

# The Heritage of Egg Color During the Development of a New Genetic Method of Improvement of Mulberry Silkworm *Bombyx Mori L.* Balanced Breed with Embryonic Z-Lethalities

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**Abstract** The mulberry silkworm *Bombyx mori L.* is one of the insects that has been domesticated and used for its own needs for 5,000 years. A mulberry silkworm is a convenient object of scientific research due to its feeding, care, and rapid reproduction. In our study, the rare C-8ngl (NGL - new genetic line) strain of *Bombyx Mori L.* mulberry silkworm was observed during ovulation, balanced by nonallelic embryonic Z-lethalities, gradually becoming F<sub>2</sub> and F<sub>3</sub> with simple silk fiber simple genetic lines. Improvements are reflected in the results. The balance of  $\ell_1$  and  $\ell_2$  embryonic non-allelic lethal attached to the Z chromosome in generations F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub> were analyzed to determine the extent to which they manifested in each generation. In each generation, the ratio of females to males was strictly controlled during the ovulation period. The results showed that in the F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub> generations, the egg ratio was balanced from 67♀:33♂ to 50♀:50♂. The theoretically expected results proved to be almost consistent with the practical results obtained by the  $\chi^2$  statistical calculation method. It follows that the correct formation of the sex ratio in each generation during C-8ngl breeding leads to the stabilization of the embryonic nonallelic lethal balance of the new genetic line obtained. Genetic schemes have been developed to show the extent to which  $\ell_1$  and  $\ell_2$  embryonic lethalities attached to the Z-chromosome in F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, and F<sub>4</sub> progeny from backcross breeding are present in each generation. By the F<sub>4</sub> generation, balancing of embryonic nonallelic genes in the selection system has been achieved.

**Keywords** Mulberry silkworm, Breed, Genetic line, Embryonic Z-lethality, Crossbreeding, Sex labeled, Translocation

## 1. Introduction

Mulberry silkworm *Bombyx mori L.* has been domesticating it for 5,000 years and using it for its own needs. A mulberry silkworm is a convenient object for research due to its feeding, care, and rapid reproduction. Therefore, in this poikilotherm and monophagous insect, several very important problems of genetics have been solved. In particular, the laws of artificial control of sex, experimental (radiation) mutagenesis, a new interpretation of heterosis, and the genetics of quantitative traits were first discovered in mulberry silkworms and the results achieved were directly put into practice.

It is well known that mutations are mainly events that directly affect an organism as a result of a complete or partial

change (disruption) of its genetic data set. V.A. Strunnikov was one of the first in his research to study the possibility of using lethal mutations in sericulture in the 1960s [1].

Currently, artificially controlled sex is only partially used in practice. The problem with their low egg production is the low cost of breeding them. However, silk fibers from male cocoons are thinner and more durable than those from female cocoons [2].

We know that mutations are mainly events that directly affect an organism as a result of a complete or partial change (disruption) of its genetic data set. In sericulture, lethal mutations are mainly used to produce male progeny. The lethal period is a series of stages that occur during the G<sub>2</sub> period before body pigmentation and mitosis [3], [4].

The occurrence and quantity of lethality depend on the strength (dose) of mutagens and the tendency of gametes to mutate (mutability), as well as the success of sex-linked lethality in the number of families and the number of larvae raised from them. Although many mutations can be caused by high doses of radiation, mutants are difficult to detect due

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to their extremely low viability [5].

Sex-linked balanced lethal (SLBL) lines solve the problem of producing only male hybrid progeny, which has an invaluable role in improving silk quality and production efficiency.

At the suggestion of Academician V.A. Strunnikov, the procedure for obtaining such series is determined as follows:

Induction of W and Z chromosomal translocation processes;

Induction of a recessive mutation attached to the Z chromosome that compensates for the dominant allele transferred to the W chromosome (Figure 1) [6].

Japanese scientist A. Ohnuma developed a complex genetic scheme to improve balanced lines on lethality based on the Moricaud breed by cross-breeding with the W-7 breed of Chinese origin, which has a high economic trait [7].

