Genomics of Insect Pests to Agriculture

Program

May 24-25, 2010
Faculty Club
The Robert H. Smith Faculty of Agriculture, Food and Environment

Sponsored by the French Embassy in Israel and the Hebrew University of Jerusalem
Monday, May 24, 2010

08:30-09:00: Venue

09:00-09:20: Opening ceremony

Welcome addresses: Prof Hanokh Czosnek (Organizer), Prof Eric Seboun (Scientific Attaché, French Embassy in Israel), Prof Shmulik Wolf (Vice Dean for Research, Faculty of Agriculture)

09:20-11:50 Session I: Lepidoptera Genomics

09:20-10:00 René Feyereisen (Centre de Recherches de Sophia Antipolis, INRA and CNRS, Antibes)  
“Conservation of synteny in Lepidoptera: the silkworm genome as model for two noctuid pests, *Helicoverpa armigera* and *Spodoptera frugiperda*

10:00-10:40 Philippe Fournier (Unité de Biologie Intégrative et Virologie des Insectes, INRA and Université Montpellier)  
“Spodoptera Genomics “

10:40-11:00 Coffee break

11:00-11:30 Ada Rafaeli (Dept of Technology and Storage of Agricultural Products, Volcani Center, Bet Dagan)  
"Molecular mechanisms underlying sex-pheromone communication in Lepidoptera"

11:30-11:40 * Racheli Bober (Dept of Technology and Storage of Agricultural Products, Volcani Center, Bet Dagan)  
"The characterization of molecular regulation mechanisms of the PBAN receptors in pest moth"

11:40-11:50 * Orly Hanin (Dept of Technology and Storage of Agricultural Products, Volcani Center, Bet Dagan)  
"The role of sex-peptide in post mating behavior of a moth"
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<tr>
<th>Time</th>
<th>Session II: Interactions with free and endosymbiotic bacteria</th>
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<td>11:50-13:10</td>
<td><strong>Joël Renaudin</strong> (Génomique Diversité Pouvoir Pathogène, INRA, Bordeaux)</td>
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<td>11:50-12:30</td>
<td>&quot;Interactions of phloem-limited bacteria with their vector insects&quot;</td>
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<td>12:30-13:00</td>
<td><strong>Einat Zchori-Fein</strong> (Dept Entomology, Volcani Center, Neve Ya’ar)</td>
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<td>12:30-13:00</td>
<td>&quot;Symbiont-based control methods and their future applications&quot;</td>
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<th>Time</th>
<th>Session III: Insect development and reproduction</th>
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<td>14:00-16:00</td>
<td><strong>Aviv Dombrovsky</strong> (Dept Plant Protection, Volcani Center, Bet Dagan)</td>
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<td>14:00-14:30</td>
<td>&quot;Profiling the repertoire of phenotypes influenced by environmental cues that occur during asexual reproduction&quot;</td>
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<td>14:30-15:00</td>
<td><strong>Benny Shilo</strong> (Dept Molecular Genetics, Weizmann Institute, Rehovot)</td>
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<td>14:30-15:00</td>
<td>&quot;What has <em>Drosophila</em> taught us about Receptor Tyrosine Kinase signaling?&quot;</td>
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<td>15:00-15:30</td>
<td><strong>Moshe Inbar</strong> (Faculty of Sciences, University of Haifa)</td>
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<td>15:00-15:30</td>
<td>&quot;Host plant manipulation by gall formers: the evolution of an extended phenotype&quot;</td>
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<th>Session IV: Insect vectors of animal diseases</th>
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<td>16:00-17:00</td>
<td><strong>Yuval Gottlieb</strong> (School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot)</td>
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<td>16:00-16:30</td>
<td>&quot;Toward symbiosis control of Culicoides biting midges, the vectors of farm animal viruses&quot;</td>
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<td>16:30-17:00</td>
<td><strong>Fouad Akad</strong> (Central Laboratory, Ministry of Health, Jerusalem)</td>
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<td>16:30-17:00</td>
<td>&quot;Molecular identification of sand fly, vector of Leishmaniasis, in Israel&quot;</td>
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Tuesday, May 25, 2010

09:00-10:10 Session V: Aphid reproduction and relation to environment

09:00-09:40 Denis Tagu (INRA and Université de Rennes, Agrocampus Rennes)
“Analysis of phenotypic plasticity of the reproductive mode in the pea aphid, using genomic resources”

09:40-10:10 Amir Ayali (Dept Zoology, Tel Aviv University)
“A possible role for the foraging gene in locust density-dependent phase polyphenism”

10:10-10:40 Coffee break

10:40-12:35 Session VI: Whitefly genomics, genetics and ecology

10:40-11:10 Henryk Hanokh Czosnek (Int Plant Science and genetics in Agriculture, Faculty of Agriculture, The Hebrew university of Jerusalem, Rehovot)
“The whitefly functional genome project”

11:10-11:40 Shai Morin (Dept entomology, Faculty of Agriculture, The Hebrew university of Jerusalem, Rehovot)
"Molecular perspective of Bemisia tabaci adaptation to synthetic insecticides and plant secondary compounds"

