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Development of a new genetic method of improving the Mulberry Silkworm Bombyx Mori L. breed balanced on Embryonic Z-Lethal

Bakhtiyar U. Nasirillaev
Institute of Sericulture, Tashkent, Uzbekistan

Murodkhuja A. Abdikodirov
National University of Uzbekistan, Tashkent, Uzbekistan

Mamura F. Khalilova
Tashkent state agrarian university, Tashkent, Uzbekistan

***Abstract*---**The problem of artificial control of sex has become important for the genetics and selection of many beneficial animals, which has not lost its relevance even in the current period. This article reveals the results of research on the improvement of the unique C-8ngl breed of *Bombyx Mori L.* mulberry silkworm balanced on embryonic Z-lethal and sex-limited in the egg stage with a simple genetic system of high-tech with fine silk features. In the experiment, sex-limited C-8ngl breed of *Bombyx mori L.* mulberry silkworm in the egg stage and balanced on embryonic Z-lethal, also sex-limited trw_2w_2 × L-67 line, as well as non-sex-limited simple L-28 line, were used. In the second stage of improvement, an analyzer crossing was performed using translocation lines sex-limited in the egg stage to obtain information on which of the double lethal was present in the obtained F_1, F_2, F_3 and F_4 generation genotype. Genetic schemes have been developed to monitor ℓ_1 and ℓ_2 embryonic lethal attached to the Z chromosome in F_1, F_2, F_3 and F_4 generations obtained by backcrossing and to show the extent to which they are present in each generation. Balancing of embryonic non-allelic genes in the selection system has been achieved having obtained F_4 generation. This new line has successfully passed laboratory tests and can only be widely used in egg production enterprises to obtain combinations of male sex hybrids.

***Keywords*---**Sex chromosome, translocation, silkworm, breed, genetic line.

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***Corresponding author**: Nasirillaev, B.U.*
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Introduction

We know that mutations are phenomena that directly affect an organism, mainly as a result of a complete or partial change (disruption) of its genetic data set. In silkworm breeding, the use of lethal mutations can enable the control of various genetic processes. A lethal mutation is a factor that leads to the premature death of an organism. In this case, the dominant lethal mutation destroys all organisms (homo - heterozygotes), while in a recessive lethal mutation only homozygous organisms die [Arefev et al 1995].

Lethal mutations and the strange puzzles that occur under their influence have prompted scientists to carry out some practical studies on them. G. Muller [Muller, 1927] developed a method for obtaining sex-linked lethal (CIB) in Drosophila: it was easy to account for lethal mutations and phenotypically manifested mutations for the Drosophila (*Drosophila melanogaster*) chromosome due to heredity. For this reason, G. Muller developed a method to account for lethal mutations in the autosome. Among the number of scientists who have improved this method through a wide range of practical experiments and introduced it to the field of silkworm breeding, the work of V.A. Strunnikov deserves special recognition [Strunnikov, 1987]. V.A. Strunnikov successfully researched mutations induced by γ-rays. Subsequently, using induced mutagenesis, V.A. Strunnikov obtained a line with two sex chromosomes marked by a recessive lethal mutation [Inge-Vechtomov, 1989].

Materials and Methods

Sex determination begins with the crossing of a wild-type female with a lethally balanced male organism. Such crossbreeding fails because it is hemizygous on female sex lethal (Figure 1). [Strunnikov, 1987].

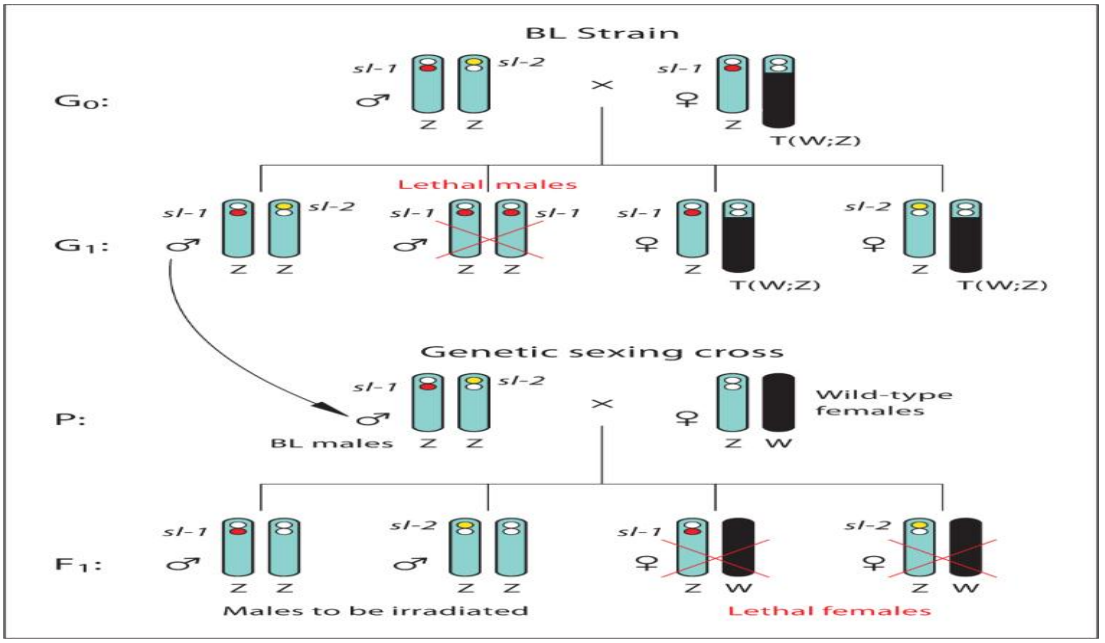

Figure 1. Scheme of genetic sexing using a balanced lethal (BL) strain suggested by Strunnikov [1975]. A segment of the Z chromosome including wild-type alleles of genes sl-1 and sl-2 has been translocated to the W resulting in a T(W; Z) chromosome.