In his experiments at a mulberry silkworm breeding plant in Shandong Province, C. Yong used the Ping 76 line of a mulberry silkworm balanced by sex-balanced lethality as a parent recipient, and a common Haoyue breed with fine silk fiber as a gene-donor material [8].

In 1996, after negotiations with activists from the Zhejiang Academy of Agricultural Sciences, lethal genes balanced based on unique, complex genetic schemes of mulberry silkworm lines were brought from the Russian Academy of Sciences [9], [10].

At present, the Uzbek Scientific Research Institute of Sericulture is conducting research on the preservation, monitoring, and creation of new hybrid combinations of translocations in the genotype of balanced progeny on Z-lethal genes.

In particular, by increasing the technological performance of the lines marked by the color of the sex egg by cross-breeding, maintaining a high level of cocoon productivity and creating new hybrid combinations, Nasirillaev B.U., Umarov Sh.R., Jumaniyozov M.Sh., M.F.Khalilova [11], Achilov Sh.I., Umarov Sh.R. [12], Nasirillaev B.U. and Abdikodirov M.A. [13], [14], [15] are conducting genetic research.

The main goal of our study is to improve the balance of the rare C-8ngl breed of mulberry silkworm on embryonic Z-lethal against the background of the main economic characteristics by transferring the technological characteristics of the L-28 line, which gives thin silk to the genotype while maintaining the lethal balance.

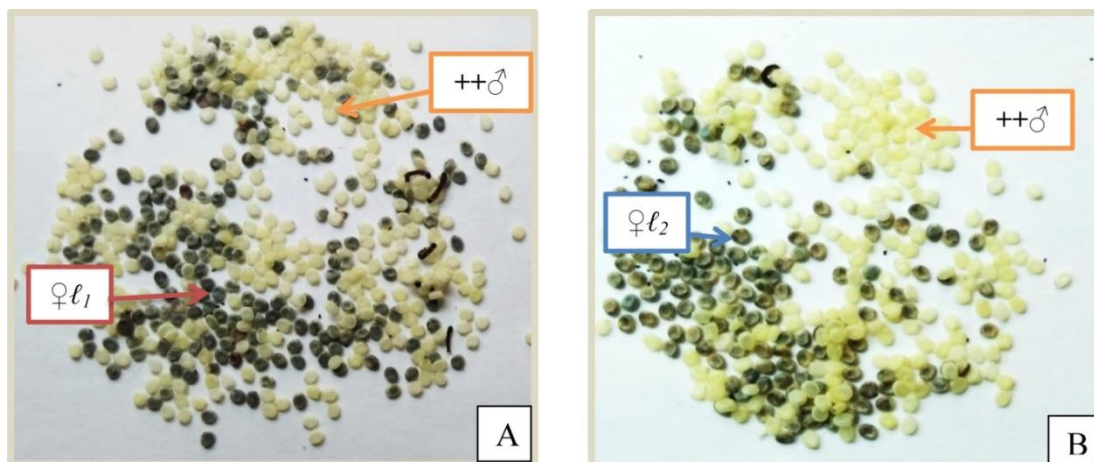
## 2. Material and Method

The experiments were conducted by scientists from the Uzbek Scientific Research Institute of Sericulture under the Committee for the Development of Silk and Wool Industry and the Department of Genetics of the National University of Uzbekistan. In the experiment, the mulberry silkworm *Bombyx mori L.* used C-8 ngl, a sex-marked, embryonic Z-lethal equilibrium, the sex-marked  $trw_2w_2 \times L-67$ , as well as the normal sex-unmarked L-28 lines.

The worms selected for the experiment were kept at a young age at a temperature of 26-27°C and relative humidity of 70-75%, and adult worms at a temperature of 24-25°C and relative humidity of 65-70% in special larvae of the Uzbek Scientific Research Institute of Sericulture. 1000 kg of mulberry leaves is used for 1 box of worms. The worms were fed with mulberry leaves of Jarariq 4, Jarariq 5, and Jarariq 6 mulberry varieties. The  $\chi^2$  method was used to mathematically and statistically evaluate the data obtained and expected in the experiment.

