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Issue Article

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DEVELOPMENT OF A METHOD FOR OBTAINING GENETIC LINES BALANCED BY TWO NON-ALLELIC Z-LETALS WITH INCREASED QUALITY INDICATORS OF COCOONS OF THE SILKWORM BOMBYX MORI L.

Abstract: *This article presents the research results to improve the unique C-8ngl breed of the silkworm Bombyx Mori L., balanced by two embryonic Z-chromosome and sex-labeled at the egg stage. Backcross crosses were carried out using a simple genetic line with high technological properties, which refers to a genetic line that exhibits desirable traits and is easy to work with in a laboratory setting. A translocated, sex-labeled trw2w2×L-67 line was also used in the experiment. At the second stage of improvement, the analyzer was crossed using a translocation line to obtain information about which of the double genotypes was present in the resulting genotype of generations* F_1, F_2, F_3, *and* F_4. *Genetic schemes were developed to monitor the embryonic genes* ℓ_1 *and* ℓ_2 *attached to the Z chromosome in generations* F_1, F_2, F_3, *and* F_4 *obtained by backcrossing and to demonstrate the degree of their presence in each generation. The balance of embryonic non-allelic genes in the breeding line was achieved by obtaining the* F_4 *generation. It was found that the newly obtained genetic line has a higher viability and quality of silk fibers than the original C-8ngl breed. This new line has passed laboratory tests and can be widely used only in food production enterprises to produce male-only hybrid combinations.*

Key words: *sex chromosome, silkworm, breed, genetic line, translocation, embryonic Z-lethality, reverse crossing, sex marking.*

Language: *English*

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Introduction

Cocoons of the silkworm species *Bombyx mori* L. They are the raw material for producing the most in-demand natural silk fibers. Worldwide, 917,650 tons of cocoons are produced annually in 21st countries. Among the countries where cocoons are grown, the leading place is occupied by China, India, and Uzbekistan. These 3 States account for 97.16 percent of the cocoon grown in the world. Other countries harvest the remaining 2.84 percent. In sericulture, based on the methods of sex management of the silkworm, when creating breeds and hybrids producing silk fibers of type 3A, 4A, and 5A, the use of genetic material containing two lethal genes in its genotype, as well as the development of effective methods for obtaining new genetic lines with their participation is one of the urgent problems.

In the world, special attention is paid to genetic research in the field of creating breeds, lines, and hybrids of silkworms that give a high yield of cocoons and, at the same time, high-quality fine silk fiber, as well as increasing the viability of larvae and resistance to various adverse factors. One of the urgent tasks is to create genetic lines with new properties based on sex-labeled breeds at the egg stage containing the genotype of the silkworm species *Bombyx mori* L. non-allelic sex-linked embryonic lethal genes, maintaining the balance of embryonic lethal genes in their genotype, as well as ensuring a 1:1 sex balance and obtaining hybrids involving these forms that meet the requirements of the modern silk industry [15, 16].

V.A.Strunnikov and S.S.Lezhenko developed the method of regulating the sex of the Silkworm based on Z-letals in 1970 [4]. V.A.Strunnikov, S.S.Lezhenko, and U.N.Nasirillaev bred male hybrids “Turon 1" and "Turon 2” with the participation of balanced paired metals and large-window breeds of Orzu, Yulduz. In order to mark the sex, S.S.Lezhenko and B.U.Nasirillaev received a new translocation [3]. Industrial hybrids “Zarafshon 2” and “Zarafshon 3” were bred by crossing sex-labeled lines with simple lines.

