Int. J. Indust. Entomol. Vol. 10, No. 1, 2005, pp. 1~10

[Review]

International Journal of *Industrial Entomology*

Parthenogenesis in Silkworm, *Bombyx mori* L.

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(Received 12 May 2004; Accepted 15 January 2005)

Parthenogenesis in mulberry silkworm, *Bombyx mori* L. acquires immense use in the development of outstanding homozygous lines with higher viability, hybrid vigour, combining ability and less phenotypic variability. It can serve as a powerful tool in controlling sex of the offsprings as well as a useful tool in selection. In fact India is the second largest silk producing country in the world next only to China and all the five types of natural silks *viz.*, mulberry, oak tasar, tropical tasar, muga and eri are produced in India. However, little information is available on the role of artificial parthenogenesis in the development of superior silkworm breeds. This paper overviews some important studies carried out on artificial parthenogenesis, and outline of different types of parthenogenesis, methods of induction of artificial parthenogenesis, factors responsible for successful parthenogenetic development, cytogenetics of artificial parthenogenesis and role of artificial parthenogenesis in silkworm breeding. Besides, an attempt is made to describe briefly about parthenogenetic engineering which includes cloning in silkworm, artificial insemination, chimeras, hybridization, chromosomal substitution and recombinant DNA in silkworm.

Key words: Artificial partheogenesis, *Bombyx mori*, Breeding

Introduction

Parthenogenesis ('virgin origin') is derived from the Greek word 'parthenos' means virgin. In many animals the egg develops into a new individual without fertilization. Such a development of the ovum without fertilization is called parthenogenesis and the individuals that reproduce by this method are called parthenotes (Retnakaran and Percy, 1985). Parthenogenesis is a normal means of reproduction in some lizards (Darevskii and Kulikova, 1961) and several insect orders except Odonata, Dermaptera, Neuroptera and Siphonaptera (Chapman, 1982). Parthenogenesis is a common phenomenon of reproduction in several animals and considered as an important tool in silkworm breeding which requires special significance and has been reviewed by many workers (Suomalainen, 1962; Tazima, 1964; Strunnikov, 1975; Cuellar, 1977; Clement and Seidel, 1981; Clement, 1982; Chowdhury, 1989; Ravindra Singh *et al.*, 1997; Klimenko, 2001). When somatic cells vegetatively reproduce it is asexual whereas haploid gametes fuse to form a diploid zygote known as sexual reproduction (Retnakaran and Percy, 1985).

Parthenogenesis is invariably the development of the unfertilized ovum. The spermatozoon, on the other hand does not develop without fertilization except in rare instances where the male gamete is artificially fertilized with a nucleus-free egg, a phenomenon known as merogony. The development of the sperm in such an enucleated egg is called androgenesis or male parthenogenesis (Retnakaran and Percy, 1985). The possibility of androgenetic development by fusion of two haploid sperm nuclei resulting in a diploid individual has been observed in silkworm (Hasimoto, 1934; Astaurov, 1936, 1937). Moreover, when two genetically different males inseminated a female, the two father's hybrids have also been obtained (Strunnikov, 1958; Astaurov, 1968).

Transfer of hereditary attributes from generation to generation takes place during the process of reproduction. It is a well-known fact that the border between any two consecutive generations passes through the activated egg which gives rise to a new organism (Klimenko, 2001). So activation is a basic input for the egg to develop into a new organism. But activation is not always caused by

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penetration of a sperm inside the egg. In natural parthenogenesis, insemination is absent and the activation is caused by other factors such as aerobic oxygen in the stick insect, *Carausius morosus* (Pijnacker and Ferverda, 1976). In other cases (wasps), the sperm penetration inside the egg is not followed by activation; the activating function is performed by the female egg laying apparatus, which strongly deforms an egg and causes activation through extension of the egg membrane. Then the male nuclear material fuses with the female pronucleus (Went, 1982). In case of natural gynogenesis (*e.g.,* some fishes), on the contrary, the sperm carries out only the activating function and the fusion of male and female nuclei does not take place (White, 1973; Schultz, 1977). Thus various factors were proved to be essential to activate the egg and established that sperm penetration inside the egg is not always the cause of its activation. So attempts to understand the activating impact of the sperm by replacement of insemination with various physico-chemical agents resulted in the discovery of artificial parthenogenesis.