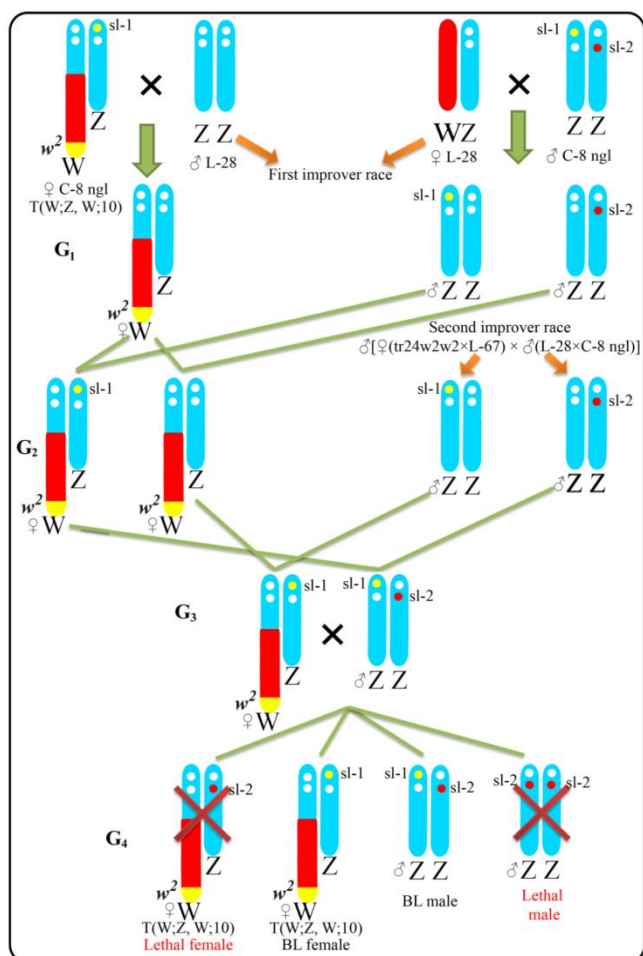
## 3. Results and Discussion

This is the first time that the C-8ngl strain-improving method, which labels gender development and is balanced by embryonic lethality, is being used for the first time as an improver for two high-productive strains and as a marker for the egg color  $+w_2$  gene on chromosome 10<sup>th</sup>. Previously, the Japanese scientist A. Ohnuma used the Chinese breed as a donor-improver, as a marker for the  $p^M$  gene on the 2<sup>nd</sup> autosomal chromosome - the skin of the worm [7].



**Figure 1.** Dying of embryos in the egg under the influence of  $\ell_1$  and  $\ell_2$  lethal genes: A- gray eggs died under the influence of  $\ell_1$  lethal ( $\delta\delta + \varnothing\varnothing$ ); B- gray eggs died under the influence of  $\ell_2$  lethal ( $\delta\delta + \varnothing\varnothing$ )

According to this method, the morphological marker attached to the sex is mottled on the body of the worm, the worms appear at the age of 3-4 years, and it is more labor-intensive to determine the sex. Genetic marking of eggs by the genetic method we developed allows the identification of lethal genes at an early stage of embryonic development, as well as significant labor and cost savings (Figure 2).



**Figure 2.** A new genetic scheme for breeding that is balanced by embryonic Z-lethalities and sex is marked during the egg stage (via the +w<sub>2</sub> gene)

During the research, egg layings prepared for the study of egg color inheritance - the progeny (lines) used as parents, and hybrid eggs laid with their participation were analyzed individually. During the analysis, the number of eggs in each egg was determined by counting the eggs individually. Unsealed and dried eggs were isolated from the analyzed eggs. The improved C-8 ngl was initially taken as the starting breed, while the F<sub>1</sub>-F<sub>3</sub> generation was taken as a control. The ratio of gray and light yellow eggs by egg color was studied in egg layings prepared for the 2019-2021 worm care season. The results are presented in Table 1.

From the three-year data in the table below, it is clear that the inheritance of the color of the eggs obtained is close to the expected result. The color of the eggs obtained by the  $\chi^2$  criterion and the ratio of males to females is very close to the

expected 1:1 ratio ( $P > 0,05$ ). The heredity of the egg color of the C-8 ngl and the sex ratio of the egg stage was found to be specific from these numbers.

