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[Review]

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Review on Silkworm (*Bombyx mori*) Sex Control in China

An-Ying Xu*, Mu-Wang Li, Ping-Jiang Sun, Yue-Hua Zhang and Cheng-Xiang Hou

Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang, 212018, P. R. China.

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Normally, silkworms, *Bombyx mori*, generate offspring by sexual activity. As we known, the hybrids of the first generation of the silkworm have higher cocoon production than pure lines. During the sericulture production, many processes are related with sex control. For example, sex sorting in the egg grainages, rearing of only male silkworm to save the mulberry leaf consumption and increase silk output and quality. Therefore it is very interested in understanding the sex control of the silkworm in theory and practice. Chinese sericultural scientists have been being engaged in the researches in the fields of artificifial parthogenesis, dispermic androgenesis, sex-limited varieties, sex linkage balanced lethal strain and high temperature sensitive male stocks for several decades and gained substantial achievement. Some of the achievements have been used in the commercial production. In this review, the authors introduced that the methods for control of the silkworm sex, and regulate the silkworm sex ratio according to different producing aim in the world and especially in China.

Key words: *Bombyx mori*, Sex control, Parthenogenesis, Androgenesis, Sex-limitation

Introduction

Most of the animals are dioecious. They reproduce their offspring by sexual activity. The offspring ratio of female and male is 1 : 1. Silkworm (*Bombyx mori* L.) belongs to the type of *ZW* sex determination, in which females sex chromosomes are *Z* and *W*, and male chromosomes are double Z chromosomes. That means Chromosome W play a key function for female determination.

Comparing with females, male silkworms have many advantages in economical merits. For example, they are robust, rear easily, and consume less mulberry leaves to produce same quantity of silk. Rearing cost can be reduced by 10% if only male silkworms are reared. Meanwhile, male cocoons have higher raw silk rate and can produce qualified silk with some advantageous characters such as finer size and less size deviation. As Huang (1980) reported that the economical benefit could be increased by 10 – 15% if only male silkworm were reared. So, rearing of only male silkworms is to be favored for both sericulture farmers and silk reeling mills. On the other hand, more females are welcomed by the silkworm eggs producers. In this regard, sex control and regulation of sex ratio are two most important subjects in silkworm rearing researches (SRI-CAAS, 1989).

Since 1930s, many Chinese scientists were engaged in the research of sex control in silkworm by different methods and have gained many fruitful results. In this review article, we briefly summarized as follows.

Parthenogenesis

Parthenogenesis means development of a new individual larva from an unfertilized egg. The occurrence of parthenogenesis is very low under natural condition. But when the unfertilized eggs were treated by some physical or chemical methods such as heating, radiation and CO_2, this ratio may fluctuate. The phenomenon of silkworm parthenogenesis was firstly recorded in the ancient Chinese book named *BOWUZHI* in which described "Tri-molting silkworms can be observed…even no sex mating between moths, the eggs can be used to rear…" The author was Zhang Hua (A. D. 232 – 300), who lived in the West Jin dynasty. However the scientific research on artificial silkworm parthenogenesis in China was started in last century.

In 1933, Zhuxian, a scientist of cell institute of Chinese Academy of Sciences successfully induced parthenoge-

*To whom correspondence should be addressed.
Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang 212-018, P. R. China.
Tel: 86-511-5616575; Fax: 86-511-5622507; E-mail: cjyz17@public.zj.js.cn

netic silkworm eggs by using of chloroform, acetic acid, saturated CO_2 and seawater, but there is no larva to be hatched. In 1950s, Zhang *et al.* (1950, 1951) dissected and extracted unfertilized eggs from virgin female silkworm moths. These eggs were incubated in hot water (47 ± 1°C) for 18 min or in 0.24% of NaCl solution for 15 min. The ratio in the parthenogenetic eggs reached to 50% and most of the hatched larvae were female. When the unfertilized eggs were incubated in –2°C – –3°C for 6 – 12 hrs, 30% of the eggs could developed into embryo stage, and the hatching ratio was lower than that of the method of incubation in warm water, but male silkworm ratio increased. Fang *et al.* (1989) incubated the unfertilized eggs in 47 ± 1°C water for 18 min, and obtained the offspring who express same characteristic as their mother. Heredity analysis with marked mutants showed that the genotype of offspring was the same as their mothers. Decade thousands of parthenogenetic silkworms were reared in different seasons and the results showed that the hatched worms are 100% of female. Breading of one of the parthenogenetic silkworm lines for 22 generations showed an induction ratio of parthenogenetic silkworms were more than 90% and hatching ratio was about 85%. At the same time, parthenogenetic silkworm line from single female moth was created. To obtain homozygotic silkworms, Fang *et al.* (1989) incubated unfertilized silkworm eggs at –11°C for 30 min to induce meiotic male parthenogenetic silkworm. Androgenesis was adopted to breed the isogenic silkworms, but the developing ratio of meiotic parthenogenesis and its hatching ratio were very low.