Artificial parthenogenesis has been induced in a wide group of animals by using a number of substances like hypertonic and hypotonic sea water, different salts like chlorides of potassium, sodium, calcium, magnecium, organic acids such as butyric acid, lactic acid, oleic acid, fat solvents namely toluene, ether, alcohol, benzene and acetone, urea, sucrose and also by irradiation of sperms with ultra-violet light or x-rays (Balinsky, 1981). Ionophores (a group of chemicals, which make cellular membranes permeable) have been found effective in inducing parthenogenetic development in animals (Steinhardt and Epel, 1974; Epel, 1977). Studies have been made on parthenogenetic activation in oocytes of various animals such as pigs by protein kinase inhibition (Mayes *et al.*, 1995), in mouse by progesterone (Imahie *et al.*, 1995) and Ca = 2 + inophore and cycloheximide (Hagemann *et al.*, 1995) and in human following cryopreservation using 1, 2-propanediol (Gook *et al.*, 1995).

Activation of unfertilized eggs of the silkworm by means of diverse physico-chemical stimuli (artificial parthenogenesis) such as various acids, alkali, electrical stimuli, and hot water (45°C) has first been demonstrated in 1886 by a Russian zoologist Tichomivov (Astaurov, 1957). In these experiments, development frequently reached the stage of egg pigmentation but hatching was never recorded because of abnormal embryogenesis. In Japan, complete artificial parthenogenesis up to larval hatching using hydrochloric acid treatments and both maturation divisions were observed in unfertilized eggs of silkworm (Sato, 1925, 1931). In the Soviet Union, during 1936 – 1940, Astaurov carried out extensive and thorough study on parthenogenesis caused by aqueous heat shock at different temperatures and protocols and he established his "sacred" formula for thermic parthenogenesis: 46°C, 18 min. In this case parthenogenetic progeny were practically all female, the maternal genotype being repeated or cloned (Astaurov, 1940).

Types of parthenogenesis

Parthenogenesis may be classified in different ways based on reproduction, sex determination and cytology. According to Suomalainen (1950) parthenogenesis can be classified as follows

Based on reproduction

Occasional or tychoparthenogenesis: In this case egg occasionally or accidentally develops through parthenogenesis and is observed in silkworm, *Bombyx mori* L. (Astaurov, 1969).

Obligatory parthenogenesis: Eggs not occasionally but exclusively without being fertilized are developed under normal conditions.

Facultative parthenogenesis: The egg, in this case can develop with or without fertilization. In the absence of fertilization it develops pathenogenetically as seen in several species of locusts (Bergerard and Senge, 1959) and in almost all races of the silkworm, facultative parthenogenesis occurs spontaneously and in normal condition undergo a limited number of cleavages and quite rarely develops up to the caterpillar stage *i.e.,* rudimentary (Tazima, 1964).

Rudimentary or incomplete parthenogenesis: Most of the unfertilized eggs start to develop parthenogenetically up to a certain embryonic stage but viable parthenogenetic progeny are not produced and is seen in certain species of mosquitoes (Kitzmiller, 1959).

Artificial parthenogenesis: The eggs have the potential to develop parthenogenetically but require a stimulus which can be provided by certain physical and chemical agents. The eggs of silkworm, for instance, can be made to develop parthenogenetically by changing the temperature (Klimenko, 1980).

Based on sex-determination

Arrehenotoky: The unfertilized eggs invariably develop into a male by parthenogenesis whereas the fertilized eggs develop sexually into the female. This type of parthenogenesis is seen in many hymenoptenrans such as the parasitic wasp, *Dohlbominus fuscipennis* (Wilkes, 1965).

Thelytoky: The unfertilized eggs in this case develop into females only by parthenogenesis and is typical of Phasmids such *Carausius morosus* (Bergerard, 1961). Astaurov

(1940, 1957) reported thelytokous progeny of silkworm where all were females resembling to their maternal type by artificial parthenogenesis.

Amphitoky or Deuterotoky: The unfertilized egg develops into either males or females and is observed in leaf-cutting ant, *Oecophylla longinoda* (Ledoux, 1950).

