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Biological Indicators of Industrial Hybrids Involved in Sex-Regulated Lines of *Bombyx Mori L.* Silkworm

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ABSTRACT

This article reveals the analysis of the biological indicators of industrial hybrids belonging to silkworm species *Bombyx mori* L. and obtained by involving new lines Line 5 labeled, Line 6 labeled, Line 31 labeled and Line 32 labeled on the color of egg and created at Uzbekistan Scientific Research Institute of Sericulture. Due to the easy separation of one component of the hybrids into the sex, it is possible to reduce the costs of egg production in the silkworm egg-producing plant by 50%. In addition, the combinations involved in hybridization allow the production of full industrial hybrids with cocoon productivity. As a result of three years of comparative testing, ♀ Line 32 lab. x ♂ Line 101 and ♀ Line 101 x ♂ Line 32 lab. combinations showed positive results. When the cocoon yield from 1 box of silkworm was 76.6 kg in a foreign hybrid, ♀ Line 32 lab. x ♂ Line 101 and ♀ Line 101 x ♂ Line 32 lab. hybrids produced 85.9 kg and 92.5 kg yield, respectively.

Keywords: silkworm, cocoon, larva, gene, hybrid.

INTRODUCTION

Silkworm is one of the first farm animals to be industrially propagated in the form of F_1 generation hybrids. Due to the contamination of local eggs produced by egg-producing enterprises with pure-bred eggs, the possibility of producing a sufficient amount of high-quality cocoon raw materials is limited. At the same time, a large amount of manual labor and financial costs are incurred for the separation of breeds for hybridization. In this regard, the importance of hybrid combinations with the involvement of new sex-labeled breeds is immeasurable, because the work of hybridization with the participation of breeds with the sex labeled on egg color is much easier and does not cost extra labor and money. Most importantly, larva hatched from 100% hybrid eggs are strong, disease resistant and highly productive, as well as being uniform in terms of shape, caliber and a number of other characteristics of the cocoons produced. As a result, the quality of silk products produced by cocoon processing enterprises will increase and the cost price of raw silk will decrease due to the reduction of dry cocoon consumption.

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Outstanding world scientists have been engaged in sex related issues of silkworm. They are Ch.Darwin, A.Wolles, A.Wesman, P.Goldschmidt, R.Fisher, G.Meller and others.

It is known that most animals have sexual polymorphism and in order to produce agricultural products, more or less of a certain species is required. For example, while a higher proportion of females is required in the production of milk and eggs, a greater proportion of males is more effective in the production of meat, wool, and silk [1,2,3].

Silkworm is the first of the farm animals to be the object of artificial sex regulation. In this regard, the first results were obtained in the 30s of the XX century. B.L. Astaurov and H. Hoshimoto developed methods of artificial parthenogenesis and experimental androgenesis for mulberry silkworm [4,5].

The solution to the problem of artificial sex regulation in animals, especially in silkworms, was first proposed by the great Russian geneticist A.S. Serebrovsky [6, 7]. He hypothesized translocation of genes which control morphological traits and located in autosomes to sex chromosomes. This theoretically advanced idea was put into practice for the first time in the history of world science by scientists from the CIS and Japan in the mulberry silkworm.

Reconstruction between the W and Z sex chromosomes under the influence of x and γ rays has been used successfully for industrial purposes in obtaining interbreeding male F_1 hybrids and in marking sex on morphological traits. By excluding the rest of the translocated autosome from the genotype with such a proportionality of translocations, the transmission of the desired autosomal trait from generation to generation in combination with sex made it possible to do so at the choice of the researcher [8].

The first system of mulberry silkworm, labeled by morphological traits, was created by the Japanese scientist Yu.Tazima under the influence of ionizing radiation [9].

Uzbek and Japanese scientists began their independent research in 1955 on the labeling of the sex of the silkworm by egg color. In 1944, Japanese scientists led by Yu.Tazima translocated the + w_2 gene located on the 10th autosome of female sex to the W chromosome, resulting in female larva hatching from gray eggs and male larva from light yellowish eggs at the expense of w_2w_2 recessive homozygous genotypes [10,11].

