Biotechnological advances in silkworm improvement: Current trends and future prospectus

ZI Buhroo, Shahina A Nagoo, Iqra Rafiq and MA Bhat

Abstract

The recent advances in Biotechnology viz., recombinant DNA techniques, genetic engineering through manipulation of desirable traits, stem cell research etc., has revolutionized research in plant and animal sciences. However, the impact of Biotechnology is yet to find a place in Sericulture owing to the fact that many industrially developed countries where Biotechnology is reaping its fruits unfortunately do not practice sericulture, this has resulted in non-attempting of biotechnological approaches in sericulture. Japan, China and India, the largest producers of natural silk in the world, have invariably made few but sincere attempts to introduce biotechnology in sericulture.

Keywords: biotechnological advances, silkworm improvement, current trends, future prospectus

Introduction

Biotechnology, in recent years, has created unprecedented opportunities in almost all the sectors. It has become the world’s fastest growing and the most rapidly changing technology.

The advent of high throughput genomics, proteomics, metabolomics and genome editing based technologies has shifted the paradigm to address the biological questions in novel ways and helped in devising new strategies to improve the crop and animal productivity. Genetic technologies applicable to plants, vertebrates and insects began to become available in the early 1980s, beginning with simple tools for inserting transgenes into genomes. Since then, there has been a steady growth in the number and sophistication of genetic technologies such that today we have the capabilities of precisely editing the DNA sequence of an organism’s genome. While these tools were often developed initially for use in popular reference organisms such as yeast, tobacco, mouse and fruit flies, however today their applications are found in a wide range of organisms including mulberry silkworm Bombyx mori as the only truly domesticated insect which has been has been intimately and completely dependent on humans for its survival and reproduction since, the legendary fall and unravelling of a cocoon in the teacup of Chinese princess Xi Ling Shi 5000 years ago. Since, the nineteenth century, B. mori has begun to serve as a reference or model organism for studies in the life sciences and has contributed many classic paradigms associated with our understanding of genetics and molecular biology [1]. While B. mori remains critically important to sericulture throughout the world, the significance of B. mori has grown with the sequencing and annotation of its genome along with the development of genetic technologies that enable the genome to be manipulated. Bombyx mori larvae have also exhibited enormous potential to be used as living ‘bioreactors’ which, after appropriate genetic modification, can produce valuable proteins, therapeutics and silk-based biomaterials. In the past 10 years, great advances have been achieved in the development of genetic technologies in B. mori, including transposon-based technologies that rely on piggyBac-mediated transgenesis and genome-editing technologies that rely on protein- or RNA-guided modification of chromosomes [2]. The successful development and application of these technologies has not only facilitated a better understanding of B. mori and its use as a silk production system, but also provided valuable experiences that have contributed to the development of similar technologies in non-model insects. These major scientific advances have greatly promoted B. mori as a model organism not only for lepidopterans but also for general biological phenomena and mysteries.

Therefore, the genetic advancements, like, molecular marker technology, association genetics and mapping, marker-assisted selection breeding, and transgenesis etc., their application and
use in genetically modifying *B. mori* with future thrust are briefly discussed in this paper.

**Silkworm Genome Sequencing**

In September 1995, two Chinese scientists of silkworm genetics, Professors Zhonghui Xiang and Zhengang Li, formally submitted a proposal to the Chinese government to sequence the silkworm genome. Subsequently, silkworm scientists in Japan started to construct a physical map of the silkworm genome based on end-sequencing of bacterial artificial chromosome (BAC) libraries. In September 2002, the International Lepidopteran Genome Consortium unanimously agreed to use the WGS sequencing method to sequence the silkworm genome. In 2003, two groups (one from China and one from Japan) independently initiated the WG Sequencing of the silkworm genome and published draft sequences of the silkworm genome with six fold and threefold genome coverage \(^{3, 4}\) respectively. These publications propelled biological research on the silkworm into a new era of genomics and functional genomics.

