Insect Pathology (ENT. 601)

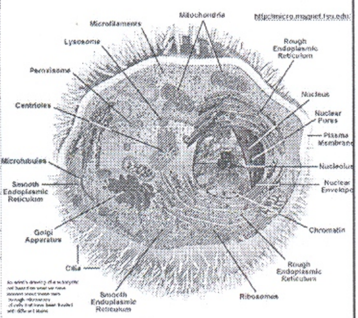
Dr. M.K. Dhillon

Division of Entomology
Indian Agricultural Research Institute (IARI),
New Delhi 110 012
Email: mukeshdhillon@rediffmail.com

Fungi: Characteristics, Phylogenetics, Classification, and Historical Aspects

Eukaryotic Cell Structure

Organelle	Structure and Function
Nucleus	Contains chromosomes and the nucleolus. Stores genetic material and controls all cellular activities.
Nucleolus	Mass of RNA located within the nucleus. Center for organizing ribosomes and other products with RNA.
Ribosomes	Granular protein particles involved with the synthesis of proteins.
Endoplasmic reticulum (ER) Rough ER Smooth ER	Membranous network continuous with the cell and nuclear membranes. ER with ribosomes attached. Involved with protein synthesis for use outside the cell. No ribosomes attached. Functions in steroid synthesis, intracellular transport, and detoxification.
Golgi apparatus	Stacked membranes and vessels that package proteins produced at rough ER.
Mitochondria	Oval organelles with membrane folds called cristae that produce ATP through the Krebs cycle and oxidative phosphorylation.
Lysosomes	Vesicles filled with enzymes that break down worn cellular components or engulfed particles.
Secretory vesicles	Membrane-bound sacs which store proteins for secretion.
Microtubules Microfilaments	Long protein fibers that function in cell support and movement.
Centrioles	Two short rods composed of microtubules located near the nucleus that are involved in movement of chromosomes during cell reproduction.

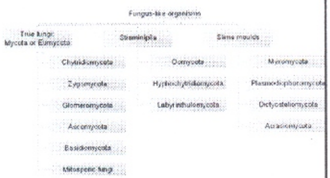


Fungal Characteristics

- Traditionally, Fungi were placed in the plant kingdom, because they have cell wall, are usually non-motile, and generate spores as a way of reproducing.
- Fungi are heterotrophic and obtain nutrients by absorption from dead organic matter (saprobes) or from living organisms (parasites).
- Fungal cells are eukaryotic, and are never photosynthetic.
- The fungal cells contain at least one nucleus in which a double membrane encloses most of the DNA.
- The DNA is closely associated with histone proteins (basic, positively charged protein), and together they form chromatin fibers.
- The nucleus also contains a nucleolus where ribosomes are assembled.
- Nuclear pores in the double membrane provide a means of communication between nuclear contents and other cytoplasmic components.
- Mitochondria, sites of oxidative degradation of nutrient molecules and of the generation of ATP, the main carrier of chemical energy, are also found in fungal cells.
- The organelles, like nuclei are surrounded by a double membrane; are about the size and shape of bacteria, contain their own DNA, and can reproduce by binary fission.

Fungi: Classification

- In 1969 Whittaker devised a five kingdom system establishing a separate kingdom for the fungi as well as for plants, animals, algae and protozoa, and bacteria.
- The higher taxa of fungi have undergone drastic revision in recent years.



- Upto the past decade, the higher taxa were generally considered as Phycomycetes, Ascomycetes, Basidiomycetes, and Deuteromycetes (Fungi Imperfecti).
- Among the several proposed revisions, we have adopted the classification by Anisworth (1973), which separates fungi into two divisions:
  - Myxomycota: For plasmodial forms.
  - Eumycota: For nonplasmodial forms that are frequently mycelial.

Entomopathogenic Fungi: Classification

- Entomopathogenic fungi are found in the division Eumycota and in the following subdivisions:
  - Mastigomycotina
  - Zygomycotina
  - Ascomycotina
  - Basidiomycotina
  - Deuteromycotina
- There are no known entomopathogenic forms in the division Myxomycota (slime molds).

