

## Dimensions of molecular entomology\*

Inaugurating the discussion meeting, M. S. Swaminathan, MSSRF, Chennai designated this as the era of Molecular Entomology, calling for millennium development courses and referred to it as 'Precision Entomology', which is the need of the hour to address specific issues. He stressed that the success of any innovation in agriculture by scientists will be evident only by its acceptance by farmers. Being the International Year of Rice, much needs to be done with regard to problems posed by such pests as the leaf folder, gall midge and the brown plant hopper. In this connection, he also made special mention of the locust invasion now threatening the crops in several countries. Uncommon opportunities are available in molecular entomology, in particular, enterprise development.

Talking on Biotechnology and Entomology, the Vice-Chancellor of the University of Madras, S. P. Thyagarajan opened up new vistas in entomology with biotechnological inputs. He summarized the investigations on functional genomics and proteomics in the field of medical entomology. Special mention was made of monoclonals and biotherapeutics, engineering insect-specific viruses, gene-enzyme pathway and phylogenomics. He emphasized that such impetus is needed in areas of Apiculture and Sericulture.

Introducing the theme, T. N. Ananthakrishnan emphasized that in recent years Biochemistry, Genetics and Molecular Biology have become essential ingredients in our understanding of entomological problems. With the molecular biology approach, a different emphasis has resulted from obtaining and handling DNA, which has provided the necessary tools for transferring, and evaluating genetic characters from a host of insects. The ability to recognize molecules is very typical of receptor sites, especially the recognition of sexual pheromone-binding proteins in the olfactory sensilla.

The significance of the sensillar-neural complexes involving recognition of molecules such as that of pheromone-binding proteins, is that the composite field of sensillomics is involved. Further, our

understanding of pheromone production has evolved from identifying biochemical pathways towards unravelling the molecular biology of key regulatory enzymes, initiating a genomic approach. A marriage of molecular genetics and genomics and behaviour is giving a new understanding of how pheromones are detected. It is readily accepted today that olfaction and taste work via sensory neurons and that odour and taste molecules stimulate these neurons by binding to receptor proteins. With chemical ecology deciphering both the chemical structure and the information content of the mediating molecules, genetically engineered cultivars which release reliable volatile organic chemicals in response to herbivore attack, have assumed significance. Metabolic engineering of volatile synthesis has come of age, in particular the formation of monoterpenes and sesquiterpenes in attracting insect natural enemies. The biosynthesis of signal molecules that convey information from one organism to another, is under genetic control and deciphered at specific receptor site and transmitted into behavioural, morphological or physiological response. Insect baculoviruses as expression vectors for neurohormonal genes, can be inserted into the genomes of crop plants, so that the transformed plants would produce the hormone and disrupt the physiological balance of phytophagous insects. The role of induced defences, some of which code for single genes such as proteinase and amylase inhibitors was also indicated, besides the insect crystal protein genes of *Bacillus thuringiensis* (*Bt*), which play an important role in resistance.

In his keynote address on 'Novel approaches in Genetic Sexing in Lepidopteran Insects', J. Nagaraju, of the Center for DNA Fingerprinting and Diagnostics, Hyderabad, indicated that the development of molecular markers has become important for construction of linkage maps, fingerprinting of strains and marker-assessed sections. A variety of techniques have been used in the silkworm to detect genome-wide polymorphism. Based on the sterile insect techniques, which rely on mass rearing and release of large numbers of genetically sterile insects into a wild population, several lepidopteran and dipteran species were tested. Indicating that molecular mechanisms of high radioresistance in Lepidoptera might induce cell recovery

system and/or DNA repair process, he suggested that the main cause of this difference could reside in the organization of chromosome in these two groups of insects. The Diptera has typical monochromatic chromosome with kinetic activity restricted to the centromere, while in Lepidoptera chromosomes are essentially holokinetic.

He outlined the approaches that are currently available to separate efficiently large number of males and females for different lepidopteran species and indicated the difficulties and constraints of these Mendelian approaches. Transgenesis cannot be carried out in several lepidopteran species and this opens up additional possibilities to develop genetic sexing strains. He outlined the development of genetic sexing strains in Lepidoptera, which are based on the construction of transgenic females carrying a dominant conditional lethal gene inserted in the female determining W-chromosome.

