International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 4 Issue 5, July-August 2020 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Development of a Method for Detecting Embryonic Sex-Linked Lethals of the Silkworm *Bombyx Moril*

Svetlana Semyonovna Lejenko[1], Baxtiyar Ubaydullayevich Nasirillayev[1], Murodxoja Alisherogli Abdiqodirov[2]

[1]Research Institute of Sericulture, Tashkent, Uzbekistan
[2]National University of Uzbekistan, Tashkent, Uzbekistan

ABSTRACT
The article presents the results of research on the development of a method for detecting sex-linked embryonic lethal in the silkworm. In experiments on obtaining embryonic sex-linked letals, we used breeds C-5 and C-12 that differ in some biological properties. Sex-linked recessive letalswere obtained by the radiation method. So was chosen as the radiation source. The impact was made with gamma rays with a power of 24 R / s at the gamma-installation of the Institute of Nuclear Physics of the Academy of Sciences of the Republic of Uzbekistan. 400 clutches were taken, obtained by crossing normal females with males descended from fathers irradiated with a dose of 5 kR and crossed with non-irradiated females. The revival in these clutches ranges from 10% to 100%. With a sample of less than 250, indicators of the content of males in lethal and non-lethal families begin to transgress. Obviously, in these classes, families with and without lethal should be mixed, but it is not possible to separate them without additional genetic analysis. All indicators of three families 175, 438, 187, with the lowest percentages of males (63.8%; 61.7%; 63.0%), were studied in detail. The percentage of males in these families, although approaching lethal, is lower than in other families. Guided by the comparative indicators of the percentage of revival of eggs in dark and light semi-clutches, therefore, all families 175 and 438 are classified as lethal.

KEYWORDS: *silkworm, lethal gene, grena, gamma rays, caterpillar*

How to cite this paper: Svetlana Semyonovna Lejenko | Baxtiyar Ubaydullayevich Nasirillayev | Murodxoja Alisherogli Abdiqodirov "Development of a Method for Detecting Embryonic Sex-Linked Lethals of the Silkworm Bombyx Moril" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-4 | Issue-5, August 2020, pp.1532-1538, URL: www.ijtsrd.com/papers/ijtsrd33217.pdf

IJTSRD33217

INTRODUCTION

It is known that in many animal species the productivity of individuals of different sex is different, and in some species only one sex produces an economically valuable product (milk, eggs, etc.). The possibility of obtaining a preferred sex in livestock, poultry, fish farming and other industries would allow using the still untapped reserves of productivity without resorting to increasing the number of livestock.

The problem of artificial regulation of the sex of animals has been and remains one of the most urgent in the genetics and selection of many farm animals. The silkworm turned out to be the first observatory on which the problem of sex regulation was solved. The sex ratio in many animal species is associated with age. There are three types of sex ratio in the time of ontogenetic development:
primary - is determined during the fertilization of eggs;
secondary - determined by birth or hatching;
tertiary - is established at a specific age.

A chance meeting of the reproductive gametes provides a primary sex ratio of 50% ♀: 50% ♂in the norm. Deviations from the normal sex ratio occur due to the different viability of gametes with different sex chromosomes. The different survival rates of embryos are the reason for the change in the secondary sex ratio. The tertiary sex ratio covers the differences in the survival of individuals of one sex or another in ontogenesis (Hutt, 1969).

THE MAIN FINDINGS AND RESULTS

The creation and breeding of sex-labeled silkworm breeds on a large scale opened up the possibility of studying another equally important issue in biology - sexual dimorphism.

It has been established that males and females in the silkworm differ in development. In monovoltine breeds, it was found that females emerge from the grène earlier than males, earlier go to perm and earlier emerge from butterfly cocoons. The author explains this phenomenon by the presence of a gene for late maturity in most mono-voltine breeds (Seiyro, 1971).

N. I. Kovalevskaya and Y. B. Filippovich (1971) managed to establish that sexual dimorphism in the proteins of the hemolymph of the silkworm, which is detected from the middle of the fifth instar, deepens towards the end of the cocoon curling and is clearly represented in the first half of the pupal phase.