Insect Nutrition & Host Plant Resistance (ENT 604)

Dr. M.K. Dhillon

Division of Entomology
Indian Agricultural Research Institute (IARI),
New Delhi 110 012
Email: mukeshdhillon@rediffmail.com

Host Plant Resistance to Insects: Basic Principles

- Resistance of plants to insects enables a plant to avoid or inhibit host selection, oviposition and feeding, reduce survival and development, and tolerate or recover from injury by insect populations that would cause greater damage to other plants of the same species under similar environmental conditions.
- Resistance in plants to insects is the consequence of heritable plant characters that result in a plant being relatively less damaged than the plant without these characters.
- Plant resistance to insects is relative, and the level of resistance is expressed in relation to resistant and susceptible genotypes. This aspect is important, since expression of resistance is dependent on insect density and environmental factors, both in time and space.

Host Plant Resistance to Insects: Basic Requirements

- Constitute host plant resistance team.
- Information on biology of the insect species & availability of pest population.
- Identify sources of insect resistance.
- Develop cost effective & reliable screening technique(s).
- Identify trait(s) associated with resistance to insect(s).
- Determine genetics and inheritance of resistance trait(s).
- Selection of appropriate breeding method.

Constitution of Host Plant Resistance Team

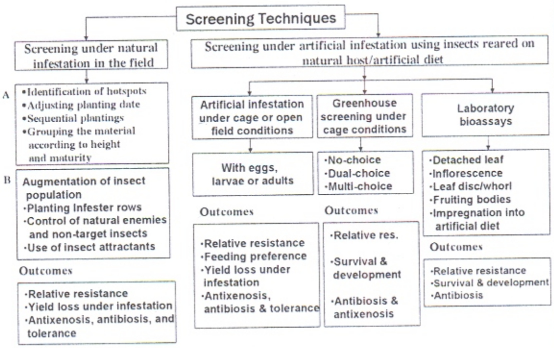
- Cooperative and interactive relationship between the Entomologist and the Plant Breeder to initiate the HPR Programme.
- Other scientists should be added to the group as the need for their contributions is recognized.
- Entomologist must lead the effort to identify sources of resistance, and also evaluate resistance breeding lines depending on need and the criteria.
- Plant breeder should provide the seed source, and later transfer the resistance genes from the identified source in the desired genetic background to achieve the status as a cultivar.

Information on Biology of Insect Species Involved

Prior to start of HPR Programme, following information should be available:

- Influence of biotic and abiotic factors on biology of the pest insect species.
- Information on behavior, especially in relation to food habits, oviposition and movement.
- Define the parameters of growth and fecundity.
- Effect of the environment on pest populations.
- Experimental design that take in to account the biological expression of important traits of the pest and the host.

Screening for Host Plant Resistance to Insects



Insect Biochemistry (ENT. 606)

Dr. M.K. Dhillon

Division of Entomology
Indian Agricultural Research Institute (IARI),
New Delhi 110 012
Email: mukeshdhillon@rediffmail.com

Some Important Enzymes & Coenzymes

- Acetyl coenzyme A (acetyl-CoA): An important molecule in metabolism, used in many biochemical reactions. Its main function is to convey the carbon atoms within the acetyl group to the citric acid cycle to be oxidized for energy production. In chemical structure, acetyl-CoA is the thioester between coenzyme A (a thiol) and acetic acid (an acyl group carrier).
- FADH2: Flavin adenine dinucleotide (FAD) is an electron accepter that is utilized in cellular respiration.
- NADH: Nicotinamide adenine dinucleotide (NADH) is a coenzyme found in all living cells (NADH, is used as a reducing agent to donate electrons).
- ATP: Adenosine-5'-triphosphate (ATP) is a multifunctional nucleotide used in cells as a coenzyme.

Lipid Digesting Enzymes

- Most of the fat eaten by an insect consists of triacylglycerols.
- Lipases secreted from the midgut and in some insects probably from symbionts, release fatty acids and glycerol from triacylglycerols.
- Emulsifying agents that enable hydrophilic enzymes to contact the hydrophobic surface of the triacylglycerol are important in digestion of lipids. Although, natural emulsifying agents are largely unknown in the insect gut; but amino acids, proteins, and fatty acylamino complexes act as emulsifiers in some insects.
- Components in the glycocalyx layer in the gut may aid in emulsifying fats and in promoting contact between lipases and triacylglycerols.

