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Molecular Marker Systems with special reference to the Silkworm *Bombyx mori* L.

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Abstract: Study on genetic diversity is critical to success in any crop breeding and it provides information about the quantum of genetic divergence and serves a platform for specific breeding objectives. Genetic diversity is a particular concern because greater genetic uniformity in silkworm can increase vulnerability to pests and diseases. Hence maintenance of genetic diversity is a fundamental component in long term management strategies for genetic improvement of silkworm which is cultivated by millions of people around the worlds for its lustrous silk. In view of the present study, genetic diversity studies carried out in silkworm using divergent methods (Quantitative traits, biochemical and molecular markers) and present level of diversity, pertaining to the literature has been reviewed. Genetic diversity is the genetic variation within species, both among geographically separated populations and among individuals within a single population. Genetic diversity is an essential aspect in conservation biology because a fundamental concept of natural selection states that the rate of evolutionary change in a population is proportional to the amount of genetic diversity present in it. Decreasing genetic diversity increases the extinction risk of populations due to a decline in fitness. Therefore, both biochemical and molecular markers have recently been employed to estimate the extent of genetic diversity present among various types of silkworm strains such as mono-, bi and multivoltines present in China, Japan, Korea, India, and several other countries.

Key words: Diversity; Breeding; Quantitative traits; Biochemical; Molecular markers

Introduction

Bombyx mori L., commonly recognized around the world as the mulberry silkworm, is characterized by a wide variability in yield and developmental traits, which have been proven through conventional genetic analysis to be of polygenic nature. Its rich repertoire of genetic resources and potential applications in sericulture and as an ideal model for biomedical research led to the initiation of genomic research.

In India, the heritage of rearing various species of silkworm, belonging to the families Bombycidae and Saturnidae, is as ancient as the Indian culture. Even today, all aspects associated with the production of silk yarn and fabrics are essential components of the rural economy in India (Datta, 1992). Globally, India is the second largest producer of silk yarn (Oommen, 2001) and most of it is produced from the low yielding multivoltine Indian races of *Bombyx mori* and (or) F_1 hybrids raised from these multivoltine and high yielding bivoltine silkworm breeds (Data 1984; Chatterjee 1993).

Today, the survival of this industry depends on increasing quantitative and qualitative production and diversification of silk products. Silk production will be increased if we use scientific rearing principles and genetic/ breeding knowledge. There are more than 3,000 silkworm lines in the world (Nagaraju and Singh 1997). In spite of such a wide silkworm genetic base, information is hardly available either on the unique features of many of these genotypes, or on the extent of genetic diversity between or within the genotypes, which in turn limit their use in the production of elite stocks for the benefit of improving quality and productivity of silk. Many genotypes have same phenotype in spite of unique genetic characteristics or the same genotypes are having different phenotype under different ecological conditions. Such similarities and differences cause problems for silkworm breeders when choosing parental genotypes for cross breeding programmes (Nagaraju and Singh, 1997). Hence, it is important that the vast genetic resources of silkworms available in different

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countries are genetically characterized and optimally used for synthesizing elite genotypes to upgrade the productivity levels in the sericulture industry. In addition, precise characterization of genotypes is extremely important in sericulture. To date, new varieties are usually described on the basis of their morphological characteristics which, as stated earlier are highly variable and environmentally dependent, thus requiring reliable techniques for genotype characterization. These limitations call for harnessing the recent developments in molecular biology for rational utilization of silkworm genetic resources and such an attempt is bound to enhance our ability to gain deeper insights into the genome of this economic insect. Thus, using DNA markers in silkworm breeding can create a new theme in sericulture section and can pave ways for molecular breeding programmes in future, as they may allow for genome based characterization, which can provide more accurate and detailed information that classical phenotypic data cannot reveal. Once, the DNA markers linked to the characters of interest are identified, they could serve as useful molecular tags to follow the characters via markers in silkworm improvement programme.

The difference in the genetic makeup of individuals has led to the identification and development of molecular markers. A molecular marker is a gene or DNA sequence with a known location on a chromosome, or a protein sequence that can be used to identify individuals or species. With the advent of molecular markers, a new generation of markers has been introduced over the last two decades which has revolutionized the entire scenario of biological sciences. DNA-based molecular markers have acted as versatile tools and have found their own position in various fields like taxonomy, physiology, embryology, genetic engineering, etc. Ever since their development, they are constantly being modified to enhance their utility and to bring about automation in the process of genetic and genomic analysis. Insects possess a vast undiscovered genetic diversity and gene pool that can be better explored using molecular marker techniques. Current trends of application of DNA marker techniques in diverse domains of insect studies show that mitochondrial DNA (mtDNA), microsatellites, Random Amplified Polymorphic DNA (RAPD), Inter-Simple Sequence Repeats (ISSRs), Expressed Sequence Tags (ESTs) and Amplified Fragment Length Polymorphism (AFLP) markers have contributed significantly towards understanding genetic basis of insect diversity and for mapping economically and medically important genes and quantitative trait loci in insects. Besides, whole genome microarray and Single Nucleotide Polymorphisms (SNPs) assays are becoming more popular to screen genome-wide polymorphisms in fast and cost-effective manner (121).

Marker Systems with special reference to Silkworm *Bombyx mori* L.

With reference to mulberry silkworm, *Bombyx mori*, more than 400 visible mutations have been placed in the linkage map (Doira, 1992) which represent 217 loci consisting of mostly morphological and a few enzyme markers. The number of loci mapped so far is insufficient for a thorough understanding of the genome and for the analysis of the quantitative trait loci (QTLs) for important commercial characters in silkworm. Hence, the development of DNA based genetic markers in silkworm was initiated in the 1990s and preliminary linkage map using RFLPs (Shi *et al.,* 1995) and RAPD map of 169 loci (Promboon *et al.,* 1995) were constructed. Later, a dense genetic map of silkworm covering all chromosomes based on 1018 RAPD markers was published (Yasukochi, 1998). Later, an AFLP map of the silkworm was constructed with 356 markers (Tan *et al.,* 2001). A genetic linkage map using 518 SSR markers was also established for *Bombyx mori* (131). Thereafter, to build a foundation for the complete genome analysis of the silkworm, *Bombyx mori*, EST database was constructed (Mita *et al.,* 2003) covering about 55% of all the genes of silkworm. A linkage map for the silkworm *Bombyx mori* was constructed based on SNPs (134). The EST data of silkworm derived from the cDNA libraries has made it possible to mine SNPs from silkworm genome (137).

