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Recent advances in silkworm biology

The silkworm, *Bombyx mori* (family Bombycidae) has been domesticated for silk production for over 4000 years, and as a result of the intense study of the silkworm, knowledge of its biology and genetics is the most advanced of any lepidopteran species.

The silkworm has long been used as a model lepidopteran for research in physiology, development, endocrinology, biochemistry, genetics and virology, providing a rich background of information on its basic biology. It was among the first eukaryotic organisms to serve as a model system for cloning genes and studying the regulation of their expression. *B. mori* was also the first insect from which a key peptide hormone, prothoracicotropic hormone (PTTH), which triggers successive moults via release of the regulatory insect steroid hormone, ecdysone was isolated. This study led to the isolation of a number of polypeptide hormones and their genes which control insect physiology and behaviour. The silkworm *B. mori* is also the first lepidopteran insect to obtain transposon-mediated germline transformants. Recent studies on immunity in *B. mori* have kept pace with the developments in other insect models, such as the fruit fly and the tobacco hornworm, *Manduca sexta*.

Besides being a lepidopteran model, *B. mori* is an insect of economic importance to countries like China, India, Thailand, Vietnam and many other developing countries. World silk production based on this insect has approximately doubled during the last 30 years in spite of man-made fibres replacing silk for some uses. China and India have been the two main producers, together manufacturing more than 50% of the world silk production each year. Silk production plays important role in the rural economies of these developing nations. Six million peo-

ple in India alone are engaged in sericulture, which is very labour intensive and provides a key to improving local quality of life. However, disease resistance and other improved traits which can augment productivity and quality are needed to be inculcated to enhance the economic benefits to the sericulture farmers. In order to be successful in this direction, a highly sophisticated knowledge of silkworm biology will be required that will enable the development of silkworm strains suitable for producing more quality silk per unit farming area, in combination with improved farming practices. Such an effort would ensure sustainability of sericulture.

In the special section of this issue of *Current Science*, I have attempted to compile reviews from experts on recent advances on a few selected areas which, in my opinion, will bear impact on practical and basic biological applications in silkworm in particular and lepidopteran insects in general.

The productivity in terms of silk quality and yield has been remarkably improved over the years in the silkworm, *B. mori*, particularly by Japanese breeders, as a result of selection and hybridization, coupled with systematic application of genetic principles. Advantages of rearing temperate silkworm strains under temperate environment aided their breeding efforts. Unlike their Japanese counterparts, Indian breeders have to struggle with a situation where temperate silkworm strains have to be reared under harsh tropical conditions coupled with poor sanitation and nutrition conditions. The Indian efforts paid off only to an extent of introducing F₁ hybrids of indigenous polyvoltine and Japanese bivoltine strains on a commercial scale. Introduction of exclusive bivoltine hybrids in the regions and seasons that are conducive to temperate silkworm rearing is now being actively pursued. The article on application of genetic principles for

silkworm improvement discusses different classical breeding methods and the genetic principles adopted in silkworm improvement programmes and also brings out some issues that are considered relevant to be addressed under Indian conditions (page 409).

When compared to the *Drosophila* genome of 180 Mb in 4 chromosomes, the genome of silkworm is 530 Mb in 28 chromosomes. Genetic crosses are routinely accomplished in silkworm, achiasmatic oogenesis implies that there is no crossing over in the heterogametic (ZW) females. Thus linkage is all or none, synteny is easy to detect, and recombination means that genes are on different chromosomes. The GC content of silkworm DNA is about 35–40%. The modern silkworm genomics is complemented by a strong foundation of classical genetics. The silkworm is second only to the fruitfly as an insect model for genetic studies, with more than 400 visible mutations identified and mapped over 200 loci. In GenBank there are currently more than 20,000 entries for the silkworm. Recently a number of molecular markers have been generated and framework maps have been prepared, BAC contigs have been built, and a high coverage of expressed genes through expressed sequence tags (ESTs) has been made, which can serve as important resources for physical map construction and to localize genes of economic and basic biological interests. Nagaraju and Goldsmith (page 415) have discussed the progress in this field in their article.

The large-scale silkworm genomics activity was started with the sequencing of ESTs by Mita and his colleagues in Japan (page 426). The entire EST data have been put on 'SilkBase' database which contains information on 32 cDNA libraries with 26,000 entries of which 9500 are non-redundant. EST-based DNA microarrays for the analysis of gene expression are an essential tool to

exploit genomic data. Drawing on the *Bombyx* EST collection, a microarray containing 6,000 independent ESTs has been prepared in Japan.