Based on cytology

Generative or haploid parthenogenesis: Generative or haploid parthenogenesis involves meiosis but the resulting individual is haploid. These haploid offsprings are usually males (arrhenotoky), whereas the females of the species are produced sexually through oviparity and are diploid. This phenomenon of halving one sex diploid and the other haploid is referred as haplodiploidy and is observed in Hymenoptera and most Thysanoptera (Elzinga, 2000).

Somatic or diploid parthenogenesis: In this type of parthenogenesis, the diploidy (2n) is regained by various types of modifications during the process of oogenesis and may be subdivided as

Apomictic or ameiotic parthenogenesis: Apomictic parthenogenesis produces females (thelytoky) that are diploid, because chromosome reduction does not occur and the offsprings are genotypically identical to the mother. It is a common type, highly stable, and found in aphids, scales, some beetles, flies, wasps and psocids (Elzinga, 2000).

Automictic or meiotic parthenogenesis: In this case, meiosis occurs but a polar body nucleus unites with the egg nucleus to restore the diploid status of the egg nucleus. Some variability between mother and offspring can result, depending upon the amount of crossover that occurred during meiosis and whether or not the egg nucleus fuses with the first or second polar body nucleus. This type of parthenogenesis is found in some scales, walkingsticks, moths and flies (Elzinga, 2000).

Schematic representation of different types of parthenogenesis in given in Fig. 1.

Methods for induction of artificial parthenogenesis in silkworm

The nature of activation or stimulation of the eggs to a resumption of the arrested maturation division has been the subject of innumerable studies. A huge amount of facts have been collected which, however, has as yet not allowed us to arrive at any general conclusions and to understand completely this phenomenon. One of the greatest obstacles in studying the process of activation is that the investigator is unable to determine whether the processes taking place in the eggs immediately the spermatozoa penetrated into them or following the action of other activating agents are the triggering mechanisms for activation or whether they are postactivational events and consequently have nothing to do with the phenomenon of activation.

The discovery of artificial parthenogenesis opened new methodical approaches to studying activation because it becomes possible to use physical and chemical agents as activators instead of spermatozoa. Various methods have been tried to induce artificial parthenogenesis in silkworm, which are as follows

High temperature: (Induction of ameiotic parthenogenesis without reduction of chromosome number)

Earlier Tichomirov (1902, 1903) observed the effect of

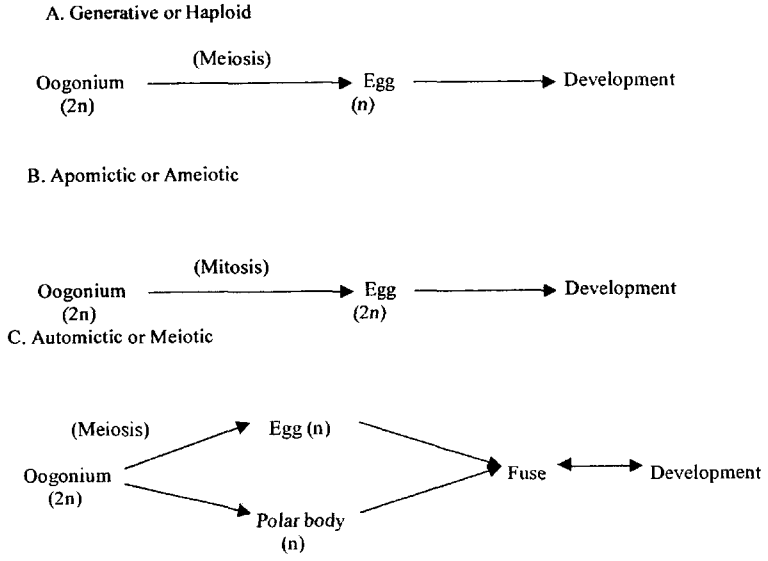

Fig. 1. Schematic representation of haploid, ameiotic and meiotic parthenogenesis.

