CHAPTER II: REVIEW OF LITERATURE
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Literature on sericulture is very extensive. Most of it is dealing with true silkworm, *Bombyx mori* Linnaeus. Reviews on the subject as it pertains to saturniids of commercial value were made by Watt (1908), Handschin (1946) and Arora and Gupta (1979). Braine (1904) and Seitz (1918) wrote books on saturniid silk culture in Sri Lanka and Germany, respectively, covering such diverse aspects as silkworm diseases, economic considerations, and even host plant cultivation. A few of the many French authors who has contributed in such field may be mentioned here such as Guérin-Méneville (1860), Fauvel (1895), Andre (1908), and Dusuzeau and Sonthommax (1897). Under his text on *Samia cynthia* (Drury) and *Antheraea polyphemus* (Cramer), Ferguson (1972) provided some good information and references about few attempts to establish silk industries based on saturniids in America.

The origin of the oak-based tasar silk production is documented in the People’s Republic of China at least to the Han Dynasty (206 BC – AD 220). Since then, this culture is so far the exclusive monopoly of the People’s Republic of China. The rearing of silkworm has been rationalized and year-old occupation by the people of China. According to historic records, domesticated Chinese oak silkworm was originated in the Province of Shandong, China about 4000 years ago (Liu et al., 2010). In 2005, about seven million kgs of cocoons (pupae) were produced in China, accounting for 90% of world production (Liu et al., 2010). This insect is also a well-known lepidopteran model system in studies of insect diapauses, photoperiod effects, and neuroendocrine regulation (Maida et al., 2000; Chang et al., 2003; Wei et al., 2008) due to its large size and pupal diapause. It is also used as a food source for human (larva and pupa) and the byproducts for the preparation of many cosmetic items.
Jolly et al. (1974, 1979) illustrated the importance of non-mulberry sericulture to the economy and culture of modern India. Yet the research on *A. proylei* and its breeds is in juvenile stage and the publication is very scanty as well. However, some reports on the study of *A. proylei*, other sericigenous insects and some allied studies have been reviewed in this chapter.

### 2.1 Morphological characterization

Singh and Singh (1988) studied the bionomics of *Antheraea proylei*. They described the characters of egg, different instars of larva, cocoon, pupa and adult. Apart from the genitalia, it is observed that the sexes may be distinguished externally by their antennae whose rami being longer in the male. Abdomen is more slender and pointed in the male. Further, the curvature of wings is more in case of males. Few morphological observations were also made by Jolly *et al.* (1974 and 1975) and Devi *et al.* (2011).

Non-mulberry moths found in India were reported by Arora and Gupta in 1979. They described 17 species of 10 genera on the basis of morphological characters such as antennae in both sexes, the shape of frons, the shape and the markings of the wings and their venation, the structures of tibial spurs, tarsal spines and male genitalia. The authors remarked that the species can be recognized by the distal ramii on the antennae. The morphological study of *Antheraea frithi* and its taxonomic traits like follicular imprint, larval body marking, number and arrangement of tubercular setae, body hair, cocoon characteristics and wing venation were studied and concluded that *A. frithi* is an intermediate form in between *A. mylitta* and *A. sivalika* on one side and *A. pernyi* and *A. roylei* on the other side (Jolly *et al.*, 1974; Jolly and Sen, 1973).

Ylla *et al.* (2005) conducted a phylogenetic analysis of 16 moon moth species using morphology, molecular biology and behaviour which was comprised of 93 characters from the larvae, pupae, cocoons and adults in all ingroup species and two outgroup species. Molecular data included 2662 nucleotides from elongation factor I-alpha (EF-α) and dopa decarboxylase (DDC) protein coding nuclear genes of six ingroups and the two outgroups. The total evidence analyses including all characters revealed the following generic relationship: (outgroups (*Argema* (*Graellsis* + *Actias*))). Character evolution indicated that the short hindwing tail evolved once and lengthened...
multiple times in different lineages of moon moths. Results supported retaining *Graellsis* as a genus separate from *Actias*.