The *P*-element mediated *Drosophila* transformation was achieved in 1982, and all subsequent efforts to employ *P*-elements as gene vectors in non-drosophilid insects failed until 13 years later when the first bonafide *Minos* transposon-mediated transformation of a non-drosophilid, the Mediterranean fruit fly, *Ceratitidis capitata* was reported (Loukiers *et al.*, *Science*, 1995, **270**, 2002). However, the transformation system used in the medfly could not be simply extended to other non-dipteran insects. The failure to achieve non-dipteran transformation prompted scientists to look for transposable elements from various non-dipteran hosts. The first success came with the discovery of *Piggy Bac*, a functional transposable element isolated from a *Trichoplusia ni* cell line. The *Piggy Bac* based vector has been used for the first germline transformation of Lepidoptera in the silkworm, *B. mori*. This success has been realized after many frustrating attempts over a number of years. Prudhomme and Couble (page 432) have provided the state-of-art knowledge on *Bombyx* transgenesis and exciting possibilities of using silkworm transgenesis for silkworm strain improvement and to study gene functions.

Many pathogens cause heavy mortality of silkworms. Among these, viral pathogens are the most destructive. Our basic knowledge about insect resistance to pathogens mainly concerns bacterial agents and there is paucity of information on viral pathogens. The early studies carried out by Watanabe and his group clearly show that genetic resistance to baculovirus is polygenic in nature. He has also discussed (page 439) genetics of resistance pertaining to the other silkworm viral pathogens

and has suggested ways and means to introduce viral resistance during silkworm breed development programmes.

The repertoire of antibacterial proteins and peptides expressed on bacterial infection in insects provides them with the ability to combat infection against a wide spectrum of pathogens defining the humoral immune response. The humoral facet of the insect host response shows an interesting originality in that it includes the rapid appearance in the cell free haemolymph of a potent antibacterial activity, which is non-specific, and non-memory type and hence the same antibacterial protein can act against a wide range of bacteria. The other important feature of such proteins is that they have very low toxicity on most animal cells. The development of this unique and sophisticated self-defence system has conferred adaptive advantage to these insects to be ecologically successful. Since the characterization of the first antibacterial protein, cecropin from the haemolymph of the Cecropia moth, *Hyalophora cecropia*, the field of insect immunology has received increasing attention which has resulted in a better understanding of innate immunity of both invertebrates and vertebrates. The silkworm, *B. mori* is known to possess considerable degree of humoral immunity by way of synthesizing a variety of induced antibacterial proteins such as cecropins, attacins and lysozyme-like proteins. Ponnuel and Yamakawa (page 447) have provided current understanding on this subject.

Lepidoteran insects and their cell lines are useful as hosts for the production of heterologous proteins by baculovirus vectors. The exploitation of silkworm, *B. mori* as a biofactory has been enabled by the development of baculovirus expression system to produce high value proteins either in cell lines, or in whole silkworms. A complete infor-

complete information on *Bombyx mori* Nuclear Polyhedrosis virus (BmNPV) has been provided by Gopinathan and his colleagues (page 455).

The phenomenon of sex determination has been and still is an enigma, in that, decades of intensive research could not bring out a clear picture of the regulation and molecular organization of sex-determining genes. The sex determination cascade is found to be a rapidly evolving system and thus a comparative analysis of sex determination has not led to a generalization of the process. Nevertheless, at least some aspects of sexual regulation are shown to have a common evolutionary origin and are conserved. For example, the downstream gene *double sex (dsx)* is the most conserved gene across the species while the way it is regulated is subjected to evolutionary changes. The review on the sex determination system in *B. mori* by Shimada and his colleagues (page 466) ascertains the idea that the downstream sexual regulators in sex determining pathways are the most ancient and that upstream regulatory genes have been recruited recently. *B. mori double sex* is controlled by an upstream regulatory system unique to this female-heterogametic insect.

The authors who have contributed to this special section have decades of experience in their respective fields and readily accepted to contribute the articles when I approached them. I sincerely thank all of them. During this period, one of the contributors, H. Watanabe, a highly distinguished virologist passed away in Tokyo because of ill health. On my personal behalf and on behalf of *Current Science* I condole his death. I hope that this special section on recent advances in silkworm biology will be useful to teachers, researchers and sericologists.

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