With regard to genome analysis in Indian silkworms, application of PCR-based markers, RAPD markers, and also DNA fingerprinting with minisatellite probes have been taken up to study the DNA profiling of silkworm genotypes (Nagaraja & Nagaraju, 1995; Nagaraju *et al.,* 1995). DNA profiling of silkworm has also been carried out using microsatellite markers, which has shown breed-specific profiles for 15 silkworms, clearly indicating the prospects of microsatellite markers for establishment of molecular Identities for distinguishing silkworm breeds (Sreekumar *et al.,* 2006). Genetic characterization by simple sequence repeats (SSR), inter-SSR (ISSR) have been taken up in 13 silkworm strains (Reddy *et al.,* 1999a, 1999b; Nagaraju *et al.,* 2001). It has been demonstrated that informativity, sensitivity and speed of the ISSR-PCR can be substantially enhanced by adding fluorescent nucleotides in the PCR mixture and the method has been termed as FISSR-PCR (Nagaraju *et al.,* 2000a, 2000b). In another study, genetic diversity among 31 silkworm strains was revealed by microsatellites (123). Molecular evaluation of bivoltine, multivoltine and mutant silkworms have been carried out using RAPD, ISSR and RFLP-STS markers to study the genetic relatedness (113). These studies have revealed distinct and unique profile that was specific to bivoltine and polyvoltine strains. The results have indicated their

potential use not only in understanding genetic relationships but also as powerful tools to generate markers that are linked to the traits of interest in silkworm.

With regard to improvement of yield attributes, marker systems have been extensively used in silkworms. RFLP analysis in Indian silkworm stocks has revealed the close association of six RFLP markers with high shell and two markers with low shell characters (Sreekumar *et al.,* 2000). DNA fingerprinting studies to search for markers associated with yield attributes in the silkworm using AFLP markers were taken up by Duverney *et al.,* (2006) (118). AFLP molecular linkage maps with a relatively high density for location of QTLs controlling the quantitative traits of silkworm cocoons were constructed using AFLP markers (123-Lu, 2004; 112,160).

Among the DNA markers, particularly PCR-based markers like RAPD were being used for genetic analysis of plant and animal genomes. RAPD analysis has several advantages over other DNA markers. These includes relatively shorter time (1-2 days) required to complete analysis after standardization; no need of prior information on the genome of an organism; availability of series of primers for analysis; minimal operational cost requirement; relatively smaller amount (=20ng) of high molecular weight DNA; simpler protocol and involvement of non-invasive sampling for tissue analysis. RAPD-PCR technique can generate species-specific fragments. These fragments are useful in developing specific 'Sequence Characterized Amplified Region' (SCAR) markers. In contrast to most RFLPs, RAPD markers are usually scored as dominant alleles since a RAPD is present only in one of the parents and amplified in the heterozygote. RAPD markers have enabled a significant advance in the ability to generate linkage maps quickly. Linkage maps of RAPD markers have been reported for Arabidopsis (Reiter *et al.,* 1992), bananas (Faure *et al.,* 1994), lettuce (Kesseli *et al.,* 1994), eucalyptus (Grattapaglia & Sederoff, 1994), chicken (Levin *et al.,* 1993) and so on. RAPD markers were used to screen near isogenic lines to locate bacterial resistance genes in tomato (Martin *et al.,* 1991; Michelmore *et al.,* 1991), down mildew resistance in *lettuce* (Paran *et al.,* 1991), genetic fingerprinting (Black *et al.,* 1992) and molecular taxonomy. RAPD was shown as a potential tool in differentiating cryptic mosquito species (Wilkerson *et al.,* 1993). RAPD technique was found as a useful tool with great resolving power to discriminate fish species (Silas *et al.,* 2005), crab species (Smith *et al.,* 1996), oysters (Klinbunga *et al.,* 2000). Molecular markers (RAPD) associated with growth, yield and origin of the silkworm was analyzed (Chatterjee & Pradeep, 2003). This study has helped in identifying a few markers and thereby opened scope of using such marker for incorporating molecular markers in the breeding program for crop improvement in silkworm. Genetic differentiation induced by selection in an inbred population of the silkworm using RAPD and ISSR marker systems has been reported (136). This investigation has showed that RAPD and ISSR primers can generate polymorphic profiles when amplified with genomic DNA of individuals of longer larval duration (LLD) and shorter larval duration (SLD) lines. The role of RAPD and ISSR markers in silkworm conservation has been elucidated (141). Further, genetic mapping of Z chromosome and identification of W chromosome- specific markers in the silkworm has been carried out (Nagaraja *et al.,* 2005). This mapping has helped in identifying and mapping sex-linked traits in the silkworm. The economic and evolutionary significance of Z- and W-linked genes in silkworm, in particular, and lepidopterans, in general has been noted. Further, RAPD markers linked to densonucleosis refractoriness gene 'nsd-1' has been identified (Abe *et al.,* 1995 & 1998). 'nsd-2' (non-susceptibility to BmDNV-2) gene was linked to the linkage group 17 of *Bombyx mori* using RFLP markers (149). 150 RAPD markers were screened for NPV resistance in *Bombyx mori* (126). Polymorphism and analysis of prothoracotropic hormone (Shimada *et al.,* 1994) and genes of diapauses hormone (Pinyarat *et al.,* 1995) has been carried out by PCR based primers.

Another class of markers, ISSRs, are DNA fragments of about 100–3000 bp located between adjacent, oppositely-oriented microsatellite regions. ISSRs are amplified by PCR using microsatellite core sequences as primers with a few selective nucleotides as anchors into the non-repeat adjacent regions (16–18 bp). About 10–60 fragments from multiple loci are generated simultaneously, separated by gel electrophoresis and scored as the presence or absence of fragments of particular size (fig. 6). Because of the multilocus fingerprinting profiles obtained, ISSR analysis can be applied in studies involving genetic identity, parentage, clone and strain identification, and taxonomic studies of closely related species. In addition, ISSRs are considered useful in gene mapping studies. The ISSR markers have been used effectively in studying the genetic relationships among silkworm strains (125). Srivastava *et al.,* (124) analyzed the genomic DNA of 15 multivoltine races, using ISSR markers, for studying their thermotolerance behavior. Chatterjee *et al.,* (127) identified ISSR markers associated with productive traits in silkworm.