Ratio of developed parthenogenetic eggs of twenty fundamental silkworm races were found to fluctuate from 18.03% to 62.84% by incubating their unfertilized eggs in 46°C water for 15 min (Zhu and Xiang, 1989). The results indicated that the parthenogenesis ratio was highest for hybrid silkworm, and the parthenogenesis ratio of Japanese strain was higher than that of Chinese strain. There was no remarkable difference among the races of the same strain. Pan *et al.* (1996a) studied the ability of parthenogenesis in 45 silkworm germplasm, and mensurated their economical characters. The results showed that the parthenogenesis ratio trends were nature-laying eggs > the eggs from dissecting; hot water treatment for 20 min > for 2 or 5 min in bivoltine races, the early layings > late layings, bivoltine > multivoltine, diapausing > non-diapausing, hybrid line > pure line, and hyper-silk-generous > low-silk-generous races. Two races, 952 and 953, were bred through the method of artificial parthenogenesis after 5 generations of selection. The egg fecundity reached 254 – 300, the ratio of parthenogenic eggs was 99.9% – 100%, and hatching ratio reached 80.1% – 83.4%. Viability and economical characters of these races exhibited good production ability.

Lu (1994) froze the silkworm ovary eggs and obtained parthenogenetic silkworms by using different temperature and incubation time. The ratio of parthenogenetic eggs was highest at 80% when the unfertilized eggs were frozen at –5°C for 60 hrs. The offspring could be created generation to generation by using freezing method, and the ratio of parthenogenetic eggs were increased along with generation increase. They also found that the parthenogenetic silk moths had good ability of mating and procreation, and further the fecundity and pigmentary egg ratio had no difference with the normal female silkworms. Wang *et al.* (1998) tried to find the best condition to induce parthenogenesis silkworm by using different water temperature and different period. The result showed that the parthenogenic ratio is highest at temperature 44 – 46 and the period for 14 – 18 min. Later, they treated 16 commercial silkworm races with warm water and and bred several parthenogenetic lines successfully.

Xu (1994) spread the rue on the head and the tail of virgin moths to stimulate layings. After the unfertilized eggs were incubated at 25°C for 12 hrs, and the eggs were irradiated by laser. Pigmentary (parthenogenetic) egg ratio accounted for 60% when they were irradiated for 5 sec. The survivor ratio was different according to different silkworm races, ranged from 96% to 12%.

Dispermic androgenesis

Since Hasimoto (1934) found that silkworm androgenesis could be induced by heat treatment, many scientists (Ostryakova-Varshaver and Astaurov, 1947, 1952, 1958; Tazima, 1967; Terskaya and Strunikov, 1974, 1975; Strunikov, 1983) have studied on the method to induce androgenetic silkworm by several stresses such as high and low temperature, CO_2, radiation, and discussed the mechanism of androgenesis. Xu *et al.* (1988, 1994a) radiated the silkworm oocyte with high dose of Co^{60}-γ radial to make the oocyte inactivity. After an eclosion of female pupae, they were mated with normal male silkworm moths. The eggs were treated in a high temperature to make two sperms combine, and then the male offspring that only express their fathers characters were obtained. When the male parents genotype was$^{Ekp}/E^{kp}$, and that of the female parents was $+^{Ekp}/+^{Ekp}$, 25 of 95 of androgenetic individuals expressed E^{kp}/E^{kp}, 46 $+^{Ekp}/E^{kp}$, 22 $+^{Ekp}/+^{Ekp}$. The ratio of these genotypes was 1 : 2 : 1. This result demonstrated that the offspring was formed from the sperms of male parent. On this base, the mechanism of androgenesis, and the relationships between irradiation dose and the induction efficiency, heat treatment period and temperature and the efficiency were discussed. Among of androgenetic silkworm lines created, and one had been generated for 16 generations. Two male silkworms which caught shape mutant markers (*ch*-chocolate newly hatched larva, *re*-red egg, or *pe*-pink egg) were used to mate to one female, and male offspring which expressed two fathers characters was obtained, and the technique of androgenesis from mix sperms of different father was established. On this base, the androgenesis lines from single male moth were bred. In these lines, all individuals were combined from the sperms of the same silkworm male moth, that meant self-crossing happened. One of the lines had been generated for six generations, and homozygosity had achieved 98.44%.