hot water for inducing artificial parthenogenesis in silkworm but he succeeded only in inducing the beginning of development (Astaurov, 1957). A decisive contribution on the study of artificial parthenogenesis was made in silkworm, *Bombyx mori* by Astaurov (1940). He found a very accurate method of obtaining worms successfully to the extent of 82% in certain egg layings. His method is simple, *i.e.,* extraction of eggs from ovarian follicles of the moth, washing and treating them with warm water at 46°C for 18 min and exposing them to a chilling of cold water, *i.e.,* instantaneous abrupt ending of heating. The results are influenced by other factors also; such as, time passed between the extraction and heat treatment, percentage of humidity, temperature and humidity at which the eggs are conserved after the treatment, the age of the moth, portion of ovary from which the eggs are taken etc. In bivoltine eggs of first generation, he obtained 25% hatching. The robustness of the larvae was not in any way inferior to those of normal larvae and all were females. Millions of larvae were produced by this method. About 27% of the worms have proved to be polyploids or more often myxoploids (2n + 4n) as reported by Chowdhury (1989). High temperature was shown to be a powerful parthnogenetic agent for induction of artificial parthenogenesis in silkworm (Astaurov, 1957). Further, extensive studies were conducted on activation of unfertilized eggs treating with warm water (Astaurov, 1967, 1978; Ohkuma, 1971; Sugai *et al.*, 1983; Murakami, 1985; Fang *et al.*, 1989; Takei *et al.*, 1990; Hirokawa, 1993, 1995; Ravindra Singh *et al.*, 1994).

Another trend in research aimed at evolving simple ways of obtaining parthenogenetic development was based on the activation of the eggs in the body of the live moth. However, attempts to activate the eggs in the moth's body with high temperature were proved unsuccessful. If the moths were warmed in air or water at various activating temperatures (40°C and higher) with exposures necessary for egg activation they died in every case. The activated eggs could be used only after their extraction from the bodies of the dead moths (Strunnikov, 1983).

Low temperature: (Induction of meiotic parthenogenesis having both maturation divisions)

Cooling of silkworm eggs to low temperature (−11°C) for 30 min caused development of only homozygous males (Terskaya and Strunnikov, 1975). According to modern classification this type of parthenogenesis by freezing at -11°C for 30 min in addition to spontaneous parthenogenesis are referred to as 'meiotic'. In all these cases, parthenogenetic progeny were mostly male. Strunnikova (1979) also tried to activate the unfertilized eggs in the body of the live moths for induction of artificial parthenogenesis and during this experiment about 70 – 85% eggs develop to the stage of pigment formation in the serosa cells and the remaining 15 – 30% of the eggs that had not begun parthenogenetic development and were still yellow in colour were separated and subjected to activation a second time. In one case they were stimulated towards meiotic and in another towards ameiotic parthenogenesis (Strunnikov, 1983). Treatment of eggs with low temperature (−11°C for 300 min) caused parthenogenetic development up to 60 – 70% and Terskaya studied stimulation of practically all the cooled eggs towards meiotic development (Strunnikov, 1983). He proposed the cytological mechanism of meiotic parthenogenesis and observed that in all the unfertilized eggs activated by low temperature (−11°C, 30 min), the maturation divisions proceed synchronously, though twice as slowly as in the standard. As a result 5 hrs after activation a 'pronucleus' is formed, as in the fertilized eggs, and three polar bodies that later degenerate. Eight hrs following activation the division of the pronucleus into two haploid nuclei (blastomeres) is completed. Then the blastomeres divide once or twice more and only after that start to fuse in pairs.

Chemical and other agents: Earlier Kawaguchi (1934) by immersing deposited unfertilized eggs at 15% HCl acid for 5 min. obtained 82 larvae, *i.e.,* 41 males and 15 females. Sato (1925, 1931) claimed to have derived at first 39 worms by parthenogenesis (23 females and 16 males) by treatment of deposited eggs, in a solution of HCl acid of specific gravity 1.04 to 1.06 heated at 40°C to 43°C for 4 to 6 min. He regarded the fact as induction due to HCl acid. Astaurov (1940), however, denied it as artificially induced, the acid only help to terminate the diapause. It was later ascertained that HCl acid alone can not induce complete parthenogenesis unless combined with simultaneous heating. Sato's result was, however, considered as a rare case of natural parthenogenesis with such high number of hatched individuals. Astaurov (1940) with the same technique obtained 222 worms with 44 males and 35 females. Silkworm eggs obtained from virgin female moths have been activated by treating with other agents *viz.,* acetic acid, hydrochloric acid, diaphenol, potassium permanganate, hydrogen peroxide, ammonia, oxygen, alternating current and also by subjection to ionophoresis (Strunnikov, 1983).