### Draft and Improved Genome Sequences

Two draft genome sequence maps for *B. mori* were constructed and published in 2004 \(^{1, 4}\). The size of the silkworm genome is estimated to be 428.7 Mb \(^{1}\) and the number of predicted genes is 18,510. Because the silkworm WGS data from the Chinese and Japanese groups were derived from the same strain, the scientists agreed to combine these two datasets to assemble an improved genome sequence. In 2007, the Chinese and Japanese groups collected and reassembled the nine fold WGS data, fosmid-and BAC-end sequences, and full-length cDNA sequences into a complete and fine genome sequence for the silkworm, with a genome size of 432 Mb and a gene count of 14,623 \(^{3}\). The reduction of the predicted gene number is undoubtedly attributable to the improvement in the integrity of the new assembly. In this fine genome assembly, 87% of the scaffold sequences and 90% of the predicted genes were mapped to all 28 chromosomes using the BAC-based integrated linkage map and the SNP linkage map \(^{6, 7}\). With this genome assembly, researchers are able to analyze the evolutionary adaptations of genes or gene families related to various biological traits of interest in the silkworm.

### Genome Characterization of Silkworm

The silkworm genome is composed of 28 chromosomes with female heterogametic constitution: ZW for female and ZZ for male. The fine assembly of the genomic sequences from male silkworm resulted in a size estimation of 432 Mbp and an estimation of 14,623 genes in the silkworm genome, which is smaller than the size (530 Mbp) estimated by Cot analysis. This difference might be due to the exclusion of the DNA sequences from the W chromosome and/or some sequence gaps. Importantly, the size of the *B. mori* genome is larger than the sequenced genomes of other insects, including *Drosophila melanogaster*, *Anopheles gambiae*, *Apis mellifera*, and *Tribolium castaneum*. The silkworm genome is \(\sim 2.5\) times the size of the *Drosophila melanogaster* genome (175 Mbp) and \(\sim 1.6\) times the genome size of *Anopheles gambiae* (280 Mbp).

### Genome analysis using Biochemical markers

The genetic variation estimated through the isozyme profiles based on differential allozymes expression in various tissues of some silkworm accesses have shown 4 allozymes (G6DP, a’ b’ esterase and acid phosphatase) that could show genetic variation through allelic frequency of allele \(^{8}\). The molecular markers, both DNA sequences and Isozymes, are useful because these do not have much negative effect on phenotype. In silkworm, out of a number of biochemical parameters, digestive amylase has been found to play significant role on the expression of various attributes. Studies have found positive correlation of digestive amylase with better digestibility and survival \(^{9}\). It has been reported by many molecular biologists that polyvoltine races contain dominant amylase gene (AC+) which is responsible for inducing hardiness in multivoltine breeds. Conversely, AC- gene is recessive in temperate breeds due to which these breeds do not tolerate much environmental fluctuations especially high temperature and humidity. Breeders, however, can transfer dominant (AC+) gene of polyvoltine breeds into temperate breeds through a suitable vector for developing hardy bivoltine races. Studies have also reported that gene controlling proteinase in digestive juice is positively correlated with dietary efficiency of silkworm. The dietary efficiency is reported better in bivoltine races. Thus, proteinase of efficient breeds could be used as a marker in improving the yield. The diversity study carried out in silkworm genotypes through isozymes like esterase, acid phosphatase, alkaline phosphatase, amylase, phosphoglucomutase, aspartate aminotransferase, malate dehydrogenase, glucose 6 phosphate dehydrogenase, and carbonic anhydrase have been reported by various authors. Among the different isoenzymes analysed, esterase was most preferred because of its diverse substrate specificity and polymorphic expression followed by acid phosphatase. Four fundamental types of esterase have been reported in silkworm and about 70% of the Japanese, Chinese, and European races investigated belong to A type and 20% to 0 type, while B type was found only in Chinese races. A higher degree of inter-strain variability has been reported on the acid phosphatase and esterase in silkworm *Bombyx mori*. Acid phosphatase has also been found to be a suitable marker for analysing the inter- and intrastain diversity and the strain differentiation. Isozyme analysis in different silkworm genotypes by different authors indicated rich genetic diversity between the genotypes and results were mainly used to separate populations and strains in order to use them in selection programs.