Application of Isozyme marker system in *Bombyx mori*

Proteins are an important, rather indispensable biomolecule of any organism. The growth and developmental processes of an organism proceed

through cascades of expression, repression and interaction of several proteins. Protein-based (isozyme) markers have greatly extended our understanding of metabolic regulation and to understand the differential gene action which is manifested in the form of differential activity of an isozyme during development. Also, isozyme analysis is an important technique for genetic variability studies. Early studies on alpha- and beta esterase isozymes revealed ontogenic variations in Drosophila (162). Similar work was done on *Bombyx mori* using acid and alkaline phosphatase isozymes (164). Developmental profiles in the isozymes of alpha- and beta-esterases were studied for embryogenesis in silkworm (163). Genetic polymorphism in 21 bivoltine silkworm races was revealed through isozyme studies (unpublished, 2008). In insects, esterase acts extensively on various kinds of substrate (161-Sudderuddin and Tan, 1973; Turunen and Chippendale, 1976) and show high polymorphism and genetic variations (161-Korochkin *et al.,* 1973, Mattiensen *et al.,* 1993). The prospects of using digestive amylase as a marker in silkworm breeding due to its wide genetic diversity, role in better digestibility and higher survival has been highlighted by Datta and Ashwath (2000). To confirm these findings, a breeding scheme was designed and high activity amylase genes from the indigenous polyvoltine di] on or parents were introgressed into the productive bivoltine parents used as recurrent parents and near isogenic lines of the recurrent parents have been developed (Ashwath *et al.,* 2001). Evaluation of hybrids developed by amylase-marker assisted selection has been carried out and on-farm trials have been conducted which indicated the superiority of GEN3xGEN2 hybrid (Ashwath *et al.,* 2002).

Marker system in other organisms

A RAPD screen successfully identified genetic markers for life span in Drosophila melanogaster on the basis of large allelic frequency differences between selected and control lines, making it a first step towards identifying QTLs of longevity (154). The phylogenetic relationship among the members of the *nasuta* group of Drosophila has been done using RAPD and ISSR polymorphisms (Nagaraja *et al.,* 2004). The investigation has revealed that phylogenetic tree generated by RAPD analysis was nearly in complete congruence with the classification based on morpho-phenotypic characters. Molecular distinction amongst varieties of mulberry was investigated using RAPD and DAMD (Direct Amplification of Minisatellite DNA) profile (Esha and Shirish, 2001). The study has shown that RAPD and DAMD data were useful in distinguishing between the nine mulberry varieties. These results were consistent with fact that mulberry was known to have highly heterozygous varieties with larger number of natural hybrids between unisexual mulberry parents.

Studies at biochemical level in silkworm.

Application of isozymes and other molecular markers help to estimate genetic diversity much more accurately than that of morphological traits. Electrophoresis identifies variation (alleles) at loci that code for enzymes (usually termed isozymes or allozymes). One advantage of allozyme loci is that they are co-dominant and heterozygotes can be scored directly. Understanding the genetic constitution of an individual in the population of races and allelic variations through isozyme studies is known to reflect the differential catalytic ability of allelic genes and their significant role in the adaptive strategy of the genotypes (Prakash *et al.*1992). The diversity study carried out in silkworm through protein profiles, enzymes and isozymes like esterase, acid phosphatase, alkaline phosphatase, amylase, phospho-glucomutase, aspartate-aminotransferase, malate-dehydrogenase, glucose 6 phosphate Dehydrogenase and carbonic anhydrase have been used to study diversity in silkworm genotypes (Staykova *et al.,* 2012).

Ashok Kumar *et al.,* (2011) while evaluating the genetic diversity and thermo tolerance among twenty-one silkworm races using metabolic enzyme profiles reported that the different genotypes clustered into eight groups and the origin and percentage were found to be closely related with clustered groups. Based on the thermo stability of metabolic enzymes, CSR_{18} was identified as thermo tolerant owing to its high protein stability and its geographical origin was found to be Kashmir region. This isozyme study clearly showed that the main genetic study variations can be identified by relating with their morphology and geographical origins. Furthermore, it is evident from the study that metabolic profiles are useful in producing reliable estimates of genetic diversity for selection of parents for the development of elite hybrids.

Ashwath *et al.,* (2001) developed Near Isogenic Lines of productive bivoltine silkworm breeds by amylase isozyme marker based selection. The evolved NILs have showed significant improvement in viability over their respective RPs without any deterioration in the yield traits. It has been clearly indicated that the prospects of using digestive amylase as a marker in silkworm breeding does exist because of its wide genetic diversity, role in better digestibility and close association with higher survival. Ashwath *et al.,* (2010) while conducting studies on Identification of RAPD markers linked to digestive amylase genes using Near Isogenic Lines of the silkworm , *Bombyx mori* reported that characterization of PCR products closely associated with the amylase gene can lead to identification of DNA sequences that may be

responsible for better digestibility and higher survivability in *Bombyx mori* which could be used for the development of improved and robust *Bombyx mori* breeds for commercial exploitation through the strategy of DNA marker assisted selection. Staykova *et al.,* (2012) investigated the polymorphism of acid phosphatase from larval haemolymph using twelve silkworm strains of various origin and reported that acid phosphatase isozymes were considered to be controlled by five co-dominant alleles. It was also found that among the different isozymes analyzed, esterase was most preferred because of its diverse substrate specificity and polymorphic expression followed by acid phosphatase. The study indicated that the acid phosphatase is very suitable marker for analyzing the inter- and intra-strain diversity and the strain differentiation.

Eguchi *et al.,* (1965) found four fundamental types of esterase and about 70 % of the Japanese, Chinese and European races investigated belong to A type and 20 % to O type, while B type was found only in Chinese races. Yoshitake *et al.,* (1965) analyzed polymorphism pattern of esterase and acid phosphates in 300 strains of silkworm and concluded that distribution of acid phosphatase and esterase was similar in European and Japanese strains and there was resemblance between Chinese and European strains. Moorthy *et al.,* (2007) carried out evaluation and selection of potential parents based on selection indices and isozyme variability in silkworm, *Bombyx mori* and observed a higher degree of inter-strain variability on the acid phosphatase and esterase. Acid phosphatase was found to be a suitable marker for analyzing the inter- and intra-strain diversity and the strain differentiation in silkworm *Bombyx mori*. Ronuqi *et al.,* (2013) carried out genetic analysis of isozymes in different silkworm genotypes to separate populations and strains in order to use them in selection programs observed polymorphism and rich genetic diversity between the genotypes studied. Staykova (2008) used isozyme patterns of 480 individuals of eight silkworm strains to assess the heterozygosity among the silkworm populations. The heterozygosity among the strains varied from 0.099 in China to 0.238 in Kinshu. Further, the study also revealed that the observed heterozygosity was much less than the expected value, which was attributed to the effect of the inbreeding.

Studies at molecular level in silkworm

The analysis of genetic diversity and the relatedness between or within species, individuals and populations is central to many disciplines of biological sciences. The development of molecular marker techniques that are based on polymorphism on DNA has greatly accelerated research in a variety of fields. The silkworm *Bombyx mori* L. has recently emerged as a lepidopteron molecular model system for diverse biological studies, including genetics, development and physiology, in addition to retaining its economic importance in silk production (Goldsmith, 1995). Nagaraju *et al.,* (1995) used banded krait minor satellite DNA Bkm-2(8) as a probe to reveal polymorphism in different silkworm races and reported that, although polymorphism level was relatively lower than the SSR-anchored PCR (Reddy *et al* .1999b), yet Bkm probe revealed very clear fingerprint profiles as such is reliable to estimate genetic diversity of silkworm germplasm based on DNA fingerprint information.