Nagraj *et al.* (1984) were able to obtain 77% hatched larvae from the hybrid J106 × Cambodge by giving post-activation care at 17°C for 96 hrs, followed by HCl acid treatment at 46°C for 5 min and incubating the eggs at 24°C till hatching. They found 77.1% complete parthenogenetic development in several generations with normal larvae up to cocoon stage.

Artificial parthenogenetic activation in silkworm eggs was observed through preservation of ovaries in liquid

nitrogen, thaawing and transplanting into 5th instar female larvae (Kusuda *et al.*, 1985). Lu (1994) has maintained generation advancement of silkworm by freezing immature ova. In another study, when ovaries were transplanted from female to male individuals, the percentage of parthenogenesis was much lower in males than in females (Sugai and Ohtsuka, 1983). Laser beam was also proved to be a successful agent for induction of artificial parthenogenesis in the silkworm (Xu *et al.*, 1990, 1995).

Combined effect of low and high temperature: The reactivity of eggs aged 24 hrs to 5 days in response to the combined action of low and high temperatures formed a new simplified technique for activating eggs to parthenogenesis. Attempts to activate the silkworm eggs through combined action of low and high temperature for induction of parthenogenesis was studied by Sugai *et al.* (1983) and Nagraj *et al.* (1984).

Though various methods were adopted earlier for induction of parthenogenesis in the unfertilized eggs of silkworm, the degree of parthenogenesis depends upon the developmental stage during which the eggs are treated and the sex of the parthenogenetic eggs depends upon the type of activation technique utilized for induction of parthenogenesis (Strunnikov, 1975, 1983). Astaurov (1957) has stated that high temperature was proved to be a powerful agent for induction of parthenogenesis and maximum parthenogenetic induction was at twelve hours after removal of eggs from the body of the moth, however, the rate of parthenogenetic induction varies in different strains (Takei *et al.*, 1990).

Factors for successful artificial parthenogenesis (thermal activation) in silkworm

A brief account of some essential factors for successful artificial parthenogenesis is mentioned below:

1. The age of an egg is usually timed after the termination of egg maturation processes and imago's emergence from a cocoon is considered as a benchmark in egg aging. Newly extracted eggs from ovarian tubes show low susceptibility whereas during first 6 – 12 hrs after extraction, the response to heat activation attains maximum but slowly decreased when the age of the egg increases. Best result has been reported in the egg that is extracted from ovarian tubes not less than 6 and not more than 24 hrs prior to activation (Astaurov, 1940).
2. The efficiency of the unfertilized eggs to respond against artificial parthenogenesis also depends upon the age of the female from whom the eggs were derived.
3. Maturity of the eggs depends upon the locations of their presence in different parts of the ovarian tubes.
4. The capacity for artificial parthenogenesis varies from one race to another (Astaurov, 1940; Takei *et al.*, 1990) and selection of highly pronounced silkworm breeds towards parthenogenesis is of utmost important. It is less marked in the European monovoltine races and most pronounced in the Asia Minor Bagdad and Eastern bivoltine races (Astaurov, 1940). In bivoltine breeds, artificial parthenogenetic development was more pronounced as compared to multivoltine breeds (Ravindra Singh *et al.*, 1994).
5. Successful artificial parthenogenesis greatly depends not only upon the activation technique and nature of the activator for induction of artificial parthenogenesis but also more precisely upon the length of time for activation (Astaurov, 1940; Strunnikov, 1975, 1983; Klimenko, 2001). The optimum exposure time depends upon the temperature. With rise of temperature by 1°C, the rate of reaction is more than doubled. So the reaction is very sensitive and every second of exposure time above 49°C is of significance. Complete parthenogenesis is most readily produced by temperature between 45 – 47°C, variation on either side diminishes the success and best results were reported when unfertilized eggs immersed in hot water for 18 min at 46°C (Astaurov, 1940).
6. Temperature and moisture during incubation of the activated eggs as well as during post-activation period are most important factors. Temperature below 19 – 20°C during development of activated eggs results in an abnormal growth and percentage of hatching decreases markedly (Astaurov, 1940).
7. Duration of cooling in water by abrupt changing of heating soon after heat activation is considered as a crucial factor for the egg to activate parthenogenetically.
8. Drying rate of eggs before and after activation enhances the unfertilized eggs to develop parthenogenetically.