From foreign scientists, Z.Xin-rong obtained a male hybrid with the participation of C-8 and S-14 lines [3]. X.Jianguo bred hybrid generations with the participation of Ping group lines obtained based on flight-balanced C-8 and S-14, sex-labeled breeds at the egg stage [6] H.Kerong worked on breeding a new breed by including sex-linked lethal genes in breeds with high economic and technological indicators [7]. F.Marec has introduced new methods into cytogenetic studies [8], A.Ohnuma has completed research conducted to improve methods for obtaining balanced embryonic lethal, sex-labeled based on skin coloring of larvae [11], W.Traut and A.Ohnuma have widely implemented improvements [12, 10]. J.Chen studied the effect of recessive non-allelic lethals linked to the Z-chromosome on abnormalities in embryonic development [5]. Currently, sex-regulated breeds are only partially used in practice. The problem of their low efficiency in egg production lies in the imperfection of these breeds [14]. The peculiarity of the unique C-8ngl breed, created by V.A. Strunnikov, is that both female and male embryos survive when crossed within the breed. When crossed with ordinary breeds of any other sex, only male individuals survive in the characterized F_1 generation [2]. Some Japanese scientists have completed their research on improving the method of obtaining balanced breeds with sex markings based on the skin color of larvae at the embryonic stage. The mixing scheme consisted of commercial lines (usually of the first generation) called "Platina Boy", which differed in their performance and high quality of silk from previous lines created in the same way [9].

The study aimed to develop a genetic method to improve the quality indicators of the Z-lethal C-8 ngl breed and obtain hybrid combinations of Silkworms, 100% male, with the participation of the C-8 ngl breed with monitoring of lethal genes.

MATERIALS AND METHODS OF RESEARCH

The object of the study was the larvaes of the F_1, F_2, and F_3 generations obtained by crossing the C-8 ngl breed of the *Bombyx mori* L. species. A silkworm balanced by paired sex-linked embryonic Z-lethal genes with the lineage Line-28, which has high technological properties, as well as new male hybrid combinations.

Methods of hybridization of the Silkworm, traits of researching genetic analysis in laboratory conditions, and applied statistical methods in processing the results obtained. This chapter provides a method for obtaining only a generation of males based on the balancing of embryonic Z-lethal and the general traits of F_1 hybrids obtained as a result of this method, "control of lethal", genetic analysis of inheritance of lethal genes, criterion 2 and processing of the obtained digital data by a biological and statistical method. In addition, a method for obtaining only the male generation based on balancing the embryonic Z-lethal genes used in experiments, reproductive indicators, signs of revival and viability, cocoon productivity, as well as methods for determining technological traits, and methods for statistical processing of results are described [1].