The strain is propagated with males heterozygous for lethal alleles (red, yellow) of the 2 genes in trans, and only this type of male offspring is viable. The genetic scheme for obtaining such lines of mulberry silkworm balanced on lethal was first developed by former Soviet scientists. Completed by Japanese scientists after long studies [Ohnuma et al. 1983]. In addition, lethal attached to ℓ_1 and ℓ_2 recessive sex and T(W; Z)+^{od} chromosome were obtained by irradiating [Z/T(W;5)+ ^{pe}] females with γ-quanta [Ohnuma, 1988]. Chinese researcher A. Y. Xu used 1,000 pupae to induce embryonic, sex-attached lethal from a dose of 4,000 R of γ-rays emitted from the Co^{60} isotope [Xu et al. 1993, 1994 1997]. In 1996, after negotiations with activists from the Zhejiang Agricultural Academy, the silkworm lines with balanced lethal genes created by unique, complex genetic schemes were brought from the Russian Academy of Sciences. The main problem was then solved using two independent, patented technologies that were developed by incorporating the combined lethal gene into the breeds with high economic and technological traits [Kerong et al 2001, 2002].

Japanese scientist A.Ohnuma has developed a complex genetic scheme that can be improved by cross-breeding with a high-yielding W-7 breed of Chinese origin to improve the traits of the line balanced on lethal created on the Moricaud breed [Ohnuma, 2005]. In recent years, some Japanese scientists have completed their research on improving methods for obtaining the lines balanced on embryonic lethal, sex-limited by larval skin color. The crossbreeding scheme consists of commercial lines called "Platina boy" (generally the first generation), which are distinguished by their productivity and high quality of silk from the previous lines, created in the same way [Ohnuma, 2006], [Trautet al 2008].

Also, as a result of molecular analyzes, in the BC_1 generation obtained by crossbreeding of S-14 line balanced on Z-lethal with the simple breed P50, mapping work has been carried out with the help of SSR markers. As a result, on the physical map of the Z chromosome, the ℓ_1 gene was found to be approximately in the range of 2,60 MB at the 19,79 MB site and ℓ_2 in the range from 0.69 MB to 17,86 MB at the 18,55 MB site [Nan et al 2010].

The study led by J. Chen focused on proteomic analysis of the ℓ_2 gene. In this case, they performed MACC-Spectrometry tests on the living (W+ ℓ_1Z+ℓ_1 ℓ_2) and (W+ ℓ_1Z ℓ_1+ℓ_2) to the lethal stage. The results suggest that ℓ_2 may be an anomalous polypeptide expressive mutant. Its expression adversely affects the processes of mitosis and morphogenesis. It has been suggested that abnormal peptide accumulation and proliferation in the cell, as well as disturbances in morphogenesis, may be important causes of embryonic death [Chen et al 2012].
In Uzbek Research Institute of Sericulture, Institute of Developmental Biology of the Russian Academy of Sciences, Tashkent State University (now the National University of Uzbekistan) has confirmed that the most effective way to obtain only male generation in mulberry silkworms is to create the breeds and lines balanced on non-allelic embryonic Z-lethal [Strunnikov et al 1969;1979].

Even though the first work in this direction has been carried out for a long time, S.S. Lezhenko et al. conducted research and studies at the Uzbek Research Institute of Sericulture in this regard. In particular, under the leadership of S.S. Lezhenko, a method has been developed for determining the lethal in the breeds balanced on lethal [Lezhenko et al 2020].

Also, B.U. Nasirillaev has been working with his students and junior researchers for several years to improve the breeds balanced on existing embryonic lethal in the laboratory and improve their economic traits [Nasirillaev et al 2021].

Work on the improvement of balanced lethal strains of mulberry silkworm was carried out earlier. But in the genetic scheme, we have created, the useful properties of the two lines are introduced into the genotype of the new line for four generations. As a result of many experiments conducted on mulberry silkworms, obtaining and balancing embryonic Z-lethal among farm animals is scientifically and methodologically based, and sex control is the only object applied to a large-scale production process.

The main goal of our study was to improve the rare C-8ngl breed of mulberry silkworm balanced on embryonic Z-lethal by maintaining the lethal balance and

basing on the transferring technological traits of L-28 and L-67 lines, which produce thin silk to its genotype. The experiments were conducted by scientists from the Uzbek Research Institute of Sericulture and the Department of Genetics of the National University of Uzbekistan. In the experiment, sex-limited C-8ngl breed of *Bombyx mori L.* mulberry silkworm in the egg stage and balanced on embryonic Z-lethal, also sex-limited trw_2w_2 × L-67 line, as well as non-sex-limited simple L-28 line, were used.