**Table 1.** Inheritance of egg color of C-8ngl breed, labeled during the breeding season of the mulberry silkworm

Years	Analyzed replications / number of eggs, pcs	Sex ratio, %		$\chi^2$
		expected	obtained	
C-8 ngl				
2020	186779	50♀:50♂	49,8♀:50,2♂	1,84
2021	66272		49,7♀:50,3♂	1,72
2022	122442		49,9♀:50,1♂	0,32

Subsequent analysis was performed to improve the C-8ngl breed in terms of technological characteristics. F<sub>2</sub> and F<sub>3</sub> are egg-laying hens. This process of improvement is characterized by the fact that the C-8ngl is balanced by double lethality, and the L-28 line, which is introduced into the population, is one of the simplest. When mixed, egg color and sex ratios are inherited in later generations due to the influence of lethal genes.

Before the following generations to improve the C-8ngl breed ♀ (C-8 ngl × L-28) × ♂ (L-28 × C-8 ngl) F<sub>2</sub> - improvement, ♀ (tr24w<sub>2</sub>w<sub>2</sub> × L-67) × ♂ (L-28 × C-8 ngl) - analytical mixing. To obtain F<sub>3</sub> progeny, female butterflies that selected  $\ell_1$  and  $\ell_2$  lethal obtained in the F<sub>2</sub> generation were mutilated with male butterflies obtained from analytical crossbreeding. Table 2 shows the results of egg color separation in generations F<sub>2</sub> and F<sub>3</sub>.

**Table 2.** Inheritance of egg color during the F<sub>2</sub>-F<sub>3</sub> generation of the C-8 ngl breed, which is labeled during the breeding season of the mulberry silkworm

Analyzed replications / number of eggs, pcs	Sex ratio, %		$\chi^2$
	expected	obtained	
Improving ♀(C-8ngl × L-28) × ♂(L-28×C-8ngl) F <sub>2</sub>			
35897	87,5:12,5	87,3:12,7	1,32
Crossbreeding under analysis			
♀(tr24w <sub>2</sub> ×L-67) × ♂(L-28×C-8ngl) (2 <sup>nd</sup> improver)			
11181	75:25	75,4:24,6	0,96
♀[♀(C-8ngl × L-28)×♂(L-28×C-8ngl)] (ℓ <sub>1</sub> Gray)× ♀[♀(tr24w <sub>2</sub> ×L-67)×♂(L-28×C-8ngl)] (ℓ <sub>2</sub> Light yellow) F <sub>3</sub>			
12613	50:50	50,25:49,75	0,34
21664	75:25	75,3:24,7	1,24
♀[♀(C-8ngl × L-28)×♂(L-28×C-8ngl)] (ℓ <sub>2</sub> Gray)× ♀[♀(tr24w <sub>2</sub> × L-67)×♂(L-28×C-8ngl)] (ℓ <sub>1</sub> Light yellow) F <sub>3</sub>			
5947	50:50	49,75:50,25	0,14
15761	75:25	75,2:24,8	0,43

From the figures in Table 2, it can be seen that the egg color in the F<sub>2</sub> and F<sub>3</sub> generations obtained to improve the C-8ngl breed is theoretically analyzed by three different separations, i.e., 87,5: 12,5, 75:25, 50:50 ratios. was made. The figures show that our figures are very close to this theoretically expected separation. The  $\chi^2$  criterion suggests that the egg color ratio in the F<sub>2</sub> and F<sub>3</sub> generations obtained to improve the C-8ngl breed is reliable ( $P > 0.05$ ).

## 4. Conclusions

Today, the development of the silk industry places high demands on the quality of mulberry silkworm silk fiber. It turns out that high-strength, thin silk is obtained from male cocoons. To create male industrial hybrids, embryonic lethal genetically balanced progeny are required. Therefore, the need and demand for the genotype of new breeds (hybrids) are always high when obtaining high-quality silk. It should be noted that the unique C-8 ngl breed has not been involved in any improvements since its inception. In the future, the task is to bring this breed of silk to the level of the highest quality type 3A - 5A. There is, of course, a need for effective improvement along the way. This method can be used not only in Uzbekistan but also by scientists from leading silkworm countries. Our study showed that the F<sub>2</sub> and F<sub>3</sub> progeny obtained to improve the C-8ngl breed balanced by double lethality were very close to the theoretically expected separation in egg color. The results were significant in that if the separation differed significantly from the theoretical ratio or was completely different, it could be concluded that the experiments were performed with great error. However, the digital data show that the work is moving in the right direction.