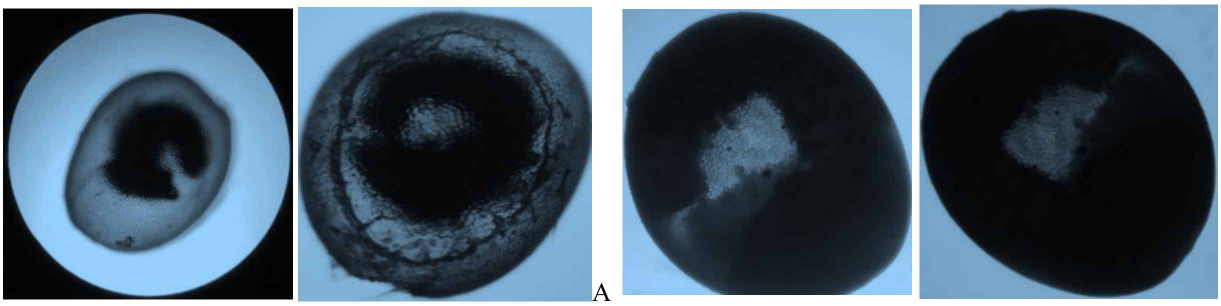
RESEARCH RESULTS AND DISCUSSION

As the results showed, a total of 186,779 pieces of eggs of the first generation of the C-8 ngl breed were analyzed, and it was confirmed that the value of $\chi 2$ was 1.84 in a ratio of 1:1. Analyses conducted using the method of $\chi 2$ criteria showed that the improved breed can be used as a starting material. It became clear that there was no significant difference between the expected ratio of gray and light yellow color of the greens and the theoretically calculated ratio (114 pieces). This result was obtained by analyzing according to the $\chi 2$ criterion of the ratio of 23903 pieces of a egg of the combination ♀(C-8 ngl×L-28)×♂(L-28×C-8 ngl). Half of the generation of females obtained in the first direction of the analyzed egg laying was killed by the lethal ℓ_1 gene. Egg clutches were split in a ratio of 3 parts gray : 1 part light yellow. In the second F_2 generation of improvement, the analysis of the ratio of eggs according to the criterion $\chi 2$ in the combination ♀(tr24w2w2×L-67)×♂(L-28×C-8ngl) of the analyzing crossing showed that the result obtained in experiments on the ratio of gray and light yellow eggs to the total number of eggs obtained in ovipositors as it is close to the theoretically expected result. Analyses carried out using the $\chi 2$ method showed that egg-laying data ($\chi 2$=0.96) can be used to determine the presence of embryonic Z-lethal in the genotype of the improved breed. A noteworthy aspect is that in the generation obtained from the combination of ♀(tr24w2w2×L-67)×♂(L-28×C-8 ngl) analyzing crossing, at the stage of this improvement, not only the embryonic flyers are determined, but also the subsequent second line of the improver after the line of the improver L-28.

For this reason, all the indicators of the larvae obtained from the combination ♀(tr24w2w2×L-

67)×♂(L-28×C-8 ngl) at the second stage of improvement were analyzed. As for the laying of eggs obtained by analyzing crossing, 16 out of 33 families obtained from two different directions had embryonic flies. Of the 16 egg clutches, the number of egg clutches containing the lethal gene ℓ_1 was 5, and the number of egg clutches containing the lethal gene ℓ_2 was 11. These 16 egg clutches were included in the next stage of improvement.

On the third day, 1/3 of the egg females (in gray egg) died at the stage of bleaching. 1/3 of the gray egg were males and could not be distinguished by heterozygosity by fig.1 Despite the somewhat low revivability of yellow-colored eggs, their viability was almost normal. All yellow eggs were homozygous for the gene of egg w_2 coloration and heterozygous for ℓ_1. Based on the above characteristics, all egg clutches obtained during the analyzing crossing were divided into two groups: those preserving the lethal ℓ_1 and ℓ_2 in the genotype. It was only on the third day that it became possible to distinguish between flying data (Fig. 1).



A B

Figure 1. A. Gray-colored genes that died under the influence of part l_1 (♂♂+♀♀)
B. Gray-colored genes that died under the influence of flying l_2 (♂♂ + ♀♀)

At the third stage of improvement, when bringing the flies into balance, they crossed according to a pre-developed theoretical scheme to combine each fly from a disjointed state into paired Z chromosomes. Moreover, before that, the ratio of prepared egg clutches was analyzed. At the same time, in the genotype of the resulting generation, the $+w_2$ gene located on chromosome 10 was homozygous and heterozygous, and gray and light yellow genes were split in two ratios – 3:1 and 1:1. Based on the slight difference between the egges obtained in practice and the egges calculated from a theoretical point of view ($\chi 2$=1.24 and 0.34, respectively), it can be seen that the error here is minimal. At the third stage of improvement, in the intermediate processes of balancing the lethal, the ratio of egg coloring in the second version of egg laying was analyzed, as was the ratio in the first version. The resulting value of $\chi 2$ in the ratios 3:1 and 1:1 was 0.43 and 0.14, respectively. The male egg clutches obtained during the analysis crossing were individually numbered and crossed once with the females of the leading egg clutch and once with the females of the lines marked by sex in the following directions:

♀[♀(C-8 ngl×L-28)×♂(L-28×C-8 ngl)] (*ℓ_1 grey*)
♂[♀(tr24w_2w_2 x L-67)×♂(L-28×C-8 ngl)] (*ℓ_2 light yellow*)
♀[♀(tr24w_2w_2 x L-67)×♂(L-28×C-8 ngl)] (*ℓ_1 grey*)
♀[♀(C-8 ngl×L-28)×♂(L-28×C-8 ngl)] (*ℓ_2 grey*)
♂[♀(tr24w_2w_2 x L-67)×♂(L-28×C-8 ngl)] (*ℓ_1 light yellow*)
♀[♀(tr24w_2w_2 x L-67)×♂(L-28×C-8 ngl)] (*ℓ_2 grey*)

