- C-10×C-12; 10- C-10×C-13; 11- C-14×C-12; 12- C-14×C-13; 13- Ipakchi1×Ipakchi2 (average)

Fig. 3. The number of normal eggs in a clutch in new hybrids in 2022



1- C-12×C-5; 2- C-12×C-10; 3- C-12×C-14; 4- C-5×C-12; 5- C-5×C-13; 6- C-13×C-5; 7- C-13×C-10; 8- C-13×C-1; 9- C-10×C-12; 10- C-10×C-13; 11- C-14×C-12; 12- C-14×C-13; 13- Ipakchi1×Ipakchi2 (average)

Fig. 4. The content of raw silk of cocoons new hybrids in 2022

Table 3. Economically valuable indicators of hybrids C-12×C-10, C-10×C-12 (2022)

No	Indicators	C-12×C-10		C-10×C-12	
		X±S ² x	as a % of control	X±S ² x	as a % of control
1	Number of normal eggs in a clutch, pcs.	615±6,7	109,8	571±6,0	102
2	Weight of normal eggs, mg	292±4,0	108,1	266±4,0	99,1
3	Viability of caterpillars, %	96,5±6,7	101,4	95,2±9,0	100
4	Cocoon weight, g	1,65±0,21	103,1	1,65±0,02	103,1
5	Shell weight, mg	362±6,4	107,4	356±3,8	105,6
6	Content of raw silk %	22,2±0,22	105,2	21,6±0,25	102,4
7	Raw silk yield,%	42,41	100,2	44,62	105,5
8	Metric number, unit.	3190	103,9	3092	100,7
9	Non-breakable filament length (m)	744	105,4	784	110

As can be seen from Table 3, all indicators of productivity signs of hybrids C-12×C-10, and C-10×C-12 exceed the control indicators. To explain heterosis by quantitative characteristics, the direct dependence of quantitative characteristics on viability is very important. Based on this position, it is not difficult to understand that in hybrids with high viability, the indicators of quantitative traits are greater than the average of parents. Consequently, there are no direct genetic mechanisms at the basis of heterosis in quantitative terms, it is indirectly determined by adaptive heterosis, or increased disability, which reduces the frequency of errors in the phenotypic implementation of the genotype. In heterogeneous material, other purely genetic causes of heterosis are possible by quantitative signs, but their specific weight is not great, as can be judged by the high level of heterosis by quantitative signs in isogenic material [21].

4. CONCLUSION

1. Determined from the genetic material of the breeds of the world collection of silkworms of the SRIS, by the ranking method, the breeds labeled by sex at the stage of the silkworm egg with the best biological indicators: C-5 W₂, C-10 W₃, C-12 W₂, C-13 W₂, C-14 W₃ for the creation of new high-heterosis hybrids.

2. The lines of the silkworm of the C-5, C-10, C-12, C-13, and C-14 breeds improved in reproductive parameters, egg hatched, caterpillar viability, weight, and content of raw-silk of cocoons were bred using traditional selection methods at all stages of development and selection by the motor activity of hatched caterpillars and male butterflies.

3. 12 hybrids have been created and tested in laboratory conditions between breeds labeled by

sex at the egg stage with an oval and elongated cocoon shape in order to determine the most productive hybrids.

4. Hybrids were identified from breeds labeled by sex at the egg stage with 100% purity of preparation, with the maximum manifestation of heterosis in reproductive and biological indicators: C-12×C-10, C-5×C-13, C-12×C-14. Hybrids are distinguished by: a high number of normal eggs in a clutch, viability of caterpillars, content of raw silk of.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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