If we analyze the research of Uzbek geneticists in this area, we can be sure that the results are better than those of Japanese researchers. V.A. Strunnikov and his colleagues were able to translocate the + w_2 gene to the W chromosome from the $+w_1$, $+w_2$, $+w_3$, $+w_4$, $+w^{os}$, $+w^{ol}$ genes on the 10th autosome of silkworm that provide pigmentation in the egg’s serous layer, and has a complementary effect. Because the new genetic system has the $+w_2$ gene on the W chromosome, female eggs are gray in color and males are pale yellow on the 10th autosome due to the presence of the w_2w_2 recessive homozygous genes [12,13,14,15].

Thus, the breed, created by a group of Uzbek scientists, sex labeled by egg color, was introduced into production as a parental component of industrial hybrids. However, these lines were created based on small-cocoon breeds, that’s why they did not produce the cocoon yield as produced by ordinary hybrids in industrial conditions, and, of course, these breeds were not used for long in production.

Taking into consideration the great importance of the marking the sex of silkworm for industry, the laboratory of "Silkworm Breeding" of the Scientific Research Institute of Sericulture conducted extensive research in the field of creating large-cocoon, high-yielding and sex-marked breeds of silkworm. Under the leadership of V.A. Strunnikov, a new simplified method of translocating a fragment of the $+w_2$ gene located on the 10th autosome to the W chromosome was developed [16]. Using this method, 5 new selection lines were created on the basis of large-cocoon “Guzal” and “Marvarid” breeds [17].

Materials and methods. The experiments in this regard were carried out in the laboratory "Silkworm breeding" of the Scientific Research Institute of Sericulture during the years 2018-2020. For the experiments planned in the development program, new 5 sex-labeled and 5 simple lines were selected.

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During the experiments, the lines prepared each year were analyzed for reproductive traits. The following indicators were determined – the average number of eggs in the egg clutch, the weight of the egg clutch, the weight of one egg and the percentage of physiological defect. Then, the egg clutch of each variant was combined, mixtures were formed, and the eggs were incubated and obtained a sufficient number of larvae to rear for hybridization.

The caterpillars of lines were reared, after their cocoon spin the cocoons were collected and according to hybrid combination scheme the following hybrid combinations were produced involving parental components of ♀ x ♂ or ♂ x ♀ form:

♀ Line 5 lab. x ♂ Line 102;
♀ Line 102 x ♂ Line 5 lab.;
♀ Line 11 lab. x ♂ Line 100;
♀ Line 100 x ♂ Line 11 lab.;
♀ Line 32 lab. x ♂ Line 101;
♀ Line 101 x ♂ Line 32 lab.;
♀ Line 11 lab. x ♂ Line 66;
♀ Line 66 x ♂ Line 11 lab.

Imported hybrid from PRCh and Uzbekistan 5 hybrid zoned in the republic were selected as comparative to these hybrids. The purpose of selecting a foreign hybrid is that this hybrid has been brought to the republic in large quantities in recent years and they are fully adapted to modern cocoon spinning machines.

Research results and their discussion. In the range of fundamental project КХФ-5-010-2012 developed in Scientific Research Institute of Sericulture, a number of new sex-labeled and simple breeding lines of silkworm were created. In the creation of these lines, synthetic selection and analytical selection methods were used, and mainly aimed at improving the technological parameters of selection. Therefore, the new lines have thin and high silkiness indicators along with the ability to spin cocoons of different weights.

Together with creating new hybrid combinations, biological and technological parameters were determined for Line 5 lab., Line 11 lab., Line 32 lab., Line 100, Line 101, Line 102, Line 103, Line 66 lines that involve in new hybrid combinatons as parental component.

The experiments began with the determination of the reproductive, i.e fecundity indicators of the lines. In 2018-2020, egg clutches laid from each of the lines were analyzed and the number of eggs in one clutch of each line, the weight of eggs in the clutch, and the weight of one egg were determined.

The data of hybrid combinations for 2018-2020 correspond to the indicators of the full maternal breed, as expected, and it is recommended to take into account the reproductive characteristics of these breeds during the hybridization process (Table 1).

Table-1
Reproductivity indicators of F_1 hybrids involving breeding lines of silkworm (2018-2020).