11:40-12:10 Michel Peterschmitt (Campus International de Baillarguet, CIRAD, Montpellier)
“Multiple genetic and ecological outcomes following contacts between invading and indigenous populations of Bemisia tabaci”

12:10-12:40 Yael Heifetz (Dept entomology, Faculty of Agriculture, The Hebrew university of Jerusalem, Rehovot)
“Making inroads into the Drosophila female reproductive system: using Drosophila as a model for studying pathways fundamental for successful reproduction”
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<td>12:40-12:50</td>
<td>* Marina Brumin (Dept Entomology, Volcani Center, Bet Dagan)</td>
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<td>“Response of the whitefly <em>Bemisia tabaci</em> to glucosinolates from <em>Arabidopsis</em>”</td>
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<td>12:50-13:45</td>
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<td>13:45-16:00</td>
<td>Session VII: Insect-virus interactions</td>
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<td>13:45-14:30</td>
<td>Nathalie Volkoff (Biologie Intégrative et Virologie des Insectes, INRA and Université Montpellier)</td>
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<td>&quot;Genomic tools to unfold host-parasitoid-polydnavirus interactions&quot;</td>
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<td>14:30-15:00</td>
<td>Nor Chejanovsky (Dept Entomology, Volcani Center, Bet Dagan)</td>
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<td>'Infection of the Mediterranean pest <em>Spodoptera littoralis</em> with the <em>Autographa californica</em> multiple nucleopolyhedrovirus, a tool to study the antiviral response of Lepidopterans&quot;</td>
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<td>15:00-15:30</td>
<td>Murad Ghanim (Dept Entomology, Volcani Center, Bet Dagan)</td>
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<td>Specific host and endosymbiont proteins are involved in the transmission of <em>Tomato yellow leaf curl virus</em> by the whitefly <em>Bemisia tabaci</em></td>
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<td>Eyal Maori (Inst Plant sciences and Genetics in Agriculture, Faculty of Agriculture, The Hebrew university of Jerusalem, Rehovot)</td>
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<td>“Turning host-virus dynamics into an anti-viral approach in bees”</td>
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<td>16:00-16:30</td>
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<td>16:30-17:30</td>
<td>Concluding remarks and discussion</td>
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## Participants

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Conservation of synteny in Lepidoptera: the silkworm genome as model for two noctuid pests, *Helicoverpa armigera* and *Spodoptera frugiperda*

E. d’Alençon (a) H. Sezutsu(b,c), F. Legeai (d), E. Permal (e), S. Bernard-Samain (f), S. Gimenez (a), C. Gagneur (a), F. Cousserans (a), M. Shimomura (c), A. Brun-Barale (b) T. Flutre (e), A. Couloux (f), P. East (g), K. Gordon (g), K. Mita (c), H. Quesneville (e), P. Fournier (a), and René Feyereisen (b)

(a) UMR 1231, INRA, Université Montpellier II, 34095 Montpellier, France; (b) UMR1301, INRA, CNRS, Université de Nice, 06903 Sophia Antipolis, France; (c) National Institute of Agrobiological Sciences, Tsukuba 305-8634, Ibaraki, Japan; (d) UMR 1099, INRA, AgroCampus, INRIA, 35042 Rennes, France; (e) UR1164, INRA Centre de Versailles, Versailles 78026, France; (f) Genoscope, Centre National de Séquençage, 91057 Evry, France (g) CSIRO Entomology, Canberra, ACT2601, Australia

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The recent assembly of the silkworm *Bombyx mori* genome with 432Mb on 28 holocentric chromosomes has become a reference in the genomic analysis of the very diverse Order of Lepidoptera. We sequenced BACs from two major pests, the noctuid moths *Helicoverpa armigera* and *Spodoptera frugiperda* corresponding to 15 regions distributed on 11 *B. mori* chromosomes, each BAC/region being anchored by known orthologous gene(s) to analyze syntenic relationships and genome rearrangements between the three species. Nearly 300 genes and numerous transposable elements were identified with LINEs and TIRs the most abundant TE classes. There was a high degree of synteny conservation between *B. mori* and the two noctuid species. Conserved syntenic blocks of identified genes were very small, however, about 1.3 genes/block between *B. mori* and the two noctuid species and 2.0 genes/block between *S. frugiperda* and *H. armigera*. This corresponds to about 2 chromosomes breaks per Mb DNA per million years. This is a much higher evolution rate than among *Drosophila* species and may be related to the holocentric nature of the lepidopteran genomes. We report a large cluster of eight members of the aminopeptidase (APN) gene family estimated to have been present since the Jurassic. In contrast, several clusters of cytochrome P450 genes showed multiple lineage-specific duplications, in particular in the lepidopteran CYP9A subfamily. Our study highlights the value of the silkworm genome as a reference in lepidopteran comparative genomics.