Two hundred thirty-seven egg clutches were prepared for the general analysis crossing. Of these, the number of egg clutches containing the lethal gene ℓ_1 was 40 out of 135, and the number of egg clutches containing the lethal gene ℓ_2 was 32 out of 102

After separating the main two improvement versions by flight, taking into account the identity of the female organisms of the first version from a phenotypic point of view, they were all fattened and crossed with butterflies of white-eyed males animated from light yellow eggs of the second version. 1/4 of the male organisms of the main II-version of improvement, or 1/2 of the male organisms animated from light yellow eggs, had paired flies that were maximally involved in the crossing process.

An analytical crossing revealed that 23 of the total 231 egg clutches of offspring with Z-lethal balanced genes from the primary egg clutches contain lethal genes ℓ_1 and ℓ_2. Thus, due to the improvement of the C-8 ngl breed, an improved line was obtained as a new analog (Fig. 3).

P $W^{+w_2+l_1}Z^{l_1},\ w_2/w_2$ × $Z^{l_1}Z^{l_2},w_2/w_2$

♀ version I F_3 ♂ version II F_3

F_4 $W^{+w_2+l_1}Z^{l_1},\ w_2/w_2$ $W^{+w_2+l_1}Z^{l_2},\ w_2/w_2$ $Z^{l_1}Z^{l_1},w_2/w_2$ $Z^{l_1}Z^{l_2},w_2/w_2$

1♀ grey 1♀ grey, dying 1♂ *light yellow*, dying 1♂ *light yellow*

Fig. 3. Obtaining an improved F4 generation.

Using a new genetic method, it is possible to transfer the properties of an improving breed (line) into the genotype of a balanced embryonic lethal breed (line) through backcross crossing. It is important to note that lethal genes must be strictly controlled from the first backcross generation to the fourth backcross generation to ensure their transmission to the next generation. As a result, the embryonic lethal genes are balanced in the fourth generation. The advantage of this new genetic method over previously created methods is that there is no need to obtain translocation under the action of gamma rays and induce embryonic ℓ_1 and ℓ_2 lethal genes. As a result, the volume of genetic breeding activities is reduced, labor productivity is increased, and labor and money are saved 2-3 times. It is possible to effectively use the recommended improvement method at silkworm breeding stations with sufficient resources.