### Genome analysis using Molecular markers

Genome analysis of *B. mori* using molecular markers has also been successfully attempted. The molecular markers, namely, RAPD, RFLP, ISSR, and SSR, have been effectively utilized in analysing the genetic diversity and phylogenetic relatedness in the domesticated silkworm *B. mori*. DNA profiles specific to diapausing and non-diapausing strains has been reported in China and India using molecular markers. RAPD based study resulted in a clear separation of two groups, one comprising of diapausing and other comprising of non-diapausing genotypes. Among the diapausing genotypes, all the “Chinese type” genotypes which spin oval cocoons grouped separately, while the “Japanese type” genotypes which spin peanut shaped cocoons were found in another group. Further genotypes, which share the same geographical origin, were grouped in the same cluster. SSR and mtDNA markers analysis revealed considerable genetic diversity among the non-diapausing silkworm genotypes that were
developed in India, China, and Bangladesh. In India, attempts have been made to develop molecular markers for high cocoon, shell weight as well as virus resistance markers and the characters are being established using divergent parents like Sarupat and CSR2. First time, eight molecular RFLP markers linked to cocoon shell character in silkworm have been identified by CSR&TI. Mysore that has prompted sincere utilization of the identified molecular markers for the successful application of directionnal breeding in silkworm for generating improved races with high silk yield. At CDFD, Hyderabad about 400 different types of microsatellite DNA markers has been developed in silkworm. Seri-Biotech Laboratory, Bangalore has succeeded in finding primers associated with characterizing low and high yielding races. These studies indicated their potential use not only in understanding genetic relationship but also as powerful tool to generate markers that are linked to traits of interest in silkworm.

**Silkworm Strain Improvement**

Since, the legendary fall and unravelling of a cocoon in the teacup of Chinese princess Xi Lingshi 5000 years ago, the mulberry silkworm, *Bombyx mori* has been intimately connected with humans. As the only truly domesticated insect, it is completely dependent on humans for survival and reproduction. As, the foundation of sericulture, the silkworm has meant economic survival for farmers and workers in the textile industry. Silk itself and the trade of silk have enriched human endeavour through art and culture, contributing to an early form of globalization for nearly 2000 years during the Silk Road Era. Beginning in the 19th century, the silkworm became a model for scientific discovery in microbiology, physiology, and genetics at a period when enzyme paradigm changes took place in our understanding of biology. Today, the silkworm plays roles in three major areas: basic research, sericulture, and biotechnology. With the recent progress in area of biotechnology, silkworm has entered into genomic era and has become a model lepidopteran organism. After the intervention of biotechnology in silkworm improvement, some of the significant achievements in genomic studies of silkworm include sex linked markers, characterization of DNA markers, construction of early linkage maps, establishment of stable germ line transformation, production of pharmaceutically important proteins, immune response proteins, and annotation of thousands of expressed sequence tags (ESTs), construction of Bacterial Artificial Chromosome libraries (BACs), identification and characterization of Z chromosome linked genes, demonstration of lack of dosage compensation, accomplishment of whole genome sequencing, identification of W chromosome specific BACs, Lepidoptera specific genes, horizontal gene transfer, and characterization of essential baculoviral genes. The development and improvement of protocol of silkworm transgenesis has opened new areas of application. The Nucleo-polyhedrosis virus is also being exploited as a vector for introduction of foreign genes. Expression of marker proteins (luciferase and green fluorescent protein) has been successfully achieved in cell lines and larval caterpillars of silkworm (*Bombyx mori*) employing recombinant *BmNPV* vector harbouring reporter genes.

**Linkage Mapping**

Genetic and molecular linkage maps provide a means of tracking inheritance of traits of interest, cloning genes for which only a phenotype is known by map position, finding transgene landing sites, and uncovering patterns of chromosome evolution. The classical linkage maps for *B. mori* consist of ~240 visible and biochemical markers on 28 linkage groups, with a recombination length of ~900 cM (23, 65). Marker density per linkage group varies from 1–3 (for 5 linkage groups) to ~20, with an average of 8 markers. Initial maps were of low-to-medium density and composed of anonymous markers genotyped by PCR (RAPDs, 169 markers) or cDNAs and known genes characterized as RFLPs (61 markers). More complete maps followed, composed of RAPDs (1018 markers), AFLPs, (356 markers), integrated RAPDs and SADFs (544 markers 35), and RFLPs based on ESTs (~200 markers 47). Many of these studies used a pair of inbred strains, C108 and p50 (also called Daizou in Japan or Dazao in China), to enable sharing of reference markers and genetic resources. p50/Daizou was also selected as the standard normal for large-scale genomic work, facilitating integration of genetic and physical maps RAPDs, AFLPs, and SADFs can be amplified by PCR, facilitating rapid largescale genotyping. However, these markers are usually strain specific and, because of their dominant inheritance, limit integration of independently constructed genetic maps. Mapping with microsatellites, of which several hundred that are polymorphic in many strains have been developed, and ESTs, which can be converted to sequence-tagged sites amplified by PCR [10,11], can circumvent these problems. Near-isogenic lines are being constructed to integrate the molecular maps with the classic linkage groups by successive backcrossing of reference strains to the standard, p50/Daizou, and will be available for all 28 linkage groups in the near future.