In the 70s, V. A. Strunnikov proposed a more effective method for obtaining only male silkworm offspring on an industrial scale, based on the breeding of a special genetic line, in which two fragments of Z and 10 - chromosomes with dominant alleles should be translocated on the W - chromosome lethal and the egg pigmentation marker gene + w2, and in the opposite Z - chromosomes in the karyotype of males, two non-allelic recessive flies are localized (Strunnikov, 1969).

The development of such an original method is possible only in the presence of hundreds of embryonic sex-linked recessive lethal localized in certain regions of the Z-chromosome. Often, spontaneous and induced changes in genes, as well as chromosome regions, disrupt the course of embryonic or postembryonic development so much that the body dies. Such mutant changes in the material bases of hereditary information are called lethal. Among lethal, a special category of so-called sex-linked lethal stands out. In animals with male heterogamy, half of the male offspring perishes from lethal, and on the contrary, half of the female offspring perishes with the female. They flew, including embryonic sex-linked ones, causing suffering to humanity and causing great harm to agriculture.

Sex-linked flying with floor flying in Drosophila played a huge role in genetics. Their use was revealed as the most convenient mutations, on which extremely important interesting research on the problems of spontaneous and artificial mutational processes was carried out. For the first time, G. Meller (1927) used recessive sex-linked flying in the development of methods for accounting for emerging mutations. He proposed an original ClB method for accounting for mutations in the sex chromosome of Drosophila. Later, to analyze the frequency of lethal mutations in the X chromosome of males, another method was developed - Müller 5 or M-5 (1962 [6]. Its advantage is that both X chromosomes of a female contain two inversions not associated with lethal In addition, both chromosomes of the female are marked with three genes: *Sc⁸, B, Wᵃ*. Males in this line are also viable. The method ClB and M-5 is widely used in various studies of genetic, cytogenetic, biochemical directions.

As for the silkworm, although this extremely useful agricultural object ranks second in terms of genetic study after the laboratory object of Drosophila, however, the works related to the production and study of lethal mutations in the literature are insufficiently covered. Embryonic *l-mse* (segmentmonster) and *l-mse* (secondsegmentmonster) severely disrupt the development of the embryo (Umeya1949a; 1950b). Two embryonic lethal mutations were noted in the works of T. Hirobe: *l-sp-lethalspindleegg* (lethal spindle) (Hirobe, 1952) and l-n (Noncolouredegglethal) - unstained lethal gene (Hirobeetal., 1952).

Y. Tanaka described a lethal (Albino) - albino or unpigmented caterpillar skin (Tanaka, 1952).

Yellow lethal - *l-y* (yllowlethal) or *l-lem^l* (lemlethal) was found in one of the lines in the Japanese collection. At the first instar, the caterpillars of this line are all normal, but in some colonies several individuals turn distinctly yellow immediately after the first molt and die within a few days due to their inability to eat. According to K. Suzuki (1948), this trait should be inherited as a simple recessive (*lim*).

B.L. Astaurov (1933a, 1934b, 1934c) was the first to study the induction of sex-linked letals in the silkworm in order to experimentally study the mutation process in this object. By the time of the beginning of his work, neither *BombyxmoriL*nor any other butterfly had ever described a single case of getting sex-linked flying. And the calculation on the possibility of the occurrence of recessive letals in the Z-chromosome, which would kill the females of the WZ structure in the silkworm, was based only on a very probable assumption, by analogy with Drosophila. It was impossible to apply a method as perfect as the ClB method for Drosophila on a silkworm, since there was not a single visible mutation linked to sex in the domestic genetic collection. Therefore, B.L. Astaurov singled out only a few families in which the content of Z-letals was supposed. However, due to the impossibility of early sex recognition (at the egg stage), the nature of the allegedly induced mutations remained unknown.

A group of scientists (Arinkumaret.al., 2013) from the Center for Genetics and Genomics of the Silkworm (India, Hyderabad) studied in detail the content of various genes in the W and Z chromosomes of the silkworm and came to the conclusion that the Z chromosome contains significantly more genes by compared to autosomam. The researchers speculate that sexual antagonism and lack of dose compensation may have led to the accumulation of many male specific genes on the Z chromosome. In addition, as these researchers note, the accumulation of genes beneficial to males on the Z chromosome occurred primarily due to translocations or tandem duplications.