Cytogenetics of artificial parthenogenesis

More interestingly, the sex progeny greatly depends upon the activation technique utilized for induction of artificial parthenogenesis (Strunnikov, 1975, 1983). For example, the parthenogenetic progeny are all females when induced by high temperature (Astaurov, 1940, 1957) whereas all males when induced by low temperature (Terskaya and Strunnikov, 1975). Both males and females have been observed by treatment of unfertilized eggs by HCl acid (Sato, 1925, 1931; Kawaguchi 1934). From those observations, Astaurov arrived at the conclusion that the parthenogenesis induced by low temperature and HCl acid in addition to spontaneous parthenogenesis, are cytogeneti-

Table 1. Nuclear behaviour in various types of parthenogenesis

Spontaneous parthenogenesis	HCl acid /Low temperature induced /Meiotic parthenogenesis	Thermal activation/ Ameiotic parthenogenesis
1st and 2nd maturation divisions are as in normal sexual reproduction.	1st and 2nd maturation divisions are as in normal sexual reproduction.	The spindle of first maturation division is destroyed only the 2nd maturation division is normal.
Meiotic divisions occur. Egg polar body fusion occurs. (Aa females from Aa female) or Blastomeric fusion occurs. (AA or aa males from Aa female)	Meiotic divisions occur. Egg polar body fusion occurs. (Aa females from Aa female) or Blastomeric fusion occurs. (AA or aa males from Aa female)	Meiotic divisions do not occur. Of the two diploid nuclei, one forms a polar body; the other migrates into the interior of the egg cytoplasm and develops into female embryo. (The daughters are all Aa like the mother)

cally different from the thermal activation. Schematic representation of nuclear behaviour in various types of parthenogenesis is presented in Table 1.

Artificial parthenogenesis and silkworm breeding

The main objective of silkworm breeding is to improve the genetic entity of the silkworm in relation to their economic utilization. Development of silkworm breeds by conventional breeding has a vital role in upgrading the quality and quantity of silk produced. Since, most of the quantitative characters in silkworm are governed by polygenes, their inheritance shows more variation and therefore, more emphasis is paid for selection of good parents by their phenotypic expression. Sometimes a suitable phenotype may not exhibit a suitable genotype and due to low heritability, the subsequent progeny may lose its unique genotype. Artificial parthenogenesis, in this regard, enables one to produce from one outbred breed hundreds of parthenoclone each of which is an exact genotypical copy of its parents (Astaurov, 1940, 1957; Strunnikov, 1983; Takei *et al.*, 1990). More interestingly, the interrelation between the success of parthenogenesis and the characteristics of the maternal parent's organism has long been noticed and the frequency of complete parthenogenesis was shown to be directly proportional to the heterozygosity of the individuals (Astaurov, 1940; Altukhov and Klimenko, 1978). Further, Ohkuma (1971) and Strunnikov (1983) have studied the origin and the mechanism of hybrid vigour by means of artificial parthenogenesis. Increased heterosis with a high tendency towards complete parthenogenesis in F_1 hybrids of parthenogenetically formed female and normal males have been reported by many workers (Strunnikov, 1986; Takei *et al.*, 1990; Ravindra Singh *et al.*, 1994).

Strunnikov (1995) has observed 30% more silk in the hybrid comprising one parthenoclone as a component. A parthenoclone-43 having egg viability of 80 – 90%, larval viability of 90 – 95%, cocoon weight ranging from 2.0 – 2.4 g., filament length of 1,300 m., silk productivity ranging from 23.0 – 24.5% and high combining ability was developed through ameiotic parthenogenesis (Strunnikov, 1995). Strunnikov (1974, 1986) has also determined high combining ability in parthenoclone-29 variants and found 15% more heterosis when the selected lines were crossed with other lines. The level of heterosis is mainly determined by the relationship between the effects of useful and harmful genes. Artificial parthenogenesis facilitates to transform a single highly heterotic genotype into different genotypical variants with varying degree of heterozygosity and ratios between useful and harmful genes. As a result, parthenoclone-29 was transformed into four genotypical variants through meiotic and ameiotic parthenogenesis and high combining ability was recorded by the number and homozygosity of the useful genes (Strunnikov, 1974, 1986).