According to Jolly and Sen (1969), the egg chorion carried distinct patterns of follicular imprints. Rebel (1925) and Döring (1955) demonstrated that chorionic sculpturing on lepidopteran eggs is taxonomically significant, *i.e.*, is species-specific, as are of shape and size. Their studies relied on light microscopes for magnification. The studies of Downey and Allyn (1981) using scanning electron microscopy (SEM) left no doubt that the chorion is complex in structure and is of considerable value in phylogenetic studies. Peigler and Stephens (1986) described and illustrated eggs of six species of *Attacus* and two allied genera using SEM.

Kawakami *et al.* (1980) studied the surface structure of the egg shell in *Antheraea yamamai* and *Antheraea pernyi* under the SEM and reported that the line structure of these two moths are very similar, and consists of two rings of the petal-like pattern around the micropyle. At the surface other than the surroundings of micropyle (general portion), the egg shell is composed of polygonal sectional part divided by the band-like structure, aeropyles are observed to be covered with the barnacle-like processes (*A. yamamai*) or the tulip-like processes (*A. pernyi*). Changing courses of structure are observed between the surroundings of micropyle and the general portion.

Attempts to classify the order Lepidoptera on the basis of the arrangement of tubercles and their setae were made as early as 1895 by Dyar. Forbes (1916) added some more information on the arrangement of tubercles of *A. mylitta*. Narashimhanna *et al.* (1969) attempted to study the tubercular setae in *Antheraea* species. The shape, number and arrangement of tubercular setae in eight *Antheraea* larvae of particular taxonomic interest have been described by Jolly (1981). Peigler (1989) reported that the coloured patch on each anal proleg is a very conspicuous marking and apparently specifically different among members of the genus *Attacus*.

Larval body colour changing pattern, tubercle characteristics, egg characteristics as well as silk characters are the important biological parameters to study the wild silkmoth *Attacus atlas* L. (Saikia and Handique, 1998). Nässig and Ragus (2001) described the larval morphology of *Loepa miranda* Atkinson in Moore
The larva differs from the other Loepa species of which the larval morphology is known in the first two instars by the polychromatic colouration and pattern (orange red on thorax and at the near end, whitish in the middle, all segments with intensive, fine black pattern). In later instars, the lateral patches are the largest of all species known so far. They also provided a hypothesis about the evolution of these camouflage patches.

Siddiqui et al. (1998) studied the phenotypic and genotypic variability in six ecotypes of Philosamia ricini for absolute silk yield and their contributing traits. A wide range of phenotypic variability was found in traits like larval weight, cocoon weight and shell weight. The associations between different economic characters of Antheraea assamensis cocoon in different commercial broods show the correlation and regression among different traits. Moreover, correlations between different variables are of great help in predicting the performance in different traits (Yadav and Goswami, 1999). In case of saturniid cocoons, it is revealed that there is a direct correlation between pupal weight and fecundity (Barah and Sengupta, 1991).

The morphology and anatomy of antennae, wing and genitalia of Agromyza obtusa was studied as taxonomic characters by Pandey (1962). The morphology and histology of antennae of Eri silkmoths (Philosamia ricini) as well as various receptors on the male and female antennae bears biological importance. Besides Johnson's organ, the sensilla, long trichoid sensilla which were absent in female are the most important characteristics of male antennae as reported by Eid et al. (1990). The sexual dimorphism in antennae is sometimes associated with the occurrence of different sense organs in Bombyx mori. The antennae of male are more complex. The dimorphism has always been considered to be an expression of the 'need' of the male to have relatively more sensilla to find his partner (Scheider, 1964). The morphology of third olfactory receptor cell of short hairs of sensilla trichoidea in Antheraea polyphemus and Antheraea pernyi and the third receptor cells of male of both the species were the factor of species isolation by which interspecific differences were evaluated (Caihong and Bestmann, 1992).