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## REFERENCES

- [1] Strunnikov V.A. Relative effect of primary radiation damage to the nucleus and cytoplasm of germ cells of the silkworm. // Cytology. - Moscow, 1960. - V.2. - No. 5. - p. 573-580.
- [2] Yong Z., Zhonghuai X., Xiaogui L. Breeding of limited tea spots in silkworm [J]. News, 1991, (4): pp.14-16.
- [3] Xuan N, Niu BL, Wang HL, Zhuang L, Meng ZQ. Mapping of the lethal genes in the sex-linkaged balanced lethal silkworm *Bombyx mori* using SSR markers. Hereditas. 2010; 32(12): 1269–74. PMID: 21513153.
- [4] Xuan N, Niu BL, Wang HL, Zhuang L, Meng ZQ. Mapping of the lethal genes in the sex-linkaged balanced lethal silkworm *Bombyx mori* using SSR markers. Hereditas. 2010; 32(12): 1269–74. PMID: 21513153.
- [5] Strunnikov V.A., Lezhenko S.S. et al. Artificial regulation of sex in the silkworm // Soob. 4. Genetics. 1979. V. 15. No 6. p. 1096-1114.
- [6] Strunnikov, V.A. (1995) Genetic engineering in the silkworm. In: Strunnikov VA (eds) Control over Reproduction, Sex, and Heterosis of the Silkworm, pp. 105-223, Harwood Academic, New York.
- [7] Ohnuma A. Establishment of the practical male-rearing technology by a balance sex-linked lethal. J. Seric. Sci. Jpn. 74 (2-3), 81-87, 2005.
- [8] Yong S., De-Wen F., Qin-Gao Z., Rui-Ying G., Guo-Jun L., Shi C., Ke-Rong H. Breeding of a male silkworm variety “Lujing ×Huayang” for spring rearing. Sericulture Science CANYE KEXUE. 2009; 35 (1). pp.165-169.
- [9] Kerong H., Xinrong Z, Hui H. Backcrossing improved silkworm sex chain balance research on the dead [J]. Seric. Sci., 2001, 27 (3): 185-188.
- [10] Kerong H., Xinrong Z., Hui H. Silkworm sex chain balance lethal line sex research on methods of controlled gene transfer [J]. Chinese Agricultural Sciences, 2002, 35 (2): 213-217.
- [11] Nasirillayev B.U., Umarov Sh.R., Jumanioyozov M.Sh., M.F. Khalilova. Prospects for the creation of new hybrid combinations with the participation of mulberry silkworm breed breeders. // Livestock and breeding work. - Tashkent, 2018. - No 2-3. p 42-44.
- [12] Achilov Sh.I., Umarov Sh.R. New lines of mulberry silkworm and their cocoon productivity indicators in the F<sub>1</sub> generation. // Livestock and breeding work. - Tashkent, 2019. - №1. pp 30-32.
- [13] Nasirillaev B.U. Sex marking at the egg stage and improvement of new translocant backcross lines of the silkworm using large cocoon donor breeds. // Proceeding of the Genetic Resources Institute of Azerbaijan National Academy of Sciences. V.VII. N2. Baku, 2018. P. 126-131.
- [14] Nasirillaev B., Abdukadirov M. Egg productivity mulberry silkworm male hybrids with the participation of a Z-lethal balanced breed. Prospects for the introduction of innovative technologies in the development of agriculture. Materials of the Int. Scientific-Practical Conference. Ferghana, Uzbekistan (2021). DOI: 10.47100/conferences.v1i1.1380.
- [15] AbdiQodirov M. Vitality indicators of F<sub>2</sub> generation in improving balanced C-8 ngl breed of silkworm by double lethal. “Modern issues of science and practice”. Materials of the Int. Sci. Prac. Conf. Washington, USA. (2021). DOI: 10.37547/iscrc-intconf12.