Discussing the molecular aspects of insect biotypes, J. S. Bentur of the Directorate of Rice Research, Hyderabad, highlighted some aspects of the genetic basis of interaction between the biotypes and the host genotypes in the wheat-hessian fly system. Based on gene-gene interaction he indicated that 31 resistance genes have so far been identified in wheat cultivars and based on the virulence pattern 14 biotypes of the fly are recognized. A new approach has been the use of genomic and recombinant DNA technologies, with target genes involved in plant-insect interactions. Available information for the rice gall midge system show similarities with the studies on wheat-hessian fly. So far 10 plant resistance genes have been recognized, targeted and mapped and 6 gall midge types have been recognized. Molecular markers have also been used to understand the evolution of gall midge biotypes. As more and more information is generated through use of molecular and genomics techniques, plant-insect interactions in general and biotype-specific resistance in particular are being better understood for a more meaningful utilization of information for developing better pest management strategies.

H. C. Sharma of ICRISAT, Hyderabad, discussing aspects of transgenic resistance to insects indicated that with recombinant DNA technology it has become possible

\*Based on the fifth Annual Discussion Meeting on Dimensions of Molecular Entomology held on 27 November 2004 in Chennai.

to clone and insert genes into the crop plants to confer resistance to insect pests. Resistance to insects has been demonstrated in transgenic plants expressing genes for  $\delta$ -endotoxins from *Bt*, protease inhibitors, enzymes, plant lectins, secondary metabolites and toxins from arthropods. The potential of plant-derived genes can be realized by developing them in combination with conventional plant resistance and novel genes. Genes conferring resistance to insects have been inserted into maize, cotton, potato, tobacco, rice, sorghum, chick pea, pigeon pea and soybean. He also highlighted the need to use this technology for improving the crop protection in the harsh environments of the tropics, where the need for increasing food production is most urgent, and thus develop a scientifically sound strategy to deploy transgenic plants for pest management to reduce pesticide use and minimize the extent of losses caused by insect pests.

M. Krishnan of the Bharathidasan University, Tiruchirapalli, discussing the molecular characterization of the vitellogenin (*Vg*) and its receptor in *Spodoptera litura* elucidated the mechanism of sequestration of *Vg* through receptor-mediated endocytotic process. He characterized the study as a prologue to molecular pest management wherein both molecular and genetic events are involved in vitellogenin synthesis, enabling adequate comparison.

A. V. Navarajan Paul of Indian Agricultural Research Institute, New Delhi, highlighted the significance of signalling molecules in the biological control of insects. The chemical cues utilized by parasitoids and predators in these processes have been recognized as interactive chemicals between organisms based on the nature of the interactions and on a cost-benefit analysis of the interaction as a kairomone, synomone or allomone. The cuticular waxes of crop varieties are found to have specific hydrocarbon profile showing specific synomonal effect on natural enemies. Breeding plant varieties having favourable hydrocarbon profile to key natural enemies will encourage their activity resulting in effective biological control. With use of kairomonal formulations, insect pheromones could be used as long-range kairomones to monitor the activity of the natural enemy in the ecosystem before and after releases. Behaviour-modifying chemicals could also be used in mass rearing laboratories to enhance 'associative learning' of the natural enemies be-

fore release to increase the host searching ability of the natural enemies.