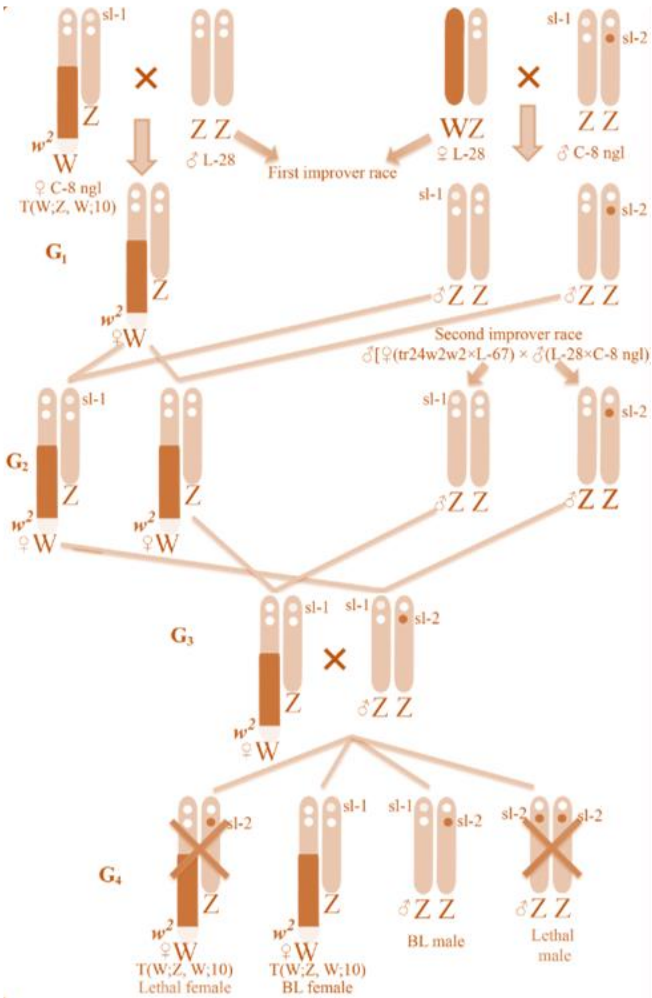


Fig. 4. Scheme of improvement of the silkworm line balanced by embryonic Z-letals, which makes it possible to obtain only the male generation [S.S.Lezhenko, B.U.Nasirillaev, M.A.Abdikodirov; 2022).

In addition, if the proposed new method of improving the necessary breeds and lines is available, it is possible to introduce it in scientific organizations of any foreign country engaged in sericulture, with identical results. Table 1 shows the biological parameters of the C-8 ngl comparator breed and the new, improved line.

Table 1. Biological indicators of larvaes at the third stage of improvement of the C-8 breed of the English silkworm

Egg Revival, %	Viability of larvaes, %	Cocoon weight,g	Silkiness, %
C-8 ngl (the comparator)			
85.0	90.9±2.26	1.96±0.01	23.1±0.13
Improvement F_3 ♀[♀(C-8 ngl×L-28)×♂(L-28×C-8 ngl)] (ℓ_2 *grey*) × ♂[♀(tr24w_2w_2 x L-67)×♂(L-28×C-8 ngl)] (ℓ_1 *light yellow*)			
82.9	91.3±3.32	1.74±0.01	23.1±0.15
Improvement F_3 ♀[♀(C-8 ngl×L-28)×♂(L-28×C-8 ngl)] (ℓ_1 *grey*) × ♂[♀(tr24w_2w_2 x L-67)×♂(L-28×C-8 ngl)] (ℓ_2 *light yellow*)			
92.6	90.67±2.53	1.6±0.02	25.4±1.44

The viability index of the C-8 ngl breed was relatively low (89.3%). The egg recovery rates included in the improvement of the first line L-28 (93.0%) and the second line tr24w2w2×L-67 (94.0%) were satisfactory. The viability of the C-8 ngl breed during the larvae period was 83.7%, the L-28 line was 91.1%, and the tr24w2w2×L-67 line was 86.8%. The proportion of larvaes that did not reach the cocoon before curling in the C-8 breed was 2.4%, in the L-28 line – 6.6%, in the tr24w2w2×L-67 line – 4.8% of the total number of larvaes. When improved in the direct and reverse generations of F1 L-28×C-8 ngl and C-8 ngl×L-28 in the embryonic period, viability was 94.6%, and viability during the larvae period was 94.9% and 91.8%, respectively. The percentage of morbidity was 2.7% and 5.2%, respectively. By the third generation, the comparator breeds C-8 ngl showed the best results in terms of fertility. In particular, the gray and light yellow eggs amounted to 300 and 334 pieces, respectively. The mass of eggs in the clutch was equal to 401 mg. This is 140-165 pieces more than the direction of the main improvement and analytical crossing. The proportion of physiologically unsuitable eggs, where there was no significant difference between the genes of the primary and analyzing areas of improvement compared to the comparator, amounted to 0.2-0.35% more.