The worms selected for the experiment were breed in wooden drawers at a young instar 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 a special cocoonery of the Uzbek Research Institute of Sericulture. The cocoonery has a size of 12x8x4 m and is equipped with a four-half cutlery. The lighting mode is provided with natural and electric lights for 12 hours per day. The source of heating of special worms consists of a gas boiler and an iron pipe register, specialized in providing a constant temperature. 1000 kg of mulberry leaves were used for 1 box of worms. The worms were fed with mulberry leaves of Jararik 4, Jararik 5, and Jararik 6 mulberry varieties. At the 2nd instars, worms were counted by 250 in 3 replications. The results obtained from the experiment were processed by mathematical-statistical methods.

C-8ngl breed sex-limited in the egg stage and balanced on Z-lethal

The female of the C-8ngl breed keeps two translocation parts on the W-chromosome: a part of the Z-chromosome with a dominant gene that provides viability in the first embryos; the second translocation is a 10th autosomal fragment that determines pigment production in the serous layer of the egg;

Although Z-chromosome contains ℓ_1-lethal in females, female embryos don't die as a result of this gene. The reason is that we can show the presence of a dominant allele of this gene in a fragment translocated to the W-chromosome.The recessive w_2 - the gene is in the homozygous state, and its effect is limited by the dominant allele w_2 -gene (+) in the region translocated to the W-chromosome. Due to this, the eggs of C-8ngl breed females are gray.

In the male genotype of the C-8ngl breed, there are two non-allelic ℓ_1 and ℓ_2 recessive lethal on the Z chromosome in the opposite position. Therefore, such male individuals are referred to as male silkworms in the balance with double Z-lethal (Chart 1).

$W^{+w_2+l_1}Z^{l_1w_2/w_2}$ $\qquad\qquad$ $Z^{l_1}Z^{l_2\,w_2/w_2}$

♀ C-8ngl $\qquad\qquad$ ♂ C-8ngl

Chart 1. Schematic view of the C-8ngl breed genotype, sex-limited in the egg stage and balanced on Z-lethal.

They survive because each recessive lethal has a dominant pair and prevents it from exerting a negative impact on its viability. However, due to the homozygous

state of the w_2-gene on the 10th chromosome, the male eggs are unpigmented and in light yellow color (Figure 2).

Figure 2. Eggs and their hatchability (inside the box with remover by cardboard) of the C-8ngl breed, sex-limited in the egg stage and balanced on Z-lethal

During inbreeding, 50% of female gray eggs in the C-8ngl breed didn't hatch under the influence of *the* ℓ_2 lethal gene, and 50% of male light-yellow eggs were under the influence of the ℓ_1 lethal gene. In the whole breed, in egg-laying, an average of 48-50% hatching was observed. As noted above, there were 25% females and 25% males in egg-laying.

On the 3rd day of mass hatching of C-8ngl, we can observe that in the mid-stage development half of the gray half egg-laying was lost. We can also see that the lethal eggs in the light-yellow dried up half egg-laying didn't hatch in the final, i.e., whitening stage of embryonic development.

tr24w_2w_2 x L-67 selection line sex-limited in the egg stage and Line-28 (simple, non-sex-limited) selection line.

The tr24 w_2w_2 × L-67 line can be used to obtain an analyzer-egg-laying from females to determine the presence of ℓ_1 and ℓ_2 lethal in the male generation. These egg-layings are used to obtain №2 order lines without ℓ_1 lethal in the genotype, as well as №3 selection lines without ℓ_2 lethal in the genotype. Tr24w_2w_2 × L-67 can also donate to the fine silk fiber trait due to its high-tech properties along with performing its analyzing function.

In the L-28 selection line, all genes are dominant - the genes that determine egg color in male and female organisms are dominant (+w_2+w_2) homozygous, as well as the genes located on the Z chromosome (L_1L_2), are also dominant.

Results and Discussions

Obtaining F_1 generation in two directions in the initial stage of improvement

A simple non-sex-limited breed was taken as the paternal component and was crossed with the females of the C-8ngl breed. The females of the first direction of the F_1 generation were needed (Chart 2).

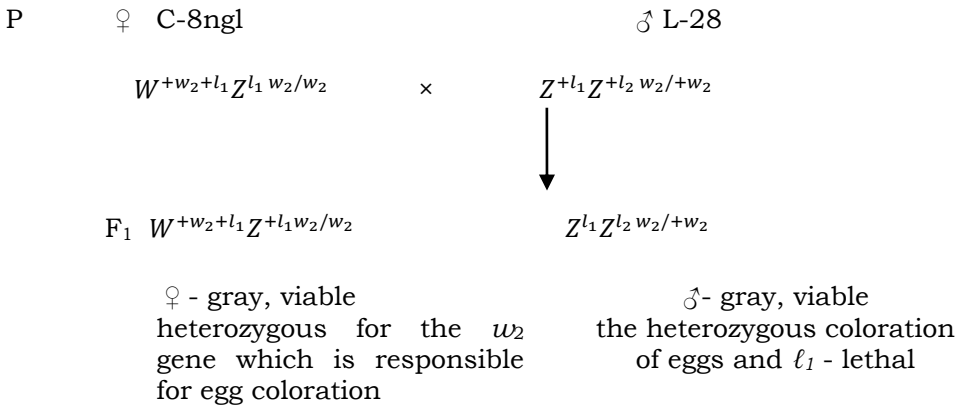

Chart 2. First crossbreeding genetic scheme for C-8 ngl breed improvement

In the second direction of crossbreeding, the L-28 line was used as the maternal component and the C-8ngl breed as the paternal component. Two different generations of males from this crossbreeding were crossed in two different directions, each with a female in the first direction.