Comparative genomic studies on insects with holocentric chromosomes


We wish to elucidate the molecular determinism and consequences of the holocentric nature of Lepidopteran chromosomes. 1. This structure was expected to induce a specific syntenic organization and this was studied by comparative analysis of 15 BACs of 3 Lep species, among which S. frugiperda (see Feyereisen’s talk). 2. We analyzed the nature and frequency of repeated elements among these regions and found an abundance of LINEs and TIRs rather than LTRs. We also observed a biased distribution in some regions affected by synteny breakage. 3. We looked for centromeric proteins in order to reveal features of the holocentric kinetochore. The discovery of an EST homolog to the human centromeric protein B in S. frugiperda prompted us to characterize that gene which has not yet been described in invertebrates. Like its human counterpart, the Sf cenp-B is related to the transposase of the pogo transposable element of D. melanogaster. The Sf CENP-B DNA binding activity in vitro, its nuclear location, its ability to bind to a retrotransposon derived sequence in vivo argue in favour of its role as a centromeric protein. 4. Chromatin immunoprecipitation was achieved against heterochromatin markers and candidate regions were identified in the sequenced BACs by qPCR with the precipitated genomic DNA as a template. Frequency of these regions and localization nearby repeated elements provide hypotheses to be further tested on the structure of heterochromatin regions, which may be related to dispersed centromeres too. In order to further elucidate this genomic aspects, our team has launched the sequencing of transcriptome and of small RNAs of S. frugiperda and we hope to also get the entire S. frugiperda genome, in the frame of an international consortium which we would like to include other Spodoptera species, and in which we hope the involvement of research teams in Israel.

Reproductive behavior involves the integration of physiological and behavioral events that synchronize male and female encounters. Receptivity in most female moths is broadcasted by the release of a unique blend of volatile sex-pheromones when they assume typical calling behaviours. This blend of sex-pheromones is derived from downstream products of fatty acid biosynthesis in the pheromone gland, situated between the ultimate and penultimate terminal segments of the abdomen. Regulation of biosynthesis of these sex-pheromones is due to a photoperiodic release of Pheromone Biosynthesis Activating Neuropeptide (PBAN), a member of the Pyrokinin (PK)/PBAN neuropeptide family characterized by a common amino acid sequence FXPRLamide motif in the C-terminus. PBAN activates pheromone production through its binding to a PBAN-Receptor (PBAN-R) and subsequent up-regulation of the fatty acid biosynthetic pathway. The PBAN-R gene was identified as a member of the G-protein coupled receptor family (GPCR), classified with the vertebrate subfamily of Neuromedin U receptors and putative binding sites are predicted through biochemical and in silico mutagenesis studies. Differential expression studies reveal localization in pheromone glands, neural tissues and male aedaeugi, a tissue homologous to the pheromone gland. Pheromone production is age-dependent and temporal differential expression levels of the PBAN-R reveal up-regulation at a critical period during pupal-adult development. Questions are raised concerning the evolutionary role of the PK/PBAN receptors belonging to the GPCR family.

The characterization of molecular regulation mechanisms of the PBAN receptors in pest moth

Rachel Bober 1 and Ada Rafaeli 2

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rachel.bober@mail.huji.ac.il

Sex pheromone production in *Helicoverpa armigera* is regulated by the timely release of a Pheromone Biosynthesis Activating Neuropeptide (PBAN). PBAN is produced by neurosecretory cells of the subesophageal ganglion, released into the hemolymph and binds to a G-protein coupled receptor in the pheromone glands of female moths where it activates sex pheromone production only in newly emerged and adult females. The PBAN receptor (PBAN-R) protein and its transcript were also demonstrated in neural tissues of adult females and the transcript was also detected and quantified in the aedaegi of the male, which is a tissue homologous to the female pheromone gland. We demonstrate temporal differential expression levels of the PBAN-R in females and males, reaching peak levels at a critical period of 5 hours post-eclosion. The role of juvenile hormone (JH) in mating and regulation of sex pheromone production in moth species has been under controversy. Previous studies implied a possible regulatory role for JH. We herein demonstrate that PBAN-R expression levels increase normally even when females are decapitated or head-ligated before peak transcript levels are reached. Furthermore, sex pheromone production can be induced by PBAN in such decapitated females. These results indicate that up-regulation, at this critical time, is not dependent on JH originating from the head. Conversely, JH injected *in vivo* at this critical period significantly inhibits PBAN-R transcript levels.

The role of Sex-Peptide in post mating behavior of a moth

Orly Hanin and Ada Rafaeli

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During copulation insect males transfer seminal peptides (Acps), produced in Male Accessory Glands (MAGs) that are implicated in female post-mating responses. Of all the Drosophila Acps whose sequences are known Sex-Peptide (Drosophila melanogaster Acp70A (DrmSP), is considered to be very important in eliciting post-mating non-receptivity and increased fecundity. Many moth species, amongst them H. armigera, signal their receptivity to mate by producing and releasing a volatile sex-pheromone blend. After mating the levels of sex-pheromone are significantly reduced. A similar reduction in sex-pheromone levels can be observed after injection of DrmSP to virgin female moths. Moreover, DrmSP stimulates Juvenile Hormone production by corpora allata in vitro in both H. armigera and D. melanogaster. JH titers significantly increase in females of some moth species after mating and the involvement of JH in mating-induced pheromone suppression has been reported. Structure activity experiments in H. armigera, using truncated fragments of Drm-SP showed that Drm-SP’s N-terminal peptides are allatotropic and C-terminal fragments are pheromonostatic in virgin females. A pheromonostatic DrmSP-like peptide was identified from male accessory glands of H. armigera. In the present study, we examine various functional roles of DrmSP in the moth. In addition, we characterize the SP receptor from H. armigera female moths.