**Molecular Mapping**

Studies have been conducted to find molecular markers that are tightly linked to traits relevant for sericulture, with the related goals of developing tools for marker assisted selection and positional cloning. RAPD or cDNA markers have been associated with the four known densenucleo virus nonsusceptibility loci, nsd-1, nsd-2, , Nid-1, and nsd-Z. Two large contigs on chromosome 17 that encompass cDNAs closely linked to Nid-1 and nsd-2 have been isolated and sequenced and are being examined for candidate genes in susceptible and non-susceptible strains. A similar strategy was used to screen for RAPD markers linked to resistance to NPV, a potentially devastating pathogen, and fluoride resistance. Studies on NPV resistance in silkworm *Bombyx mori* has revealed that one SSR primer viz. BmSat117 and one genic primer viz. Nag 65 showed association with NPV resistant phenotype. During the study, twelve genotypes have also been reported to be resistant and susceptible to NPV and indicated presence of the markers in almost all individuals in heterozygote form. The SSR primer BmSat117 also showed association with land races reported to be NPV resistant viz. Pure Mysore, Nistari (D), GNM an; d Hosa Mysore, while, the primer Nag 65 showed association with Nistari(D), Pure Mysore’ and partly with Hosa Mysore and C. nich. Apart from the above two primers, five genic primers viz. Nag 34, Nag 55, Nag 57, Nag 84 and Nag 88 from CDFD Hyderabad also revealed association with NPV resistant divergent parents viz. Sarupat and CSR2, while, Nag 34 showed association with C. nich, GNM and Nistari. Towards introduction of DNA markers to desired breeds, Sarupat (as resistant parent)
and CSR2 (as susceptible parent) were reared and crossed obtaining F1 progeny. The back cross [F1 females were crossed with susceptible male (CSR2) lines BC1 and BC3 revealed presence of the markers in heterozygous state. DNA marker to distinguish virulent microsporidia species from non-virulent species has been identified. Screening of flacherie affected mulberry silkworm samples from farmers' fields through PCR technique indicated widespread prevalence and severity of DNV-2 in the samples, thus associating DNV-2 as a major pathogen with flacherie disease. Screening of silkworm germplasm races through PCR based technique revealed genes for resistance to DNV2 in few multivoltine and one bivoltine [KA] mulberry silkworm germplasm races. A multiplex PCR system was developed for the first time to simultaneously detect *Nosema bombycis*, Nuclear Polyhedrosis Virus (NPV) and Denso Nucleosis Virus (DNV) that infect silkworms and the technique is now being used as a service to the P3 basic seed farms of CSB.

To identify potential silkworm races/ breeds specific to thermo-tolerance for their effective utilization in breeding programme, analysis of genomic DNA samples of 15 selected polyvoltine silkworm races with 15 selected ISSR primers revealed that, the 15 silkworm races grouped into five and one isolate. Further analysis could also classify the silkworms clearly into three groups viz. susceptible, moderately tolerant and tolerant for thermal stress based on pupation rate. Analysis also revealed that one DNA marker 80S showed highly significant correlation with thermo tolerance. Progress has also been made in assigning AFLPs , RAPDs and inter-simple sequence repeat markers to QTL for characters such as larval growth rate and pupal and cocoon weight. A suite of additional fingerprinting tools has been developed for these applications.

**Genetic Engineering in Silkworm**

**Systemic RNAi**

In *B. mori*, the systemic RNAi method of injecting dsRNA into animals has been widely used in *vivo*, although the success of systemic RNAi depends greatly on the developmental stage [47]. Analysis of the gene function involved in embryonic development through the injection of dsRNA into embryos appears to be very successful. The analysed genes subjected to systemic RNAi are involved in the processes of pigmentation [12] and testis development (95). Interestingly, short interfering RNA (siRNA) might be more efficient than long dsRNA for the induction of an RNAi response during *B. mori* embryogenesis [13]. The second most suitable stage for efficient RNAi appears to be the last larval stage just prior to pupation, and the injection of the dsRNA of critical genes involved in the 20E signal transduction pathway prevents pupation, delays cell death and larval tissue remodelling, and causes lethality [10]. To date, there are no successful reports on systemic RNAi during the feeding larval stages.