Aparna Dutta Gupta of the School of Life Sciences, University of Hyderabad, discussed aspects relating to the identification of a novel regulatory protein from haemolymph of *Corcyra cephalonica*. The activity of acid-phosphatase (ACP) in insect fat bodies is stimulated by the steroid hormone 20-hydroxyecdysone (20E) *in vivo*. However, in *in vitro* cultured fat bodies, a factor from the haemolymph is required to enhance the ACP activity, which was identified as a 19 kDa protein (HP19) from the haemolymph of *Corcyra cephalonica*. Co-culture, immunoblot and *in situ* immunodetection studies revealed that the protein is synthesized by hindgut associated lobular fat body (HGLFB) of larvae and is released into the haemolymph. Although the protein is found to be present in haemolymph of all stages of final instar larvae, only the late last instar larval (LLI) haemolymph has the potentiation ability, suggesting that the protein gets activated at this stage. Active HP 19 inhibited the fat body tyrosine kinase activity as well as 20E-induced phosphorylation of the hexameric receptor protein and blocked a sequestration of hexamerins. Immobilizing HP 19 *in vitro* by injection HP 19 antibody to last instar larvae led to the development of non-viable larval-pupal or pupal-adult intermediates, suggesting that the protein has the role in post-embryonic development, by regulating a few of the 20E-mediated interactions. Cloning the HP 19 cDNA was done by immunoscreening a HGLFB-cDNA expression library. Analysis of the amino acid sequence showed that HP 19 belongs to the family of glutathione S-transferase (GST) like proteins. However, affinity purified GST from *Corcyra* failed to show any mediation effect on 20E stimulated ACP activity and HP 19 lacked GST enzymatic activity.

B. K. Tyagi of the ICMR Centre for Research in Medical Entomology, Madurai, highlighted the need for an understanding of the genomic sequence of mosquitoes. The principal thesis of genomic discovery revolves around the basic plan of the double-stranded molecule and there are 278 m base-pairs in the genome of the *Anopheles* mosquito. Written in this DNA are over 13,000 genes (a similar number to that of the fruit fly, *Drosophila melanogaster*, whose genome has already been decoded) that the mosquito cells used as templates to make proteins. In view of the tremendous amount of variation in the

physiology and behaviour between *Anopheles* and other major vector mosquito groups, researchers are now engaged in deciphering the genome of *Aedes aegypti*, the carrier of the deadly yellow fever and dengue infections. Vector mosquitoes like *Anopheles stephensi* (another serious vector for malaria), *Culex quinquefasciatus* (vector of lymphatic filariasis) and *Aedes albopictus* (vector of dengue in South Asia, and several kinds of viral infections in different part of the world, including the Americas) are being targeted next.

A. Sen of the National Chemical Laboratory, Pune discussed aspects relating to electroantennogram responses of *Trichogramma chilonis* and *Chrysoperla carnea* to plant volatiles, and highlighted the need for assessing molecular aspects of sensory signals. The wide spectrum of response obtained with males and females of both species suggests that the olfactory system in such polyphagous species is not only tuned to specific plant odours but also to other compounds irrespective of their host range, thereby reflecting differential tuning of receptors on the antennae. Since host plant odours are a complex blend consisting largely of compounds generally distributed among plant species, the difference in response to the major group of compounds suggests that using plant cues probably enhances its orientation towards a plant species on which the likelihood of finding a host is increased. Current research focuses on the role of induced plant volatiles using the GC-EAD technique that would further elaborate volatiles that are specific to a particular host-plant complex.

G. T. Gujar of Indian Agricultural Research Institute, New Delhi highlighted aspects of the molecular basis of genobiotic resistance in insects. Selection of insects of the high doses of genobiotics over the generations is a genetic trait. Insecticide resistance being a recessive trait helps in having a heterozygotic susceptible population. Insecticide resistance is both metabolic and biophysical. Degradative enzymes like mixed function oxidase, esterase, glutathione transferases and amidases participate in imparting resistance to insecticides. The characterization of enzymes and receptor proteins have helped in the development of biochemical diagnostic tools, such as PAGE for esterases in pyrethroid resistance. These have also given rise to DNA-based genotypic techniques, such as bi-directional PCR amplification of specific alleles, single-standard conforma-

tional polymorphism (SSCP) and minisequencing. Studies carried out on *Bt* resistance have shown the possibility of involvement of single gene or limited alleles. Characterizing the gene responsible for *Bt* resistance in *H. virescens* has enabled efficient DNA-based screening for resistant heterozygotes by directly detecting the recessive allele. Similar studies on identification of DNA markers to monitor *Bt* resistance in the American bollworm, *Helicoverpa armigera* are being carried out, to provide further impetus on efforts to develop *Bt* resistance management tactics.