Interactions of phloem-limited bacteria with their insect vectors the example of *Spiroplasma citri*

Marc Breton, Fabien Labroussaa, Nathalie Arricau-Bouvery, Marie-Pierre Dubrana, Sybille Duret, Brigitte Batailler, Laure Béven, Colette Saillard, and [Joël Renaudin](mailto:renaudin@bordeaux.inra.fr)

INRA and Université Victor Segalen Bordeaux 2, UMR 1090 Génomique Diversité Pouvoir Pathogène, F-33883 Villenave d’Ornon, France.

*Spiroplasma citri*, the causal agent of citrus stubborn disease, belongs to the class *Mollicutes*, a group of wall-less cell organisms related to low G+C Gram positive bacteria. The spiroplasmas inhabit the phloem sieve tubes and are transmitted by the phloem sap-feeding leafhopper vector *Circulifer haematoceps* in a persistent and propagative manner. *S. citri* GII3 contains low-copy-number plasmids pSci1-6, predicted to encode determinants of insect transmission (not detected in insect non-transmissible strains). Despite the strong similarities between their replication regions, pSci1-6 coexist in the spiroplasma cells. By using a curing/replacement strategy based on incompatibility of plasmids having identical replication regions, we constructed *S. citri* mutants with various plasmid contents. Transmission of these *S. citri* plasmid mutants through injection into the leafhopper vector revealed that a pSci6-encoded protein of unknown function was essential for transmission to occur. In contrast, the adhesion-related proteins (ScARPs) encoded by pSci1-5 were not required, even though they were found to improve the transmission efficiency. Successful transmission of *S. citri* relies on its ability to establish close interactions with the leafhopper cells to cross the gut epithelium and invade the salivary glands. *In vitro* protein overlay assays revealed specific binding of the *S. citri* phosphoglycerate kinase (PGK) to the leafhopper actin, in agreement with confocal microscopy observations showing that spiroplasmas co-localized with the actin filaments. Competitive binding and internalization assays using the leafhopper Ciha-1 cell culture system showed that exogenous PGK had no effect on spiroplasmal attachment to the leafhopper cell surfaces but inhibited internalization of *S. citri*.


Symbiont-based control strategies and their future applications

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Pest and disease management poses significant challenges for the medical and agricultural communities. In addition, public concern over pesticide use and more stringent environmental regulations create the need for new technologies. Bacterial symbiosis is prevalent in arthropods that can be devastating pests and efficient disease vectors. One new approach to control arthropod pest populations or to reduce vector competence is by symbiont-based control strategies (SCS) that are environmentally friendly. Molecular techniques have revealed the diversity of microbial symbionts associated with insects, and have promoted the swift establishment of applied research on invertebrate-symbioses. For example, SCS are currently being developed to control Chagas disease vectored by triatomine bugs, Pierce’s disease vectored by glassy winged sharpshooters and mosquito-vectored diseases. A successful symbiont-based control project requires ample, system-specific information, such as the diversity, distribution, localization, transmission pathways and transmission rates of each symbiont, as well as the interactions among symbionts within a single host and the interactions between the host and symbiont genotypes. The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) is a minute insect that ranks among the most noxious pests attacking field and greenhouse crops around the world. The whitefly harbours the primary symbiont *Portiera aleyrodidarum*, which is located in the bacteriocytes, specialized cells housing bacteria. Additionally, *B. tabaci* may harbour several secondary symbionts, including *Rickettsia, Hamiltonella, Wolbachia, Arsenophonus, Cardinium* and *Fritschea*. In order to develop a SCS for that pest, an attempt is been made to collect relevant data on that system.

Profiling the repertoire of phenotypes influenced by environmental cues that occur during asexual reproduction

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The aphid *Acyrthosiphon Pisum* population is composed of different morphs such as winged and wingless parthenogens, males and sexual females. The combined effect of reduced photoperiodicity and cold in fall triggers the apparition of sexual morphs. In contrast they reproduce asexually in spring and summer. In our current study, we provide evidence that clonal individuals display phenotypic variability within asexual morph categories. We describe that clones sharing the same morphological features which arose from the same founder mother constitute a repertoire of variants with distinct behavioural and physiological traits. Our results suggest that the prevailing environmental conditions influence the recruitment of adaptive phenotypes from a cohort of clonal individuals exhibiting considerable molecular diversity. However, we observed that the variability might be reduced or enhanced by external factors but is never abolished in accordance with a model of stochastically produced phenotypes. This overall mechanism allows the renewal of colonies from a few adapted individuals that survive drastic episodic changes in a fluctuating environment.

Making inroads into the female reproductive system: using *Drosophila* as a model for studying pathways fundamental to successful reproduction

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Reproduction and its regulation are critical components of any successful insect pest management scheme. In the past few decades, considerable strides have been made in advancing our knowledge of the major physiological and morphological events that characterize oogenesis, spermatogenesis, and embryogenesis, all fundamental to mating and reproduction. Nevertheless, the physiological and genetic properties fundamental to fecundity and fertilization are poorly understood. Similarly, we know little of the molecular processes, both genetic and physiological, that immediately follow mating. To uncover the mechanisms that underlie mating-induced changes, we conducted comparative microarray, proteomic, morphological and functional studies to characterize the reproductive tract of unmated and mated *Drosophila* females. We show that mating triggers molecular changes and active tissue remodelling in the female reproductive tract that mediate its progression to a mature functional state. Our long term goal is to achieve a systems level understanding of the mechanisms that underlie the transition from an unmated to a mated state and how miss-regulation contributes to infertility. These studies provide valuable knowledge of the factors leading to functional reproductive tissues in *Drosophila* as well as other insects and mammalians. This knowledge will also promote new ideas for insect pest control.