Ukrainian scientists (Paliieal., 1996) studied the influence of ooplasm, as well as some stress factors of early ontogenesis (temperature, hypoxia, etc.) on the recombination frequency and fertility of the silkworm. On hybrids of two breeds with the line-analyzer *re*, *pe* the anti-recombinogenic effect of high-temperature shock effects in the sub-embryonic period of the silkworm was first established. On the part of these researchers, the peculiarities of the chromatin of diapausing silkworm embryos in normal and parthenogenetic development were also investigated. The authors found a direct correlation between the number of interphase chromatin grains and the number of chromosomes in the nucleus, after which they studied the polyploidization of cells in the embryo at the stage of diapause. For the first time, on crushed preparations, primary germ cells (or germ cells) of a diapausing embryo were identified, characterized by less compact chromatin, especially in the zygotic variant of development (Klimenkoi et al., 2011a; 2012b; Klimenko, 2001).

Currently, research work on the regulation and marking of sex in the silkworm has been intensively developed at the molecular and chromosomal level, to which the results of the work of Japanese, Indian and Chinese scientists can be attributed (Chen, 2002).

In the work of Japanese scientists (Khirokyetal., 2010), a key stage for sex determination in the silkworm was studied. Thus, it was found that the gene responsible for sex is expressed over a limited period, i.e. during the early embryonic stage. To study the key stages of sex determination in this insect, the researchers focused on the expression of the *Bmdsx* gene (*BombyxmoriL.* sex switch gene) and *BmIMP* (a gene specifically expressed in males, involved in the splicing of the *Bmdsx* gene in males).

As you know, in the silk industry, males are more superior to females in that they show positive economic characteristics, for example, low food consumption and a higher yield of silk thread. In their work on the creation of a transgenic genetic control system for sex determination in the silkworm (Anjiangetal., 2010), a construct was developed where a positive feedback loop regulated sex - a specific alternative splicing, which led to a high level of expression of a tetracycline - repressed trans-activator only in females. Transgenic insects showed female lethality during the embryonic and early larval stages of development, resulting in male silkworms.

From the above review, it follows that there have been no direct studies on farm animals focused on the targeted production of lethal mutations, and even more so on their use in artificial sex regulation. Literary sources contain only information about the harmful effect of lethal mutations. Discovered by researchers flying determine various anomalies in humans and animals, inevitably leading to death. The identified lethal mutations are mostly spontaneous. Among them, sex-linked flew was not so frequent.

Thanks to the research carried out, the silkworm turned out to be the first object among farm animals, which solved the scientific and methodological issues of obtaining and balancing embryonic Z- lethal with their subsequent use in sex regulation on a large industrial scale.

The aim of the study is to obtain a large number of embryonic recessive Z- lethal.

Materials and methods

The research was carried out at the Uzbek Research Institute of Sericulture and at the Department of Genetics of the National University of Uzbekistan. In the experiments, we used the breeds and lines of the silkworm *BombyxmoriL*, bred in the Uzbekistan Research Institute of Sericulture.

In experiments on obtaining embryonic sex-linked lethal, we used breeds C-5 and C-12, differing in some biological properties. The choice of such breeds was determined by the fact that they are genetically marked by sex at the egg stage. Each clutch of these breeds was split according to the color of the eggs into two colors - dark and light in a 1: 1 ratio. Dark-eyed female caterpillars hatch from dark-colored grenades, male white-eyed caterpillars hatch from non-pigmented (light) eggs.

Already on the third day after oviposition, when the serous membrane is pigmented, the sex can be freely determined. Ultra-early sex diagnostics greatly simplifies the method of detecting embryonic lethalities attached to the floor. There is no need for cumbersome, labor-intensive feeding F_b. Experiments on sex-marked breeds at the egg stage make it possible to analyze a significant number of F_b (Z-chromosomes exposed to radiation) and to obtain guaranteed embryonic sex-linked lethal.

Sex-linked recessive flying was obtained by the radiation method. So was chosen as the radiation source. The impact was made with gamma rays with a power of 24 R / s at the gamma-installation of the Institute of Nuclear Physics of the Academy of Sciences of the Republic of Uzbekistan. The exposure was carried out directly on mature sperm and eggs that did not undergo reduction divisions (at the pupal stage). Letters were obtained according to the following scheme (Fig. 1).