Talebi *et al.,* (2011), studied RAPD markers for understanding the genetic variability among the four silkworm races namely C_{108}, NB_4D_2, Pure Mysore, Nistari and their hybrids and reported that the lowest percentage of polymorphism (0.0%) was observed when amplified by OPA-01 and OPA-02. The cluster analysis based on UPGMA (unweighted pair group method with arithmetic mean) method based on the dissimilarity index values has generated the dendogram separating the bivoltine and multivoltine races into two groups and has revealed that Pure Mysore is further away from the group.

Ramesha *et al.,* (2010) carried out nutri-genomic analysis of mulberry silkworm (*Bombyx mori* L.) strains using polymerase chain reaction simple sequence repeats (PCR-SSR) to gain better understanding on genotyping of certain nutri-genomic gene loci in nutritionally efficient silkworm breeds/hybrids and reported that these results showed that a single yet varying size amplified band in all four parental silkworm strains (RMG_4,RMW_2,RBD_1 and RBO_2) and two clear bands in the hybrids (RMG_1 x RBD_1 and RMW_2 x RBO_2) with different molecular weight from three PCR-SSR primers loci viz ; F_{11139}, F_{10429} and F_{110705} . The PCR-SSR results demonstrated homozygosity in newly evaluated nutritionally efficient parental silkworm strains and heterozygosity in hybrid and confirm their being nutritionally efficient with superior nutrigenomic traits. Jain *et al.,* (2010), studied RAPD marker system in insects and reported that RAPD markers are well suited for genetic mapping, plant and animal breeding application and DNA fingerprinting, with particular utility for studies on population genetics and have become the most common yardsticks for measuring genetic differences between individuals, within and between related species. Sanjeeva Reddy *et al.,* (2009), while exploring the genetic variability in *Bombyx mori* L. With molecular markers reported that there was distinct grouping between the multivoltine and bivoltine races when grouped with marker generated by ISSR-PCR and the 2D diagram of the PCA (principle component

analysis) of the marker generated by the different ISSR primers helped to visualize the two major clusters which included the multivoltine and bivoltine separately. Awasthi *et al.,* (2008) studied the molecular evaluation of bivoltine, polyvoltine and mutant silkworm (*Bombyx mori* L.) with RAPD, ISSR and RFLP-STS markers and reported that on clustering with UPGMA and PCA, RAPD and ISSR markers clearly discriminated the bivoltine and multivoltine and a multivoltine Tamil Nadu white occupied the positions among bivoltine, since it has bivoltine parentage. Boropolu, an original land race from North-East, India and Feng Shang, a Chinese silkworm strain also showed a close genetic relationship.

Dalirsefat *et al.,* (2007) estimated the genetic diversity in three Iranian native silkworms (*B. mori* L.) strains and three commercial Japanese lines using AFLP markers and reported that the distinct clustering of these two sets of strains and lines reflect the differences of the geographical origin, morphological, qualitative and quantitative traits associated with them. The study also revealed high genetic diversity (75.4%) between the strains. Srivastava *et al.,* (2005) carried out genetic analysis of silkworm (*B. mori* L.) through RAPD markers and reported certain silkworm stocks like Kalimpong-A, C-nichi, Nistari and Mysore Princess were having higher genetic distance from others and thus could be used for heterosis breeding and also in breeding programmes aimed at introgressing hardy genes into the bivoltine high yielding stocks. Li *et al.,* (2005) estimated the genetic relationship among 31 diverse silkworms (*B. mori* L.) strains with SSR markers and reported that SSR markers are an efficient tool for fingerprinting cultivars and conducting genetic diversity in silkworm. Chatterjee and Tanushree (2004) carried out molecular profiling of Silkworm biodiversity in India and assessed molecular diversity among *Atheraea mylitta*, *A. assama*, *A. pernyi*, *A. proylei*, *A. roylei* and *Phileosomia Cynthia* with 11 ISSR and 8 non-random primers on agarose gel and established closest relationship between *A. pernyi* and *A. proylei.*

Mita *et al.,* (2003) first initiated intensive sequencing on the silkworm genome using expressed sequence tags (EST's). Recently this group (Mita *et al.,* 2004) and second group (Xia *et al.,* 2004) reported whole gene shotgun sequencing in silkworm. Nagaraju *et al.,* (2001) studied comparison of multilocus RFLP's and PCR-based marker systems for genetic analysis of the silkworm (*B. mori* L.) and reported that the ISSR-PCR produced 39 fragments of which 76.98 per cent were polymorphic. The highest diversity index was observed for ISSR-PCR (0.957) and the lowest for RAPD's (0.744). The RAPD, ISSR-PCR and RFLP assay clearly separated the diapausing and non-diapausing silkworm varieties. These are discussed in terms of choice of appropriate marker technology for different aspects of silkworm genome analysis.

Singh *et al.,* (2011) investigated the RAPD profile of isolated DNA from Eleven morphologically distinct strains of muga silkworm *Antherea assama* and reported that RAPD technique is sensitive enough to detect differences and high degree of polymorphism between strains of muga silkworms in which differentiation is not always possible morphologically. Their study further revealed clear domestication between the wild and domesticated muga silkworm strains based on the dendogram generated. Kartik Neog *et al.,* (2010), studied analysis of genetic diversity of Muga silkworm (*Antheraea assamensis*, Helfer; lepidoptera; Saturnidae) using RAPD-based molecular markers and reported that although there lies little morphological differences among the collected Muga silkworm populations, the populations are highly polymorphic which might have enabled the silkworm to survive under restricted geographical location i.e. North-East region of India only but under adverse climatic conditions for a longer period. Mirhosseini *et al.,* (2007) studied the genetic relationship within and among seven Iranian native silkworm strains determined by DNA fingerprinting by using AFLP markers. The study revealed that the variability of DNA fingerprints within and among silkworm strains could provide an essential basis for breeders in planning cross breeding strategies to produce potentially heterotic hybrids in addition to contributing in conservation programmes. In a study carried out by Velu *et al.,* (2008), mutant silkworm stocks were evaluated to determine their genetic relationship using ISSR Primers. The dendogram produced using the Unweight Pair Group Method with Arithmetic Mean (UPGMA) and Cluster Analysis resulted in the formation of one major cluster and six sub-clusters, separated 20 mutant silkworm strains belonging to the same origin and similar voltinism. The findings of this study revealed considerable polymorphism among strains and indicate that ISSR markers can be efficiently utilized to analyze phylogenetic relationships and heterozygosity in silkworm. With the help of 16 million single nucleotide polymorphism (SNP) markers, identified using the genomic information of 40 domesticated and wild silkworm strains, the domestication events and subsequent genetic differentiation in *Bombyx mori* have been worked out by Xia *et al.,* (2009). The study revealed significant genetic divergence between wild and domesticated strains of *B. mori.* The genetic variation was measured using the population size scaled mutation rate which was significantly smaller in domesticated strains (0.011) when compared to the wild strains (0.013). The rate of heterozygosity in domesticated strains was two times lower than that in wild varieties (0.003 and

0.008, respectively), which is most likely attributed to inbreeding experienced by domesticated lines. The study also shows clear genetic separation between domesticated and wild varieties and relatively less gene flow between them.