Hybrids	Number of eggs in the clutch, pcs	C_v,%	Weight of eggs in the clutch, mg	C_v, %	Weight of one egg, mg	C_v, %
Line 5 lab. x Line 102	610,5±19,6	14,0	383±13,8	15,9	0,627±0,0026	2,8
Line 102 x Line 5 lab.	455±18,0	18,1	225±9,9	20,2	0,492±0,0060	5,3
Line 11 lab. x Line 100	583,5±17,6	15,2	307,5±10,7	17,0	526±0,0065	6,8
Line 100 x Line 11 lab.	528±7,2	10,8	291±4,8	13,2	0,550±0,0030	3,6

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Line 32 lab. x Line 101	678,3±12,7	13,3	374,7±7,1	15,4	0,552±0,0049	7,0
Line 101 x Line 32 lab.	657,3±8,2	12,2	392±5,5	13,0	0,609±0,0149	7,4
Line 11 lab. x Line 66	620,3±11,0	10,2	331±10,2	15,5	0,534±0,0048	6,6
Line 66 x Line 11 lab.	601±8,1	11,5	359±5,8	12,5	0,597±0,0038	6,8
Uzbekistan 5 (comparative 1)	757,6±19,1	15,0	486,3±13,6	15,6	0,643±0,0060	5,7

On the number of eggs in the clutch Pd=0,999;
On the weight of egg clutch Pd=0,999;
On the weight of an egg Pd=0,236-0,999;

Before analyzing the figures in Table 1, it should be noted that the test of industrial hybrids did not cover exactly 8 different combinations. Hybrid combinations with the best indicators were included in the experiments, according to the simplification of the experimental work and the initial data. The main criterion here is the indicators of the parental lines. Therefore, the data of hybrids involving Line 5 lab., Line 11 lab. and Line 100, Line 103 lines are of 1-2 years.

The fecundity of industrial hybrids replicates the fecundity of the maternal component as noted above, and the hybrids with high fecundity are the combinations Line 32 lab. x Line 101 (678.3 pcs, 374.7 mg) and Line 101 x Line 32 lab. (657.3 pcs, 392 mg). The fecundity of tetra-comparative hybrid Uzbekistan 5, which used as a standard hybrid, is slightly higher – 757.6 pieces and 486.3 mg, respectively. These figures are, of course, explained by the fecundityof the large-cocoon breeds “Orzu” and “Yulduz” that make up this hybrid. Having considered the newly proposed hybrids were obtained with the participation of medium-cocoon breeds, it can be concluded that their egg productivity fully meets the requirements of egg-producing enterprises.

Based on the analyzes performed on reproductive traits, the Line 32 lab. x Line 101 and Line 101 x Line 32 lab. industrial hybrids were found to be the hybrids with the highest egg productivity.

The eggs hatching of the new lines and the viability of the larva are of great importance in the large-scale incubation and care of the larva of the hybrid combinations created with their participation in the future. Therefore, in our experiments for 2018-2020, we determined the parental lines, egg hatching and larva survival of the hybrids obtained with parental involvement.

While the hatching of the eggs of the mulberry silkworm determines viability at the embryonic stage, the survival of the larva indicates postembryonic viability. In most cases, egg hatching and larva survival are mutually exclusive selection traits, and the higher the egg hatching, the more viable the larva will be and their higher resistance to various adverse conditions.

The main goal of the research is to create new productive, industrial hybrids with high technological indicators. Therefore, comparative silkworm rearing was carried out by hatching the new combinations in the laboratory. Zoned Uzbekistan 5 and foreign hybrid were taken as comparative hybrids. In our experiments during the years 2018-2020, eggs of new hybrid combinations along with the lines and comparative standard foreign and local hybrid eggs were hatched, and the viability of the larva was determined (Table 2).

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Table-2
Egg hatching and larva survival in new hybrid combinations (2018-2020)

Lines	**Egg hatching, %**	**Larva survival, %**	**Percentage of disease, %**
Line 5 lab. x Line 102	97,5	85,0	6,3
Line 102 x Line 5 lab.	88,7	92,1	2,0
Line 11 lab. x Line 100	96,0	87,8	6,0
Line 100 x Line 11 lab.	96,3	87,1	4,5
Line 32 lab. x Line 101	97,0	92,2	4,9
Line 101 x Line 32 lab.	97,2	92,9	1,8
Line 11 lab. x Line 66	94,7	89,4	4,4
Line 66 x Line 11 lab.	95,7	86,9	5,4
Uzbekistan 5 (comparative 1)	90,7	89,4	3,7
Foreign hybrid (comparative 2)	96,4	87,8	3,7

On egg hatching percentage (Pd=0,485-0,993);
On larva survival Pd=0,850-0,952;
On disease percentage Pd=0,294-0,987.