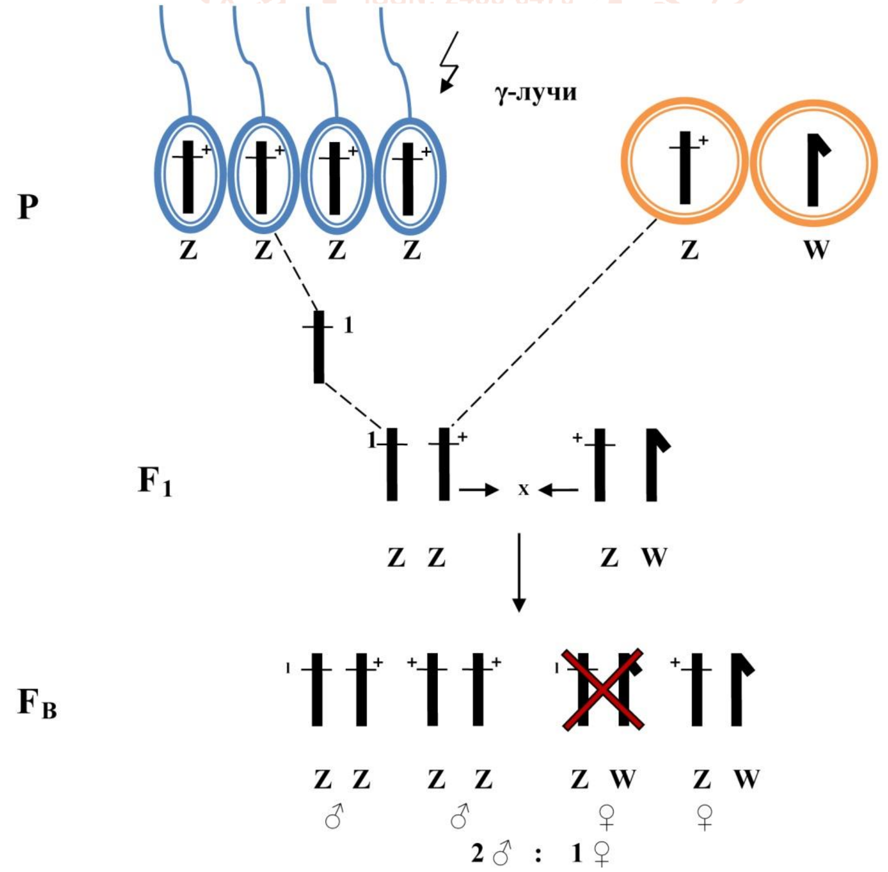

Figure 1. Scheme of obtaining sex-linked lethal in a silkworm

White-eyed males at the pupal stage were irradiated with doses of 5-10 kR. Male butterflies emerging from cocoons were crossed with non-irradiated females. In the male offspring from them, induced lethality is preserved in one of the Z chromosomes; therefore, the first generation green hair (F1) was divided by sex (by the color of the eggs) and only male caterpillars hatched from light eggs were fed. The resulting male butterflies were crossed again with non-irradiated females of sex-marked breeds. Each obtained clutch was divided by color into females and males. Two parchment bags of the same masonry with different genders were fastened and incubated in place, i.e. under the same conditions.

After the end of hatching of caterpillars from eggs, both half-clutches were compared in terms of the percentage of eggs alive. If the revival of the dark grène is approximately 50% of the revival of the light one, taken as 100%, then in this clutch one can suspect the presence of an embryonic sex-linked recessive fly, because half females will probably die due to the fly transmitted to her from her father, localized on the Z-chromosome.

The presence of sex-linked lethal in suspicious clutches was further checked by the hybrid logical method, i.e. male caterpillars (light gren) from suspicious clutches were fed by families and then analyzed by offspring. Half of the clutches obtained from males of a lethal colony should be lethal, the other half normal (Fig. 1).

Results

Detection of embryonic sex-linked lethal

The frequency of lethality depends on the dose of the mutagen and the mutability of the germ cells. The success of detecting embryonic sex-linked lethals is mainly determined by the size of the sample (family) of caterpillars hatched from eggs, which in turn depends on the fecundity of the female, the percentage of F_b egg revival obtained from irradiated parents, and the relative revival of Grena with male and female embryos. The higher the percentage of revitalization of the grena, the more it is used for the analysis of hatched male and female caterpillars with the same number of eggs in a clutch.