Yamamoto *et al.,* (2008) mapped 1,755 single nucleotide polymorphism (SNP) markers from bacterial artificial chromosome (BAC) end sequences onto 28 linkage groups of silkworm using a recombining male backcross population, yielding an average inter-SNP distance of 0.81 cM (about 270 kilobases). A test of synteny using Oxford grid analysis with more than 500 silkworm genes revealed six versus 20 silkworm linkage groups containing eight or more orthologs of *Apis* versus *Tribolium*, respectively. The integrated map contains approximately 10% of predicted silkworm genes and has an estimated 76% genome coverage by BACs. Hence, this assignment of nearly 10% of predicted silkworm genes to 28 chromosomes will not only facilitate construction of accurate scaffolds and annotation of the silkworm genome, but also provide a valuable resource for testing microsynteny in Lepidoptera and other insects Further, this study provides a new resource for improved assembly of whole-genome shotgun data, gene annotation and positional cloning, and will serve as a platform for comparative genomics and gene discovery in Lepidoptera and other insects.

Reddy *et al.,* 2010 carried out an investigation to analyze the diversity in different silkworm races using Inter Simple Sequence Repeat (ISSR) molecular marker for the selection of diverse parents. Among the primers used for ISSR analyses, the primer ISSR2 and ISSR3 generated highest number of fragments. All the primers exhibited 100 per cent polymorphism across 30 silkworm races analysed. The similarity coefficients ranged from 0.33 to 1.00. Of the pairwise combinations, NB7 and Kollegal Jawan showed the lowest similarity index (0.33), whereas the highest similarity index was recorded between C. Nichi and P4D3 (1.00), followed by CSR3 and CSR2xCSR4 (0.98). The mean similarity index was 0.68. The 2D diagram of the PCA analysis of the markers generated by the different ISSR primers helped to visualize the two major clusters which included the multivoltines and bivoltines separately. The grouping of bivoltines in the PCA analysis clearly showed higher similarity among bivoltines as compared to the multivoltines. Since, the combining ability of Pure Mysore was reported excellent with bivoltine races for hybridization and also of its high amylase activity, it was selected as donor parent for the F_1 cross. CSR2 was selected as the other parent because of its better quantity and quality parameters. Superiority over the parents with regard to biological parameters was found with F_1 (Pure Mysore × CSR2). Srivastava *et. al.*, 2011 used Inter simple sequence repeats (ISSR) and random amplified DNA polymorphism (RAPD) markers to estimate the genetic diversity among 14 tropical silkworm races (*Bombyx mori* L.) for identifying potential parents to be used for hybrid preparation for commercial exploitation. High polymorphism (70.91 and 74.70%) was revealed by ISSR and RAPD markers. The dendrograms generated, using unweighted pair group method using arithmetic average from these markers, grouped the 14 silkworm races into two major groups, which corroborates the differences in cocoon characteristics. Discriminant function analysis of ISSR and RAPD markers identified three functions for cocoon weight and two functions for shell weights, respectively. Step-wise multiple regression analysis identified six ISSR markers (834_{500}, 884_{1700}, 884_{1850}, 827_{1500}, 840_{1500} and 789_{1250}) and seven RAPD markers (834_{500}, 885_{900}, 810_{1400}, 884_{900}, 836_{1500}, 789_{1250} and 762_{1700}) significantly associated with cocoon and shell weights. The genetically divergent parents, identified through this study, can be used for the preparation of hybrids for commercial utilization.

Williams *et al.,* (1990) reported that RAPD markers are well suited for genetic mapping, for plant and animal breeding applications, and for DNA fingerprinting, with particular utility for studies of population genetics. Chandrakanth *et al.,* (2014) studied genetic relationships among ten silkworm strains comprising of five each of bivoltine and polyvoltine utilizing 15 SSR markers. The dendrogram generated by UPGMA based on 15 microsatellite loci differentiated silkworm strains into two major groups. Group I consisted of bivoltines and group II contained polyvoltines. Further the bivoltine group was subdivided based on cocoon shape and polyvoltine group was subdivided based on the cocoon colour spun by the silkworm strains. This study was also able to identify divergent parent which can be used in further breeding programme. Arunkumar *et al.,* (2009) developed Microsatellite markers for the Indian golden silkmoth, *Antheraea assama* and reported that these microsatellite markers will be useful resources for population genetic studies of *A. assama* and other closely related species of saturniids. Cheng *et al.,* (2004) estimated the frequency of single nucleotide polymorphism (SNP) diversity in a silkworm strain Diazo to be $1.3x10^{-3}$ by sequence diversity and reported that SNP analysis can provide a fundamental description of nucleotide diversity of the silkworm genome and may play an important role in further genetic analysis and functional genomics of the silkworm and other lepidopteron insects. Li *et al.,* (2010) studied genetic diversity and molecular phylogeny based on comparative analysis on 41 mitochondrial genomes of *Bombyx mori* and *Bombyx mandarina* from China and Japan and reported 347 Single Nucleotide Polymorphism (SNPs) in the

mitochondrial genome. A phylogeny inferred from these whole genome SNPs resulted in a well classified phylogeny tree, confirming that the domesticated silkworm, *Bombyx mori*, had most recently diverged from the Chinese wild silkworm rather than from the Japanese wild silkworm. The results of this study strongly implied that the Chinese wild silkworm *Bombyx mandarina* is the most recent ancestor of domesticated silkworm.

Reddy *et al.,* (1999) carried out genetic diversity studies in 13 silkworm strains based on microsatellite markers. The dendogram generated by UPGMA analysis resolved the 13 silkworm strains into two groups; one comprising of diapausing strains and the other non- diapausing strains. The microsatellite based analysis reflected the geographical, pedigree and morphological relations of strains used in this study. Prasad *et al.,* (2005) carried out survey and analysis of the silkworm, *Bombyx mori* based on microsatellite markers. The microsatellite map generated using these polymorphic markers resulted in 8 linkage groups. *Bombyx mori* microsatellites were the most conserved in its immediate ancestor, *Bombyx Mandarina* followed by the wild saturnid silkmoth, *Antheraea assama*. The results of this study provide an opportunity to use SSR markers for investigating the wide range of genetic diversity that exists in the wild species outside the gene pool of domesticated silkworm, *Bombyx mori*. Vijayan *et al.,* (2010) assessed genetic diversity among 13 tropical non-diapausing strains of silkworm, *Bombyx mori* genotypes with SSR and mtDNA-SSCP markers. The study revealed presence of considerable genetic diversity among 13 silkworm genotypes. Based on the phenotypic and genetic analysis, BL2, Kollegal Jawan and Mysore princess was identified to have high potential to be used as parents for developing high yielding summer specific strains for commercial exploitation.