β-Oxidation

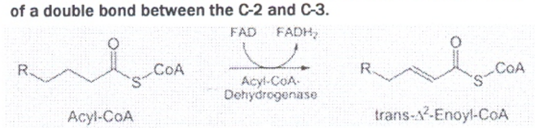
- Beta oxidation is the process by which fatty acids, in the form of Acyl-CoA molecules, are broken down in mitochondria and/or in peroxisomes to generate Acetyl-CoA, the entry molecule for the Citric Acid Cycle.
- The beta oxidation of fatty acids involve three stages:
  - ✓ Activation of fatty acids in the cytosol.
  - ✓ Transport of fatty acids into mitochondria.
  - ✓ Beta oxidation proper in the mitochondrial matrix.
- Fatty acids are oxidized by most of the tissues in the body. However, the brain, erythrocytes, and adrenal medulla cannot utilize fatty acids for energy requirements.

Activation of Fatty Acids

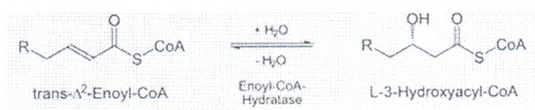
- Free fatty acids can penetrate the plasma membrane due to their poor water solubility and high fat solubility.
- Once in the cytosol, a fatty acid reacts with ATP to give fatty acyl adenylate + inorganic pyrophosphate.
- This reactive acyl adenylate then reacts with free coenzyme A to give fatty acyl-CoA ester + AMP (Adenosine monophosphate).
- The fatty acyl-CoA is then reacted with carnitine to form acylcarnitine, which is transported across the inner mitochondrial membrane by a translocase enzyme in the membrane.
- Once inside the mitochondria, each cycle of β-oxidation, liberate a two carbon unit-acetyl CoA, in each sequence of four reactions.

Reactions During Each Cycle of β-Oxidation

1. Dehydrogenation by FAD: The first step is the oxidation of the fatty acid by Acyl-CoA-Dehydrogenase. The enzyme catalyzes the formation of a double bond between the C-2 and C-3.



2. Hydration: The second step is the hydration of the bond between C-2 and C-3 by Enoyl CoA hydratase. The reaction is stereospecific, forming only the L-isomer.



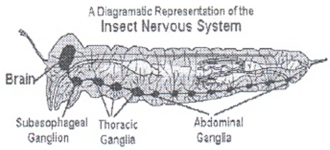
Biochemistry of Insecticides Action (ENT 607)

Dr. M.K. Dhillon

Division of Entomology
Indian Agricultural Research Institute (IARI),
New Delhi 110 012
Email: mukeshdhillon@iari.res.in

Nervous System of Insects

Nervous system: Nervous system is the mechanism of coordination & control of different life processes of the body of the insects. Entire nervous system is derived from Ectoderm and is the organization of ganglia and nerves.



Ganglia: The ganglia are the mass of nervous tissue and mainly consist of two types of cells – neurons and the surrounding cells (neurogleal cells).

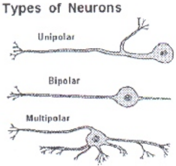
Neuron: Neuron is the functional unit of nervous system, which is differentiated into – Nerve cell (Neurocyte or perikaryon with nucleus and protoplasmic extensions), and Nerve fibers.

Nervous System of Insects

The longest prolongation of the nerve cell is known as Axon, while shorter ones are known as Dendrites. A branch from Axon near the neurocytes is called Collateral. Axon is the process of a nerve cell that carries impulses away from it. Nerve fibers which carry impulses to the central nervous system (CNS) are termed afferent nerve fibers, and those carrying impulses from the CNS to periphery are known as efferent nerve fibers. Dendrite is the process which collects the impulses from other neurons and carries them towards the cell body. It is generally short with many branches, and contains Nissl granules.

Both Axon and Collateral end in very fine branches. From the other side of the neurocytes are given off several protoplasmic projections or extensions. If a neurocyte has a projection only on one side than it is known as Unipolar or Monopolar. If it has on both sides known as Bipolar, while more than two projections is the Multipolar.

Types of Neurons



Nervous System of Insects

Nerve: A collection of several neurotic strands form a nerve. On functional basis the nerves are divided into i). Sensory neurons, ii). Motor neurons, and iii). Internuncial or association neurons.

i). Sensory neurons: The sensory neurons are also known as afferent neurons. They carry impulses from the receptors to central nervous system (CNS) and are concerned with perception of impulses. Dendritic fibers of the nerve may have contact with sensilla on the cuticle, while Axon makes contact with CNS.

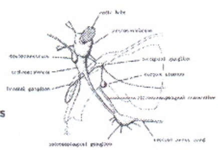
ii). Motor neurons: Motor neurons are also known as efferent neurons, and are concerned with conduction of impulses from CNS to the effectors (muscles).

iii). Internuncial or association neurons: These neurons link sensory and motor neurons with in the CNS through synapses. Synapses are the site at which Axonic end of a neuron comes in close approximation with dendritic end of another adjacent/following neuron is known as Synapse. There is no direct contact between two neurons at synapse (leave a gap of 100A°).