In our research, we decided to gradually study the individual most important factors for the success of the method. First of all, we established an extremely low sample (the number of individuals in a family), which ensures an accurate differentiation of families into carriers and non-carriers (lethal and non-lethal). Knowing the average number of eggs in a clutch, having established the percentage of egg revival under different irradiation conditions, one can thus establish the required sample size, which will make it possible to determine the optimal irradiation conditions without resorting to extremely complex experiments that reveal the irradiation conditions directly from the number of lethal obtained.

In normal clutches, the sex ratio among hatched caterpillars corresponds on average to 50% ♀: 50% ♂, while in lethal clutches it should shift to 33.3% ♀: 66.7% ♂. However, this average sex ratio has statically regular deviations in individual clutches. These deviations depend on the sample size: the larger the number of eggs in the analyzed clutch sample, the smaller the deviation from the theoretically expected sex ratio.

Table 1 shows the allowable biological statistics (D. Snedekor, 1961) fluctuations in the percentage of male caterpillars in normal and lethal clutches with different sample sizes.

Table 1 Fluctuations in the percentage of males in clutches of normal and lethal colonies depending on the sample size

Samplesize, n	Permissible fluctuations in the percentage of males in clutches	
	normal ($\bar{X}$=50%)	lethal ($\bar{X}$=66,7%)
50	31-69	47-85
100	37-63	54-79
150	38,7-61,3	55,6-77,4
200	40,4-59,6	57,3-75,8
225	41,3-58,7	58,75
250	42-58	59-74
300	42,5-57,6	59,4-73,6
350	42,8-57,2	59,8-73,2
400	43,2-56,8	60,2-72,8
500	44-56	61-72

According to Table 1, it should be concluded that when analyzing for lethality, in order to obtain accurate results, samples with a number of revived caterpillars of at least 225-300 pieces are needed, then there will be no overlap between the highest percentages of males in normal clutches and the lowest percentages in lethal ones. Based on these statistical indicators, we calculated the extremely low percentage of egg revival, which is permissible for identifying lethal.

If the average number of eggs in a clutch is taken as 500, then in lethal clutches will hatch:
at 100% revival 250 ♂+ 125 ♀ = 375 tracks;
80% animation 200 ♂+ 100 ♀ = 300 caterpillars;
60% animation 150 ♂+ 75 ♀ = 225 caterpillars;
50% animation 125 ♂+ 63 ♀ = 188 caterpillars;

whereas in normal clutches with a revival equal to
100% hatch 500 caterpillars;
80% will hatch 400 caterpillars;
60% hatch 300 caterpillars;
50% will hatch 250 caterpillars.

According to the above calculation, for reliable isolation of non-lethal clutches, the extremely low revival can be 50%, including if the analyzed clutch has at least 500 eggs. However, in lethal clutches, 25% fewer eggs are always revived due to the death of half of the females, and thus the number of analyzed hatched caterpillars, with potentially identical other possibilities, is also reduced by 25. In this regard, only with revival above 60% can one relatively accurately determine the presence of analyzed families.

In the lethal lines obtained by us and sufficiently stabilized with good egg revival and normal fertility, the division of families into lethal and non-lethal is carried out quite easily and accurately.

Figure 2 shows families by the percentage of hatched males in the lethal lines we obtained that have passed several generations of reproduction.