Through the study of sexual dimorphism, wing span, external morphology of the silkworm, Anaphe venata Butler (Lepidoptera: Notodontidae) in adult form could
confirm the species status (Ashiru, 1990). Nässig (1991) attempted the new morphological aspects of Antheraea Hubner and reclassified the taxonomic and phylogenetic revision of all taxa within genus Antheraea frithi and its taxonomic status with other Antheraea species in repeat of follicular imprint, larval body marking, number and arrangement of tubercular setae, body hair, cocoon characteristics and wing venation.

Bulcázar-L and Vázquez-G (1994) reported a new sub-species of Antheraea polyphemus: Antheraea polyphemus tuxtalensis from Mexico in which morphological characters are differed from other sub-species of A. polyphemus. The characters are crenulate tegmen, principally that of the hindwing; black ring of the eyespot in hindwing extends basally across the middle of the discal cell, as opposed to that of the forewing; a blue ‘crescent’ of strewn scales present on the eye spot on the ventral side of forewing; apical angles of both of wings falcate; yellow ground colour.

2.2 Rearing performances

Several studies have been conducted to evaluate the superior breeds and promising ones are selected for commercial exploitation and for further breeding programmes (Singh and Rao, 1993; Raju and Krishnamurthy, 1993). Among the various evaluation methods used in silkworms, Multiple Trait Evaluation Index (E.I.) method of Mano et al. (1993), has been extensively used for selection of superior breeds and hybrids (Begum et al., 2000; Rajalakshmi et al., 2000; Singh et al., 2001; Kariappa and Rajan, 2005; Rao et al., 2006; Singh and Kumar, 2008; Begum et al., 2008; Lakshmanan and Suresh Kumar, 2012; Lakshmi et al., 2012; Devi et al., 2013).

Kumaresan et al. (2007) studied the genetic variation and genetic diversity among fifty-eight polyvoltine silkworm genotypes of Bombyx mori by using ten economic traits. It revealed that the single shell weight showed higher genetic variation such as Phenotypical Coefficient of Variation, PCV% (17.20%), Genotypical Coefficient of Variation, GCV% (12.93%) and heritability (56.5%) followed by single cocoon weight, shell ratio and matured larval weight. The D² (Mahalanobis’ distance) statistics revealed nine clusters with substantial inter- and intra- cluster distances. The genotypes included in different clusters varied from 1 to 16. The genotype Pure Mysore
was included in isolated cluster which indicates its longer adaptation. They reported that the genotypes included in cluster VIII and IX showed optimum genetic distance along with cluster mean emphasized for utilization in the silkworm breeding programmes.

Hussain et al. (2010) carried out a study to calculate the ranking of eleven pure silkworm lines for breeding and cocoon production. It was calculated using the Multiple Trait Evaluation Index Method of Mano et al. (1993). On the basis of the index values M-101(57.55), PAK-3(52.22), PFI-2(52.04), PFI-1(51.14) and PAK-4(50.52) were identified as good performer for various economically important traits and were selected for field trials, hybridization and other breeding programmes. The traits used by them for evaluation were fecundity, cocoon yield, pupation rate, cocoon weight, shell weight, shell ratio, filament length, raw silk (%) and neatness.

Pal and Moorthy (2011) studied variability in larval and cocoon traits in 19 genotypes of bivoltine silkworm, *Bombyx mori* L. Characters like larval weight, silk gland weight, cocoon weight, shell weight and shell ratio have shown higher variability than the other characters. Significant and high positive correlation was observed between larval weight, silk gland weight and cocoon weight. Both larval body length and cocoon length has positive correlation with shell weight. Silk gland weight also has positive correlation with larval weight, cocoon weight and shell weight. The clustering using Euclidean distance reflects differences of the origin and traits associated with them.

2.3 Cytogenetical characterization

It can be noted that the comparative karyology has some obvious advantages over other methods used in taxonomic studies of insects and other animals. In particular, chromosomal characters are essentially morphological, and therefore they can be analysed in approximately the same way as other morphological features. Moreover, some characters of the karyotype (such as the number of chromosomes, chromosome arms, nucleolar organizers, and heterochromatic blocks) can possess only discrete values, allowing one to recognize easily most cases of the intraspecific chromosomal polymorphism as well as hybridization between forms with different chromosome numbers. Finally, methods of chromosomal analysis are relatively inexpensive and
allow vast material to be examined in a short space of time. Thus, they represent promising methods of screening in both laboratory and natural populations of insects.