If we analyze the hybrid combinations, we can see that their hatching rate was average 88.7-97.5% on three-year observations (Rd = 0.485-0.993). Egg hatching in the comparative standard hybrid and foreign hybrid was 90.7% and 96.4%, respectively. Line 32 lab. x Line 101 (97,0%), Line 101 x Line 32 lab. (97,2%), Line 11 lab. x Line 66 (94,7%) and Line 66 x Line 11 lab. (95,7%) hybrid combinations manifested higher results on egg hatching compared to other hybrids. This rate in comparative local and foreign hybrids was noted to be 90,7%-96,4%, as the figures in table-3 show their lower results by 0,8-3,4% than in new hybrids.

If we consider the larva survival indicators in hybrid combinations, it should be noted that there is a wide variability and insignificant heterosis. The survival and morbidity rates in the new hybrids were 85.0–92.9% (Pd = 0.850–0.952) and 1.8–6.3% (Pd = 0.294–0.987), respectively. In comparative Uzbekistan 5 and foreign hybrid these indicators showed 87,8-89,4% and 3,7% respectively. The results on survival of tested combinations corresponds to egg hatching, that is, Line 32 lab. x Line 101, Line 101 x Line 32 lab., Line 11 lab. x Line 66 ва Line 66 x Line 11 lab. hybrid combinations had a slight higher survival potential. This is confirmed by the morbidity rate (1.8-4.9%) of larva in these hybrids during their cocoon-spinning stage.

The weight and silkiness of the cocoon is one of the most important traits among mulberry silkworm farm traits. Twenty or thirty years ago, the selection focused on mainly large cocoon breeds, but in recent years, much attention has been paid to the cocoon silkiness and technological indicators of new lines. Considering this, in our previous selection work, we tried to increase their silkiness and leading technological properties by maintaining our sex-labeled and simple lines at the level of medium-cocoon breeds.

The cocoon weight of industrial hybrids is an important indicator that determines cocoon yield. It is generally observed that the cocoon weight of the hybrid offspring is relatively heavier than that of the parental components. The cocoon productivity of the new hybrids over three years of laboratory testing is given in table 3.

Table- 3

Cocoon yield of hybrids (2018-2020).

Hybrids	**Cocoon weight** $\bar{X} \pm S\bar{x}$**, g**	**Weight of cocoon shell** $\bar{X} \pm S\bar{x}$**, mg**	**Silkiness** $\bar{X} \pm S\bar{x}$**, %**

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Line 5 lab. x Line 102	2,22	520	23,4
Line 102x Line 5 lab.	2,25	566	25,1
Line 11 lab. x Line 100	2,13	495	23,2
Line 100 x Line 11 lab.	2,35	563	23,9
Line 32 lab. x Line 101	2,15	508	23,6
Line 101x Line 32 lab.	2,28	508	22,3
Line 11 lab. x Line 66	2,03	484	23,7
Line 66 x Line 11 lab.	2,01	453	22,5
Uzbekistan 5 (comparative 1)	2,22	488	21,9
Foreign hybrid (comparative 2)	1,92	419	21,8

If we look at the cocoon productivity figures of the new hybrid combinations in Table 3, we can see that they have a higher cocoon weight than the parent lines (2.01–2.35 g). In comparative hybrids this rate made 1.92-2.22 g (Pd=0,591-0,999). As in 2018-2020, cocoon weight of hybrids in Line 32 lab x Line 101, Line 101 x Line 32 lab and Line 66 x Line 11 lab hybrid combinations on average results was 2.15-2.35%, a bit higher compared to other hybrids, that is, 0.13-0.23 abs. g higher than in comparative hybrids (Pd=0,973-0,999).

The silkiness of live cocoons determines the amount of raw silk obtained from the raw material of the produced cocoon, and this indicator is one of the main farm traits of industrial hybrids. The analysis of the silkiness of hybrid combinations in table 3 shows that most of our new hybrids have a higher silkiness potential than foreign and local standard hybrids. The indicators on this trait in Line 102 x Line 5 lab (25,1%), Line 32 lab x Line 101 (23,6%), Line 101 x Line 32 lab (22,3%), Line 100 x Line 11 lab (23,9%) hybrids can confirm our opinion.

Cocoon yield is the most important criterion in evaluating hybrids. The more viable the hybrid worms are, the more their number is maintained, and at the same time if the heavier cocoons are wrapped, the higher the cocoon yield. Therefore, we calculated the yield of cocoon and cocoon shell of the newly tested hybrids (Table 4).