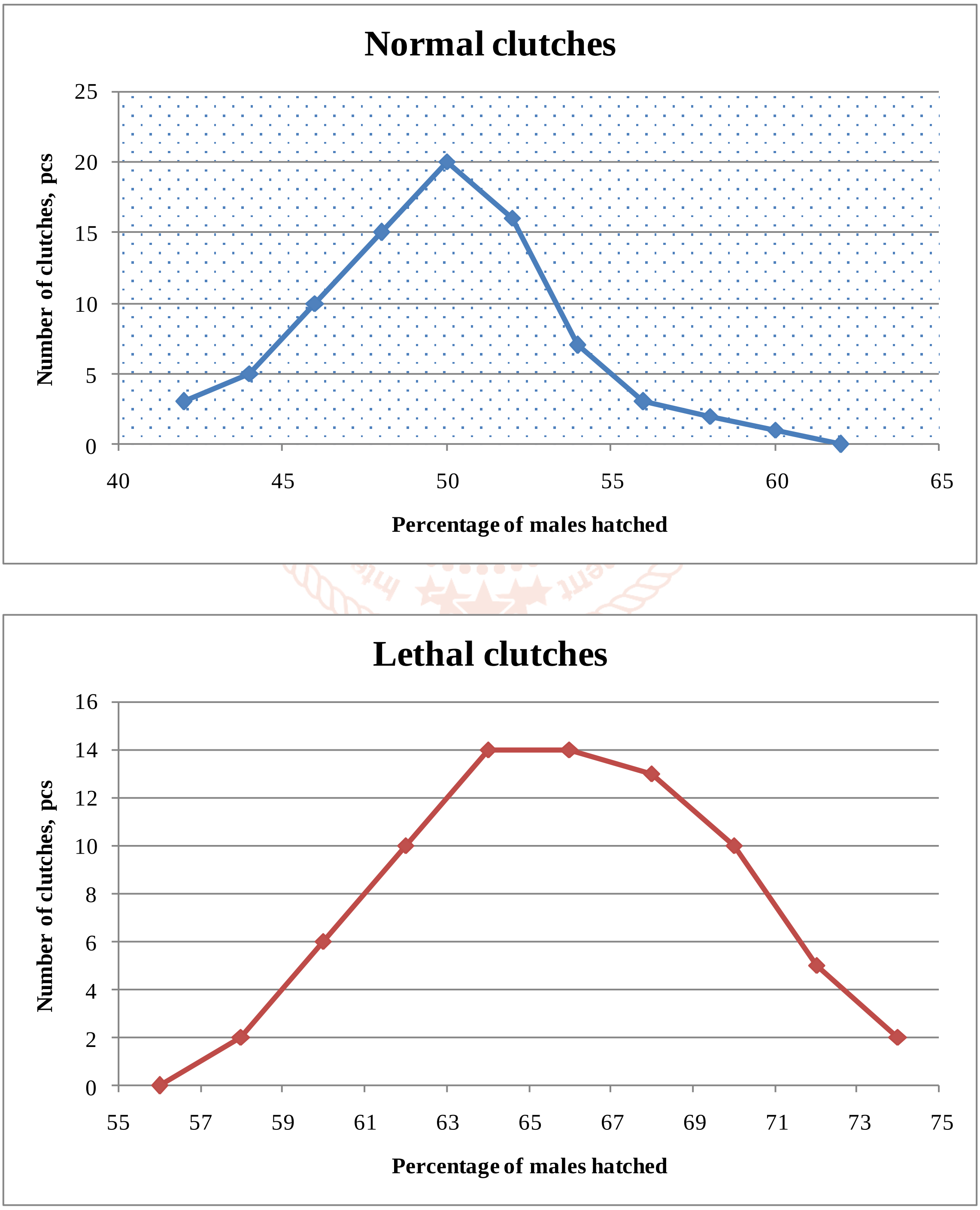

Figure 2 Distribution of families of lethal lines by percentage of males in a sample of 220 hatched caterpillars.

The presented data very clearly demonstrate the division of families into two groups - non-lethal and lethal. Practically, with a sample of 250 and higher dates; there is no transgression between the variation series of these two groups.

In the event that lethal colonies are re-identified in Fb, then this operation is significantly complicated due to the reduced revival of eggs in this generation, and this, as already noted, will lead to a decrease in the sample size of revived caterpillars, the sex ratio of which is a criterion for the presence family.

The influence on the accuracy of the identification of lethal families and other factors not foreseen a priori is not excluded. In this regard, we decided to check the accuracy of the isolation of lethal families using factual material. For this purpose, 400 clutches were taken, obtained as a result of crossing of normal females with males descended from fathers irradiated with a dose of 5 kR and crossed with non-irradiated females. The revival in these clutches ranges from 10% to 100%.