One of the most common reasons for chromosome study is to determine the identity of organisms (de Melo et al., 2000). Cytogenetic analysis has contributed greatly to the studies of phylogeny, speciation mechanisms and genetic variability of many plants and animal group. Chromosomal analysis can be used to reveal and identify sibling species, as well as to identify immature phases of insects. Studies of insect chromosomes may reveal cases of hybridization between forms with different karyotypes (Gokhman and Kuznetsova, 2006). White (1973) emphasized the use of studying the chromosome of insects since cytogenetics has contributed to insect systematics in several ways as species which are not always morphologically differentiated, can be ascertained as distinct species when difference is noted in their karyotype.

The studies on chromosomes of the silkworms have been attempted, but the identification was limited to only several chromosomes because of the chromosomes’ features, coarseness of the preparation technique and the lack of appropriate materials for preparation (Traut, 1976). In fact, the schematic standard index for the characterization of the silkworm has not been yet suggested. Due to the dot-shaped mitotic metaphase chromosomes, several researchers have been tried the preparation using meiotic pachytene chromosomes that are relatively long and obtaining the meiotic cell specimen has been one of the more difficult tasks for well spread preparation (Friedlander and Wahrman, 1970; Luciani, 1975; Traut, 1976; Hwang et al., 2004).

Meiosis, a highly conserved process in eukaryotes, plays a central role in the life cycles of all sexually reproducing organisms. The meiotic studies of the genus which is wild or extensively cultivated for various economic purposes are thought to be desirable because the analysis of chromosomal associations (especially the pairing behaviour of chromosomes) during meiosis provides sufficient information on the mechanism of evolution and potentials for genetic recombination present in the species (Sinha and Sinha, 1977). Chromosome number, karyotype and meiotic pairing behaviour at metaphase-I in hybrid species can provide useful information for the assessment of taxonomic relationship (Stace, 2000).
Study of karyology of silk moths belonging to the order Lepidoptera has been carried out by various authors (Dederer, 1907, 1915; Friedländer and Wahrman, 1970; Narang and Gupta, 1979; Gupta and Narang, 1981; Ibotombi et al., 1991; Daimon et al., 2012; Packiam, 2013). They have laid the foundation of lepidopteran cytogenetics. The chromosomes of lepidoptera species are small, numerous and uniform in both shape and size (Robinson 1971; Bedo, 1984). One of the most important cytogenetic characteristics of lepidoptera is the holokinetic nature of their chromosomes. Holokinetic organization in lepidopteran species was claimed by many (Suomalainen, 1953; Virkki, 1963; Barry et al., 1967; Bauer, 1967; Emmel and Trew, 1973; Emmel et al., 1973; Maeki, 1980; Murakami and Imai, 1974; Ennis and Sohi, 1976; Mitsuhashi, 1995; Traut, 1986). Wrensch et al., 1994 reported that the holokinetic nature of chromosomes is supposed to facilitate chromosomes fusions and fissions.

Among saturniids, the haploid chromosome number is lowest in Philosamia cynthia (n = 13) (Dederer, 1907, 1915; Traut and Mosbacher, 1968) and highest in Antheraea pernyi (Kawaguchi, 1933; Jolly et al., 1970) and Antheraea proylei with a value of n = 49. The modal chromosome number of the family Saturniidae is n = 31, which is mostly found in Antheraea species. Dederer (1907, 1915); Narang and Gupta (1979) described the meiotic cycle of Philosamia cynthia. The achiasmatic mechanism, a characteristic of Philosamia cynthia ricini has been reported by Narang and Gupta (1979). The sex chromosome mechanism in Philosamia cynthia is XX♂ : XY♀ (Traut and Mosbacher, 1968; Ennis, 1976) and XX♂ : XY♀ and XX♂ : XY♀ in the Titabar and Borduar-Dhanubhanga populations respectively (Narang and Gupta 1979; Gupta and Narang, 1980).