A molecular assisted approach was used by Hou *et al.,* (2013) for breeding silkworm strains with high silk production and resistance to densonucleosis virus and reported that SSR markers are efficient tools to select silkworms resistant to densonucleosis virus. Li *et al.,* (2006) carried out linkage and mapping analysis to the densonucleosis non-susceptible gene nsd-Z in the silkworm *Bombyx mori* using SSR markers. Their investigation revealed considerable genetic diversity among silkworm germplasm and reported seven SSR markers linked nsd-Z. Pradeep *et al.,* (2011) carried out genetic analysis of scattered populations of Indian Eri silkworm, Samia *Cynthia ricini* by SSR markers and found 98% inter-population and 23-58% intra-population polymorphism. It was inferred from the study that the genetic distances among the eri populations increased with the increase in altitude and geographical distances. They further reported that deforestation and exploitation has led to the fragmentation of habitats and scattering of populations of the economically important eri silkworms in North East India. Further, Patchy habitats prompted low genetic variability, high linkage disequilibrium and colonization by new sub-populations. Radjabi *et al.,* (2012) studied the interspecific biodiversity of Six Iranian local races of silkworm *Bombyx mori* L., by ISSR molecular markers. Results of this study revealed that five Primers yielded 81 scorable bands with fragment size between 250-300 base pairs. Maximum of the amplified bands showed in UBC_{807} Primer which has maximum percentage of polymorphism among Primers.

Sreekumar *et al.,* (2011) worked to detect of Single Nucleotide Polymorphism (SNP) DNA markers linked to cocoon traits in mulberry silkworm. Out of 240 Primer Pairs used, 48 Primers revealed distinct Polymorphism between the parents which was confirmed by the co-dominant expression of PCR products in F1 generation. However, only one base pair 04124 was found to show clear cut differences in the amplified products. Akir *et al.,* (2010) carried out molecular analysis of Turkish silkworm breeds by RAPD-PCR method and reported that diapausing and non-diapausing local breeds were separated successfully and the percentage of Polymorphic loci were determined higher in non-diapausing breeds and lower in diapausing breeds. It was further reported that the results obtained from the study can be used for the improvement programs of Turkish silkworm breeds in future. Prasad *et al.,* (2003) studied comparative phylogenetic evolution and inverted terminal repeate (ITR) conservation of mariner elements (MLEs) from Indian Saturniid silk moth, *Anthereae mylitta* and observed that *Anthereae mylitta* MLEs got phylogenetically classified under Cecropia sub-family and cluster closely with the elements from other Bombycidae super-family members implying vertical transmission from a common ancestor. They further reported that information on MLEs in silk moths and recent advancement in the marnier-mediated transgenesis will help to test the feasibility of transforming silk moths with a foreign gene of economic importance using mariener based vectors. Narumol Thananata (1997) studied differentiation and identification in morphologically different silkworm Bombyx mori strains using RAPD-PCR technique and reported that RAPD-PCR technique is efficient tool in silkworm differentiation and identification studies which could separate five morphologically different races into diapausing and non-diapausing varieties. An investigation on RAPD profiles of Romanian silkworm genotypes was carried out by Furudi *et al.* (2009) in which amplification products resulted by RAPD pointed out significant differences between strains and hybrids at genotype level. The study proved that RAPD technique can be successfully applied for characterization of *Bombyx mori* genotypes.

A comparative assessment of genetic diversity and genetic structure based on RAPD markers for three silkworm species viz; *Bombyx mori* L., *Anthereae pernyi* and Samia *Cynthia ricini was* done by Liu *et al.* (2010). The study showed highest levels of genetic diversity between *Anthereae pernyi* and *Bombyx mori* at the species level. However, at the strain level, Anthereae pernyi had relatively highest genetic diversity and *Bombyx. mori* had lowest genetic diversity. An investigation to analyze genetic diversity and phylogenetic relationship among some races and hybrids of Romanian *Bombyx mori* was carried out by Furudi *et al.* (2011) using RAPD markers. The DNA profiles from 8 hybrids and races was amplified with 35 highly polymorphic RAPD primers, of which 21 markers generated polymorphic bands that were used to analyze genetic phylogeny and diversity. Further, a total of 921 polymorphic bands were detected and UPGMA cluster analysis grouped silkworm strains on the basis of their origin, obtaining a dendogram reflecting their genetic relationship. Li *et al.,* (2007) while analyzing genetic relationship among different ecotypes of silkworm strains using ISSR markers observed that univoltine, bivoltine and multivoltine strains clustered separately based on their origin. The results of this study revealed that ISSR amplification is a valuable method for determining genetic variability among silkworm varieties and is an efficient technique for characterizing the large number of silkworm strains held in national and international germplasm centers. Estimating the genetic variability with microsatellite markers among six populations of Indian golden Saturniid silk moth (*Anthereae assama*), Arunkumar *et al.* (2012) observed highly significant diversity in one of the populations and reported that *Anthereae assama* inhibiting the WWS-region was genetically divergent from the five populations studied. He also reported that this study laid base for further investigation to identify and study such populations from muga silkworms as well as other habitat to generate information useful in conservation of dwindling muga culture in North East India. While estimating the genetic relatedness within and among different silkworm verities using Bkm-derived probe, Sharma *et al.,* (1999) observed a high degree of genetic similarity within each variety studied. The dendogram generated using UPGMA resolved silkworm varieties into two major clusters. One group comprised of non-diapausing varieties and the other comprised of diapausing varieties. The results of this study demonstrated that variability of DNA fingerprints within and among silkworms and can provide an essential basis for crossbreeding programs to produce potentially heterotic hybrids. Shivashankar *et al.,* (2012) while conducting genetic diversity analysis of Eri silkworms by RAPD markers observed low genetic distance between the Eri silkworm populations and reported that the range of genetic diversity and low genetic distance between the Eri silkworm populations is attributed to restricted environmental acclimatization .He further reported that RAPD technique is sensitive enough to detect differences between accessions of Eri silkworms were differentiation is not always possible morphologically.