Inbreeding depression among the inbred lines is a major setback in maintaining and multiplying the pure parental stocks. Artificial parthenogenesis, on the contrary, can facilitate to evolve a line with restored diploidy of lower inbreeding depression and can be reared for several years without any reduction in quantitative characters (Astaurov, 1957) and therefore, it is possible to establish a new colony from a single individual through artificial parthenogenesis (Cuellar, 1977). The desired type of silkworm either entirely females (completely heterozygous) or predominantly males (homozygous) can be produced by application of various methods of activation and can be served as a useful tool for controlling the sex of the offsprings (Strunnikov, 1975). Moreover, male cocoon shells with rich silk content, are of great demand to the silk industry and there is a challenge to the breeders to evolve superior hybrids with only males. Hybrids between parthenogenetic females and males of sex-linked lethal producing only males would be beneficial in this regard.

Hirokawa (1990, 1995) has identified some high parthenogenetic lines showing average hatchabilities (86 –

91%) through selection of high parthenogenetic lines by hot water treatment and practical utilization of F1 hybrids of the silkworm, *Bombyx mori*. There was no difference in pupation rate and cocoon yield between parthenotes and F1 hybrids. Though the quantitative characters of parthenogenetic strains were inferior to those of the normal races, the economic characters in F1 hybrids of the cross between parthenogenetic Japanese strain females and normal Chinese race males were the same as those of normal F1 hybrids whereas more hybrid vigour over mid parent and better parent values for effective rate of rearing and cocoon characteristics was recorded in the hybrid resulting from a female parthenote NB7 and a bisexual male NB18 and its reciprocal than in the bisexual hybrid of the respective breeds (Ravindra Singh *et al.*, 1994).

Importance of artificial parthenogenesis in silkworm has been realized by most of the breeders and it acquires a special significance in the development of superior silkworm breeds. In conventional breeding, the offsprings receive only a random half of alleles from either of the parent; therefore, results cannot be predicted accurately specially in the case of low heritable characters (Seidel and Brackett, 1981). It may be possible to predict the results through application of new breeding strategies like parthenogenesis, androgenesis, cloning etc. in the development of outstanding genotypes possessing high hybrid vigour, combining ability, viabilility and less phenotypic variability (Strunnikov *et al.*, 1982; Strunnikov, 1986; Takei *et al.*, 1990; Shinbo *et al.*, 1991).

Parthenogenetic engineering

To accelerate the artificial parthenogenesis further, a number of new genetic approaches that require increasing levels of technological sophistication have been developed during the last few decades. Transgenesis has been recently put into practice in the silkworm (Klimenko, 2001). This deals with the genetic manipulation of individual cells by introducing not only foreign genes but in the same way, any organic and non-organic agents, cellular components, somatic or germ-line nuclei or cells at different stages of embryogenesis or in other ways specifically altering the genetic composition of those cells. In this context, the future prospects of artificial parthenogenesis through the application of transgenesis for several research directions are summarized below

Cloning in the silkworm (Nuclear transplantation): Now it is possible to produce genetically identical individuals by inserting the nucleus of one cell into another, before or after destroying the original genetic complement. These occur by separation of embryos or parts of embryos early in the development but well after fertilization has occurred. But it is true that egg can be activated rather than fertilization by an effective activator and therefore, nuclear transplanted unfertilized egg may be activated to make the genetic copies of any outstanding genotype and all the basic problems of transnuclear cloning in mammals could be studied using *Bombyx mori* L., with any silkworm genotype (not only female as in parthenocloning), in principle, being cloned (Klimenko, 2001).

Artificial insemination: Microinjection of mature spermatozoa or spermatocytes (at different stages of differentiation) into the unfertilized eggs with a subsequent egg activation of the meiotic type (for example, $-11^{\circ}C$ for 30 min) can be used for direct artificial insemination in insects and also to approach some problems of insemination and fertilization in general (Klimenko, 2001). With this technology, continued use of valuable sire after his death is possible and can be served as a convenient experimental system in silkworm to realize the importance of egg cytoplasm in relation the penetrance and expression of a gene in a deeper way.