Obtaining solo sex silkworms by means of heredity methods

Sex limited lines: Sex limited lines means that some characters are expressed only in one sex. W chromosome is absolutely possessed by female in silkworm, and there is no exchange between W and Z chromosomes. If some genes are located at W chromosome, these genes would only express in females. If the segment with one gene was translated to W chromosome, the character, which is expressed by this gene would only behave in females. This phenomenon could be happened in nature mutant or by artificial induction.

Striped sex limited: Striped sex limited means that silkworm sex could be identified by their worm body mark of stripes. Tazima (1941) obtained the first line of the silkworm, sex-marked by the integumentary pattern of the larvae. In this experiment, the females carrying a translocated dominant gene ($+^P$, P^{sa}) in the W chromosome by using X radiation were crossed with the plain males (*p*, *p*), the next generation expressed characteristics of sex-limited silkworm strain. Sun (1959) made use of the character of sex limited strain and bred Zhen-3, Zhen-4 whose larva sex could be identified by their mark, the females had normal mark but the males were markless. These breads can be reared in commercial size. The male or female silkworms can be put on mountages separately according to their larvae mark so as to reel male or female cocoons respectively.

Newly hatched larvae color sex limited lines: Huang (1984a, 1984b) irradiated 269 individual female pupa (genotype: W/Z^{+sch}) of variety "134a" with 10.32 C/kgd X rays. These female moths were mated to sex-linkage character *sch* males (Z^{sch}/Z^{sch}). Among 40,495 F_1 individuals, the female mutants was found that their +sch segment of chromosome Z had translocated to chromosome W. Then these individuals were back-crossed to sex-linked inherited character *sch* male successively, and the sex-limited newly hatched larvae body color lines were created. In these lines, sex of individuals could be distinguished by their newly hatched larvae body color, whose genotype of female was W^{+sch}/Z^{sch}, the newly hatched larva expressed black color, and that of male was Z^{sch}/Z^{sch}, the newly hatched larva expressed chocolate color. After years of breeding, commercial sex-limited newly hatched larvae body color lines were bred. Their economical characters were similar to the silkworm races for summer-autumn rearing and the aim of rearing was achieved only in the male silkworms by discarding the black newly hatched larva.

Sex limited fluorescent colors of silkworm cocoon: Silkworm cocoon filament is composed of protein, and contains some structures which can irradiate colors when these structures were irradiated by ultraviolet radiation. They absorb photons and stand in the situation of blazing, and release energy when they are renewed. If the energy meets the energy of visible light, it produces fluorescence. Silkworm cocoon has several natural colors, such as white, yellow, red and green. When silkworm cocoons are irradiated by ultraviolet radiation, they shed white, yellow or purple fluorescence. Normally, there is no relationship between cocoon fluorescent color and silkworm sex, but female cocoon fluorescence color of some special silkworm races showed purple or white, and that of male showed yellow.

In 1979, Dong34 was investigated for its cocoon fluorescent color in Sericultural Department, Southern China Agricultural University. After 3 generation of selection, sex of new Dong34 could be identified by the cocoon fluorescence. Its male cocoons showed yellow fluorescence, while female cocoons showed purple fluorescence. The exactness achieved was 99.4%.

Liu (1996) bred a fluorescent hybrid silkworm verity "Yingguang Chunyu". The results indicated that all the yellow fluorescent cocoons were male and the white fluorescent cocoons were female when their cocoons were screened under 365 nm ultraviolet rays. The veracity was more than 98%.

The cocoon fluorescent colors of 348 varieties had been observed under 365 nm ultraviolet radiation by Chen *et al.* (1988). There was obvious difference in fluorescence between male and female cocoons. The former strongly showed yellow fluorescence, while white and purple fluorescence was dominant in the latter. Five fundamental races were discovered that all of their male cocoons showed yellow fluorescence, while female cocoons showed purple fluorescence. Three lines of sex-mark fluorescence were bred, and their accurate ratio approached 100%.

