Introduction

Parthenogenesis (‘virgin origin’) is invariably the development of unfertilized ovum. Such a development of the ovum without fertilization is called parthenogenesis and the individuals that reproduce by this method are called parthenotes or parthenoclones. 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 the mulberry silkworm, *Bombyx mori* L. (Hasimoto, 1934; Astaurov, 1936, 1937). Two father’s hybrids have also been obtained by inseminating two genetically different males in a female (Strunnikov, 1958).

Transfer of hereditary attributes from generation to generation takes place during the process of sexual propagation and is the predominant mode of reproduction in eukaryotes. However, in several insect orders, parthenogenesis is a normal mode of reproduction except Odonata, Dermaptera, Neuroptera and Siphonaptera (Chapman, 1982). In almost all breeds of the silkworm, spontaneous parthenogenesis is rare and in normal condition, undergoes a limited number of cleavages; quite rarely develops up to the caterpillar stage (one case in $10^{5-6}$) and seems to be rudimentary (Tazima, 1964). Various strategies of reproduction such as parthenogenesis, androgenesis, gynogenesis or polyploidogenesis can be induced in silkworm by artificial manipulation (Astaurov, 1967).

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 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 female egg laying apparatus strongly deforms an egg and causes activation through extension of the egg membrane and performs the activating function. Then the male nuclear material fuses with the female pronucleus (Went, 1982). In case of natural gynogenesis (some fishes), 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 have been proved to be essential for the activation of the egg and established that sperm penetration inside the egg is not always the cause of its activation. 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 and magnecium, organic acids such as butyric acid, lactic acid and oleic acid, fat solvents namely, toluene, ether, alcohol, benzene and acetone 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 mulberry silkworm by means of diverse physico-chemical stimuli such as various acids, alkali, electric stimuli and hot water has first been demonstrated in 1886 by a Russian zoologist, Tichomirov (as reviewed by Astaurov, 1957). Many experimental treatments have since been shown empirically to attain this goal such as action of acetic acids, hydrochloric acids, diaphenol, potassium permanganate, hydrogen peroxide, ammonia, oxygen, low temperature, alternating current and also by exposure to ionophoresis (Strunnikov, 1983). Astaurov (1940) was the first to design a suitable and reliable method of thermal parthenogenesis for activating silkworm eggs towards ameiotic parthenogenesis. Utilization of artificial parthenogenesis in the silkworm has been reviewed by many workers (Strunnikov, 1975; Choudhury, 1989; Ravindra Singh et al., 1997; Klimenko, 2001; Gangopadhyay et al., 2005). Recently, the genetic effects on parthenogenetic characters of the silkworm have been analyzed (Yongqiang et al., 2004) and the possibility of artificial parthenogenesis in the management of transgenic population of the silkworm has been put forward (Grenier et al., 2004).

In silkworm females are heterogametic (ZW) and there is no crossing over in ameiotic parthenogenesis. A controlled heat shock applied to unfertilized eggs can disrupt the metaphase I spindle and prevent the reductional division, giving rise to diploid female pronuclei identical to the mother’s genotype (Klimenko, 1982, 1990). Thus, parthenogenetic daughters are genotypically identical and stable clones can be derived from a given moth. The discovery of artificial parthenogenesis opened new methodical approaches to study 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 mentioned briefly as follows-
**High temperature:** (Induction of ameiotic parthenogenesis without reduction of chromosome number)

Tichomirov (1902, 1903) observed the effect of hot water for inducing artificial parthenogenesis in silkworm but he succeeded only in inducing the beginning of development. A decisive contribution on the study of artificial parthenogenesis was made in silkworm by Astaurov (1940). He found a very accurate method to obtain worms successfully at the extent of 82 % in certain egg batches. His method was 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 cold water. The results were influenced by other factors such as time passed between the extraction and heat treatment, temperature and humidity at which the eggs were conserved after the treatment, the age of the moth, portion of ovary from which the eggs were 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. Further, extensive studies were conducted by activating 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; Gangopadhyay and Ravindra Singh, 2004).

**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 as 'meiotic'. In all these cases, parthenogenetic progeny were mostly males. 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 proceeded synchronously, though
twice as slowly as in the normal. As a result, 5 h after activation a 'pronucleus' was formed,
as in the fertilized eggs, and three polar bodies later degenerated. The division of pronucleus
into two haploid nuclei (blastomeres) was completed 8 h after activation. The blastomeres
divided once or twice more and then started to fuse in pairs.