Chimeras (Mosaicism): A chimera is an animal comprised of cell lines from a variety of sources. The occurrence of mosaicism is a well-know fact in the silkworm (Toyama, 1906; Ikeda, 1908; Takahashi, 1914; Tanaka, 1916; Yagi, 1924; Tamazawa, 1977a, b; Ravindra Singh *et al.*, 1990). Chimeras can be formed artificially by fusing two or more early embryos or by adding extra cells to blastocytes. Astaurov thermoactivation might result in fusion of recipient and donor diploid female pronuclei and formation of a tetraploid (the donor is *B. mori*) or an amphidiploid (if the donor is a different species) cleavage nucleus. Polyploid of increasing degree could be obtained with this technique and could be useful to provide a genetic tool for better understanding of development and maternal-fetal interactions.

Hybridization: To expand the opportunities of hybridization in insects, microinjection of spermatozoa of one species into the unfertilized eggs of another species followed by artificial egg activation could be exploited to overcome the inability to perform interspecific crosses (Klimenko, 2001). The approach could be valuable in preserving endangered species, or to learn more directly about the evolutionary relationship between genes/chromosomes, etc. in closely related species (Klimenko, 2001).

Chromosomal substitution in the silkworm: Chromosomal substitution is a technique generally employed in breeding to substitute only one pair of homologous chromosomes at will for a corresponding chromosomal pair taken from a different breed or even species. An example of such breeding is provided by the introduction into the genome of wheat of a small chromosomal segment of *Aegilops* along with gene for resistance to rust (Sears and

Rodenhiser, 1948). Artificial meiotic parthenogenesis permitted us to produce predominantly homozygous male from its heterozygous mother and strictly homozygous male by chromosomal substitution in the domesticated silkoworm of the 28th autosomal pair (aa) recessive for the homologous autosomes (AA) of the wild silkworm, *Bombyx mandarina* Moore was reported by Strunnikov (1983). Sometimes, recombination of chromosomes by substitution of its homologue may result in a reduction of quantitative characters of economical value due to the presence of harmful gene, along with useful non-additive gene as well. Such a problem can be overcome by translocation technique and is possible for silkworm also. With this chromosomal substitution technique, the actual role played by all genes contained both in the displaced and the introduced chromosomal pair can be analyzed deeply.

Recombinant DNA: The ability towards parthenogenesis varies among the different breeds and its occurrence is controlled genetically (Murakami, 1988; Takei *et al.*, 1990; Ravindra Singh *et al.*, 1994). The introduction of foreign DNA which is responsible to induce higher parthenogenetic ability from a corresponding race (for example Cambodge) by recombinant DNA technology into an economical viable breeds of less parthenogenetic ability is of special interest in this regard. But prior to exploitation of recombinant DNA technology in animal breeding, it is essential to identify gene loci on chromosomes, *i.e.*, genetic mapping. However, the mechanics of changing the DNA molecules of farm animals directly have not yet been worked out. Although the plasmid methods used in bacteria may not be applicable, but with the advanced knowledge in genetics and by the help of modern sophisticated technology it can be assumed that the stabilization of superior breeds along with ability to complete parthenogenesis may be successful in near future.

Conventional properties of farm animals, such as their rate of growth and other commercial metric traits, have been manipulated by selected breeding for thousand of years, resulting in the specialized breeds and bear little resemblance to their ancestors. For example, due to continuous domestication and efforts to evolve superior breeds with desirable traits by conventional breeding make the domesticated silkworm, *B. mori* (n = 28) different form its ancestor, *B. mandarina* (n = 27) genotypically. Against this background, the novel aspect of transgenic technology is the ability to introduce additional genes, form other sources and, therefore, an individual can be used as a source of a variety of useful products. Experimental deactivation of freshly inseminated eggs and artificial reactivation of unfertilized eggs clearly depicts the future prospects of artificial parthenogenesis to further study the mechanism of artificial activation of the egg in combination with microsurgical delivery of different genetic, nuclear and cellular material which in turn to be known as parthenogenetic engineering and will allow us to make mature oocyte a cytogenetic reactor for creating and cloning a wide spectrum of genotypes (Klimenko, 2001).

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