Mariamamma Jacob of the University of Kerala, Thiruvananthapuram highlighted the insect cell, tissue and organ culture as a tool in molecular endocrine research. Studies on the hormonal regulating mechanisms of spermatogenesis of *Oryctes rhinoceros* showed that testis fragments underwent meiosis in the presence of 20-hydroxyecdysone. Electrophoresis (SDSPAGE) of culture media, before and after spermatogonia culture showed new proteins in response to ecdysone added in the culture. Spermatocytes differentiate to spermatozoa when brain, corpus cardiacum (cc) and corpus allatum (ca) from adults are added in culture, thus giving evidence that other hormones/neuronal factors are

essential for spermatogenesis. Most of the lepidopteran studies show that ecdysone is the only hormone promoting spermatogenesis.

Aspects relating to genetic and molecular implications in the improvement of *B. thuringiensis* were discussed by J. S. Kennedy of the Tamil Nadu Agricultural University, Coimbatore. He illustrated five different techniques for using genetically engineered *Bt* endotoxin gene to combat insect pests. These include spores and crystals, bioencapsulation, epiphytes or microbes that colonize the roots or leaves of plants, endophytes or microbes that live inside plant tissue and transgenic plants.

Delivering the valedictory address, T. M. Manjunath, Consultant, Monsanto Research Centre, Bangalore discussed opportunities and challenges afforded by transgenic crops. Transgenic technology offers exciting opportunities to develop plants with new traits, which include resistance to biotic stresses, pests, diseases, herbicides as well as abiotic stresses, drought, heat, cold, salinity. Transgenic technology also helps in increasing crop yield and enhancing quality traits such as nutrition and shelf-life of fruits, vegetables and flowers. Transgenic crops bestowed with any of these traits will significantly contribute towards improving sustainable agriculture. Similar

economic and environmental benefits from transgenic crops had been reported from other countries also. Transgenic crops with various other traits are at various stages of experimentation in several countries. 'Golden Rice' fortified with vitamin A and iron, mustard with increased beta-carotene, potato with enhanced protein content, fat free oil, corn with enhanced animal feed products like lysine, edible vaccines are good examples.

S. Chelliah, Tamil Nadu Agricultural University, Coimbatore, while moderating the open session concluded that younger scientists should take up shores on molecular entomology, since there is need for a paradigm shift from traditional study of insects to address specific problems posed by biotypes and races of insect species for which molecular tools may be used in the diagnostics of biotypes. He emphasized once again that any success in agricultural revolution through innovative approaches is possible only if it is accepted and adopted by farmers.

**T. N. Ananthkrishnan**, Flat No. 6, 'Dwaraka' 22 (New No. 42), Kamdar Nagar, Nungambakkam, Chennai 600 034, India e-mail: ananththrips@yahoo.com

## RESEARCH NEWS

### Obesity and gut flora: Link revealed?

*Parag Vaishampayan, Milind Patole and Yogesh S. Shouche*

The spreading epidemic of obesity is engulfing not just industrialized countries but developing nations as well<sup>1</sup>. It is also associated with other disorders like type II diabetes, cardiovascular pathology, hypertension and non-alcoholic fatty liver diseases. India is no exception to this and weight gain and obesity are beginning to pose a growing threat to the health of citizens<sup>2</sup>. The reasons attributed to this are industrialization, urbanization, increased living standards, change of diet and life style. But in their recent publication, Backhed *et al.*<sup>3</sup> make a startling revela-

tion that our gut microbes are responsible for fat deposition in our body.

Influence of gut microbes on human health is not new, it was realized long back<sup>4</sup>. Their sheer number in the gut milieu speaks of their value in human health. In a normal healthy adult, there are as many as  $10^{23}$  microbes in the gut, this is about 10 times the total number of cells present in the human body. There is increasing belief that this microbiota should be viewed equivalent to an 'organ', exquisitely tuned to carry out metabolic functions that we are unable to perform ourselves. Like any

other branch of microbiology, the components of this microbiota remained poorly defined due to the limitations of being able to culture them in the laboratory. Recently developed approaches based on direct amplification and sequencing of genes like 16S rRNA estimate that total number of different species present in the gut could be 500–1000, this again is equivalent to 100 times more genes than the human genome<sup>5</sup>.

The most prominent member of this community is a Gram-negative anaerobic organism called *Bacteroides thetaiotami-*