**Chemical and other agents**

Sato (1925, 1931) by treating 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 and Kawaguchi (1934) by
immersing deposited unfertilized eggs at 15 % HCl acid for 5 min induced parthenogenetic
development. Astaurov (1940) with the same technique obtained 222 larvae with 44 males
and 35 females and denied it as artificially induced, the acid only help to terminate the
diapause. It was later ascertained that HCl acid alone couldn’t induce complete
parthenogenesis unless combined with simultaneous heating. Sato's result was, therefore,
considered as a rare case of natural parthenogenesis with high number of hatched
individuals. Parthenogenetic activation in silkworm eggs was observed through preservation
of ovaries in liquid nitrogen, thawing and transplanting into 5th instar female larvae (Kusuda
et al., 1985). Lu (1994) has maintained several generations 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 Otsuka, 1983). Laser beam was also found effective 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 between 1 to 5 day(s) in response to the combined
action of low and high temperatures formed a new simplified technique for activating the
eggs towards parthenogenetic development. Attempts were made to activate the eggs through combined action of low and high temperature for induction of parthenogenesis (Sugai, et al., 1983; 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). High temperature has been proved to be a powerful agent for induction of parthenogenesis and maximum parthenogenetic induction has been observed at 12 h after removal of eggs from the body of the moth (Astaurov, 1967) however, the rate of parthenogenetic induction varied in different strains (Takei et al., 1990).

Silkworm is one of the earliest organism to which genetic principles were applied for enhancing its economic potential. The credit for introducing F1 hybrids with a clear demonstration of their superiority over parental genotypes goes to Toyama (1906) in Japan. Latter, several attempts have been made to analyze the practical significance of heterosis by means of introducing F1 hybrids through cross breeding strategies (Harada, 1961; Ravindra Singh et al., 1990, Nagaraju et al., 1996). Manifestation of heterosis in F1 hybrids is the result of elimination of the action of harmful recessive genes with totally dominant genes separately inherited from both parents and favourable effects of some alleles in the heterozygous state (Strunnikov, 1986). With increased heterozygosity, hybrid vigour can be realized when the offspring performs well above the average of their parents. A knowledge of the extent and magnitude of heterosis helps in the isolation of superior segregates.

Various crossing systems like diallel, line × tester, double and three way crosses were tried to know the genetic effects in the utilization of hybrid vigour in silkworm (Sengupta et al., 1974; Narasimhanna et al., 1976; Das et al., 1997). Extensive studies have
been carried out on the analysis of combining ability in the silkworm in order to select promising parents and hybrids (Satenaahalli *et al*., 1989; Subba Rao and Sahai, 1989; Rajalakshmi *et al*., 1997; Raghavendra Rao *et al*., 2002; Ravindra Singh *et al*., 2000; 2001; 2003; 2005).

In addition to the use of controlled hybridization, the improvement of silkworm breeds for silk production can be achieved by systematic and continuous selection (Miyahara, 1978). Performance of F1 hybrids depends upon proper selection of suitable parents and genetic divergence present between them (Ravindra Singh *et al*., 2003). Efforts aiming at improving the productivity and quality of silk have yielded a good number of inbred lines (Datta, 1984). Selection of suitable silkworm breeds / hybrids based on the multiple traits is useful in silkworm breeding (Narayanaswamy *et al*., 2002). Attempts have been made for the selection of superior polyvoltine breeds (Kariappa and Rajan, 2005), polyivoltine × bivoltine hybrids (Vidyumalata *et al*., 1998, Mal Reddy *et al*., 2002) and bivoltine silkworm breeds / hybrids (Rajalakshmi *et al*., 2000).

Inbreeding depression among the inbred lines is a major setback in maintaining and multiplying the pure parental stocks. Artificial parthenogenesis can facilitate to develop a line with restored diploidy of lower inbreeding depression and therefore, it is possible to establish a new colony from a single individual (Cuellar, 1977). The interrelation between the success of parthenogenesis and the characteristics of the silkworm parent 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). The desired type of silkworm either entirely females (completely heterozygous) or males (predominantly homozygous) can be produced by application of various methods of activation and can serve as a useful tool for controlling the sex of the offsprings (Strunnikov, 1975).
Cocoon size is an important parameter from the standpoint of silk production, development as well as evaluation of commercial hybrids (Nakada, 1994). Uniform cocoons enhance the performance of semiautomatic and automatic reeling machines to get uniform filament size (Mano, 1994). Ravindra Singh et al. (1998) have emphasized the importance of cocoon size uniformity in India. Recently, studies on cocoon size uniformity in polyvoltine × bivoltine hybrids of the silkworm tolerant to high temperature and humidity have been carried out (Umadevi and Raghavendra Rao, 2006).

Since, improvement of qualitative and quantitative characters in a desired direction is a continuous process, there is further scope to identify superior breeds / hybrids through combining ability studies. Exploitation of hybrid vigour to a greater extent depends on the homozygous nature of the breeds involved (Strunnikov, 1986; Nacheva et al., 1999). Artificial parthenogenesis has been employed to develop homozygous silkworm breeds to harness heterosis (Strunnikov et al., 1982; Strunnikov, 1986; Takei et al., 1990; Ravindra Singh et al., 1994, 2004). Mechanism of hybrid vigour through artificial parthenogenesis has been reported (Ohkuma, 1971). The present study has been undertaken to develop superior breeds / hybrids of the silkworm through the application of artificial parthenogenesis coupled with conventional breeding techniques with the following objectives-

- Identification of silkworm breeds / hybrids with high parthenogenetic ability.
- Development of homozygous silkworm breeds with parthenogenetic origin.
- Assessment of practical advantages of parthenogenesis like viability, heterosis, combining ability and phenotypic variability.