Nervous system of Insects

The nervous system consists of:

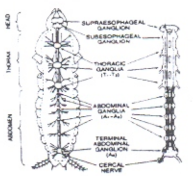
Central nervous system (CNS): It forms the major portion of the nervous system, and is distinguished into 3 regions – Brain (cerebral ganglions or supra oesophageal ganglion), Sub-oesophageal ganglion, and Ventral nerve cord.



Components of the insect brain

Stomodeal or visceral or sympathetic nervous system: This is mainly associated with the alimentary canal and is divided into – Oesophageal or stomodeal nervous system, Ventral stomodeal nervous system, and Caudal stomodeal nervous system.

Peripheral nervous system: It consists of the nerves radiating from the ganglia of the central and sympathetic nervous system. The Axons of the sensory neurons of the receptors run from the periphery of the insects inwards to the CNS, while the Axons of motor neurons go outwards to the muscles and glands from the CNS. Thus, this includes the nerves trunks radiating from ganglia and the distal branches along with terminal organs of motor and sensory fibers contained in the trunk.



Entry of Insecticides in the Insect Body

The insecticides enter the insect body either of the following routes: Cuticle, ii) Mouth, iii) Spiracles, or iv) Other exposed sensory organs

The site of entry generally depends on the type of insecticides. For example
High vapor pressure insecticides – spiracles/antennae
Lipophilic insecticides – cuticle/ plasma membrane
Highly polar insecticides – mouth
Hydrophilic insecticides – aqueous pores of plasma membrane

Penetration of insecticides through cell: The cell is surrounded by a membrane known as plasma membrane, which is an important barrier for the entry of xenobiotics. The water soluble (hydrophilic) compounds are readily penetrated through the aqueous pores (also known as filtration), while the oil soluble (lipophilic) compounds are easily penetrated through the plasma membrane (passive diffusion down a concentration gradient).

Penetration of insecticides through cuticle: The insecticides pass thorough the cuticle to the haemolymph in the body cavity in which it then circulate to reach the nervous system. Recently, it has been viewed that the insecticides migrate laterally in the integument and reach tracheae.

Advances in Insect Physiology (ENT. 608)

Dr. M.K. Dhillon

Division of Entomology
Indian Agricultural Research Institute (IARI),
New Delhi 110 012
Email: mukeshdhillon@iari.res.in

L-1: Chemical Communication in Insects

Communication

- Communication may be defined as any exchange of information between individuals.
- For members of the Human species, communication is an essential part of all social interactions. We communicate through speech, written language, sign language, body language, Morse code, and many other cultural and technological inventions. There is even some evidence that we use body odor in communication. Our brains are uniquely adapted for symbolic communication, but most of our "language" skills are acquired through learning.
- Insects also have many ways to communicate but, unlike humans, their language is almost entirely innate. Each individual is born with a distinctive vocabulary that is shared only with other members of its own species. Learning plays little or no role in the ability to produce these signals or to understand them.
- Thus, an insect may send a communication signal by doing something (e.g. make a noise, release a chemical, or flash a light) or the signal may simply be an inherent part of the insect's physical makeup (e.g. wing pattern, body color, or surface chemistry). In either case, the signal must elicit some behavioral change in order for a human observer to recognize its existence.

Why Do Insects Communicate?

- Some form of intra-specific communication is a prerequisite for any behavior that involves the participation or cooperation of two or more individuals. Intentionally or not, insects may also communicate with members of other species (inter-specific communication). The adaptive value of these communication signals may include:
  1. Recognition of kin or nestmates.
  2. Locating or identifying a member of the opposite sex.
  3. Facilitation of courtship and mating.
  4. Giving directions for location of food or other resources.
  5. Regulating spatial distribution of individuals – aggregation or dispersal; establishing and maintaining a territory.
  6. Warning of danger; setting off an alarm.
  7. Advertising one's presence or location.
  8. Expressing threat or submission (agonistic behaviors).
  9. Deception/mimicry.

How Do Insects Communicate?