Gupta and Narang (1981) worked on cytogenetics of A. compta, a wild silkworm and A. assamensis, a semi-domesticated silkworm, which produces golden-hued muga silk. At mitotic metaphase, the chromosomes were spherical and rod shaped. Presence of two pairs of satellite chromosomes has been reported in both the species. However, primary constrictions (localized centromeres) were observed in the chromosomes of A. compta. The sex chromosome system was found to be XX♂ : XY♀ in A. compta and XX♂ : XO♀ in A. assamensis.
2.4 Molecular characterization

Assessment of genetic variation among silkworm is useful for predicting potential genetic gain in a breeding programme and setting up appropriate cross breeding strategies. Traditionally, morphological and phenotypic characters have been used for this purpose. In recent years, DNA markers are considered to be the most common means for measuring genetic diversity between individuals or within related species or population because of their abundant polymorphism and the fact that they are independent of environmental conditions (Behura, 2006).

DNA fingerprinting, first described by Jeffreys et al. (1985), is now commonly used to study genetic variability and to analyse pedigree relationships in a wide variety of organisms including insects (Nybom, 1991; Blanchetot and Gooding, 1994; Dallas, 1988; Georges et al., 1988). Several investigations have also been established using various molecular techniques to analyse the silkworm (Nagaraju et al., 2002; Nagaraju and Goldsmith, 2002; Chatterjee and Pradeep, 2003; Chen et al., 2003; Lu et al., 2003; Cheng et al., 2004; Lu et al., 2004; Miao et al., 2005; Goldsmith et al., 2005; Mirhoseini et al., 2007).

DNA-based molecular markers such as random amplified polymorphic DNA (RAPD) (Nagaraja and Nagaraju, 1995; Xia et al., 1998; Lu et al., 2002), amplified fragment length polymorphism (AFLP) (Lu et al., 2001), restriction fragment length polymorphism (RFLP) (Sethuraman et al., 2002), simple sequence repeats (SSR) (Shen et al., 2004; Li et al., 2005) and inter simple sequence repeats (ISSR) (Chatterjee and Mohandas, 2003) have been widely adopted in silkworm genetic diversity studies. In general, these studies provided the positive information to enhance the understanding of silkworm phylogeny.

ISSR has already been used in numerous organisms for genetic characterization (Reddy et al., 1999; Cano et al., 2005) to assess genetic diversity (Qiu et al., 2004; Wang and Wang 2005; Lu et al., 2006 and Zhang et al., 1994;), to identify genetic trait loci (Zietkiewicz et al., 1994; Ratnaparkhe, 1998; Blair, 1999 and Arcade et al., 2000) and for understanding phylogenetic and/or interspecific relationships (Wolfe and Liston, 1998; Reddy et al., 1999; Wolfe and Randle, 2001; Wu et al., 2005). Several families of
Lepidoptera have been investigated using ISSR markers: Noctuidae, Pyralidae, Pieridae and Sphingidae (Lunque et al., 2002; Hundsdoerfer and Wink, 2005).

Reddy et al. (1999) analyzed thirteen diverse strains of silkworm using ISSR-PCR where strain specific pattern was shown to be inherited and segregated in a Mendelian fashion. The dendrogram revealed two distinct groups, one comprising non-diapausing and another comprising diapausing strains. The results suggested that the ISSR method is potentially useful for genetic fingerprinting of silkworm genotypes and as a mapping tool for the silkworms. The fact that ISSR method resolves diapause and non-diapause strain-specific amplification products makes it useful to augment the marker resources in the silkworm genome mapping programme. The strain-specific profiles and pattern similarity within the strains make this method invaluable in addressing problems involved in breeders’ rights, genetic homozygosity of the strains, marker-assisted breeding and cross-breeding strategies.