**Transgenic Silkworm Techniques**

By using a piggyBac transposon-derived vector with an EGFP reporter under the control of the Act3 promoter, stable germline transformation was accomplished in *B. mori* more than 10 years ago [18]. Germline transformation has also been achieved using a Minos transposon-derived vector [19]. Recently, a germline transformation with a visible dominant marker for body colour changes was established and provided convenient selection [20]. In addition to the conventional microinjection procedure, sperm-mediated [21] and electroproportion-mediated somatic transgenesis for germ cell transformation has been developed [22]. A binary GAL4/UAS system has been developed to unravel the spatiotemporal expression and function of genes in *B. mori* and is based on the piggyBac-mediated germline transformation. In addition to the globally expressed Act3, the promoters of Fibroin light chain [23, 24], Ser1, sex pheromone receptor BmOR1 [25], and SP2 [17] can be used to create PSG, MSG, BmOR1-expressing neuron, and fat-body-specific GAL4 lines, respectively. Various GAL4 lines with tissue- or stage-specific expression patterns have also been generated with the enhancer trapping system [17]. In the field of applications research, silk glands of silkworms have been developed as a fine bioreactor for recombinant protein production using various promoters of Ser1, fibroin light chain, fibroin heavy chain, and P25 derived transgenic expression vectors [26]. Cre and FLP recombinases are the best-characterized tyrosine-catalysed integrases that enable the post integration manipulation of transgenes or endogenous chromosome fragments flanked by loxP and FRT sites. Cre and FLP-mediated site-specific recombination systems have also been established to generate transgenic silkworms harbouring loxP and FRT sites and fluorescence reporter genes [27]. The Cre-loxP and FLP-FRT are promising genetic tools for the future analysis of gene functions.

**Gene Function Studies with Transgenic Tools**

Through overexpression or knockdown, transgenic silkworm technology has been used to study the biological function of various genes involved in JH biosynthesis and degradation [28], ecysis triggering hormone signalling [29], 20E signalling [30], pheromone signalling [25], and colour determination [31]. Several transgenic lines with improved silk quantity [23], prolonged pupal duration [30], and improved resistance to pathogenic viruses have been obtained [32].

**Genome Editing Using Engineered Nucleases**

The complete functional removal of a target gene of interest or the disruption of a certain genomic element has been a long-term challenge in silkworm research. An efficient and precise genome editing methodology to directly manipulate the desired chromosomal context without the pre insertion of a landing site is urgently needed. Several encouraging reports have recently demonstrated the possibility and feasibility of using zinc finger nuclease (ZFN) to achieve targeted gene disruption in the silkworm. A direct microinjection of custom ZFN mRNAs into embryos led to the successful mutagenesis of the endogenous epidermal colour locus BmBlos2 but failed for the locus Bmwh3. However, it is a sizable challenge to produce custom ZFNs with high specificity and activity. Transcription activator-like effector nucleases (TALENs) targeted to endogenous BmBlos2 were generated, and TALENs could induce both mosaic and germline mutations with remarkable frequency. More importantly, TALEN can also be used to generate complicated genome engineering, such as large chromosomal deletions that could be transmitted to subsequent generations [11]. With the rapid progress and more frequent application of TALEN in the silkworm, TALEN will surely become a routine method for silkworm genome editing and will eventually contribute greatly to silkworm research.
Protein production using transgenic silkworms

Transgenic silkworms have also been used for recombinant protein production. To establish transgenic silkworms, two different systems, involving the use of an attenuated recombinant baculovirus or a piggyBac transposon-derived vector, were adopted. A method combining the two systems was also established. Human type III procollagen and feline interferon were produced in cocoons using transgenic silkworms. Human μ-opioid receptor was expressed in the silk glands and fat bodies of transgenic silkworms, which were screened by the GAL4/UAS system. Its expression level was comparable to that obtained in the baculovirus expression system using SF-9 cells.

BmNPV bacmid development and its applications

To construct a recombinant baculovirus containing a gene of interest, cultured cells must be transfected with the baculovirus and the transfer vector. Moreover, this system requires the isolation and amplification of the recombinant baculovirus. It takes a long time (3–6 months) to prepare the high-titer recombinant baculovirus solution, and these steps are very tedious and troublesome. A novel system for the efficient production of recombinant AcMNPV was reported, which is based on site-specific transposition in Escherichia coli. Luckow and colleagues constructed a recombinant baculovirus vector (bacmid) that can replicate in E. coli as a large plasmid. This system is known as the Bac-to-Bac Baculovirus expression system, and various kits based on this Bac-to-Bac system have been distributed from Invitrogen Corp. This system reduces the time required to isolate and purify a recombinant baculovirus and allows the simultaneous construction of multiple recombinant baculoviruses. Studies have been done and a BmNPV bacmid have been constructed and established the Bac-to-Bac system using BmNPV. GFPuv was expressed with only the injection of BmNPV bacmid DNA into silkworm larvae and pupae. This bacmid system provided rapid expression of recombinant proteins, since it did not require the preparation of a Baculovirus solution by transfection, as compared to the Baculovirus expression system using cultured cells. Furthermore, this BmNPV bacmid system dramatically reduced the time needed for recombinant protein production by silkworm expression.