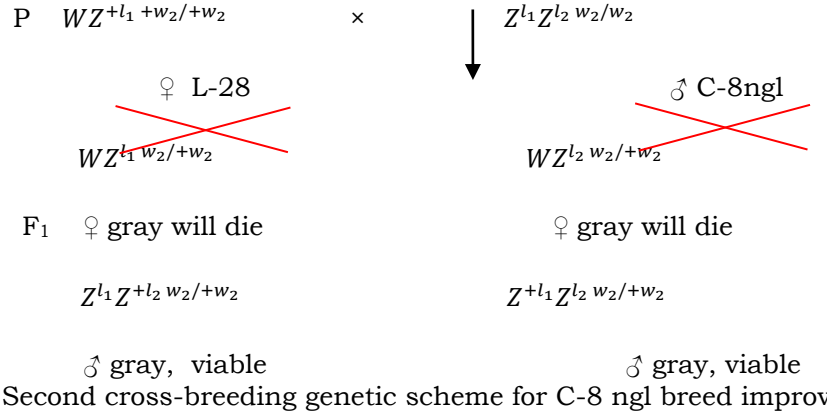

Chart 3. Second cross-breeding genetic scheme for C-8 ngl breed improvement

Obtaining the second F_2 generation of the improvement

To obtain F_2 generation to improve the C-8 ngl breed, 1st direction female moths were crossed with male moths with recessive ℓ_1 and ℓ_2 lethal in the genotype

obtained from the 2 nd direction. All-female generation eggs obtained from the F_2 generation in the 1st direction were gray, and although half of them kept l_1 lethal in their genotype, their viability was normal. Half of the F_2 generation males of the 1st direction kept l_1 lethal in their genotype, and viability was normal. The ¾ part of males of the F_2 generation was gray, while the ¼ part was light yellow (Chart 4).

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

♀F_1(I direction) ♂ F_1 (II direction)

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

1♀ gray 2♀ gray 1♀ gray

$W^{+w_2+l_1}Z^{l_1\ +w_2/+w_2}$ $W^{+w_2+l_1}Z^{l_1\ w_2/+w_2}$ $W^{+w_2+l_1}Z^{+l_1\ w_2/w_2}$

F_2 1♀ gray 2♀ gray 1♀ gray

$Z^{+l_1}Z^{+l_2+w_2/+w_2}$ $Z^{+l_1}Z^{+l_2\ w_2/+w_2}$ $Z^{+l_1}Z^{+l_2\ w_2/w_2}$

1♂ gray 2♂ gray 1♂ light yellow

$Z^{l_1}Z^{+l_2+w_2/+w_2}$ $Z^{l_1}Z^{+l_2\ w_2/+w_2}$ $Z^{l_1}Z^{+l_2\ w_2/w_2}$

1♂ gray 2♂ gray 1♂ light yellow

Chart 4. F_2 generation genotypes in the first direction of the improvement

Since half of the females of the F_2 generation keep ℓ_2 lethal in their genotype, they died in the 2nd direction of the improvement in the middle stage of embryonic development. Lethal eggs became gray and dried up. The fact that half of the males of the second direction in F_2-generation kept ℓ_2 lethal gene in their genotype did not affect the viability of the normal state. ¼ part of F_2 generation males became light yellow, ¾ part gray (Chart 5).

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

♀ F_1 (direction I) ♂ F_1 (direction II)

$W^{+w_2+l_1}Z^{+l_1\ +w_2/+w_2}$ $W^{+w_2+l_1}Z^{+l_1\ w_2/+w_2}$ $W^{+w_2+l_1}Z^{+l_1\ w_2/w_2}$

1♀ gray 2♀ gray 1♀ gray

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

1♀ gray , will die 2♀ gray , will die 1♀ gray , will die

F_2 $Z^{+l_1}Z^{+l_2+w_2/+w_2}$ $Z^{+l_1}Z^{+l_2\ w_2/+w_2}$ $Z^{+l_1}Z^{+l_2\ w_2/w_2}$

1♂ gray 2♂ gray 1♂ light yellow

$Z^{+l_1}Z^{l_2+w_2/+w_2}$ $Z^{+l_1}Z^{l_2\ w_2/+w_2}$ $Z^{+l_1}Z^{l_2\ w_2/w_2}$

1♂ gray 2♂ gray 1♂ light yellow

Chart 5. Genotypes of F_2 generation in the second direction of the improvement

Analyzer crossing in the second stage of improvement

In the second stage of improvement, an analyzer crossing was performed using translocation lines sex-limited in the egg stage to obtain information on which of the double lethal was present in the obtained F_2 generation genotype. Half of the female generation obtained in the 1st direction of analytical egg-laying died under the influence of the l_1 lethal gene. Egg-layings were separated in the ratio of 3 gray: 1 light yellow (Chart 6).