Nagaraju et al. (2001) dealt with DNA fingerprinting assays in estimation of genetic diversity within and between populations. He used RFLP, RAPD, ISSR and SSR for genetic characterization and examined 13 diverse silkworm strains. All four approaches successfully discriminated the 13 silkworm varieties but differed in the amount of polymorphism detected. The usefulness of each system was examined in terms of number of loci revealed and the amount of polymorphism detected. The RAPD, ISSR-PCR and RFLP assays clearly separated the diapausing and non-diapausing silkworm varieties. These results were discussed in terms of choice of appropriate marker technology for different aspects of silkworm genome analysis. RAPDs have been used to examine genetic variation in both geographically distinct ecotypes and highly inbred lines of silkworm accessions. The study, interestingly, resulted in diapausing and non-diapausing genotype specific PCR products. The dendrogram based on data from amplification using 40 oligonucleotides in thirteen highly divergent silkworm genotypes was consistent with the known geographical and breeding history of silkworm populations.

Kar et al. (2005) studied the genetic diversity in the wild and semi-domesticated populations of Daba ecorace of *Antheraea mylitta* to ascertain the distribution of variability within and among population of semi-domestic bivoltine
(DB), trivoltine (DT) and nature grown wild population (DN) with inter-simple sequence repeat (ISSR) markers. For the individual populations, the percentage polymorphism was observed higher in the wild populations in comparison to the bivoltine and trivoltine semi-domestic populations. From the dendrogram, considerable intra and inter-populations was found in all three populations. The population structure analysis further suggested that the semi-domestic population of Daba ecotype are at the threshold of differentiating themselves. The Daba population of *A. mylitta* is of much importance for conservation as well as utilization in systematic breeding programme.

Vijayan *et al.* (2006) studied with DNA fingerprinting to detect genetic variation in different insect species not only between populations, but also between individuals within population. ISSR system was used to assess genetic diversity and differentiation among six commercially exploited *S. cynthia ricini* populations. Twenty ISSR primers produced 87% of inter population variability among the six populations. Based on genetic diversity, these populations were considered as different ecotypes and *in situ* conservation of them were recommended. These studies explicitly reported the importance of repeats and transposable elements in the molecular phylogenetic analysis of domesticated and wild silkworm.

Arunkumar *et al.* (2006) derived the molecular phylogeny of *A. proylei*, *A. roylei*, *A. pernyi*, *Theopilia religiosa*, *B. mandarina* (Chinese and Japanese) and *B. mori* (Nistari and ND4D2) using the three mitochondrial genes, 12S rRNA, 16S rRNA and COI and the control region. Maximum analyses showed two distinct clades, one consisting of moths from Bombycidae family and the other from Saturniidae family. The maximum likelihood analyses for complete mitochondrial genome sequences of *B. mori* (strains Aojuko, C108, Backokjam and Xiafang), Japanese and Chinese strains of *B. mandarina* and *A. pernyi* revealed two distinct clades, one comprising of *B. mori* strains and other with *B. mandarina* and *A. pernyi* forming an outgroup. Pairwise distances revealed that all of the strains of *B. mori* studied are closer to Chinese strains than to Japanese *mandarina*. They concluded that the wild species of Bombycidae family, *T. religiosa*, whose phylogenetic status was not clear, clustered together with the other bombycid moths. They also reported that analysis of the interspecific hybrid, *A. proylei* gave evidence for paternal inheritance of mitochondrial DNA.
Velu et al. (2008) used amplified Inter simple sequence repeats (ISSR) markers to determine genetic relationships among mutant silkworm strains of *Bombyx mori*. A total of 113 markers were produced among 20 mutant strains of which 73.45% were found to be polymorphic. The dendrogram illustrated the phylogenetic relationship among 20 mutant silkworm strains forming one major cluster containing 6 sub clusters showing that all the strains originated from same origin and similar voltinism as they belong to bivoltine. The use of ISSR markers revealed highest polymorphism (73.45%) with the length of band ranging from 250 to 3,000 bp and the number of band was 3-14, which indicated the available range of heterozygosity in mutant strains. The study indicates that ISSR markers can be effectively utilized to analyze phylogenetic relationship and heterozygosity in silkworm.