What has *Drosophila* taught us about Receptor Tyrosine Kinase signaling?

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Receptor tyrosine kinases (RTKs) represent one of the central routes for transfer of extracellular signals into cells, to control their developmental fate, proliferation or migration. Dimerization of receptors following interaction of ligands with the extracellular domains, leads to dimerization of the intracellular kinase domains, and transphosphorylation of tyrosine residues. These residues serve as docking sites for proteins that relay the signal into the cell, most notably through the Ras/MAP kinase pathway. Analysis of the pathway in vertebrates has been complicated by the presence of multiple receptor types from each family, the parallel activation of several intracellular signaling pathways, and the fact that these pathways are also activated by other receptor types. In *Drosophila*, the picture is simplified since each RTK type has only 1-2 members within its sub-family, and the RAS/MAP kinase pathway appears to be the predominant output, thus allowing linear propagation of the signal. Utilization of the receptors as an entry point for genetic studies has allowed the identification and characterization of each signaling pathway at its critical and unique biological junction. Among the *Drosophila* RTKs, some mediate a single developmental decision (e.g. Torso or Sevenless). Others, like the EGF receptor, mediate numerous decisions throughout embryonic and post-embryonic development. It seems that the common Ras/MAP kinase pathway can be utilized to trigger these different outputs, because the “context”, i.e. the repertoire of transcription factors within the cell receiving the signal, is different in each case. Particularly revealing was the ability to follow the activated state of RTK pathways *in situ*, by utilizing an antibody detecting the activated, double phosphorylated form of MAP kinase, thus providing a developmental atlas of RTK activation.

One paradigm emerging from the studies of the EGF receptor in *Drosophila* is the existence of inducible negative feedbacks, which restrict the range (but not the duration) of activation. Surprisingly, in spite of the evolutionary conservation at the level of the ligands, receptor, and downstream elements, the strategy for ligand processing is distinct in invertebrates. The unique biological properties of the protease (termed Rhomboid) will be described. Alterations in the intracellular compartments where Rhomboids are localized, modulate the level of EGF receptor activation, thus adjusting the pathway to distinct biological scenarios.

Host plant manipulation by gall formers: the evolution of an extended phenotype

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Indirect, plant-mediated interactions (negative or positive) between herbivores is common and important. We investigated such interactions between a leaf-galling aphid, Synanthrodes betae and the folivorous pistachio processionary moth (Thaumetopoea solitaria). Both species exclusively share the leaves of the common Pistacia atlantica trees across the Middle East. The aphids induced leaf galls in early spring in which they reproduce until the fall. In the spring the caterpillars feed on the same leaves; occasionally, leavening the plant totally defoliated. It is expected therefore, that both insects will be engaged with complex interactions.

In a set of field and laboratory experiments we found that: 1. The caterpillars repeatedly avoid the galls while consuming the leaves creating intact "trimmed" galls. 2. The galls are protected physically, but primarily chemically from the moth. 3. In GC-MS analysis we found higher levels of mono- and sesquiterpenes in the galls that may contribute to such defense. 4. Following moth feeding, the level of mono- and sesquiterpenes were significantly higher the galls; this indicate differential induce resistance in the gall. 5. In the field, gall density on defoliated shoots increased (X 4) as compared to caterpillar-free shoots. This was due to the compensatory growth of P. atlantica following caterpillar's defoliation which extended the availability of young leaves for the galling fundatrices. 6. Trimmed galls maintained strong sink for nutrients from alternative sources as reflected by the unaffected reproduction of the aphids in these galls. Molecular analyses based on mitochondrial COI and COII genes (1952 bp), for the evolution of 14 species of gall-forming aphid species showed that similar gall types are induced at similar sites on different Pistacia hosts suggesting control of the aphids on gall traits.

Toward symbiosis control of Culicoides biting midges, the vectors of farm animal viruses

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Culicoides (Diptera: Ceratopogonidae) transmit many major viral diseases, including bluetongue (BT), which is listed among the most damaging by the World Organization for Animal Health (OIE) and recently has emerged in completely unexpected areas (Northern Europe). Understanding why sympatric species have different vectorial capacity is key information when developing control strategies. Despite significant effort to elucidate the vectorial capacity of certain Culicoides species, and the critical basis of variability in infection, almost no attention has been given to symbiotic interactions between the vector and its bacterial tenants. It is now established that bacterial symbionts have major influences on their host biology, ranging from altering modes of reproduction to protecting them against natural enemies. Other important traits that can influence vectorial capacity, such as resistance to high temperature and feeding patterns, have been shown to be symbiont-dependent.