Signs of viability had higher rates. In particular, if the indicator of embryonic viability of the comparator was 85.0%, then in the direction of improvement, consisting of the offspring of females, this indicator was 82.91%; in the second direction, consisting of the generation of males, it set the upper limit from 82.91%. Only the gray-colored females of the first improvement direction in the larvae stage had a relatively good viability index of 91.3%. As expected, the ratio of larvaes that, under the influence of any factors, could not curl healthy cocoons to healthy larvaes was 1.89% only in the offspring of F3 males animated from light yellow eggs of the second direction. It should be noted that the caliber of cocoons in two main directions has achieved a significant increase in shell weight and silkiness. Indeed, as a result of the enlargement of cocoons and softer than usual shells and poles of cocoons of the C-8 ngl breed, taken as a comparator, it was once again demonstrated on digital data to accelerate the process of reducing silkiness, as well as a severe change of pedigree in the improved F3 generation, the highest level of silkiness was observed in the first group of gray color – 25.4 %.

Table 2. Technological indicators of cocoons at the third stage of improvement of the C-8 breed of the Silkworm

Traits	C-8 ngl	F_3 Improvement grey (♀ ℓ_2 × ♂ ℓ_1)	F_3 Improvement *light yellow* (♀ ℓ_1 × ♂ ℓ_2)
Dry cocoon weight, г	0.791	0,761	0,686
Output of raw silk, %	35.70	49,37	52,29
The output of silk products, %	44.38	53.59	54.21
Unrolling the cocoon, %	80.44	91.08	86.97

The length of the continuously unwound fiber, m	258	967	1033
The total length of the silk fiber, m	1071	1358	1592
Metric number of fiber, m/g	3145	3056	3374

If we analyze directly the most important technological traits of the cocoons obtained, then among them, which are considered essential, the yield of raw silk from the C-8 ngl breed was 35.7%. The female organisms that developed from the gray eggs of the F_3 generation gave 49.37%, and male organisms – 52.29% of silk (Table 2).

In the C-8 ngl breed, the total length of the silk fiber (1071 m) was shorter than the generations of males and females obtained from improvement by 287-521 m, respectively. The metric number of the fiber, i.e., thickness, in the newly obtained male cocoons of the F_3 generation was 3374 m/g, and compared with the comparator C-8 ngl, the result was higher by 229 m/g.

The work was carried out on monitoring by dividing the family into groups to build a genealogy of families of the C-8 ngl breed prepared for the spring season. The results are presented in Tables 3 and 4.

Table 3. Indicators for monitoring details and the percentage of liveability in the female generation of the C-8 eng breed

Female ♀♀				
quantity of eggs, pieces				**percent revived, %**
total	**revived**	**non-reviving lethal**	**non-revived**	
357±3.02	132±1.54	197±2.70	19±0.06	37,0±0.10
340±6.94	151±4.11	168±5.11	20±1.94	45,0±0.67
325±4.46	138±5.11	173±3.20	14±1.44	46,0±0.49
Average				
341±9.24	140±5.61	179±8.95	17±1.91	43,0±2.86

As the analysis of three-year monitoring results showed, the resulting generation of females had a liveliness index below 50%, where the high limit was 47% and the lower limit was 37%. The balance of female generation details obtained within the breed was not shifted in either direction. We can also observe that the generation of males obtained within the breed has undergone unexpected changes. In some families, the survival rate of males was 54%. This indicates a violation of the balance of deaths in the family. This family was only accepted by attracting a generation of males to grow in the next season.

Table 4. Indicators of mortality monitoring and the percentage of vivacity in the male generation of the C-8 ngl breed

Male ♂♂				
quantity of eggs, pieces				**percent revived, %**
total	**revived**	**non-reviving lethal**	**non-revived**	
399±1,14	144±0,40	184±1,37	19±0,86	37,0±0,17
374±9,67	169±6,14	141±4,84	14±5,99	45,0±0,91
343±6,52	138±6,59	181±3,47	24±2,98	43,0±0,86
Averege				
372±6,13	150±6,57	154±4,86	19±2,89	42,0±2,42

The following 5-table shows the biological indicators of 100% male hybrids obtained with the participation of the C-8 Engl breed as the paternal form and new lines as the maternal form.