Table-4
Cocoon and cocoon shell yield of hybrid combinations (2018-2020).

Hybrids	**Yield per one box of silkworm, kg**		**Relative to foreign hybrid, %/%**
	cocoon	**cocoon shell**	
Line 5 lab. x Line 102	81,1	18,9	105,9/113,2
Line 102 x Line 5 lab.	82,7	20,8	108,0/124,6
Line 11 lab. x Line 100	81,7	19,0	106,7/113,8
Line 100 x Line 11 lab.	88,7	21,2	115,8/126,9
Line 32 lab. x Line 101	85,9	20,3	112,1/121,6
Line 101x Line 32 lab.	92,5	20,6	120,8/123,4
Line 11 lab. x Line 66	77,2	18,0	100,8/107,8
Line 66x Line 11 lab.	75,2	17,0	98,2/101,8
Uzbekistan 5 (comparative 1)	82,3	17,5	107,4/104,8
Foreign hybrid (comparative 2)	76,6	16,7	100,0

Analyzing the cocoon and cocoon shell yields in table 4, we can see that the newly created industrial hybrids have a much higher cocoon yield and are significantly superior to foreign comparative hybrid. According to the three-year indicators, the highest cocoon yield was noted in Line 101 x Line 32 lab (92.5 kg; 20.6 kg), Line 32 lab x Line 101 (85.9 kg; 20.3 kg) hybrids. While in local standard hybrid this rate was 82.3 kg; 17,5 kg, in foreign hybrid 76.6 kg; 16.7 kg, respectively. It should be particularly noted that the average cocoon yield of a comparative foreign hybrid in production condition of the republic does not exceed 59.0 kg.

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High cocoon yields are obtained mainly due to heavy cocoons and live larva. This means that these indicators must be in demand level in parental breeds at the beginning of the hybridization.

CONCLUSIONS

The problem of mulberry silkworm sex regulation has been a scientific issue that has been studied for many years. Uzbek scientists have been able to obtain translocations that regulate several sexes, and since the 1960s they have been able to fully control the sex of local breeds of silkworm. However, due to the fact that the breeds of that period were small cocoons, the hybrid combinations obtained with the participation of these sex-labeled breeds did not meet the production requirements. As a result, those created breeds and hybrids could not find their place in practice.

At present, genetic scientists of the Uzbek Scientific Research Institute of Sericulture have obtained new translocations that can mark the sex in the genotype of the large-cocoon “Marvarid” and “Guzal” breeds. In Line 5 lab, Line 6 lab, Line 11 lab, Line 31 lab, and Line 32 lab lines, the female worms hatch from gray eggs and male worms in light yellow eggs and these lines serve as inbred lines for several generations. In the population of these lines, in addition to important features and characteristics such as cocoon shape, weight, there is a wide variability in the cocoon silkiness and technological indicators. The aim of this research work is to identify new hybrid combinations that allow the production of 100% hybrid industrial eggs of mulberry silkworm for industry. In the proposed hybrids, the sex of one parental component is separated into 50% females and 50% males in the early stages of ontogeny during the egg stage. As a result, there will be no problem with differentiating breeding cocoons in the activities of the egg-producing company, i.e 100% hybrid eggs will be obtained.

According to the results of the three-year comparative analysis, Line 101 x Line 32 lab (92.5 kg; 20.6 kg), Line 32 lab x Line 101 (85.9 kg; 20.3 kg) hybrids were found to be considerably superior to local standard hybrid (82.3 kg; 17.5 kg) and foreign hybrid (76.6 kg; 16.7 kg) on cocoon yield per one box of silkworm. Fecundity (egg productivity) of breeds and lines is also an important indicator for egg-producing enterprises. The standard Uzbekistan 5 industrial hybrid consists of 4 components, which includes large-cocoon local “Orzu” and “Yulduz” breeds, and, of course, the mother moths of this hybrid are fecund and recognized as the most fertile hybrids in Uzbekistan. However, mother moths of industrial hybrids Line 32 lab x Line 101 and Line 101 x Line 32 lab that manifested high results in our study, lay average 657-678 pieces eggs and this rate is recognized as positive result for egg-producing enterprises in the condition of Uzbekistan.

These hybrids meet the production requirements for their fecundity, high cocoon yield and cocoon silkiness, as well as larva viability and can be recommended for production.

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