Lethal families determined in the egg-layings of F_2 generation obtained from analyzer crossing

Table 1.

Geneology of families	l_1	l_2
1(49)	+	
2(2)		+
3(22)		+
4(45)		+
5(47)	+	
6(23)	+	
7(8)	+	
8(37)		+
9(27)		+
10(12)		+
11(34)	-	-
12(17)		+
13(41)		+
14(38)	-	-
15(72)		+
16(10)	+	

As can be seen from this table, families with the ℓ_1 lethal gene in the received families amounted to five units. The number of families with ℓ_2 embryonic lethal was nine units. And the number of families that did not have lethal was two units. The revival in them was as usual. Families with acquired ℓ_1 and ℓ_2 lethal were united. The lethal gene did not affect the viability of the light yellow eggs. 1/3 of the gray eggs died during the whitening phase of development.

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

♀tr24w$_2$w$_2$ × L-67 ♂ F_1 (direction I)

$W^{+w_2}Z^{+l_2\ w_2/+w_2}$ $W^{+w_2}Z^{+l_2\ w_2/w_2}$ $W^{+w_2}Z^{l_1\ w_2/+w_2}$ ~~$W^{+w_2}Z^{l_1\ w_2/w_2}$~~ 1♀ gray

1♀ gray 1♀ gray , will die 1♀ gray , will die

BC_1F_2

$Z^{+l_1+l_2}Z^{l_1 w_2/+w_2}$ $Z^{+l_1+l_2}Z^{l_1\ w_2/w_2}$ $Z^{+l_1+l_2}Z^{+l_2 w_2/+w_2}$ $Z^{+l_1+l_2}Z^{+l_2\ w_2/w_2}$

1♂ gray 1♂ light yellow 1♂ gray 1♂ light yellow

Chart 6. F_2 generation genotypes obtained from the 1st direction of analyzer crossing

Half of the female generation obtained in the 2nd direction of egg-laying production from analyzer crossing died under the influence of the ℓ_2 lethal gene. ½ of the male generation obtained in the 2nd direction of egg-laying production from analyzer crossing kept ℓ_2 gene in the genotype and was found to be viable. This recessive lethal didn't affect the vitality of light yellow eggs. (Chart 7).

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

♀tr24w$_2$w$_2$ × L-67 ♂ F_1 (direction II)

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

1♀ gray 1♀ gray 1♀ gray , will die 1♀ gray , will die

BC_1F_2

$Z^{+l_1+l_2}Z^{+l_1 w_2/+w_2}$ $Z^{+l_1+l_2}Z^{+l_1\ w_2/w_2}$ $Z^{+l_1+l_2}Z^{l_2\ w_2/+w_2}$ $Z^{+l_1+l_2}Z^{l_2\ w_2/w_2}$

1♂ gray 1♂ light yellow 1♂ gray 1♂ light yellow

Chart 7. F_2 generation genotypes were obtained from the first direction of analyzer crossing

Obtaining F_3 generation in two different directions at the third stage of improvement

Egg-layings obtained from the analyzer crossing were placed in the hatching three days before the main egg-laying. On the 3rd day, 1/3 of the females (in gray eggs) died during the whitening phase. 1/3 of the gray eggs were males and could not be distinguished for their heterozygosis on ℓ_1 lethal (Figure 3). Although the viability of the yellow eggs was slightly lower, their viability was almost normal. All yellow eggs were homozygous for the egg color of the w_2 gene and heterozygous for ℓ_1 lethal. All egg-layings obtained from analyzer crossing were divided into two groups based on the above characteristics: keep ℓ_1 and ℓ_2 in the genotype. These lethal could be distinguished only on the 3rd day.

Eggs of genotypes ℓ_1 and ℓ_2 were grouped into four groups according to their origin from gray and yellow eggs:

- a gray egg hatched worms with ℓ_1 lethal;
- a gray egg hatched worms with ℓ_2 lethal;
- a yellow egg hatched male worms with ℓ_1 lethal;
- a yellow egg hatched male worms with ℓ_2 lethal.

We only needed males from egg-laying obtained from the analyzer crossing. They served as both analytical and improving factors.
The main egg-layings were grouped into four groups considering the gray and yellow color of eggs with ℓ_1 and ℓ_2 lethal genotypes and based on analyzer egg-laying analysis:

- a gray egg hatched worms with ℓ_1 lethal;
- a gray egg hatched worms with ℓ_2 lethal;
- a yellow egg hatched male worms with ℓ_1 lethal;
- a yellow egg hatched male worms with ℓ_2 lethal.
-