Table 5. Biological indicators of male F1 hybrids obtained by crossing the C-8 Engl breed with ordinary breeds (lines)

Hybrids	Egg revival, %	Viability of larvaes, %	Cocoon weight, g	Silkiness, %
Istikbol	90.2±3.48	92.9±1.23	1.86±0.04	24.99±0.55
L-66xC-8 ngl	89.2±4.61	93.8±1.75	1.77±0.04	25.87±0.37
L-28xC-8 ngl	87.3±5.16	90.4±2.33	1.78±0.09	27.2±0.55
L-203xC-8 ngl	88.2±2.10	91.5±3.05	1.85±0.08	25.6±0.44
L-207xC-8 ngl	87.0±1.52	92.9±1.73	1.77±0.04	25.1±0.37
Uzb-5 (control)	85.2±3.13	92.9±1.17	2.20±0.09	21.7±0.63

It can be seen above that among the male hybrids, the Istikbol hybrid, with a high viability of 90.2%, showed a good result. The hybrid L-66hC-8 ngl can be cited as a satisfactory result among generations of male hybrids with a high proportion of larvaes that have achieved cocoon hatching (93.8%). The next indicator is the percentage of morbidity, measured by the ratio of the number of larvaes that failed to curl a healthy cocoon to the total number of healthy larvaes. The results of the three-year experiment showed that this result was different. In particular, the L-66hC-8 ngl combination was distinguished by the presence of only 1.91% of unhealthy cocoons among the total number of cocoons. The analyzed data of the Uzbekistan 5 hybrid, taken as a comparator, showed that, despite their fertility, the larvaes' viability was insufficient. Firstly, Uzbekistan 5, taken as a comparison, has giant cocoons due to its tetrahybrid origin. This, in turn, affects the weight of the cocoons. In particular, this hybrid differed by a cocoon weight of 2.22 g from other male hybrids, parent breeds, and lines. L-207xC-8 ngl and Istikbol (1.86-1.89 g) were closer to this hybrid with relatively similar indicators. Approximately the same trend was observed for the mass of the cocoon shell. In particular, tetrahybride Uzbekistan 5 was 503 mg, whereas the closest indicator was found in L-28hC-8 ngl and amounted to 489 mg. The Marvarid breed also had a relatively high cocoon mass (441 mg). The ratio of the cocoon shell's mass to the cocoon's total mass, i.e., The property of high silkiness, was clearly manifested by the male hybrid combination L-28xX-8 ngl (27.2%). The L-28 line, taken as the parent line, had an indicator of 24.7%.

Conclusion

1. It was noted that starting from the second stage of improvement, an analytical crossing was carried out; out of 33 families, five families containing the lethal gene ℓ_1 and 11 families containing the lethal gene ℓ_2 were identified. As a result of the analyzing crossing carried out at the third stage of improvement, the presence of the lethal ℓ_1 gene in the genotype of 40 out of 135 families and the presence of the lethal ℓ_2 gene in the genotype of 32 out of 102 families was proved. Of 231 families, 23 were identified as having achieved balance by the F_4 generation, containing paired chromosome.

2. It was revealed that the viability (92.63%) and high metric number of silk fiber (3374 m/g) of the new line obtained as an analog of the C-8 ngl breed allow it to be recommended for drainage enterprises.

3. It was noted that the embryonic and postembryonic viability of F_1 hybrid offspring at the level of 85.2-95.5% and 85.0-93.5%, respectively, means that there is no negative influence of 2 non-allelic lethal genes ℓ_1 and ℓ_2 in their genotype.

4. It was revealed that the yield of raw silk from cocoons (46.99-50.43%), the total fiber length (1214-1356 m) and the metric fiber number (thinness) (2859-3213 m/g) show significant superiority in quality of cocoons of male hybrids for production.

5. It has been thoroughly proven that the lethal genes ℓ_1 and ℓ_2 in the genotype of male F_1 hybrids do not negatively affect the economic characteristics and viability of the Silkworm.

6. It is noted that the high technological performance of silk fiber unwound from cocoons of industrial male hybrids increases the possibility of obtaining silk skeins of type 4A and 5A in Uzbekistan.

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