Devi *et al.,* (2012) subjected six populations of Anthereae proyeli and Anthereae frithi Moore to Inter Simple Sequence Repeat (ISSR) marker analysis and found very high polymorphism among the breeds and reported that ISSR markers are suitable to study intra and inter specific variation in this group of insects. He further reported that the findings made in this study are of much importance for germplasm conservation as well as breeding of these economically important insects since, the study revealed phylogenetic relationship among *Anthereae proyeli* and its breeds. An investigation carried out by Reddy *et al.,* (2010) using ISSR markers to analyze the diversity in different silkworm races for selection of diverse parents resulted in distinct grouping between the multivoltine and bivoltine races into two major clusters separately. Further, the grouping of bivoltines by Principle Component Analysis (PCA) clearly showed higher similarity among bivoltines as compared to multivoltines and the combining ability of Pure Mysore was reported excellent than bivoltine races for hybridization and owing to its high amylase activity, it was selected as donor parent for the F_1 cross while as CSR_2 was selected as the other parent, owing to its better qualitative and quantitative parameters. Reddy *et al.,* (1999b) while analyzing the fingerprints of diverse silkworm strains using SSR-anchored PCR found that strain specific amplified products resolved silkworm stocks into two distinct groups one comprising of non- diapausing and other comprising of diapausing strains. They reported that this study established that ISSR- anchored PCR method is potentially useful for genetic fingerprinting of silkworm genotypes and as a mapping tool in the silkworm.

Correlation Studies

Hirata (1974) examined the correlation between the amylase activity of the larval digestive juice and several quantitative characters in strains of the mulberry silkworm *Bombyx mori* which were reared under three different nutritional conditions viz; normal leaves, hardened leaves and artificial diet. Since there was variation in the amylase activity of their larval digestive juice, the larvae were separated into two classes' i.e., high amylase activity group and low amylase activity group. In all the experiments, cocoon weight, shell weight, shell ratio percentage, cocoon shell productivity per day in 5th instar and survival rate were higher in the larvae of high amylase activity than in those

of the low activity. These differences were to be remarkable when the larvae were reared on hardened leaves or on the artificial diet. In the rearing experiments of larvae of F_1 hybrids between Japanese and Chinese strains, the differences in these quantitative characters were remarkable on account of heterosis.

Chatterjee *et al.,* (1989) studied the variability of digestive amylase in the mulberry silkworm *Bombyx mori* L. Genetic variability in digestive amylase activity was investigated by them in 64 multivoltine and 39 bivoltine breeds or combination. Larval weight, cocoon weight and shell weight were found to be negatively correlated with amylase activity which was positively correlated with effective rate of rearing. Majumder and Bose (1991) reported the relationship between amylase and quantitative characters in silkworm, *Bombyx mori* L. The amylase in digestive juice had the beneficial effect on some quantitative characters; especially its high activity on the survival rate when the larvae were reared on hardened leaves. Chatterjee *et al.,* (1992) worked out the genetic variability of amylase activity of Indian races and other exotic breeds of the silkworm, *Bombyx mori* and its role in the expression of yield components. Existence of high and low amylase activity in the blood of non-hibernating (low yielding) and also hibernating (high yielding) silkworm breeds did not show any apparent relationship between enzyme activity and yield parameters. In the digestive system, enzyme activity was found to be higher in the later stages of development. Further, Indian traditional breeds were found to have the highest activity followed by Indian evolved breeds and Chinese breeds of low yield. The activity was found to be much lower in evolved high yielding bivoltine breeds of Indian and univoltine breeds of Europe and Japan. The enzyme activity analyzed for 5^{th} instar showed a significant negative correlation with the larval weight, larval duration, weight of cocoons and shell weight but a strong positive correlation (r= +0.47 and + 0.81) respectively for the 10^{th} and 15^{th} feeding sets with the effective rate of rearing, which is a measure of survival in sericulture.

Chatterjee *et al.,* (1993) carried out a detailed study on six biochemical markers viz., amylase, invertase, alkaline phosphate, protease (PH 7.0), protease (PH 10.0), trehalose and four yield attributes using multiple regression analysis to investigate their relationship in the mulberry silkworm, *Bombyx mori* L. It was reported that amylase showed high significant correlation ($p<0.001$) with all four yield parameters whereas trehalose gave a negative correlation ($p<0.05$) with the effective rate of rearing during season- I. However, other independent variables did not show any significant correlation with any of the yield parameters during season-I. During season- II, effective rate of rearing was found to be positively correlated with both digestive amylase and digestive alkaline protease. Larval weight on the other hand showed a negative correlation with the protease at pH 7 and 10 but had a strong positive correlation with the alkaline phosphate. Both single cocoon weight and single shell weight were negatively correlated with the protease activity at pH 7 and pH 10; however positive correlation were observed with alkaline phosphate for the same two characters. This study suggested the importance of digestive amylase as suitable marker for the survival of the silkworm and revealed the inability of other biochemical markers to affect this relationship.

Murugesh *et al.,* (2011) investigated the differential amylase activity in the silkworm, *Bombyx mori* in relation with biological and yield traits. They found that amylase activity in the digestive juice was about 10 10 to 15-fold higher in multivoltines than bivoltines. Positive significant correlation ($p=0.01$) was recorded between digestive amylase activity and effective rate of rearing (0.812). Negative correlation was recorded between amylase activity and biological and yield related traits of the silkworm, *Bombyx mori* L. Mirhosseini *et al.,* (2010) studied identification of AFLP markers linked with cocoon weight genes in silkworm (*B. mori* L.) and reported that content genomes (amount and combination) in each F2 generation are different. Mirhosseini *et al.,* (2009) constructed linkage map for silkworm based on AFLP markers and reported that AFLP amplification is highly reproducible, the development of an AFLP linkage map provides an invaluable tool for studying silkworm genetics, such as identification of strain specific markers for tracking allele frequency changes and QTL analysis for economically important traits. Pradeep *et al.,* (2007) studied molecular markers for Biomass Traits; association, interaction and genetic divergence in silkworm *Bombyx mori* and reported that identification of several potential markers that continue to develop genetic characteristics of silkworm population and reveal genetic divergence within and low yielding strains could have potential practical utility in prospective silkworm breeding programme.

Chatterjee and Mohandas (2003) identified ISSR markers associated with productivity traits in silkworm (*B. mori* L.) and reported that the present result along with that obtained by homologous multilocus probes offers an approach for identifying a set of DNA markers projecting a significant association with specific yield components. Such markers need to be tested for their use in marker assisted breeding programmes for modifying the yield potential of silkworm. Nagaraju *et al.,* (1997) assessed the genetic diversity of diapausing and non-diapausing silkworm (*B. mori* L.) by DNA profiling through RAPD markers and reported that RAPD analysis showed potential

to become a viable tool for analysis of genetic variation among populations and for identifying molecular markers for economic trait of silkworm. Nagaraja and Nagaraju (1995) studied the genome fingerprinting of diapausing and non-diapausing silkworm genotypes using random arbitrary primers and reported that RAPD technique could be used as a powerful tool to generate genetic markers that are linked to traits of interest in the silkworm.