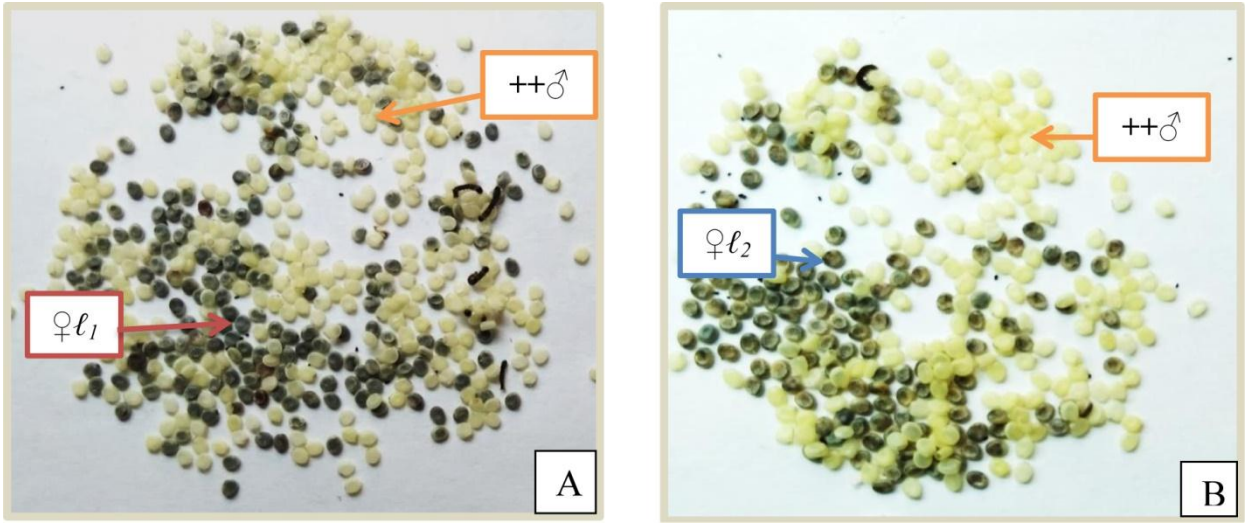

Figure 3. Dying of embryos in the egg under the influence of $\ell_1$and ℓ_2 lethal genes**:**
A- gray eggs died under the influence of ℓ_1 lethal (♂♂ + ♀♀)
B- gray eggs died under the influence of ℓ_2 lethal (♂♂ + ♀♀)

For the improvement, only males are needed from egg-laying obtained from analyzer crossing. Females will no longer be needed. Males in the main egg laying can be used two times. The males of egg-laying from analyzer crossing were numbered each and crossed once with the main egg-laying females and once with the females of sex-limited line in the following directions:

♀[♀(C-8ngl×L-28)×♂(L-28×C-8ngl)] (ℓ_1 gray)
♂[♀(tr24w$_2$w$_2$ x L-67)×♂(L-28×C-8ngl)] (ℓ_2 light yellow)
♀[♀(tr24w$_2$w$_2$ x L-67)×♂(L-28×C-8ngl)] (ℓ_1 gray)
♀[♀(C-8ngl×L-28)×♂(L-28×C-8ngl)] (ℓ_2 gray)
♂[♀(tr24w$_2$w$_2$ x L-67)×♂(L-28×C-8ngl)] (ℓ_1 light yellow)
♀[♀(tr24w$_2$w$_2$ x L-67)×♂(L-28×C-8ngl)] (ℓ_2 gray)

Obtaining main egg-laying F_3 (direction I)

P $W^{+w_2+l_1}Z^{+l_1}\ w_2/+w_2$ × $Z^{l_1}Z^{+l_2}\ w_2/+w_2$
♀[♀(C-8ngl×L-28)× ♂[♀(tr24w$_2$w$_2$ x L-67) ×
♂(L-28×C-8ngl)] (ℓ_2 gray) ♂[L-28×C-8ngl)] (ℓ_1 light yellow)
$W^{+w_2+l_1}Z^{l_1}\ w_2/+w_2$ $W^{+w_2+l_1}Z^{l_1}\ w_2/w_2$ $W^{+w_2+l_1}Z^{+l_2}\ w_2/+w_2$ $W^{+w_2+l_1}Z^{+l_2}\ w_2/w_2$

1♀ gray 1♀ gray 1♀ gray 1♀ gray

F$_3$

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

1♂ light yellow 1♂ gray 1♂ gray 1♂ light yellow

Chart 8. Obtaining F$_3$ generation in the first direction of the improvement

Obtaining main egg-laying F$_3$ (direction II)

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

♀[♀(C-8ngl×L-28)× ♂(L-28×C-8ngl)] (ℓ_1 *gray*) ♂[♀(tr24w$_2$w$_2$ x L-67) × ♂(L-28×C-8ngl)] (ℓ_2 *light yellow*)

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

~~1♀ gray , will die~~ 1♀ gray , will die 1♀ gray 1♀ gray

F$_3$

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

1♂ light yellow 1♂ gray 1♂ light yellow 1♂ gray

Chart 9. Obtaining F$_3$ generation in the second direction of the improvement

Analyzer crossing in the third stage of improvement

In the third stage of improvement, an analyzer crossing was performed using translocation lines sex-limited in the egg stage to obtain information on which of the double lethal was present in the derived F$_3$ generation genotype. Egg-layings obtained from analyzer crossing were placed in hatching three days before the main egg-laying. On day three, in gray eggs of a direction I of the analyzer crossing, 1/3 of the females died during the whitening phase. While 1/3 of the gray eggs were males and could not be distinguished for their heterozygosity on ℓ_1 lethal. Although the viability of the yellow eggs was slightly lower, their viability was almost normal. All of the yellow eggs were homozygous for the egg color w_2 gene and heterozygous for ℓ_1 lethal.