The resulting clutches were distributed according to the percentage of males contained in them. On the variation series, the boundaries of statically permissible fluctuations in the percentage of males are plotted for the given sample sizes. With a sample of less than 250, indicators of the content of males in lethal and non-lethal families begin to transgress. Obviously, in these classes, families with and without lethal should be mixed, but it is not possible to separate them without additional genetic analysis. There were 12 families (3.0%) in the transgressive zone. Of these, the lethal nature of nine families is beyond doubt, while three families located near the border may belong to either group, despite the fact that the percentage of males in them allows them to be ranked as lethal families.

A detailed description of these 12 families is presented in Table 2.

Table 2 Characteristics of clutches suspected of flying

Clutch numbers	DarkGrena (♀♀)			LightGrena (♂♂)			Totalhatchedcaterpillars	Caterpillarhatchedpercentage
	numberofeggs, pcs	numberofrevivedcaterpillars	livelinessofcaterpillars,%	numberofeggs, pcs	numberofrevivedcaterpillars	livelinessofcaterpillars,%		
157	106	17	16,0	135	71	52,5	88	80,6
603	117	39	33,3	125	97	77,5	136	17,3
487	125	41	32,8	138	94	68,1	135	69,6
278	157	52	33,1	150	97	64,6	149	65,1
51	123	48	39,0	121	103	85,1	151	68,2
96	142	53	37,3	134	105	78,3	158	66,4
118	96	40	41,7	110	97	88,1	137	70,7
512	158	72	45,5	156	143	91,7	215	66,5
261	98	41	41,9	109	94	86,5	135	72,7
175	111	43	38,7	121	76	62,7	119	63,8
438	226	75	33,2	210	121	57,7	196	61,7
187	135	54	40,0	163	92	56,4	146	63,0

The indicators in Table 2 clearly confirm the lethal nature of the first nine families. We tried to study in more detail all indicators of three families 175, 438, 187, which have the lowest percentages of males (63.8%; 61.7%; 63.0%). The percentage of males in these families, although approaching lethal, is much lower than in other families. Guided by the comparative indicators of the percentage of revival of eggs in dark and light semi-clutches, therefore, families 175 and 438 would still be classified as lethal.

As for the 187 family, the percentage of the revival of the dark ones in it is slightly less than the revival of the light grena, however, the preponderance in the number of light eggs compared to their number in the dark semi-clutch is significant.

It is these two factors - a slightly larger sample of eggs in a light semi-clutch, as well as their somewhat better revival in total, lead to a significant increase in the percentage of hatched males. If we make a miscalculation of the last indicator with an equal number of eggs in semi-clutches of dark and white eggs, then the percentage of males among hatched caterpillars will approach the norm (58.6%) and now this clutch can be unmistakably classified as normal.

CONCLUSION

Thus, the theoretical approaches to solving the problem of revealing letals and the actual experimental data perfectly coincide, and due to this it became possible to propose simpler ways to develop conditions for the induction of letals and their revealing.

From all that has been said, a fairly clear plan for the study of acceptable irradiation conditions emerges. Naturally, the higher the radiation dose, the more Z-letals arise. However, the increase in doses cannot be unlimited. Their limiting or optimal value should be determined by the viability in F1, and the viability of eggs in Fb should be especially decisive when determining the percentage of hatched males in order to detect flew. As the theoretical calculations and direct studies described above have shown, it must be at least 50% for non-lethal clutches and at least 60% for lethal clutches if there are at least 500 eggs in them.

In this case, practically all the arising flights are caught. However, even with a sample of 100 to 250 dates, a significant number of lethal families can still be captured.

But with a sample from 50 to 100, more than half of all lethal families should be lost, and with samples below 50, it is practically impossible to identify lethal families.

REFERENCES

[1] Astaurov B. L. (1933) An artificial mutation in the silkworm (*Bombyxmori L.*). Message 1. Experience in obtaining floor-coupled letals by the action of radium rays. // Biological journal. – Tashkent. 2. - No. 2-3. - pp. 116-131.

[2] Astaurov B. L. (1934) Artificial mutations in the silkworm (*Bombyxmori L.*). Message 2. Further data on the appearance of floor-linked letals under the action of radium γ-rays. // Biological journal. – Tashkent. 3. - pp. 27-38.