- Like all other animals, insects use their five senses (taste, touch, vision, olfaction, and hearing) to acquire information about their environment; and any of these sensory modalities may serve as a pathway for the exchange of information.
- Taste and touch are both contact senses - exchange of information can occur only when two individuals are touching one another.
- Vision, olfaction (smell), and hearing are remote senses – information signals may propagate through the air or water over considerable distances.

Chemical Communication in Insects

- Chemical communication plays an important part in the lives of insects, particularly in lives of those that live in groups or social organizations.
- The insects rely more heavily on chemical signals than on any other form of communication. These signals are often called semiochemicals or infochemicals, being used by insects to communicate with each other and with other organisms. Insects pick up semiochemicals through receptors that intercept and decode the signal. Semiochemicals are categorized as pheromones and allelochemicals.
- Pheromones are a category of semiochemicals which are used for communication between individuals of the same species.
- Allelochemicals are signals that travel from one animal to some member of a different species. These include defensive signals such as repellents, compounds used to locate suitable host plants, and a vast array of other substances that regulate inter-specific behaviors.
- Allomones benefit the sender – a repellent, or defensive compound.
- Kairomones benefit the receiver – an odor that a parasite uses to find its host.
- Synomones benefit the sender and receiver - plant volatiles that attract insect pollinators.

Recent Trends in Entomology (ENT 609)

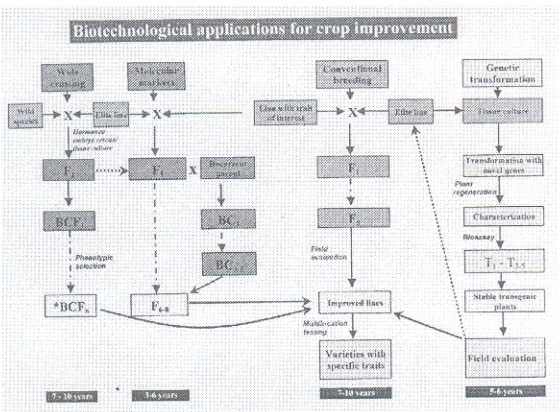
Dr. M.K. Dhillon

Division of Entomology
Indian Agricultural Research Institute (IARI),
New Delhi 110 012
Email: mukeshdhillon@iari.res.in

Genetic Engineering: What it Does !

Plant Breeding	Genetic Engineering
➢ Uses limited gene pool	➢ Unlimited gene pool
➢ Simple skills sufficient	➢ Requires highly technical skills
➢ Gene regulation native	➢ Gene regulations modified
➢ Imprecise transfer of genes	➢ Precise gene transfer
➢ Integration homologous	➢ Gene integration random
➢ Function of genes unknown	➢ Known gene function

Products of genetic engineering are not conceptually different than those from conventional breeding.



Genetic Transformation: Methods

Components for developing transgenic plants are:
i) reliable tissue culture and regeneration systems
ii) preparation of gene constructs and transformation with suitable vectors
iii) efficient transformation techniques to introduce genes into the crop plants
iv) recovery and multiplication of transgenic plants
v) molecular and genetic characterization of transgenic plants
vi) transfer genes of interest into elite cultivars
vii) evaluation of transgenic plants for their effectiveness.

Although several approaches have been tried successfully for genetic transformation of crop plants, only i). *Agrobacterium*-mediated gene-transfer, ii) Microprojectile bombardment with DNA or biolistics, and iii). Direct DNA transfer into isolated protoplasts, have been used widely to introduce genes into a wide range of crop plants.

Genetic Transformation: Candidate Genes for Insect Resistance

- Cry genes from *Bacillus thuringiensis.*
- **Plant metabolites:** Flavonoids, terpenoids.
- **Enzyme inhibitors:** Protease inhibitors (SBTI, CpTi), alpha amylase inhibitors (lectin-like, knottin-like, cereal-type, Kunitz-like, gamma-purothionin-like, and thaumatin-like).
- **Enzymes:** Chitinase, lipoxigenase.
- **Plant lectins:** snowdrop lectin (GNA), *Concanavalin A*, avidin, *Alliun sativun* leaf lectin (ASAL).
- **Toxins from predators:** Neurotoxin genes from scorpion, spiders.
- **Neuropeptides and peptidic hormones:** *Aedes aegypti* (L.) (Trypsin Modulating and Oostatic Factor, *Aea-TMOF*), insect neuropeptide from *M. sexta*, allatostatin (*Manse-AS*).