Using 16S rDNA gene base analyses, we initiated a profile of the bacterial community in several Israeli and American vectors and non-vectors of BTV. These Culicoides species were identified morphologically, however, in order to specify vector-symbiont relationship we will have to reveal the taxonomic status of certain group species using genomic data.

Molecular identification of sand fly, vector of Leishmaniases, in Israel

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Protozoal parasites of the genus Leishmania are transmitted by sand fly bites to humans and animals. Three major forms of disease are caused by these parasites: cutaneous leishmaniasis, responsible for disfiguring skin wounds; mucocutaneous leishmaniasis and the potentially fatal visceral leishmaniasis, involving internal organs such as the spleen and liver. More than 2 million human are infections annually by leishmaniasis around the global; it is endemic in more than 88 countries and prevalent also as an imported disease in non-endemic regions due to travel and tourism. These Leishmania species have diverse reservoir hosts and sand fly vectors. In Israel there are 14 species of sand flies of the genus Phlebotomus. The list includes: P. sergenti, P. arabicus vectors for L. tropica, P. papatasi, P. neglectusi vectors of L. major and P. syriacus, P. tobbi, P. perfilewi, P. alexandri vectors for L. infantum. Sand fly morphological and molecular classification, ecological records of each species and its possible involvement in Leishmania transmission are discussed.

Analysis of phenotypic plasticity of the reproductive mode in the pea aphid, using genomic resources

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The success of aphids as pests is related to their peculiar life history traits, notably the variation in their reproductive mode between asexual and sexual reproduction which allows extremely high rates of population increase and fast adaptation to environmental change. Aphids reproduce by a viviparous clonal process. Embryos develop within the ovaries of aphid females from diploid oocytes that escape meiosis, in the absence of males. Viviparous parthenogenesis allows adaptation of aphids to local environmental changes by phenotypic plasticity. Aphids seasonally change their reproductive mode to survive cold winters by producing over-wintering sexual eggs in autumn. In autumn, the decrease of day-length is a sufficient and necessary signal to trigger a developmental switch. When parthenogenetic, a female adult aphid contains embryos at all stages of differentiation and development, ensuring that at least some embryos (among the youngest) will respond to an environmental trigger. The reproductive tract within embryos of aphids submitted to short day conditions switches from the production of diploid oocytes to the production by true meiosis of functional gametes. In the frame of the International Aphid Genomics Consortium, we have developed genomic resources for the pea aphid *Acyrthosiphon pisum* to identify genetic programs that are regulated during the switch of reproductive mode. We show that in aphid heads, cuticular proteins as well as proteins involved in neuronal physiology are regulated by the shortening of photoperiod. Based on these observations, we proposed the hypothesis that the dopamine and the insulin pathway might regulate the photoperiod response.

The locust *foraging* gene and a possible role for cGMP-dependent protein kinase (PKG) in locust density-dependent phase polyphenism

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Locusts have been one of the most important agricultural pests since the earliest recorded times. During plague, the Desert locust may spread over some 29 million km², extending over or into parts of 60 countries with the potential to damage the livelihood of a tenth of the world's population. Locust phase polyphenism is an extreme example of environmentally-induced behavioral plasticity. In response to changes in population density locusts dramatically alter their behavior, from solitary and relatively sedentary behavior to active aggregation and swarming.

Our knowledge of how genes act on the nervous system in response to the environment to generate behavioral plasticity is limited. A number of recent advancements in this area concern a specific gene family: *foraging* (*for*), which encodes a cGMP-dependent protein kinase (PKG), and feeding and locomotion-related behaviors. While gregarious locusts are notorious for their destructive feeding and long term migratory behavior, little is known about the molecular and genetic basis of this striking phenomenon. In this work we identified and cloned the *for* gene of the desert locust (*Schistocerca gregaria*). We compared its expression in the brain of gregarious and solitary-reared locusts. We also determined the phylogenetic relationship between the locust PKG and other known PKG proteins in insects. FOR expression was found to be confined to neurons of the anterior midline of the brain - the pars intercerebralis. The PKG activity of gregarious locusts was found to be significantly higher than that of solitary ones. Differences in PKG activity were sex specific, with higher PKG activity found in males than in females, in both solitary and gregarious locusts. Our findings are correlated to well-established phase-related behavioral differences and thus lay the ground work for functional studies of the locust *for* gene and its possible relations to locust phase polyphenism.

The *Bemisia tabaci* Functional Genome Project

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Despite the economic and scientific importance of *Bemisia tabaci*, the genome of the whitefly and its expression have not been investigated on a large scale. To address this general shortage of information, we have constructed several cDNA libraries from viruliferous and non-viruliferous whiteflies. A cDNA spotted microarray containing 6,000 entries was constructed and used for gene expression studies. In parallel a catalog of proteins is in the process of being established. The set of sequences developed makes available the first DNA sequence database for an important pest of agricultural crops. Its availability allows the investigation of important questions regarding whitefly biology, development, and comparative biology. It will also facilitate studies to elucidate the genetics underlying gene expression in pest- and non-pest biotypes, and the basis for virus-vector specificity, resistance to insecticides, and plant host preferences for this cryptic species. The *B. tabaci* microarray has allowed analyzing patterns of gene expression during several important processes in the life of the insect: 1) development, 2) acquisition of *Tomato yellow leaf curl virus* TYLCV, 3) parasitization by wasps, 4) comparative biotype-specific gene expression under heat stress.