[3] Astaurov B. L. (1935) Artificial mutations in the silkworm (*Bombyxmori L.*). Message 3. Experience of obtaining mutations by the action of X-rays. // Biological journal. – Tashkent. 4. - No. 1. - pp. 39-80.

[4] Klimenko V. V., Hoayuan L. (2012) Chromatin in the diapause of the duckwormBombyxmori L.: thermal partenogenesis normal development. //Cytology.– Tashkent. 54. - No. 3. - pp. 218-229.

[5] Klimenko V. V., Hoayuan L., Prokhorova E. A., Tigunkova A.E. (2011) Experimental silkworm triploids and the origin of natural polyploidy in bisexual animals. // Factors of experimental evolution of organisms. - pp. 65-68.

[6] Kovalevskaya N. I., Filippovich Y. B. (1971) Sexual dimorphism in the content of soluble proteins in the tissues and organs of the silkworm (*BombyxmoriL.*), Studied by polyacrylamide gel electrophoresis. // Silk. –Tashkent. - No. 3. - p. 20.

[7] Moeller G. (1962) Radiation genetics. – Moscow. Ed. Academy of Sciences of the USSR. - p. 368.

[8] Seyiro M. (1971) Comparative development of male and female butterflies. //Silk. – Tashkent. - No. 4. - p. 37.

[9] Strunnikov V. A. (1969) Getting male offspring from the silkworm. // Reports of the USSR Academy of Sciences. - Tashkent. 189. - No. 5. - pp. 1155-1158.

[10] Hutt F. Animal Genetics. – Moscow. 1969. - pp. 363-368.

[11] Anjiang Tan, Guoliang Fu, Li Jin, Qiuhong Guo, Zhiqian Li, Baolong Niu, Zhiqi Meng, Neil I. Morrison, Luke Alphey, Yongping Huang. Transgene-based, female-specific lethality system for genetic sexing of the silkworm, *Bombyxmori. //Japan, 2010.*[http://www.ncbi.nlm.nih.gov/pubmed]

[12] Arinkumar K. P., VitaK., NagaraguJ. (2013)The silk worm Zchromosomeisenrichedintestis-specificgenes. //6thBacsa international conference «Bulding Value Chaines in Sericulture», «Biserica» 2013.Padua Italy.April 7th. - pp. 42-44.

[13] Chen Y. (2002) Conservation status of silkworm genetic resources in China. Expert consultation on promotion of global exchange of sericulture germplasm resources. // Satellite session of XIXth ISC Congress, 21st -25th, September, Bangkok, Thailand. - pp. 77-81.

[14] Hirobe T., Takatsu T.andOhi H. (1952) Onna new linkage of the silkworm between lethal non-hybernated (*l-n*), noglue (*Ng*) and Yellow cocoon (C). // Jap. J. Genet, №27. - p. 231.

[15] HirobeT. (1952) on lethal spindle egg in the silkworm. //J. Seric. Sci. Jap, V. 21. - p. 122.

[16] Khiroky S., FugakuA.,Masataka G. Suzuki. (2010) Identification of the key stages for sex determination in the silkworm, Bombyxmori L.[http://silkwormmori.blogspot.com]

[17] KlimenkoV. V. (2001) Partenogenesis and cloning in the silkworm *BombyxmoriL.*: Problems and prospects. InsectBiotechnol.Sericologi, №70. - pp. 155-165.

[18] Muller H.J. (1927) Artificial transmutation of the gene. Science, 169. - p. 66.

[19] PaliiS. S., Klimenko V. V. (1996) Crossover frequencies in the silkworm *BombyxmoriL.*are under the influence of stresses experienced in early ontogenesis. //Sericologia. France, №3.6. - pp. 363-373.

[20] Suzuki K. (1948, 1950) On the inheritance of yellow silkworm which appears after the first moult. //Genet. №23. - p. 51; №25. - pp. 95-99.

[21] TanakaY. (1952) Silkworm Genetics.Shokabo, Tokyo. - p. 302.

[22] Umeya Y. (1949) Influence of environment on the inheritance of segment monstar. //Zool. Mag. Tokyo. V. 58. -pp. 94-95.

[23] Umeya Y. (1950) The role of cytoplasm in the incidence of heridaey segment monster. //Jbid. V. 59. - pp. 272-277.