Development and Deployment of Genetically Modified Crops for Pest Management

- The *Bt* toxin gene was cloned in 1981, and the first transgenic plants were produced by mid-1980s (Barton et al. 1987; Fischoff et al. 1987; Vaeck et al. 1987).
- Since then, *Bt* genes conferring resistance to insects have been cloned and inserted into several crop plants such as maize, rice, wheat, sorghum, sugarcane, cotton, potato, tobacco, broccoli, cabbage, chickpea, pigeonpea, cowpea, groundnut, tomato, brinjal, and soybean (Hilder and Boulter 1999; Sharma 2009; ILSI Research Foundation 2010).
- Among these Bt-transgenic maize, cotton, rice, potato, and tomato have been commercially deployed for insect pest management in different countries of the world.
- Commercial cultivation of genetically engineered crops has increased from 1.7 million hectares in 1996 to over 148 million hectares in 2010, being grown by over 15.4 million farmers in 16 developing and 9 industrialized countries, of which insect-resistant transgenic crops are grown on more than 45 million ha in several countries of the world.

Insect Genetics & Molecular Biology (ENT 610)

Dr. M.K. Dhillon

Division of Entomology
Indian Agricultural Research Institute (IARI),
New Delhi 110 012
Email: mukeshdhillon@iari.res.in

Application of Molecular Techniques in Diagnosis and Molecular Biology

The modern tools of biotechnology can be used for:

- Detection and identification of insect pests
- Insect biotypes
- Understand genetic diversity
- Population structure
- Tri-trophic interactions
- Insect-plant relationships
- Insect metabolism and development
- Study insect phylogeny
- Predator - prey relationships

Diagnosis of Natural Enemies of Insect Pests

- Correct taxonomic identification is essential for effective pest management, particularly for identification of natural enemies.
- RAPDs, SSRs, and allozymes have been widely used for such studies.
- RAPDs have been used for distinguishing populations of coccinellid beetles from different geographical areas, while RFLP and mtDNA markers have been used as diagnostic markers for distinguishing insect populations or migration.
- RAPDs have also been used to differentiate between colonies of *Ageniaspis citricola*, which showed differences in life cycle and behavior.

Diagnosis of Natural Enemies of Insect Pests

- Allozymes have been used for differentiating *Aphidius ervi* attacking pea aphid, *A. pisum* and nettle aphids, *Microlophium carnosum*.
- Enzyme electrophoresis has been used to monitor parasitism of the aphid, *Sitobion avenae*, and RAPDs to identify two endoparasitic wasps, *Diaeretiella rapae* and *Lysiphlebius testaceips* within the bodies of their aphid hosts.
- The PCR-RFLP of mitochondrial COI gene has been used to detect parasitism of mirid bugs, *Lygus lineolaris* by *Peristenus* spp.
- Species specific ITS2 and 16S primers have been used to determine the establishment of the parasitoid, *Aphelinus hordei* for the control of Russian wheat aphid, *Diuraphis noxia*, and of the eggs of *L. lineolaris* by *Anaphes iole*.

PCR Primers Used for Detecting Parasitoids

Species	Region
Ageniapsis citricola	Actin genes
Aphelinus abdominalis, Cotesia congregate, Diaretiella rapae	Microsatellite
Aphelinis hordei	ITS-2, 16s rDNA
Aphelinis ervi	Microsatellite, CO I-II, CO B
Lydella thompsoni, Pseudoperichaeta nigrolineata	CO I
Nasonia vitripennis, Spalangia calcitrans, Trichomalopsis sarcophagae, Trichogramma ostriniae	ITS-1
Lysiphlebus testaceipes, Trichogramma deion, T. dendrolimi, T. pretiosum, T. turkestanica	ITS-2
Peristenus digoneutis, T. australicum, T. minutum	ITS-1, ITS-2

Detection of Insect Biotypes

- Molecular tools have been used for solving routine taxonomic and ecological problems regarding biotype or cryptic status of insect pests.
- Sequences of ITS2 region have been used to separate the Australian and Taiwanese *Ageniaspis fuscicollis* populations.
- The use of molecular markers has been found to be useful for characterizing strains to determine genetic origin and identity of the parasitoids for use in pest management, and detection of alien strains/species.
- Allozymes, RFLP, RAPD, microsatellite, and mtDNA based markers have been used for differentiating biotypes and sympatric species.
- Real time qualitative PCR using SYBR green assay with melting curve analysis has been developed to identify these two symptomatic species of tephritid fruit flies, *Bactrocera philippinensis* and *B. occipitalis*, and can be used as a rapid detection technique in quarantine inspection.