Behavioural and genomic responses of *Bemisia tabaci* to over-expression of the phenylpropanoid-biosynthesis pathway in *Nicotiana tabacum*

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Phenylpropanoid metabolism provides plants with thousands of compounds widely used in the anti-microbial and anti-herbivore defense. We have produced tobacco (*Nicotiana tabacum*) plants that over-express the MYB transcription factor *Pap1* (Production of Anthocyanin Pigment 1), which activates the phenylpropanoid-biosynthesis pathway. These plants allowed investigating the effect of high levels of phenylpropanoids / flavonoids on host selection, oviposition, development, survival and gene expression of *Bemisia tabaci* (Hemiptera: Aleyrodidae).

In choice experiments *B. tabaci* females (B biotype) preferred wild type (wt) plants over *Pap1*-transgenic plants. On the other hand, percentage of egg hatching was higher and nymphal development rate faster on *Pap1*-transgenic than on wt plants. In no-choice experiments, higher number of adults survived long periods of feeding on *Pap1*-transgenic plants than on wild type plants. Also, higher number of eggs were oviposited on the transgenic than on wt plants.

We used a spotted *B. tabaci* cDNA microarray to compare the expression patterns of 6000 ESTs between *B. tabaci* adults fed for 6 h on *Pap1*-transgenic plants or wt plants. Adults feeding on transgenic plants showed lower expression of mitochondrial, metabolism (proteins, carbohydrates, lipids), cytoskeleton and defense response genes and higher expression of genes involved in protein biosynthesis and immune defenses. Analyses of wt and transgenic plants SA- and JA-dependent defense signaling pathways suggested that the differences in insect performance might be related to enhanced expression of the SA pathway in the transgenic plants.


Multiple genetic and ecological outcomes following contacts between invading and indigenous populations of *Bemisia tabaci*

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The whitefly *Bemisia tabaci* is a pest of vegetable, ornamental and field crops. It is also the vector of several economically important plant viruses as Tomato yellow leaf curl virus. The comparison of cytochrome oxydase 1 sequences of a global collection of *B. tabaci* has revealed 12 well resolved genetic groups with a strong geographic structure. Based on the considerable genetic diversity together with distinct biological parameters and particularly varying abilities to interbreed, it was proposed that *B. tabaci* represent a cryptic species complex. Due to human activities, a genetic group, believed to be originating from the sahel-like regions of Middle Eastern Mediterranean/ North Africa/ Asia Minor, was very largely dispersed outside its geographic origin. This group, mainly consisting of individuals inducing physiological disorders on infested plants, is commonly referred to as the biotype B. As *B. tabaci* is a worldwide pest, the dispersed biotype B was often introduced into agroecosystems containing indigenous populations of *B. tabaci*. The objective of this study was to analyze the genetic and ecological outcomes of such contacts between the invading biotype B and indigenous populations in different regions of the world. In the Caribbean islands we have observed the almost complete displacement of the indigenous populations. In Reunion Island, we have detected the introgression of genetic material from the indigenous populations into biotype B. In Morocco, we have observed the coexistence of the indigenous populations and biotype B without any clear evidence of gene flow.


Response of the whitefly *Bemisia tabaci* to glucosinolates from *Arabidopsis*

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Generalist insects such as the sweet potato whitefly can feed on hundreds of plant species, which produce a great diversity of defensive secondary metabolites. It is thus crucial for the insect to cope with this diversity of toxic materials by using a wide array of defense response system. Cruciferous plants and *Arabidopsis thaliana* produce glucosinolate-derived secondary metabolites as defensive materials. We investigated the response of *B. tabaci* to feeding on artificial and natural indole glucosinolates (GLs). For natural glucosinolates we used *A. thaliana* wild type Columbia-O (Col-0) and Landsberg erecta (Ler) and mutants that lack or over accumulate these materials. As artificial glucosinolates we used the final breakdown products in wild types, indole-3-carbinol (I3C) and indol-3-acetonitrile (IAN) produced in Col-O and Ler respectively. Choice experiments on treated cotton with IAN and I3C showed deterrent effect on *B. tabaci* adults, while non-choice experiments showed a significant effect on fecundity. Microarray analysis comparing the genomic response of *B. tabaci* to feeding on artificial diets containing IAN or I3C revealed a significant effect on gene expression after feeding with IAN, and a minor effect after feeding with I3C. Consistently, fecundity was significantly higher on Col-O and *cyp79B2cyp79B3* (lack GLs) and lower on Ler, *atr1D* (over accumulate indole GLs) and *tgg1 tgg2* (myrosinase-deficient). Thus, IAN has a stronger effect on *B. tabaci* than 13C. The genomic response of *B. tabaci* adults fed on the different *A. thaliana* backgrounds, digestion and breakdown of GLs in the digestive system and the behavioral response of adult *B. tabaci* fed on wild type and mutant *A. thaliana* plants using the Electrical Penetrating Graph (EPG) will be further investigated.
Genomic tools to unfold host-pathogens interactions.