Sex linkage balanced lethal strain

Balanced lethal means that two non-allelic recessive lethal genes (Z^{l1} *or* Z^{l2}) locate on ZZ chromosomes respectively, so this male worms wont be died because the corresponding dominant gene covers up recessive lethal gene (Z^{l1}/Z^{l2}). If anyone of the lethal gene becomes homozygous Z (Z^{l1}/Z^{l1} *or* Z^{l2}/Z^{l2}), the organism will die. If Z^{l1}/Z^{l2} cross with WZ^{+}, the male offsprings (Z^{l1}/Z^{+} or Z^{l2}/Z^{+}) will survive but females (Z^{l1}/W or Z^{l2}/W) will die. By using this method, Strunnikov *et al.*1969obtained the Sex linkage balanced lethal stocks firstly.

Xu *et al.* (1993, 1994b, 1997) irradiated 1,000 female pupa using 4,000 R Co^{60}-γ irradiation to induce sex-linkage lethal genes. These female moths were mated with sex-linked chocolate males Z^{sch}/Z^{sch}. All of the F_2 eggs were wrapped separately, and their newly hatched larva color and the hatching rate were investigated. If the hatching rate of the batch was lower or equal to 75% and the ratio of chocolate to black newly hatched larva was about 2 : 1, the batch was reserved and the chocolate and black newly hatched larva were reared separately. Sex of these silkworms was investigated immediately after the 4th ecdysis. If all of the black newly hatched larva were male, and the ratio of male to female was 1 : 1 in the chocolate newly hatched larva, they judged that there was a recessive mutant gene located on one of Z chromosomes in male of this batch. Forty-four recessive mutants which are located on Z chromosome had been obtained, and were tested by *sch* and *od* gene using the method of three point cross to calculate their genetic distance respectively. Seventeen of them were demonstrated to be located between *os* (0.0) and *sch* (21.5), and seven of them were between *sch* (21.5) and *od* (49.6). Four translation lines of $Z \rightarrow W^{\frown +sch}$ had been obtained in 1997 by irradiation methods, and their relationship with those recessive mutants had been analyzed. Methods of androgenesis and the translocation lines had been used to acquire balanced lethal male silkworms.

Two sex linked balanced lethal lines, S-8 and S-14 had been introduced from Development Biology Research Institute, Russian Academy of Sciences to Sericultural Research Institute, Zhejiang Academy of Sciences in 1996. Nowadays, a series of sex-linkage lethal balanced lines are in use for commercial production, the male silkworm of these lines accounted for 99%.

High temperature sensitive male stocks

Pan *et al.* (1992) discovered that the embryos of the individuals which express sex linkage chocolate gene (Z^{sch}/Z^{sch} or W/Z^{sch}) were sensible to and could not survive in high temperature and desiccative environment. When *sch* males (Z^{sch}/Z^{sch}) was mated to xin9 females (normal, genotype: W/Z^{+}), and their offspring was incubated at high temperature (30°C) and desiccative (R. H. 60%) environment, females (W/Z^{sch}) could not hatch (hatching ratio: 3.27%) but males (Z^{+}/Z^{sch}) could hatch (hatching ratio: 99.66), so that silkworm sex could be controlled. But all of the offspring of their reverse mating (sch♀ × Xin9♂) could hatch. Lin (1996) obtained the same results using Hua1 and improved *sch* as materials.

Lin (2000) validated the *sch* sensibility to high temperature and desiccative environment using the sex-limited lines $Z \rightarrow W^{\frown +sch}$ as materials. The eggs were incubated at 30°C and 60% of R. H., females ($W^{\frown +sch}/Z^{sch}$) hatched normally, but male (Z^{sch}/Z^{sch}) of them could not hatch. The ratio of hatched female to male reached 94.86 to 5.14.

Zhu (2001) transmitted *sch* gene into economic silkworm races, after 8 generations of backcrossing, two races for rearing only male silkworms, Xia *sch* and Qiu *sch*, were bred. These races could be used as economic silkworm races and were reared in sericulture areas. Male silkworms accounted for 95% when F_1 eggs were incubated in given environment.

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