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

♀[♀(tr24w$_2$w$_2$ × L-67) × ♂(L-28×C-8ngl)] ♂[♀(tr24w$_2$w$_2$ × L-67) × ♂(L-28×C-8ngl)] (ℓ_1 light yellow)

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

~~1♀gray , will die~~ 1♀ gray , will die 1♀ gray 1♀ gray

BC$_2$F$_3$ $Z^{+l_1+l_2}Z^{+l_2w_2/w_2}$ $Z^{+l_1+l_2}Z^{+l_2\,w_2/+w_2}$ $Z^{+l_1+l_2}Z^{l_1\,w_2/w_2}$ $Z^{+l_1+l_2}Z^{l_1\,w_2/+w_2}$

1♂ light yellow 1♂gray 1♂ light yellow 1♂ gray

Chart 10. F$_3$ generation of analyzer crossing

On day three of analyzer crossing in the gray eggs of direction II, 1/3 of the females died at much earlier stages of embryonic development. 1/3 of the gray eggs were males and were indistinguishable for their heterozygosity on ℓ_2 lethal.

All of the yellow eggs were homozygous for the egg color of the ω_2 gene and heterozygous for ℓ_2 lethal.

Obtaining F_3 analytical egg-laying (direction II)

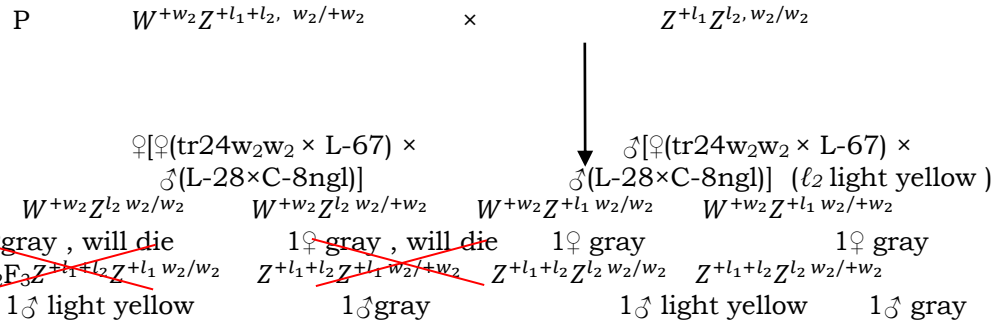

Chart 11. F_3 generation of analyzer crossing

Obtaining F_4 generation in the second direction at the fourth stage of improvement

At this stage, the egg-layings obtained from the analyzer crossing were used only to detect the presence of lethal and then destroyed. After separating the two main directions of improvement by lethal, taking into account the phenotypic homogeneity of the female organisms in the direction I, all were cared for, bred, and crossed with white-eyed male moths hatched from the light yellow egg in direction II. In direction II of the main crossing of improvement, 1/4 part of the male organisms, or the ½ proportion of male organisms hatched from the light yellow egg, were found to have double lethal and were involved in the crossbreeding process as much as possible.

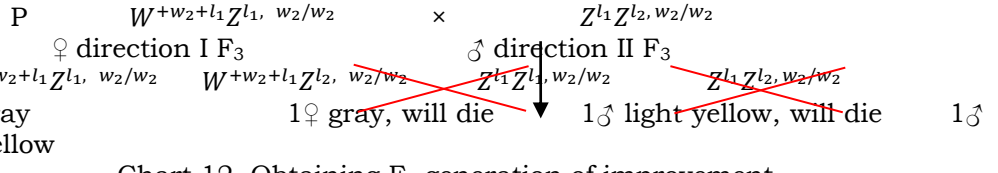

Chart 12. Obtaining F_4 generation of improvement

Analyzer crossing at the fourth stage of improvement

Analyzer crossing was performed to determine whether white-eyed male moths hatched from light yellow eggs in direction II had double lethal or ℓ_1 lethal. This led to a process of crossbreeding in two different directions.