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The complex of species composing the \textit{Spodoptera} genus includes some of the most important lepidopteran pests of agricultural crops in the world. We are particularly interested in two species, \textit{S. frugiperda} (fall armyworm) and \textit{S. littoralis} (cotton worm). Both are polyphagous and cause important damages in Central and South America for \textit{S. frugiperda} and in south of Europe for \textit{S. littoralis}. For both, populations may be controlled by a set of pathogens such as viruses, bacteria or parasitic wasps. In the laboratory, we focus on the densoviruses, the bacteria from the \textit{Photorhabdus} and \textit{Xenorhabdus} genera, and the parasitic wasp \textit{Hyposoter didymator} associated with a polydnavirus. Using genomic approaches, we aim at understanding the strategies these pathogens have developed to modulate or interfere with the transcriptional program of host cells in order to develop into the host (evasion of the host immune responses, entry into host cells). Thanks to genomic resources that we have developed, transcriptomic and proteomic approaches have been undertaken in our laboratory to understand the detail of the interactions between \textit{S. frugiperda} and these pathogens. The programs currently under investigation will be exposed.


Infection of the Mediterranean pest *Spodoptera littoralis* with the *Autographa californica* multiple nucleopolyhedrovirus, a tool to study the antiviral response of Lepidopterans

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*Spodoptera littoralis*, a Mediterranean insect pest is highly resistant to infection by the baculovirus AcMNPV. Oral infection of 2nd instar larvae require high viral dose to cause 50 % mortality of the insect population. Analysis of infected larvae showed that the insect immune system reacts to orally-acquired viral particles by encapsulating them in the insect midgut, blocking the propagation of the virus. *S. littoralis* fed orally with recombinant AcMNPV bearing genes from the *cys*-motif and *vankyrin* families of the immunosuppressive polydnavirus *Campoletis sonorensis* were highly susceptible to AcMNPV showing high mortality rates. Construction of GFP-tagged versions of the above recombinant baculoviruses indicated that the enhanced infectivity of these recombinant baculoviruses was due to enhanced accessibility and propagation of the viral particles through the insect body. Our data suggest that these effect is probably the result of the expression of specific polydnavirus genes affecting the insect immune system that blocked the baculovirus infection. We believe that this system opens up a new concept to enhance the use of baculoviruses for pest control and is an interesting model to study host-pathogen relationships using genomic tools.

Specific host and endosymbiont proteins are involved in the transmission of Tomato yellow leaf curl virus by the whitefly Bemisia tabaci

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Tomato yellow leaf curl virus (TYLCV) (Geminiviridae: Begomovirus) is exclusively vectored by the whitefly Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae). TYLCV transmission depends upon a 63-kDa GroEL protein produced by the vector's endosymbiotic bacteria. B. tabaci is a species complex comprising several genetically distinct biotypes that show different secondary-symbiont fauna. In Israel, the B biotype harbors Hamiltonella, and the Q biotype harbors Wolbachia and Arsenophonus. Both biotypes harbor Rickettsia and Portiera (the obligatory primary symbiont). The aim of this study was to determine which B. tabaci symbionts are involved in TYLCV transmission using cultures of B. tabaci populations collected in Israel. Virus-transmission assays by B. tabaci showed that the B biotype efficiently transmits the virus, while the Q biotype scarcely transmits it. Yeast two-hybrid and protein pull-down assays showed that while the GroEL protein produced by Hamiltonella interacts with TYLCV coat protein, GroEL produced by Rickettsia and Portiera does not. Employing in-vivo and in-vitro-synthesized GroEL proteins from all symbionts and artificial feeding through membranes showed that interaction between GroEL and TYLCV was found to occur in the B, but not Q biotype. Microarray analysis comparing viruliferous and non-viruliferous whiteflies from the B and the Q biotype identified several host proteins with possible roles in virus transmission. HSP70 was a strong candidate and was further investigated. Taken together, Hamiltonella GroEL and host proteins seem to affect virus transmission by B. tabaci, however, other symbionts from both biotypes do not seem to be essential for transmission of this virus.


Turning host-virus dynamics into an anti-viral approach in bees

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Bee colony losses, in particular Colony Collapse Disorder (CCD), have become a major global economic concern due to the dominant pollination contribution of these insects to a wide range of food crops. Israel Acute Paralysis Virus (IAPV) is a bee-affecting non-retroviral RNA dicistrovirus which has been strongly associated with CCD. A segment of IAPV was found to be incorporated into the bee's genome and bees harboring an integrated viral segment exhibit a virus resistant phenotype. The exchange of genetic information between IAPV and its host is reciprocal and a bee sequence was found fused to IAPV defective-RNA (dRNA) within purified virus particles. IAPV virions also carry other types of dRNAs: Some of them are recombinants of different genomic parts of IAPV and others are recombinants of IAPV and another dicistrovirus RNA. Interestingly, among some of the dRNAs population the sense oriented strand has recombined with its complement forming hairpin and stem-loop structures. Finally, we report on restraining IAPV infection by feeding bees with double-stranded RNA (dsRNA), as an efficient and applicative way of controlling this viral disease. The possible dynamics of reciprocal sequence exchange between IAPV and its host leading to association with CCD, as well as the potential of controlling bee diseases and CCD by recruiting RNAi-technology are discussed.