Functional Proteins in Silkworm for Biomedical Application

Haemolymph contains hemocyanin. Hemocyanin is large copper-containing protein that transports oxygen in the haemolymph of many arthropod and mollusc species, they are haemolymph plasma proteins which functions in defence against microbial infection. Phenol oxidase an enzyme stored in haemocytes, and their function is melanisation process of after body injury for stop the blood loss. Some times haemocytes are also playing an important role of immune function to protect. Recently silkworm is used for assessing the therapeutic effects of chemicals, drug discovery, screening for immune stimulatory agents, detecting test of poisons for drugs and quantitatively evaluating therapeutic effects. Pyriproxyfen residue is changes the level of sugar, urea, uric acid, cholesterol, total protein, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in haemolymph. The changes of haemolymph protein metabolism after treatment of organophosphorus insecticides are identified in haemolymph.

The neurohormones are secreted from the central nervous system by the neurosecretory cells of the pars intercerebralis of the brain into the haemolymph to regulate various aspects in a diverse group of insects. The findings of silkworm haemolymph profiling reveals the complex mechanisms involved as evident from the insect physiological mechanisms. It is very relevant to analyse and understand the complex proteins in silkworm Bombyx mori. Though till recently it was only looked at as a silk producing organism, with the advent and of natural materials and bio-resourceable materials for biomedical applications. The present need of considering the use of silkworm as a model animal for genomic as well as pharmaceutical applications industrially will be more efficacious considering the ethical and functional aspects. The function of same as mammalian insulin, Bombyx mori bombyxin stimulates the phosphorylation of InR and Akt. Similar to insulin in mammals, bombyxin also regulates sugar and lipid metabolism in Bombyx mori. 30K protein of silkworm haemolymph have been reported to exhibit anti-apoptotic activity in various mammalian and insect cell systems and could have therapeutic potential in diseases related to apoptosis. They isolated and characterized the apoptosis inhibiting compounds from silkworm haemolymph.

Antimicrobial Peptide Families

AMPs are mainly effector molecules in the immune system that play direct roles in killing and eliminating infectious microorganisms. AMPs are composed mainly of four families: cecropins, gloverins, moricins, and attacins. The silkworm has the largest Cecropin gene family among all the sequenced insect genomes. Thirteen Cecropin genes, including two Cecropin A, six Cecropin B, one Cecropin C, one Cecropin D, one Cecropin E, and two Ebocin genes, were identified in the silkworm genome. Eleven Cecropin genes are localized on chromosome 11 as a cluster, which suggests that these genes are derived from an expansion through gene duplication. The Gloverin family contains four gloverin genes, named BmGlov1 through BmGlov4; three of these genes are located on chromosome 28, and the other is located on chromosome 17. All the Gloverin genes are induced in the larval fat body after an immune challenge by Escherichia coli. Moricins exhibit wide antibacterial activity against gram-negative and gram-positive bacteria. One Bmмор and eight BmMorLs (BmMorLA1, BmMorLA2, and BmMorLB1–6) have been identified in the silkworm genome. Two Attacin genes are located in tandem on chromosome 6, whereas single Defensin and Lebocin genes are located on chromosomes 13 and 10, respectively. Cecropin, Attacin, and Defensin are evolutionarily conserved in insects, whereas Lebocin, Gloverin, and Moricin are specific to Lepidoptera.

Future Perspectives

With the genomic elucidation of silkworm, even more laboratories around the world will be interested in its utilization. After years of effort, the silkworm B. mori has been developed into one of just a few insect systems for which the most advanced genetic technologies are available. The application of these technologies has not only facilitated the functional analysis of B. mori genes, and enabled the sophisticated genetic modification of silkworms to improve their commercial value, but also contributed to the development and use of similar technologies in non-model insects. Enhanced capabilities for genetically manipulating B. mori.