Singh *et al.,* (2011) while studying the RAPD profile of morphologically different strains of muga silkworms reported that among the eleven muga silkworm strains, three high yielding strains were genetically different from the remaining strains. Li *et al., (*2013) carried out genetic analysis of quantitative trait loci for cocoon and silk production in *Bombyx mori* and identified a total 14 QTL's for the economically significant silk quality characteristics viz., cocoon filament length, whole cocoon weight, pupal weight, silk filament weight and cocoon shell weight. These fourteen QTL's were found to be distributed on 5 linkage groups (linkage group 1,14,18,23 and 25). Their findings highlight that QTL's for the same characters are linked which indicate the quantitative inheritance of these characters. The results of their study provide an excellent foundation for the map based cloning of major genes that control silk production and marker assisted selection for improving silk quality of economically important silkworm strains.

Pereira *et al.,* (2013) utilised RAPD technique to verify genetic polymorphism and divergence as well as susceptibility to *Bombyx mori* Nucleopolyhedrosis virus (BmNPV) and reported that there is marked difference in diverse characteristics including geographical origin, body weight, larval span, cocoon weight and other biological traits. Using AFLP markers for characterizing 23 silkworm strains composed of 12 Japanese and 11 Chinese strains, Gavaria *et al.* (2006) observed that Japanese strains were more heterogeneous than the Chinese strains. Group analysis showed the separation of the strains according to their geographical origin. The study further revealed a significant association of AFLP markers with the productive traits of silkworm. However, Correlation analysis between the bands and productive characters showed both positive and negative association. The outcome of the study suggests that such markers might help in programs of marker assisted selection. Yu *et al.,* (2011) conducted an analysis of the nucleotide diversity based on melanin synthesis pathway genes between domesticated and wild silkworm strains with various body colors and reported that the tyrosine hydroxylase gene is related to silkworm domestication. Yamamoto *et al.,* (2006) constructed a linkage map for *Bombyx mori* by surveying the segregation patterns of 534 SNPs and found a total of 1,755 SNPs on an expanded linkage map were successfully positioned. The SNP markers segregated into 28 linkage groups, with a total recombination length of 1,413 cM, 26 of the SNP linkage groups were assigned to classical silkworm chromosomes 1 to 26, defined by morphologic markers (for example, cocoon color and larval markings) and protein polymorphisms (for instance, haemolymph proteins). They reported that each of the SNP markers is directly linked to a specific genomic BAC clone and to whole genome sequence data and some of them are also linked to EST data. The study revealed that SNP linkage will prove a powerful tool for investigating silkworm genome properties, mutations, mapping and map based cloning of genes of industrial and agricultural importance. Zhan *et al.,* (2009) reported an improved method for constructing silkworm SSR genetic maps with more informative loci based on new mapping populations. Using this approach, they localized QTL for whole cocoon weight, cocoon shell weight, cocoon shell ratio, and pupal weight. Based on the data set, twelve candidate loci involved in whole cocoon weight (CW), cocoon shell weight (CSW), cocoon shell ratio (CSR), and pupal weight (PW) were identified. Of these, six QTL were confirmed by independent analysis. QTL1 on Chromosome 1 had the most significant contribution for the three traits, accounting for 29.38, 27.75, and 27.96% of the phenotypic variation in CW (LOD = 15.49), CSW (LOD = 14.85), and PW (LOD = 14.64). The other putative QTL (Q2 and Q3) associated with CW and PW had relatively weak effects (LOD of about 3), which were localized in the neighboring region of S2304. QTL4 for CSW was mapped to Chromosome 23, which also contributed a relatively small effect (r2 = 5.23%, LOD = 3.30). For CSR, two QTL (Q5 and Q6) were identified on Chromosome 18 and 19 that accounted for 6.54 and 8.28% of the phenotypic variance, respectively. This work suggests that the integrated map produced is a highly efficient genetic tool for the high-throughput mapping of single genes and QTL and offers a greater number of markers and polymorphisms; thus, it may be used as a resource for marker-assisted breeding of productive silkworm lines.

Li *et al.,* (2010) while carrying out the mapping of major quantitative trait loci of silkworm cocoon reported that there are eleven effective QTLs whose contribution rate is over 10%. Among them two QTLs account for over 20% of the contribution rate controlling the whole cocoon weight (cw-33), cocoon shell weight (sw-33) and these two QTLs could be very helpful in improving cocoon traits. Thirteen stocks of silkworm *Bombyx mori L.* were characterized by Sethuraman *et al.* (2002) using six multilocus RFLP

markers and observed a high level of polymorphism (98%). The study placed thirteen silkworm stocks into two major clusters, one contained high yielding stocks and the other contained the low yielding stocks. Further, adopting multiple regression analysis, the RFLP markers associated with economically important characters were identified. The study indicated that RFLP markers are highly useful in molecular mapping, genotype characterization and marker assisted selection thus, create an opportunity of using germplasm stocks directly for isolating specific RFLP bands for their use in marker assisted selection. Zhao *et al.,* (2010) used SSR markers to identify and map thermo tolerant gene (KN) in two silkworm strains viz; Dong34, a thermo tolerant strain and OU17, a highly susceptible strain reported that five SSR markers were linked to thermo tolerance in silkworm and is present in 8th linkage group.

Conclusion

The most important traits of sericulture, as in agriculture, are not controlled by a single gene but the concerted action of several genes (polygenic or quantitative traits) and non-hereditary factors. Dissecting such traits require substantially enhanced efforts on the part of silkworm geneticists. Recent developments in silkworm genome analysis provide tools and techniques which, coupled with conventional breeding will help silkworm geneticists and breeders to perform such a task. Further, the developments in transgenic silkworm technology, application of DNA markers for strain characterization and construction of linkage maps, and understanding the genetics of viral resistance provide requisite tools that can expedite further silkworm improvement**.** The large collections of carefully maintained silkworm mutations and practical breeding stocks are valuable resources that have only begun to be exploited to their full potential. The well-studied genetic resources of *B. mori* make it an ideal reference for the lepidoptera, where comparative genetics and genomics can work together to elucidate conserved evolutionary pathways and their diversification; identify new genes and gene systems as targets for transgenesis. Besides, it leads to basic research on new genome-based approaches for the exploitation of other economically important species such as *Anthereae* and in the control of pest species. The study placed the silkworms into two distinct groups consisting of six diapausing and seven non-diapausing genotypes. The genotype, Pure Mysore was found to be the most divergent genotype among the genotypes studied.

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