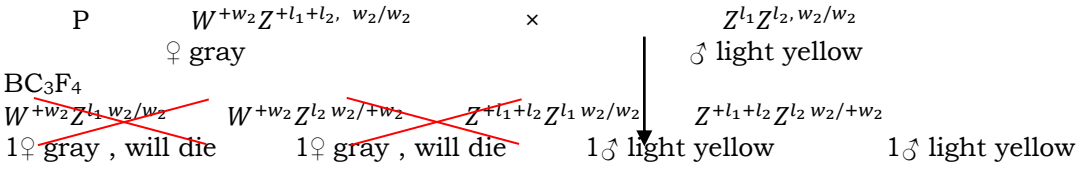

Chart 13.F_4 generation of analyzer crossing

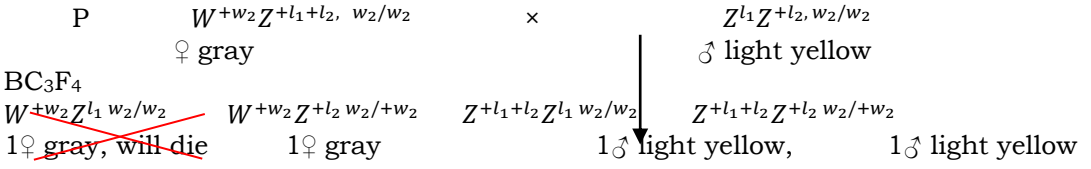

Chart 14. F_4 generation of analyzer crossing

The achievement of this new genetic method is that, while maintaining the balance of genes in the genotype of existing lethal retaining breeds, it is possible to create a silk-giving breed that meets the requirements of sericulture. The point is that the unique C-8ngl breed properties that are improving are not at the required level of sericulture in the 21st century. More precisely, 3A type of silk fiber can not be removed, because of the low technological characteristics of the silk fiber of that breed. Creating such a breed, first of all, takes a lot of time and resources. Second, you must complete the sequence of genetic selection processes and spend high cost.

Using the new genetic method arises an opportunity to transfer the characteristics of the improving breed (line) to the genotype of the breed (line) balanced by embryonic lethal by backcross. The noteworthy aspect is that embryonic lethal genes from the first backcross generation to the fourth backcross generation must be kept under strict control, to ensure that the latter generation of the sketch is given. As a result, in the fourth generation, embryonic lethal genes come into balance. The effect of this new genetic method is that translocations are not obtained under the influence of γ-rays and induction of embryonic ℓ_1 and ℓ_2 lethal genes are not required. As a result, the scope of genetic-selection activities which are carried out will be reduced, the productivity of work will increase, labor and money will have economized 2-3 times.

Serious attention will be paid to this, studies on the sex of mulberry silkworms and with lethal genes should be conducted in a special laboratory, far from ordinary breeds, either by qualified genetic scientists or under their supervision. However, the improvement method which we offer can be effectively utilized in mulberry silkworm breeding farms with sufficient resources. The condition is that should keep the egg color and sex ratio under control, as well as the balance of embryonic lethal genes in each generation. If there are the desired breed and lines the offered improvement input can be used in the desired foreign state silk scientific organizations and can obtain an ident result.

This created C-8ngl breed improvement method is being implemented for the first time using the $+\omega_2$ gene marker, which determines the color of the eggs contained in the 10th autosomal chromosome, precisely as an improvement from two highly productive breeds. Before this, the Japanese scientist A.Ohnuma used the *pM* gene, located on the 2nd autosomal chromosome, as a marker for the carpal appearance of the skin cover of the worm, as a donor from the Chinese breed, which has a high yield. [Ohnuma A., 2005]

According to this method, the morphological marker- the carpal appearance of the skin cover of the worm-attached to the genus appear at the 3rd - 4th age of the worms, and more time and work will be spent on determining the genus. Marking sex by egg color in the genetic method proposed by us allows us to identify the lethal genes at the initial stage of embryonic development and is not subject to large amounts of work and cost. Currently, the development of the silk industry puts high demands on the technological performance of mulberry silkworm silk fiber. As it turned out, the most qualitative, thin silk is obtained from the male-sex cocoon. To create male-sex industrial hybrids, balanced breeds with two lethal genes are considered essential. Therefore, when obtaining high-quality silk, there is always a need and demand for a new breed and hybrids genotype. It should also be noted that since the creation of the unique C-8 ngl breed genotype, no genes have been introduced to this genotype. In the future, the task is set to bring the silk of this breed to the level of type 3A - 5A of the highest quality. On this path, of course, there is a need for effective use of improvement methods. In addition to Uzbekistan, this method can be used by scientists from leading countries.

Within the framework of improving the balanced C-8ngl breed on embryonic lethal genes, theoretically expected results were achieved in practically every generation. It is convenient and easy to use this method – it is carried out with the involvement of an optional breed (line), in a short period the lethal genes are balanced and high technological properties are collected in a single genotype. Given the above, it can be recommended to genetic, selective research on the creation of new breeds and hybrids of the silkworm.

Conclusion

Based on the results of the experiment it can be concluded as follows:

- 3-year results showed that the reproductive signs of the mulberry silkworm had no adverse effects on embryonic Z-lethal genes, the effect of which was not observed.
- As an analogue to the C-8ngl breed which is obtained in this new system with high viability indicators and satisfactory silk fiber quality make it possible to recommend it to seed-breeding enterprises.
- It is necessary to pay serious attention to the complex genetic schemes used in the improvement of the C-8ngl breed, in which the genus is celebrated, balanced by double-letals, and at each stage to ensure that the balance of the syncretic letals is strictly controlled in order
- Based on the genetic method of the new proposed improvement, it has been proven that some families of the balanced embryonic ℓ_1 and ℓ_2 letal genes in

the genotype of the improved pure breed will be separated in the fourth generation.

All of the above genetic schemes describe the monitoring of ℓ_1 and ℓ_2 lethal genes in the process of introducing thin and high-quality silk feature through a simple system (without any genetic changes in sex-limiting or controlling) into the C-8 ngl breed population, which is sex-limited and balanced on ℓ_1 and ℓ_2 lethal. In addition, having obtained the F_4 generation, and while maintaining the genetic structure of the improved C-8 ngl breed, its economic traits have manifested new characteristics. In the population of this genotype, it is possible to create an almost new breed by conducting analytical selection-breeding work from generation to generation.

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